“Ik meet het succes van iemand niet aan hoe hoog hij klimt, maar aan hoe hoog hij weer opveert als hij de bodem raakt.” (George S. Patton)

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Bioactive compounds in leek (*Allium ampeloprasum* var. *porrum*): analysis as a function of the genetic diversity, harvest time and processing techniques

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences

Dutch translation of the title:

Bioactieve componenten in prei (*Allium ampeloprasum* var. *porrum*): analyse in functie van de genetische diversiteit, oogsttijdstip en verwerkingstechnieken

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ABBREVIATIONS

AA	L-Ascorbic Acid
AAPH	2,2'-azobis(2-methylpropionamidine) dihydrochloride
ACSO	*S*-alk(en)yl-L-Cysteine Sulfoxides
AD	Air-Drying
ANOVA	Analysis of Variance
AOAC	Association of Analytical Communities
AUC	Area Under the Curve
APS	5-adenylsulfate
APSR	APS reductase
ATPS	ATP sulfurylase
CHI	Chalcone Isomerase
CHS	Chalcone Synthase
DHA	L-dehydroascorbic acid
DP	Degree of Polimerisation
DPPH	2,2-Diphenyl-1-picrylhydrazyl
dw	dry weight
EL	Entire Leek
ET	Electron Transfer
FA	Flavonoid Aglycones
FAO	Food and Agriculture Organization
FD	Flavonoid Diglycosides
FD	Freeze-Drying
FDLP	Freeze-Dried Leek Powder
FM	Flavonoid Monoglycosides
FEH	Fructan Exohydrolase
FOS	Fructooligosaccharides
FT	Fructosyl Transferase
fw	fresh weight
FRAP	Ferric Reducing Antioxidant Power
GC	Gas Chromatography
GCS	Glutamylcysteine Synthase
GDP	Guanosine Diphosphate
GS	Glutathione Synthase
GSPC	γ-L-glutamyl-S-(trans-1-propenyl)-L-cysteine
HAT	Hydrogen Atom Transfer
HPAEC-PAD	High-Performance–Anion-Exchange Chromatography with Pulsed Amperometric Detection
HPLC	High-Performance Liquid Chromatography
I	Invertase
I	Isorhamnetin
I3G	Isorhamnetin 3-O-glucoside
ILVO	Institute for Agricultural and Fisheries Research
JHI	James Hutton Institute
K	Kaempferol

K3G	Kaempferol 3-O-glucoside
K4'M	Kaempferol 4'-methylether
LAB	Lactic Acid Bacteria
LAVA	Logistieke en Administratieve Veilingassociatie
Lb	*Lactobacillus*
LF	Lachrymatory Factor
LOD	Limit of detection
LOQ	Limit of Quantification
LTS	Long Term Storage
MALDI-TOF	matrix assisted laser desorption/ionisation time-of-flight
MAP	MestActiePlan
MRL	Maximum Residue Level
MRM	Multiple Selection Monitorings
MS	Mass Spectrometry
ms	Male Sterile
nd	Not Detected
N	Nitrogen
NMR	Nuclear Magnetic Resonance
OAS	O-acetylserine
OASTL	OAS thiollyase
OP	Open Pollinated
OPA	Ortho-phtaldiadehyde
ORAC	Oxygen Radical Absorbance Capacity
PA	Phenolic acids
PAL	Phenylalanine Ammonia Lyase
PCA	Principal Component Analysis
PL	Packaged Leek
Q	Quercetin
Q3G	Quercetin 3-O-glucoside
Q34'G	Quercetin 3,4'-O-diglucoside
ROS	Reactive Oxygen Species
RP	Reversed Phase
RWD	Refractance Window Drying
SAT	Acetyltransferase
SD	Standard Deviation
SiR	Sulfide Reductase
TLC	Thin Layer Chromatography
TP	Total Phenol
TPTZ	2,4,6-tripyridyl-S-triazine
USDA	United States Department of Agriculture
VITO	Vlaamse Instelling voor Technologisch Onderzoek
VLAM	Vlaams Centrum voor Agro- en Visserijmarketing
VLM	Vlaamse Landmaatschappij
WHO	World Health Organizatio

CHAPTER 1. GENERAL INTRODUCTION AND OBJECTIVES

1.1 General introduction

Leek (*Allium ampeloprasum* var. *porrum*) is predominantly a European crop with significant cultivation in Turkey (9000 ha), France (5800 ha), Belgium (4800 ha) and Poland (4400 ha). Although worldwide, Indonesia is the largest producer of leek as stated by FAO[1]. In Belgium, leek is one of the most important vegetables cultivated outdoors accounting for 16% of the total field agricultural production value. It is grown for its cylindrical pseudo stem, which is blanched white from growing underground and is made up of long leaf bases. The white shaft is used in many culinary preparations, whereas the green leaves are considered inferior and are, therefore, usually only used in soups or discarded during harvesting and processing of the fresh produce for the market. With regard to its health aspects, epidemiologic studies elucidated the reduction of the risk of prostate, colorectal, stomach and breast cancer upon the consumption of leek. These health benefits are linked to a range of phytochemicals, including 4 important chemical groups, *i.e*. (1) *S*-alk(en)yl-L-cysteine sulfoxides (2) polyphenols, (3) vitamins and (4) fructans.

The present study focuses on the analysis of these compounds in leek as a function of 3 main parameters, including genetic diversity, harvest time and processing/potential valorisation techniques. The results of the present study can recommend leek growers to use specific cultivars, types and practices to maximise their crop's content of specific health-promoting compounds. The availability of data on antioxidant levels of leek can be considered as an important criterion for selection of genotypes from a gene bank for use in crop improvement or other research-related or commercial activities. Therefore, an understanding of the health-promoting compounds of leek can lay the foundation to develop even healthier varieties. Additionally, the results of this dissertation can serve to recommend consumers how to maintain the maximum amount of antioxidants when preparing leek at home. Moreover, the study can stimulate the valorisation of the green leaves of leek or by-products of other plant material in general.

The outcome of this study can bridge the missing information about leeks, because many reports focus on health-promoting compounds in related *Allium* species, including onion, garlic and shallot. Therefore, the results reported in this dissertation are original and can be of importance for leek production in Flanders and beyond and as such strengthen its market position.

[1] Food and Agriculture Organization

1.2 Objectives

The objective of this PhD thesis is to develop knowledge on the presence of health-promoting compounds in leek in order to complement to the many reports focusing on these compounds in other related *Allium* species. The aim is, on the basis of novel scientific knowledge on health-promoting compounds in leek, to stimulate innovation in leek breeding, production, marketing and the consumption pattern of leek.
The specific research questions connected to this PhD research project were: (1) Which bioactive compounds are present in the white shaft and green leaves of leek? (2) Is there a difference in bioactive compound concentration among the range of current, commercial and old leek cultivars? (3) Is there a difference in leek type (summer, autumn winter) with regard to its antioxidant properties, harvested in their respective harvest season? (4) Does harvest time have an influence on the antioxidant concentration? (5) Where is leek, based on its content of health-promoting compounds, situated in the *Allium* genus? (6) Can we see a change in antioxidants upon post-harvest processing at the farm and refrigerated storage? (7) What is the amount of remaining antioxidants after domestic cooking processes? (8) How can we stabilise and valorise the amount of leek by-products generated during the currently used harvesting and processing methods? And finally, (9) what is the influence of these stabilisation processes on the content of antioxidants?

In this study, bioactive compounds (*S*-alk(en)yl-L-cysteine sulfoxides, polyphenols, vitamins and fructans) were analysed in leek as a function of different parameters, including genetic diversity, harvest time and processing/potential valorisation techniques. The schematic diagram in Figure 1.1 represents the different chapters within the present study and their coherence.

Chapter 2 gives a summary concerning the current position of leek production in Belgium and abroad. A distinction is made between leek as a crop and leek as a food product. Moreover, the presence and properties of bioactive compounds is discussed in Chapter 2.
Chapter 3 describes the experimental design, including the selected plant material, the sampling procedure and further sample preparation for each experiment. The analytical and statistical methods are discussed in this chapter as well.
Chapter 4 describes the influence of leek tissue and leek cultivar on content of different bioactive compounds present in the white shaft and green leaves. Thirty leek cultivars were investigated, a selection which was based on type of cultivar, manner of breeding and seed company.

Chapter 5 discusses the role of harvest time on the antioxidant properties of leek, in order to give further insight into the differences reported in Chapter 4. For this part, leek hybrids were harvested at 4 time points from September until March.

Chapter 6 presents the comparison between leek and some of its related species, such as onion, shallot, bunching (Welsh) onion, chives, Egyptian and Chinese leek with regard to the antioxidant properties. The outcome of this study can elucidate the position of leek within the *Allium* genus as several studies report high antioxidant levels in members of the same family of leek.

Chapter 7 focuses on the effect of post-harvest processing and storage on the antioxidant properties of leek. In this chapter, the antioxidants were measured from harvest until 13 days of refrigerated storage – 'from harvest to fridge'.

In addition, *Allium* species are usually consumed after a heat treatment, which can have an effect on the antioxidants. The application of domestic cooking processing was discussed in **Chapter 8** – 'from fridge to fork'.

Because of the restricted culinary application of the green leek leaves and the change in demands, a large part remains unused, resulting in a large quantity of valuable biomass. Ways to stabilise the green leaves are needed if further valorisation is envisaged. Therefore, the application of some alternative value-adding processing and preservation methods such as fermentations and drying were investigated in **Chapter 9**. Fermentation and drying were performed in different ways and compared with regard to the end antioxidant properties.

Chapter 10 provides a general discussion and perspectives for further research, while the summary at the end of this dissertation lists the most prominent results.

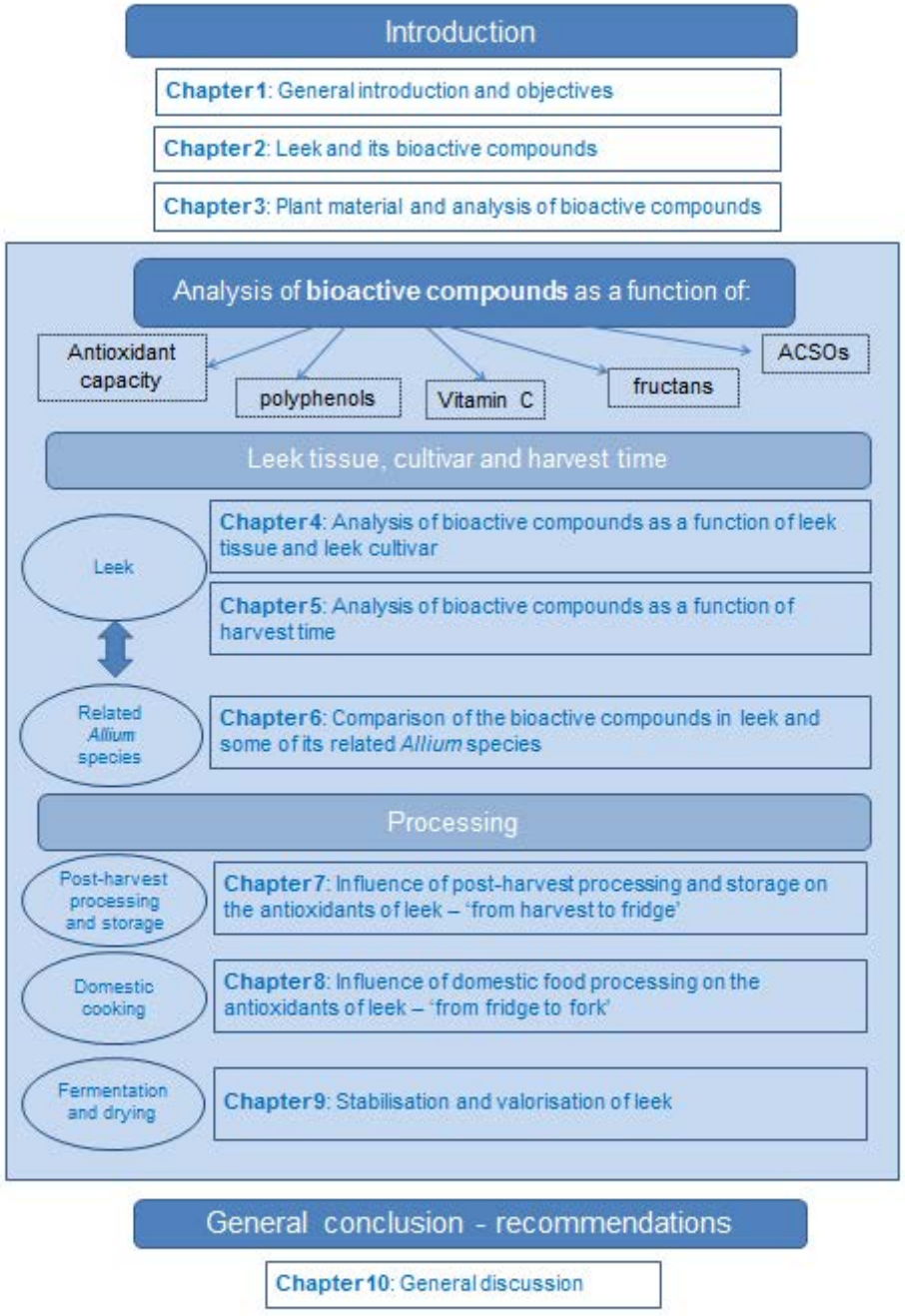


Figure 1.1 Structural organisation of the present study

CHAPTER 2. LEEK AND ITS BIOACTIVE COMPOUNDS

2.1 Introduction

Leek (*Allium ampeloprasum* var. *porrum*) belongs to the *Alliaceae* family and is one of the most important vegetables cultivated outdoors in Western Europe, especially in Belgium. In addition to their economic importance, they are a source of several phytochemicals, including 4 important chemical groups that have perceived benefits to human health, *i.e*. the *S*-alk(en)yl-L-cysteine sulfoxides, polyphenols, vitamins and fructans. In this chapter, 3 topics will be discussed, *i.e.* (1) leek as a crop, (2) leek as a food product and (3) the main bioactive compounds of leek.

2.2 Leek as a crop – from 'seed to harvest'

2.2.1 Taxonomy and history of leek

Leek is a monocotyledonous plant of the *Alliaceae* family and belongs to the *Allium* genus, a word which originates from the Greek "αλεω", which means to avoid, referring to its offensive smell (Boswell, 1983). The *Allium* genus, one of the largest plant genera, includes about 700 species comprising numerous economically important vegetables such as *A. cepa* (onion), *A. sativum* (garlic), *A. ampeloprasum* (elephant garlic), *A. fistulosum* (bunching onion), *A. schoenoprasum* (chive) and *A. ascalonicum* (shallot), primarily used for their unique flavours (Block, 2010). However, there are numerous of cultivated vegetable *Alliums* of more regional importance, including kurrat (*A. ampeloprasum* var. *kurrat*) which is eaten as a pickled leaf primarily in Egypt, rakkyo (*A. chinense*) and Chinese chives (*A. tuberosum)* (Jones and Mann, 1963). Leek *(A. ampeloprasum* var*. porrum*) and kurrat *(A. ampeloprasum* var*. kurrat)* are cultigens of wild forms of *A. ampeloprasum* and are therefore members of the same species. These wild forms differ from leek and kurrat by the production of a large bulb which consists of 2 cloves (Jones and Mann, 1963). Wild plants of this species occur in the Mediterranean area from Portugal and northwest Africa in the west to Turkey, Syria, northern Iraq and western Iran in the east (De Wilde-Duyfjes, 1976; Stearn, 1978). According to Masefield et al. (1969), *A. ampeloprasum* is also a native of the Atlantic islands of the Azores, Canaries, Cape Verdes and Madeira and possibly native in a few areas on the southern coasts and off-shore islands of England and Wales. Nowadays, *A. kurrat* is the Middle-Eastern cultivated leek, while *A. porrum* is predominantly a European crop.

2.2.2 Position of leek in agriculture and the market

Significant cultivation of leek in Europe is found in Turkey (9 000 ha), France (5 800 ha), Belgium (4 800 ha) and Poland (4 400 ha) (De Clercq et al., 2003; Block, 2010, Eurostat, 2012). In 2010, 23 300 ha were dedicated for cultivation of leek in Europe, where 1 ha typically comprises 150 000 leek plants (Eurostat, 2012). Worldwide, Indonesia is the largest producer of leek followed by Turkey, France, Belgium, China and Poland (FAO, 2012).

Figure 2.1 shows the evolution of the area used for cultivation of leek in Belgium from 1965 until 2011. Until the 1980s, leek was cultivated on approximately 3 000 ha, which increased significantly during the 1990s. From the year 2000, the cultivated area has stabilised around 4500 ha. Belgium's leek production did not increase significantly over the past 10 years, with the current production being 169 600 tonnes (Eurostat, 2012).

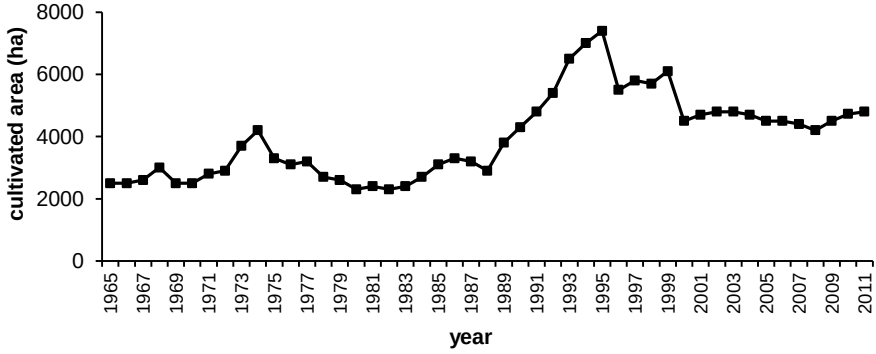


Figure 2.1 Cultivated area (ha) of leek in Belgium from 1965 until 2011 (Eurostat, 2012)

Nowadays, leek is one of the most important field vegetable crops in Belgium, accounting for 16% of the total field agricultural production value (Platteau et al., 2010). The main area of Belgiums leek production is situated in the region of West Flanders, comprising more than 75% of the leek production (Platteau et al., 2010).

In 2011, approximately half of the leek production (77 106 tonnes of fresh leeks) was exported from Belgium mainly to France, Denmark, Poland, Sweden and Spain, accounting for 8% of the fresh vegetable export value in Belgium (VLAM[2], 2011).

[2] Vlaams Centrum voor Agro-en Visserijmarketing

2.2.3 Cultivation of leek

Leek is grown in Belgium for its cylindrical pseudo stem, which is blanched white from being grown underground; it is made up of long leaf bases. The basal plate or disk, a much suppressed stem, is located at the base of the pseudo stem where its apical meristem gives rise to leaves (van der Meer and Hanelt, 1990). Leek is a biannual crop, which means that the plant develops in the first year and after vernalisation in winter it flowers in the second year. In commercial cropping, leek is grown as a short-lived annual (Burt, 2011).

During growth in the field, the relative growth rate of leek plants per effective degree-day is low compared to other vegetable species (Tsouvaltzis et al., 2010). The base temperature for growth of leek has been reported to be 5.9 ± 0.7 °C and the growth rates of leeks increase linearly with temperature between 6 and 20 °C (Brewster and Sutherland, 1993).

2.2.3.1 Leek breeding

Leek is a heterozygote cross-pollinated plant with 20% self-fertilisation (De Clercq et al., 2003). The first leek cultivars were **landraces** (the result of mass selection), propagated by open pollination (OP) and were highly variable in agronomic and morphological traits. The method of mass selection is shown in Figure 2.2. Cycles can be repeated until the desired result. Subsequently, local landraces or **breeder selections** (the result of mass selection and family selection), adapted to different climates and market demands, were developed in many European countries from Bulgaria to Ireland and in other parts of the world (*e.g.* Middle East).

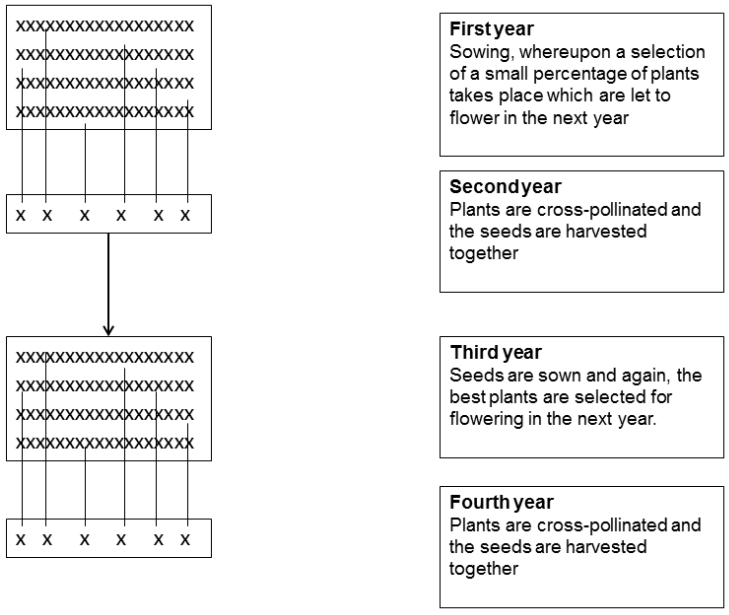


Figure 2.2 Positive mass selection (Engelen, 2003)

In the case of family selection, harvest of the seeds takes place on each plant separately. The result is called a 'half sib family', if the mother plant is known but not the father or a 'full sib family', when both mother and parent plants are known (personal communication De Clercq).

During the second half of the 20th century, many new leek cultivars were developed. These cultivars were maintained by OP after selection for specific traits, including winter-hardiness, long shafts, erectness of the leaves, dark leaf colour, disease resistance and uniformity and further referred as **commercial OP cultivars** (Havey and Leite, 1999). However, lack of uniformity was a major limiting factor in the marketability of the crop (Silvertand, 1996). Nowadays, F1 hybrids are gaining popularity among growers, due to their improved uniformity, higher yields and improved disease resistance compared with OP cultivars (De Clercq and Van Bockstaele, 2002). In order to maintain the uniformity of the crop, prevention of selfing in hybrid seed production is needed. Leek is, however, a self-incompatible crop. Therefore, it is advantageous to use plants that are male sterile (ms) in the production of **hybrids** of leek. Male sterile plants do not produce (or less) pollen. The combination of male sterile plants with a pollination population results in a first generation, called F1 hybrids. In fact, these F1 hybrids are pseudo-hybrids as it is the result of a controlled pollination.

The following steps are performed to obtain F1 leek hybrids (personal communication De Clercq):

1) Selection of the parent lines
    a. Tracing ms in F2-F3 and maintaining this seed parent via bulbils
    b. Selection of pollinator parent and maintaining via bulbils
2) Test crossings
    a. Production of top crosses: the specific combining ability of 1 male with several female lines is evaluated
3) Evaluation
    a. First evaluation of the new hybrids on 1 location
    b. Evaluation of the new hybrids on more locations

4) Introduction of a new cultivar, via scaling-up the number of plants for seed production

The first F1 hybrids*, i.e.* Parton, Upton and Carlton (Nunhems) were developed in 1995.

Leek types

The selection and breeding efforts have resulted in different types of leek cultivars, each specifically adapted for growth during a specific part of the leek production season (Table 2.1). More specifically, the cultivation of leek is divided according to the time of harvest, into 3 periods: summer, autumn and winter period. A further subdivision can be made into very early, early and normal summer, early and late autumn and normal winter and late winter cultivation (Silvertand, 1996).

Table 2.1 Distinctive features of leek types (De Clercq et al., 1999)

Distinctive features	**Leek types**		
	summer	**autumn**	**winter**
Leaf colour	yellow-green	pale-green	blue-green
Shaft length	long (30-50 cm)	medium (24-29 cm)	short (18-23 cm)
Maturity	very fast growing	fast growing	slow growing
Winter hardiness	no tolerance to frost	some tolerance to frost	good tolerance to frost

2.2.3.2 Sowing of leek

Seeds of leek have an angular shape and are black coloured (De Clercq, 2008). 1 gramme contains 300 to 400 seeds (Grubben and Denton, 2004). Sowing starts most of the time indoors as hard frost can be detrimental for young plants. The sowing method is pneumatic with precision seed (1.8-2.0 mm, germinative power of minimum 90%), whereupon seeds are covered with 1.5 cm of soil (personal communication De Clercq). Sowing of summer leek cultivars starts in December and continues until March. The autumn cultivars are sown in March and the winter cultivars from April to the middle of May.

2.2.3.3 Transplanting leek

After 10 to 12 weeks upon sowing, a selection of the young plants (thickness 5-10 mm) are transplanted to the production field. Three ways of planting are established in Belgium: (1) the gross of the leek in West Flanders is planted on ridges (1 row of leek plants for each ridge). Growth on ridges is preferred because of the fast growth due to the fast heating-up of the soil. Moreover growth on ridges gives the opportunity for deeper planting, which allows the production of a longer white shaft. (2) It is also possible to grow leek on a flat field, which need to be earthed up during growth. (3) Typical in the region of Mechelen is the growth of leek on beds (1.2 m width and 30 cm height, 4 rows of leek plants for each bed), without the need of earthing up.

2.2.3.4 Soil and fertilisation

Leeks may be grown successfully on a wide range of soil types but because of the unbranched roots, sandy loam soils are the most suitable for plant growth in Belgium (sandy clay soils in the Netherlands) and result in above average yields (Silvertand, 1996). Soil pH of at least 5.8 is most desirable. The soil should be prepared with green manure ploughed down or farmyard manure to enhance organic content and provide nutrients and extra moisture holding ability for the crop. Leeks require about 200-250 kg nitrogen (N) hectare^{-1}. Phosphate requirements of leeks are not very substantial and applications of 50 to 100 kg P_2O_5 hectare^{-1} are adequate. Potassium requirements are moderate and 150 to 200 kg K_2O hectare^{-1} as sulfate of potassium is adequate (Baker, 1998). MestActiePlan (MAP) in Flanders requires a maximum of 320 kg N hectare^{-1} year^{-1} and 75 kg P_2O_5 hectare^{-1} year^{-1} for the cultivation of leek (VLM, 2012).

2.2.3.5 Pests and diseases

Leek can be attacked by many pests and diseases. Onion fly (*Delia antique*), thrips (*Thrips tabaci*) and leek moth (*Acrolepiosis essectella*) are the most important pests. Rust (*Puccinnia allii*), white tip (*Phytophthora porri*) and purple blotch (*Alternaria porri*), are the diseases most frequently observed in leek (Silvertand, 1996). Leaf blotch (*Cladosporium allii-porrii)* and black stripe (*Leptotrochila porri*) are more rare (personal communication De Clercq).

2.2.3.6 Harvest and storage

When leek plants are mature, harvest can take place. In Belgium, the summer leek types are harvested from June until October, the autumn types from September until January and the winter types from January until April. Physical size should meet market requirements for thickness and length.
Different leek harvest machines are developed over the years, from a simple lifting knife to a semi-automatic lifter-harvester machine, with the possibility to shorten the leaves and cut off the roots in the field. For processing purposes (frozen industry), leek is trimmed on the field followed by cutting the roots. For the fresh market, leeks are harvested without trimming, which will take place at the farm (personal communication De Clercq).

In order to fill up the gap in the sales market during May and June, late winter leek plants are harvested in April and stored in cold rooms. Each subsequent week, a part is taken out to clean and pack for sale (personal communication De Clercq). Alternatively, freshly harvested leek plants are imported from southern European countries (Silvertand, 1996). As a result, leek is supplied to the fresh market year-round.

2.3 Leek as a food product – from ‘harvest to fork’

2.3.1 Preparation for the market

2.3.1.1 Peeling, washing and sorting

Firstly, the outer leaves are removed at the farm and the leeks are placed on a transporting belt, going through a washing line. Simultaneously, the leaves and roots are trimmed. For fresh market delivery, leek is commonly packaged in 10 kg boxes, sorted for uniform thickness and colour. The length is about 55 cm in cages of 60 cm (personal communication De Clercq).

As a result, leek production is faced with biomass wastes and by-products, especially linked to the green leaves. Two fractions of the unused green leaves can be established, a first fraction, which is left behind on the field during harvesting (especially leek for industry), while a second fraction is removed during processing at the farm. For example, Groentenhof (Bornem, Belgium), growing 50 ha of leek, obtained a waste of green leaves ranging from 400 to 600 tonnes of green leaves a year (personal communication Croket). This amount of waste corresponds with 8 to 12 tonnes per hectare. When extrapolated to the Belgian leek production (4800 ha), it means 38 400 to 57 600 tonnes in 2012. This biomass is usually brought back onto the field, however, this large quantity of plant biomass could be valorised given an adequate stabilisation method and an identified use of the derived product.

2.3.1.2 Fresh-cut leek

The demand for fresh-cut vegetables, both for retail and food service applications, has grown tremendously over the past few years and has led to an increase in the quantity and variety of products available for the consumers. Leek may provide a challenge as a minimally processed vegetable, because it is a vegetable that requires minimal processing before consumption (Hong and Kim, 2001). Attempts have been made to develop minimally processed leek. This involves removal of roots and decayed leaves, and pseudo stem trimming to different sizes. However, the major problems that arise are inner leaf extension, discolouration of the cut surface as well as fresh weight loss, which causes a rapid loss in the overall market quality of the product (Tsouvaltzis et al., 2007; Tsouvaltzis et al., 2010).

2.3.2 Commercialisation

Leek production falls into 2 segments: leek for the fresh market and leek for the food processing industry. Around 80% of harvested leek plants are sold for direct consumption, whilst 20% is stored under refrigeration, frozen, dried or destined for the preparation of ready-made dishes (VLAM, 2012). A lot of companies in Belgium produce frozen vegetables and commercialise leek and leek derived frozen products, such as Ardo, Pinguin-Lutosa, Dujardin Foods, d'Arta, Pasfrost, Begro, Dejaeghere, Dicogel, Hesbayefrost, Horafrost. Most of these companies are concentrated in West Flanders, and are grouped in VeGeBe (Vereniging van Groenteverwerkende Bedrijven). Leek plants for the fresh market are mostly sold at vegetables auctions and subsequently find their way to the consumer via markets and the retail sector. Four vegetable auctions are situated in Belgium including BelOrta (Zellik, Kampenhout and Sint-Katelijne-Waver, a

fusion of Mechelse Veilingen and Coöbra), REO veiling (Roeselare), Veiling In-Co (Hoogstraten) and Limburgse Tuinbouwveiling (Herk-de-Stad) (LAVA[3], 2012). Only these auctions offer the Flandria quality mark. Flandria is the Belgian quality label for fruits and vegetables. Since its inception in 1995, the Flandria label has given added value to fresh products. This seal of approval guarantees quality and freshness plus concern and care for the environment during their cultivation (LAVA, 2012). This Belgian quality mark is comparable with the Q&S (Qualität und Sicherheit) system in Germany.
Table 2.2 shows the current assortment of leek on the Belgian and Dutch market. A distinction is made between the fresh and the frozen market.

Demands for leek specifications differ from country to country (Neefs and Meulemeester, 2010). In Denmark, for example, thin leek is required and especially organically grown leek. In Japan, on the other hand, thick leek shafts are preferred (personal communication, De Neef). In England, leek leaves are cut away and only pseudo stalks are meant for trade. In many other countries including Poland, whole leek plants with leaves are a marketable product where the leek leaves are used as an addition to soups or as a component of vegetable salads (De Clercq et al., 1999). Germany and Japan have high requirements concerning pesticide residue levels (Neefs and Meulemeester, 2010). In addition to the demands for each country, each individual buyer has its own wishes with regard to packaging, size, quality and pesticide residue.

[3] Logistieke en Administratieve Veilingassociatie

Table 2.2 Assortment of leek in Belgium and the Netherlands (Neefs and Meulemeester, 2010; van den Elzen, 2012; d'Arta, 2012; Ardo, 2012)

Fresh			Frozen
Loose	**Packaged**	**Chopped**	**Chopped**
 **Entire leek** Length: 56 cm With or without roots	 **Flow pack** Length: 20 cm, 36 cm, 40 cm, 56 cm 1 or more plants in 1 bag With or without roots	 **White-green** 4 mm, 10 mm	**White-green** (40/60-50/50-70/30) **White leek** rings (10 mm, 15 mm) cubes
 **Semi-entire leek** Length: 40 cm, 36 cm With or without roots	 **Bundle** Length: 56 cm		
 **White shaft** Length: 20 cm	 **Netting bag** Length: 56 cm Weight: 2 kg, 3 kg, 5 kg, 10 kg With or without roots		
	 **EPS box**		
	 **Carton box**		
	 **Plastic**		
	 **IFCO**		

2.3.3 Trends

Three important lifestyle trends have led to a remarkable evolution in leek production, commercialisation and consumption: (a) the demand for convenience as a result of the increasing small families, their limited time because of a time consuming job, living in a small apartments etc.; (b) consumers wish to cook with top-quality products when devoting the time to cooking. Cooking programmes on television have a great influence on this trend; (c) frequency of dining out has increased. These trends were the onset for a diversification in leek production, such as selling the white shaft separately. But also the development of the Flandria label for leek is a result of these different trends (Neefs and Meulemeester, 2010).

2.3.4 Consumption

Leek is usually consumed after a cooking process, such as in soups, oven dishes, stewed with béchamel sauce etc., but leek can also be consumed in fresh state in salads (Compernol and De Ryck, 2011). In the bachelor thesis of Compernol and De Ryck (2011), 618 Belgian persons were subjected to a survey concerning the habits and preferences of consumption of leek. Seven percent of the participants do not eat leek at all, mainly because of the taste. In total, 23% of the leek consuming group eats leek once per year, 45% once in a month, and 26% eat leek once in a week. A very small percentage of the group (6%) consume leek twice a week or even more. One-fourth of the participants only consume the white shaft of leek, mainly because of the toughness and bitterness of the green leaves. The leek consuming group buy leek, mostly in the fresh state as the entire leek plant, while a little part bought a fresh, washed, chopped or frozen mixture. Only 4% of the participants claimed to buy leek as plastic packaged fresh white part.

Information on consumption quantities of vegetables and especially of leek is limited, although data can be found on the purchased amount. Table 2.3 shows the average amount of individual vegetable, including leek, bought in 2009 and 2010 in Belgium, expressed as kg capita^{-1}.

Table 2.3 Average weight of vegetable purchased per capita in Belgium, in 2009 and 2010 (VLAM, 2011)

Vegetable	2009 (kg capita^{-1})	2010 (kg capita^{-1})
Tomato	10.44	9.91
Carrot	9.55	9.58
Onion	6.88	6.60
Chicory	6.34	6.25
Lettuce	3.73	4.04
Leek	**2.70**	**3.10**
Mushroom	2.09	2.34
Pepper	2.07	2.29
Cauliflower	1.85	1.92
Courgette	1.84	1.78

Table 2.3 demonstrates the increasing importance of leek, in addition to the top 5 vegetables (tomato, carrot, onion, chicory and lettuce) (VLAM, 2011). In 2009 and 2010, 2.70 kg and 3.10 kg leek was purchased per capita in Belgium, which was higher than the purchase of cauliflower and courgette but lower than tomatoes, carrots and onions. In 2010, leek represented 5.0% of the volume of vegetables used in the consumer's kitchen (Neefs and Meulemeester, 2010). In comparison, in Germany the purchase of leek in 2010 was 1.3 kg capita^{-1} (VLAM, 2011).

2.4 Chemical composition of leek and its bioactive compounds

The main components of 100 g fresh leek include, in addition to water, carbohydrates (5.0-11.2 g), proteins (1.6-2.2 g), fat (0.1-0.4 g), dietary fiber (1.0-2.3 g) and mineral components such as K (248-347 mg), Ca (48-75 mg), Mg (10-11 mg), Na (5-9 mg), Cu (0.06-0.30 mg), as well as vitamins, such as vitamin C and vitamins of the B group and other secondary metabolites, which can have a positive influence on human health (Grzelak-Blaszczyk et al., 2011).

Research on the nutritive and health benefits of leek is scarce but the medicinal value of the other *Alliaceae* (*e.g.* garlic and onion), has been recognised for thousands of years (Carson, 1987; Augusti, 1990; Lawson, 1998). Eating raw onions helps to reduce cholesterol levels because they increase levels of high-density lipoproteins; it also helps to control coronary heart disease, thrombosis and blood pressure. Onions are also used in the treatment of anaemia, urinary disorders, bleeding piles and teeth disorders.

Several researchers have found that onion has an anti-cancer effect, platelet anti-aggregating agent, anti-hypercholesterolemia, anti-ulcer and anti-gastric cancer activity (Mitra et al., 2012). Moreover, epidemiologic studies elucidated the reduction of the risk of prostate, colorectal, stomach and breast cancer upon the consumption of *Allium* species, including leek (Bianchini and Vainio, 2001; Hsing et al., 2002). Moreover, a population case-control study (238 case subjects and 471 control subjects) conducted in Shanghai, revealed a significantly lower risk of prostate cancer upon consumption of leek more than 10 g day^{-1} compared to a consumption less than 2.2 g day^{-1} (Bianchini and Vainio, 2001; Hsing et al., 2002).

These health benefits are attributed to a range of bioactive compounds present in *Allium* species (Havey et al., 2004). Bioactive compounds are extra nutritional constituents that typically occur in small quantities in food, which vary widely in chemical structure and function and are beneficial to consumer's health (Kris-Etherton et al., 2002). Four classes of bioactive compounds are found in *Allium* species. **Organosulfur compounds (including *S*-alk(en)yl-L-cysteine sulfoxides),** responsible for the typical organoleptic parameters of the species in the *Allium* genus, are implicated as contributing in part to the *Allium* health-promoting properties (Singh and Shukla, 1998; Mostafa et al., 2000; Yin et al., 2002; Xiao et al., 2005; Lanzotti, 2006). In addition, **polyphenolic compounds** are another group of secondary metabolites who constitute a major class of phytochemicals found in *Alliums* and consist of 3 major subclasses, that is phenolic acids (and their derivatives), flavonoids (and their glycosides), and flavonoid polymers (proanthocyanidins or condensed tannins) (Grotewold, 2006). *Allium* species also contain significant levels of **vitamin C**, the most important vitamin in fruits and vegetables for human nutrition and **fructans**, oligo- and polysaccharides consisting of short chains of fructose units with a single D-glucosyl unit at the non-reducing end.

These four groups of bioactive compounds in *Allium* species will be discussed in the next sections. Although not discussed, lutein, β-carotene, vitamin E and B may also contribute to the health-promoting properties of these species (Hart and Scott, 1995; Proteggente et al., 2002; Muir et al., 2007).

2.4.1 The *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs)

Allium vegetables have been shown to have beneficial effects against several diseases, including cancer (Bianchini and Vainio, 2001; Hsing et al., 2002). The protective effect appears to be partly related to the presence of organosulfur compounds and mainly allyl derivates, which inhibit carcinogenesis in the forestomach, oesophagus, colon, mammary gland and lung of experimental animals. Organosulfur compounds modulate the activity of several metabolizing enzymes that activate (cytochrome P450s) or detoxify

(glutathione S-transferases) carcinogens and inhibit the formation of DNA adducts in several target tissues (Bianchini and Vainio, 2001).
All *Allium* species produce sulfur-containing compounds. Up to a few percent of the dry weight may be non-protein sulfur amino acids, better known as *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs). These amino acids are enzymatically formed by hydrolysis of the corresponding γ-glutamyl-*S*-alk(en)ylcysteine storage dipeptides (Lancaster and Shaw, 1989). It has been estimated that approximately 75% of the sulfur in *Allium* species occurs as ACSOs or the storage form γ-glutamyl-ACSOs. These non-volatile, odourless ACSOs are the precursors both of flavours, odours and the lachrymatory factor (LF), *i.e.* propanethial sulfoxide (Griffiths et al., 2002; Bloem et al., 2004; Jones et al., 2004).

When the tissue of *Allium* species is damaged, ACSOs are cleaved by the endogenous enzymes alliinase and lachrymatory factor synthase, to yield unstable alk(en)yl sulfenic acids, pyruvic acid and ammonia. Alk(en)yl sulfenic acids rearrange non-enzymatically to form thiosulfinates that contribute to the flavour perceived. Pyruvic acid and ammonia are non-flavour products of the enzymatic reaction (Lancaster and Kelly, 1983) (Figure 2.3). The lachrymatory factor is formed only from 1-propenesulfinic acid following from hydrolysis of the ACSO *trans*-*S*-1-propenyl-L-cysteine sulfoxide. The LF is highly reactive and hydrolyses to propionaldehyde, sulfuric acid and hydrogen sulfide. It is also the precursor of several sulfur derivatives (Bianchini and Vainio, 2001).

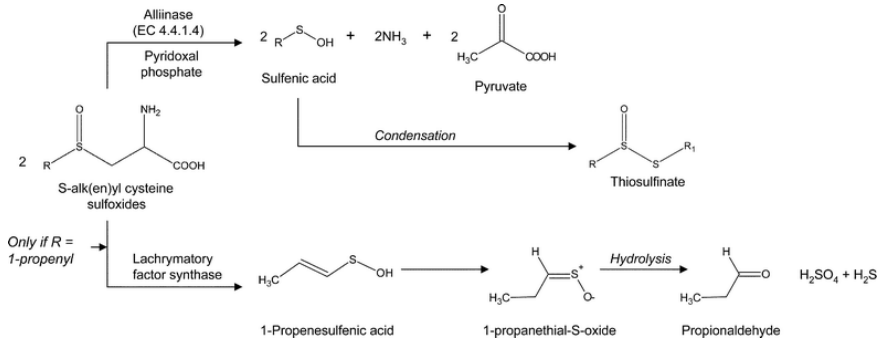
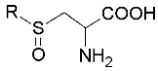


Figure 2.3 Formation of flavour compounds in *Allium* species from the precursors *S*-alk(en)yl-L-cysteine sulfoxides; with 1-propanethial-S-oxide = lachrymatory factor (Rose et al., 2005)

ACSOs occur in many plant families, such as *Alliaceae* and *Brassicaceae* vegetables, but also in fungi and algae (Kubec and Dadakova, 2008). Ten ACSOs have been reported in *Allium* species (Table 2.4), *i.e.* (1) *S*-methyl-L-cysteine sulfoxide (methiin, present in *Alliaceae* and some *Brassicaceae*), (2) *S*-propyl-L-cysteine sulfoxide (propiin), (3) *trans*-*S*-1-propenyl-L-cysteine sulfoxide (isoalliin; characteristic for onion), (4) *S*-(2-

propenyl)-L-cysteine sulfoxide (alliin; characteristic for garlic), (5) *S*-ethyl-L-cysteine sulfoxide (ethiin), (6) *S*-butyl-L-cysteine sulfoxide (butiin), (7) *S*-(3-pentenyl)-L-cysteine sulfoxide, (8) *S*-(1-butenyl)-L-cysteine sulfoxide (homoisoalliin), (9) *S*-(methylthiomethyl)-L-cysteine sulfoxide (marasmin) and (10) *S*-(2-pyrrolyl)-L-cysteine sullfoxide (Kubec et al., 2000; Kubec et al., 2002; Dini et al., 2008; Kubec et al., 2010; Kubec et al., 2011; Kucerova et al., 2011). The content of some of these compounds in *Allium* species is presented in Table 2.5.

Table 2.4 Chemical structure of 10 identified *S*-alk(en)yl-L-cysteine sulfoxides and their presence in *Allium* species

General structure	Identification subgroup	Trivial name	*Allium* species	Ref.
	$R = CH_3$	methiin	leek, garlic, onion, Chinese chive, bunching onion	Lundegardh et al. (2008); Yoo and Pike (1998); Kubec et al. (2000); Kubec and Dadakova (2008); Yamazaki et al. (2011)
	$R = CH_2CH_3$	ethiin	*Allium cepa* var. *tropeana*	Dini et al. (2008)
	$R = CH_2CH_2CH_3$	propiin	leek, onion	Kubec and Dadakova (2008); Hovius and Goldman (2005)
	$R = CH_2CH=CH_2$	alliin	leek, garlic, Chinese chive, onion	Kubec and Dadakova (2008); Yamazaki et al. (2011); Hovius and Goldman (2005)
	$R = CH=CHCH_3$	isoalliin	leek, garlic, onion, Chinese chive, bunching onion	Lundegardh et al. (2008); Kubec et al. (2000); Kubec et al. (2000); Yoo and Pike (1998); Kubec and Dadakova (2008); Yamazaki et al. (2011)
	$R = CH_2CH_2CH_2CH_3$	butiin	*Allium cepa* var. *tropeana*	Dini et al. (2008)
	$R = CH_2CH_2CHCHCH_3$	*S*-(3-pentenyl)-L-cysteine sulfoxide	*Allium cepa* var. *tropeana*	Dini et al. (2008)
	$R = CHCHCH_2CH_3$	homoisoalliin	*Allium siculum*	Kubec et al. (2010)
	$R = CH_2SCH_3$	marasmin	*Allium stipitatum*	Kubec et al. (2011)
	R = 	*S*-(2-pyrrolyl)-L-cysteine sulfoxide	*Allium giganteum*	Kucerova et al. (2011)

Most *Allium* species do not contain all of these ACSO compounds and the composition is species-specific (Lancaster and Kelly, 1983; Yoo and Pike, 1998). It is also found that

alliinase acts quickly, but differently on the individual ACSOs, such that some of the flavour precursors are more completely degraded than others (Lancaster et al., 1998).

Table 2.5 Content of ACSOs in different *Allium* species

***Allium* species**	**tissue**	**isoalliin**		**alliin**		**methiin**	
		mg g^{-1} dw	**Ref.**	**mg g^{-1} dw**	**Ref.**	**mg g^{-1} dw**	**Ref.**
Leek	shaft	23.0	Lundegardh et al. (2008)			1.5	Lundegardh et al. (2008)
		37.4	Yamazaki et al. (2011)			7.7	Yamazaki et al. (2011)
Garlic	cloves	1.3	Yamazaki et al. (2011)	21.7	Yamazaki et al. (2011)	3.9	Yamazaki et al. (2011)
Onion	bulb	21.1	Yamazaki et al. (2011)			3.5	Yamazaki et al. (2011)
Bunching onion	leaves	34.8	Yamazaki et al. (2011)			7.1	Yamazaki et al. (2011)

Only isoalliin and methiin are found in leek (Lundegardh et al., 2008; Kubec and Dadakova, 2008). However, in the study of Kubec and Dadakova (2008), propiin and alliin are reported to be present in trace amounts. Yamazaki et al. (2011) determined the distribution of flavour precursors in 7 *Allium* vegetables including leek, and only found isoalliin, methiin and cycloalliin in significant amounts. However, cycloalliin does not contribute to flavour because of the absence of alliinase sensitivity.

2.4.1.1 Biosynthesis

Figure 2.4 shows the biosynthesis pathway of *S*-alk(en)yl-L-cysteine sulfoxides in *Allium* species (Leustek and Saito, 1999; Masamura et al., 2011). However, there is still considerable uncertainty about several stages, the relationship between ACSOs and γ-glutamyl peptides (γGP) relatives and whether the same pathway is followed in all tissues (Jones et al., 2004).

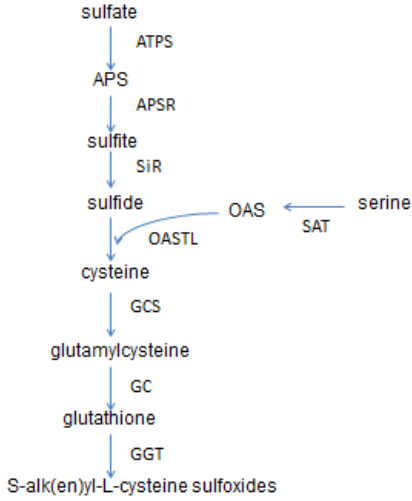


Figure 2.4 Biosynthesis of *S*-alk(en)yl-L-cysteine sulfoxides in *Alliums* (Jones et al., 2004)

Initially, SO_4^{2-} (sulfate) is transported across the root plasma membrane, whereupon it accumulates within plant cells. In order for SO_4^{2-} to be utilised for cysteine biosynthesis, it must be converted to the intermediate compound 5-adenylsulfate (APS). This reaction is catalysed by the enzyme ATP sulfurylase (ATPS) in plastids. APS can then be used by APS reductase (APSR) to form sulfite prior to its conversion to sulfide by the enzyme sulfide reductase (SiR). Next, cysteine is formed from the reaction of sulfide with O-acetylserine (OAS), a process catalysed by the enzyme OAS thiol-lyase (OASTL). OAS is derived from the acetylation of serine by the action of the enzyme serine acetyltransferase (SAT). Some of the cysteine is exchanged for glutathione catalysed by glutamylcysteine synthase (GCS) and glutathione synthase (GS). Subsequently, some of the cysteine and synthesised glutathione forms ACSOs through a chain reaction including decarboxylation, oxidation, and transpeptidation. Lancaster and Shaw (1989) suggested that the biosynthesis of ACSOs proceeds in onion via γ-glutamyl peptide intermediates (Masamura et al., 2011). Two possible pathways are proposed to obtain ACSO from glutathione or cysteine (Figure 2.5) (Jones et al., 2004). Pathway (a) illustrates the participation of glutathione, which is methylated, and then through loss of glycine, oxidation and finally loss of the γ-glutamyl group converted to methyl cysteine sulfoxide. Pathway (b) shows an alternative route via direct methylation of O-acetyl serine to yield methyl cysteine sulfoxide (Jones et al., 2004).

(a) (b)

Figure 2.5 Two proposed pathways for the biosynthesis of methyl cysteine sulfoxide (Jones et al., 2004)

There is limited information about where the flavour precursors are synthesised in the plant cell (Figure 2.6) (Jones et al., 2004). Derived from studies on onion, glutathione was identified in the chloroplasts and cytoplasm, while ACSOs and γGPs were located within the cytoplasm only (Lancaster and Shaw, 1989).

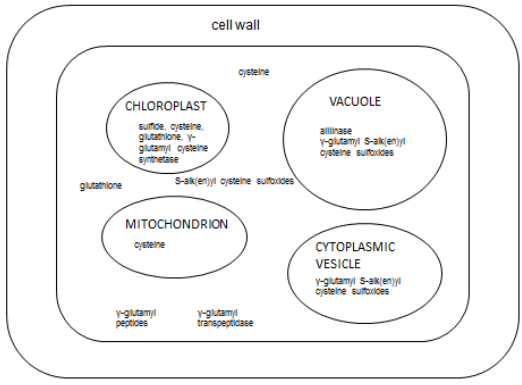


Figure 2.6 Subcellular location of biosynthetic intermediates, flavour precursors and alliinase in *Alliums* (Jones et al., 2004)

2.4.1.2 Physiological role in the plant

ACSOs play a role in defence against pests and predation, particularly in the overwintering bulb. Cysteine sulfoxides in combination with the enzyme alliinase are thought to be responsible for chemical protection against herbivores (Keusgen et al., 2002). High levels of cysteine sulfoxides have also been shown to have antibacterial and antifungal properties which are probably beneficial during extreme biotic conditions (Fritsch and Keusgen, 2006). In addition, they function as carbon, nitrogen and sulfur storage and transport (Lancaster and Boland, 1990; Jones et al., 2004).

2.4.1.3 Factors influencing the ACSO content

Several factors can influence the *Allium* ACSO content including biotic and abiotic parameters, which are discussed below.

2.4.1.3.1 Cultivar and tissue type

Differences in ACSOs exist among onion (*Allium cepa* L.) cultivars when grown under similar conditions. These differences can be attributed to the heterogeneity found within the *Allium cepa* germplasm (Kopsell et al., 1999; Lee et al., 2009). Moreover, the content of flavour precursor compounds in onion is significantly influenced by cultivar × location interaction (Lancaster et al., 1988; Lee et al., 2009).
In addition, there are differences in ACSO levels in one plant. Leaves of onion contain more methiin than the bulbs. Bulbs will contain more isoalliin than the roots and leaves. The outer fleshy layers and the top and bottom sections of the onion bulb contain the highest content of ACSOs. The lowest levels are observed in the dry brown skin (Bacon et al., 1999). Total ACSO concentrations in leek (cv. Tadorna) decrease acropetally and mature upper leek leaves contain lower levels of ACSOs (Doran et al., 2007).

2.4.1.3.2 Pre-harvest factors

Fertilisation. Mineral fertilisation (N 190 kg ha^{-1} and S 21 kg ha^{-1}) is responsible for an increase in ACSO levels in leek by 37% compared to unfertilized leek, whereas fertilisation with direct incorporation of red clover (*Trifolium pratense* L.), mulch, or red clover biodigestate (anaerobically digested red clover biomass) has no influence on the ACSO level of leek (Lundegardh et al., 2008). SO_4^{2-} and nitrogen availability are known to have an influence on onion flavour, thus also on the ACSO content, as ACSOs play a role in flavour production (Randle et al., 1995; Randle, 2000). It is known that sulfur

supply negatively influences the APS reductase (APSR) activity at bulbing of onion, while the ATP sulfurylase (ATPS) is positively influenced (Thomas et al., 2011).

Temperature. The total ACSO content and the individual ACSO content in onions (cv. Granex 33) increase linearly in response to a higher temperature. The ACSO content of onion bulbs grown at 15.6 °C were roughly a third of those growing at 32.2 °C (Coolong and Randle, 2003).

Irrigation. Irrigation regime influences onion flavour (Randle et al., 1995; Randle, 2000).

Light. Onions grown under normal long-day conditions or under short-day conditions, extended with incandescent light, show an increase in ACSO levels in the leaves in early development, followed by a decline in the leaves and a simultaneous increase in the bulbs.

Harvest time. To our knowledge, no studies report the influence of harvest time on ACSO content in *Allium* species.

2.4.1.3.3 Post-harvest factors

In *Alliums*, most of the sulfur is stored in the form of ACSOs. This sulfur is taken up from the soil by the roots as sulfate and thus, after harvest, no further increase can occur. If there is an increase, it should be the result of rearrangement of total sulfur to form ACSOs (Bacon et al., 1999).

Storage. The level of methiin in different onion cultivars decreases significantly upon 6 months of cool storage, while isoalliin content increases, but differences are cultivar dependent. The increase in isoalliin upon storage suggests an increased γ-glutamyl transpeptidase activity, as in the biosynthetic flavour pathway, γ-glutamyl transpeptidase is responsible for the hydrolysis of the γ-glutamyl moiety from γ-L-glutamyl-S-(1-propenyl)-L-cysteine sulfoxide to produce isoalliin (Kopsell et al., 1999). In another study, methiin concentrations remained unchanged when onion bulbs were stored at 5 °C, 24 °C or 30 °C for 5 months, while isoalliin continuously increased upon storage at 5 °C (Yoo et al., 2012).

Processing. Blanching garlic in hot water (90 °C for 5 min) hardly affect the individual organosulfur compound content, while fermentation and packaging steps negatively affect the levels of the ACSOs in garlic (Beato et al., 2012).

2.4.1.4 Analysis of ACSOs: state of the art

Numerous methods for quantitative determination of ACSOs have been developed. A leading role among these methods plays high-performance liquid chromatography (HPLC) determination after precolumn derivatisation, with ortho-phthaldialdehyde/tertbutylthiol being the most frequently used derivatisation reagent (Krest et al., 2000). Alternatively, ACSOs can be quantified by gas chromatography (GC) after derivatisation with ethyl chloroformate and reduction of the thermolabile sulfoxide group by sodium iodide (Kubec et al., 2000). ACSOs can also be determined by capillary electrophoresis. This method is based on extraction of these sulfur amino acids by methanol, their derivatisation by fluorenylmethyl chloroformate and subsequent separation by micellar electrokinetic capillary chromatography (Kubec and Dadakova, 2008).

2.4.1.5 ACSOs and health

The ACSO compounds and their derivatives show a number of activities that are beneficial to human health, such as antidiabetic, antihyperglycemic and antiplatelet activities (Kumari et al., 1995; Romanramos et al., 1995; Ali et al., 1999). The degree of the respective contributions of these organosulfur compounds to bioactivity depend on their alkyl moieties, given in Table 2.4 (Yamazaki et al., 2011).

Supplements. Regulid (Figure 2.7), a dietary supplement commercialised by BioXtract, contains allyl sulfides and vinyldithiins coming from garlic (*Allium sativum* L.). In garlic, allicin is formed upon the reaction of alliin with alliinase. Allicin is the main precursor of various other transformation compounds, such as allyl sulfides and vinyldithiins. BioXtract uses allyl sulfides to stabilise human weight after a diet and avoid yo-yo dieting but also uses to help preventing the metabolic syndrome (BioXtract, 2012).

Figure 2.7 Organosulfur supplement, extracted from garlic (BioXtract, 2012)

2.4.2 Polyphenolic compounds

In addition, a wealth of other classes of compounds, such as polyphenolic compounds, also referred as polyphenols, are also suggested to contribute to the health-promoting properties of the *Allium* species (Lanzotti, 2006). Polyphenolic compounds are secondary metabolites who constitute a major class of phytochemicals found in plants (Erdman et al., 2007) and may contribute to the bitterness, astringency, colour, flavour and odour of the products (Naczk and Shahidi, 2004). They are synthesised by plants during normal development and in response to stress conditions such as infection, wounding and UV radiation (Beckman, 2000). Polyphenolic compounds exhibit a wide range of physiological properties such as anti-allergenic, anti-artherogenic, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, cardioprotective and vascodilatory effects (Benavente-Garcia et al., 1997; Samman et al., 1998; Manach et al., 2005). Over 8000 polyphenolic compounds have been isolated from different natural products and consist of 3 major subclasses: **phenolic acids** (and their derivatives), **flavonoids** (and their glycosides) and **flavonoid polymers** (proanthocyanidins or condensed tannins).

Phenolic acids contain a phenolic ring and an organic carboxylic acid function and consists of 2 subgroups, the hydrobenzoic and hydroxycinnamic acids.

Flavonoids (Figure 2.8) constitute the largest group of plant polyphenols and consist of 2 aromatic rings (A and B rings) linked by a 3-carbon chain that forms an oxygenated heterocyclic ring (C ring).

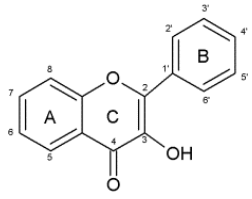


Figure 2.8 General structure of a flavonoid molecule

Based on the degree of oxidation and saturation in the heterocyclic C-ring, the flavonoids may be divided into 8 groups (flavan, flavanone, flavones, flavonol, dihydroflavonol, flavan-3-ol, flavan-4-ol and flavan-3,4-diol) (Grotewold, 2006). The hydroxyl functional groups found on all 3 rings are potential sites for links to carbohydrates, and if bound to 1 or more sugar molecules, they are known as flavonoid glycosides, whereas those that are not bound to a sugar molecule are called aglycones. The structural complexity of flavonoids is further increased with the linking of acetyl and malonyl groups to the sugar conjugates (Beecher, 2003; Erdman et al., 2007). Flavonols are a major group of

flavonoids, which occur mainly in the form of glycosides in plants. In vegetables, quercetin glycosides predominate, but glycosides of kaempferol, luteolin and apigenin are also present. Fruits almost exclusively contain quercetin glycosides, whereas kaempferol and myricetin glycosides are found in trace amounts (Herrmann, 1988). The common sugar residues are glucose and galactose, but rutinose, xylose, arabinose and rhamnose are also found.
The third subclass of the polyphenols comprises the flavonoid polymers such as proanthocyanidins, which are oligomers of flavan-3-ols.

Allium species are rich sources of flavonols (Table 2.6). The primary flavonols in onion and shallot include quercetin 3,4'-O-diglucoside, quercetin 3-O-glucoside, quercetin 4'-O-glucoside, isorhamnetin 4'-O-glucoside and quercetin aglycone (Bonaccorsi et al., 2008; Lee and Mitchell, 2011a). Quercetin 3,4'-O-diglucoside, quercetin 4'-O-glucoside and free quercetin are reported to constitute 68% of the total phenolic content in onion and over 85% of the flavonoid content in the bulb (Williamson et al., 1996). Quercetin 3,4'-O-diglucosides and quercetin 4'-O-glucosides in *Alliums* are also thought to contribute to the health-promoting properties of these species (Lanzotti, 2006). Previous investigations have demonstrated that kaempferol is the main flavonoid aglycone in leek (Hertog et al., 1992a). Fattorusso et al. (2001) isolated flavonoid glycosides in leek based on the aglycone kaempferol, including kaempferol 3-O-glucoside, kaempferol-3-O-neohesperidoside, kaempferol 3-O-[2-O-(trans-3-methoxy-4-hydroxycinnamoyl)-β-d-galactopyranosyl]-(1→4)-O-β-d glucopyranoside and kaempferol 3-O-[2-O-(trans-3-methoxy-4-hydroxycinnamoyl)-β-d-glucopyranosyl]-(1→6)-O-β-d-glucopyranoside.

Table 2.6 Presence and content of flavonols in *Allium* species

Flavonoid sub class	**Trivial name**	***Allium* species**	**Flavonol mg 100 g^{-1} dw**	**Ref.**
Flavonol	quercetin	shallot leek	2	Bonaccorsi et al. (2008); Wiczkowski et al. (2008); Hertog et al. (1992a)
	quercetin 3-O-glucoside	shallot onion bunching onion		Bonaccorsi et al. (2008); Wiczkowski et al. (2008); Lee and Mitchell (2011b); Zill-e-Huma et al. (2011); Parvu et al. (2010)

	quercetin 3,4'-O-diglucoside	shallot onion	11-556	Bonaccorsi et al. (2008); Lee and Mitchell (2011b); Wiczkowski et al. (2008); Lee and Mitchell (2011b) Zill-e-Huma et al. (2011)
	kaempferol	onion leek	29.5	Galdon et al. (2008) Hertog et al. (1992a)
	kaempferol 3-O-glucoside	onion leek		Muminova et al. (2006); Fattorusso et al. (2001)
	isorhamnetin	onion		Marotti and Piccaglia (2002)
	Isorhamnetin 3-O-glucoside	onion		Bonaccorsi et al. (2005)

2.4.2.1 Biosynthesis

Flavonoids are products of the phenylpropanoid pathway (Figure 2.9). The central enzyme in phenylpropanoid metabolism that directs carbon from aromatic amino acids to the phenylpropanoids is phenylalanine ammonia-lyase (PAL), which forms cinnamic acid from phenylalanine. The biosynthesis of flavonoids occurs in organized multi-enzyme complexes, 'metabolons', and the transport of flavonoids from the site of synthesis to final destinations such as vacuoles or cell wall, requires specific transferases and membrane transporters (Winkel-Shirley, 2001). The first enzyme committed to flavonoid biosynthesis is chalcone synthase (CHS), which condensates 3 acetate units from malonyl-CoA with p-coumaroyl-CoA. The resulting 4,2',4',6'-tetrahydroxychalcone (naringenin chalcone) is rapidly converted to naringenin by the enzyme chalcone isomerase (CHI). These first 2 enzymes of the flavonoid pathway are found in plants almost ubiquitously. However, the enzymes that catalyse the subsequent steps of flavonoid pathway vary from 1 plant species to another, giving rise to different flavones, flavonols, anthocyanins and/or proanthocyanidins.

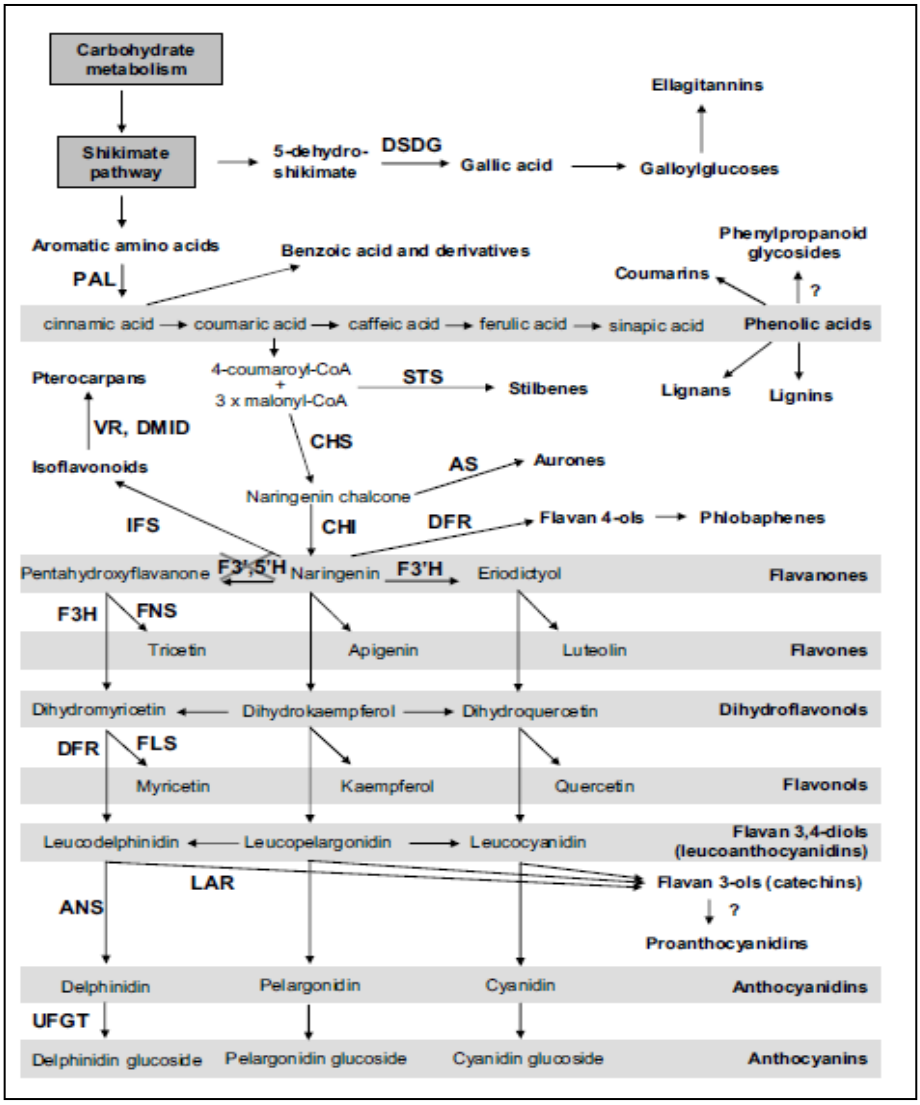


Figure 2.9 Biosynthesis of polyphenolic compounds. PAL, phenylalanine ammonia-lyase; STS, stilbene synthase; CHS, chalcone synthase; CHI, chalcone isomerase; F3',5'H, flavonoid-3',5'-hydroxylase; F3'H, flavonoid-3'-hydroxylase; F3H, flavanone hydroxylase; FNS, flavones synthase; FLS, flavonol synthase; ANS, anthocyanidin synthase; IFS, isoflavone synthase; DSDG, dehydroshikimate dehydrogenase; AS, aureusidin synthase; UFGT, UDP-glucose flavonol 3-O-glucosyl transferase; VR, vestitone reductase; DMID, 7,2'-dihydroxy-4'metohoxyisoflavanol dehydratase; LCR, leucoanthocyanidin reductase. The flavonoid pathway enzymes marked on the left side downstream from the compound pentahydroxyflavanone function also on the pathways downstream from naringenin and eriodictyol at the corresponding step (Hanhineva et al., 2008)

2.4.2.2 Physiological role in the plant

Flavonoids play an important role in plants as physiologically-active components, including stress-protecting agents, attractants, feeding deterrents, signalling materials between plants and soil micro-organisms and defence materials against biotic and abiotic stresses (Harborne and Williams, 2000; Treutter, 2006). Polyphenols play a role in protecting plants from both insect and mammalian herbivores, by acting as phytoalexins or by increasing food astringency, thus making food unpalatable (Bravo, 1998). Flavonoids generally absorb in the 280-315 nm region and thus are capable of acting as a UV filter, thereby protecting the underlying photosynthetic tissues from damage. The flavonoids most frequently cited as being UV-protective are flavones and flavonol glycosides having hydrocycinnamyl acylation linked through sugars (Harborne and Williams, 2000).

2.4.2.3 Factors influencing the polyphenol content

2.4.2.3.1 Cultivar and tissue type

A lot of studies demonstrate a significant influence of cultivar on the content of polyphenols in different crops including onion, artichoke, potato, faba bean and tomato (Mogren et al., 2007b, Al-Weshahy and Rao, 2009; Chaieb et al., 2011; Vallerdú-Queralt et al., 2011; Lombardo et al., 2012).

Flavonols and flavones are located predominantly in the leaves and in the outer parts of the plants, while only trace amounts can be found below the soil surface (Hertog and Hollman, 1996). In contrast to other vegetables, the highest amounts of quercetin in onions are found in the parts below the surface, more specifically in the outer dry scales (Patil et al., 1995). As a result, the greatest loss of flavonoids takes place when onions are peeled (Ewald et al., 1999).

2.4.2.3.2 Pre-harvest factors

Fertilisation. Nitrogen fertiliser do not affect the flavonoid content or composition in onion neither at start of storage nor during 5 months of cold storage, which means that it may be possible to grow onions with limited nitrogen leakage without reduced yield or polyphenol concentration (Mogren et al., 2007b).

Light. Flavonoids can either degrade or increase in the presence of light depending on the state of the food (fresh or processed). Light causes a stress signal which enhances the flavonoid synthesis in fresh foods (Cisneros-Zevallos, 2003). The effect of light and

mainly the photo-degradation of polyphenols depends upon different factors such as the light wavelength, the pH, the concentration and the structure of polyphenols (Ioannou et al., 2012).

Harvest time. Mogren et al. (2007a) reported an annual variation in quercetin content of onion at lifting and observed that late lifting time resulted in higher quercetin content.

2.4.2.3.3 Post-harvest factors

Processing. With increased processing heating temperatures and exposure times, the total contents of polyphenols and flavonoids in onion increased (Woo et al., 2007). Mechanical actions such as cutting and slicing increase oxidation, which can lead to a decrease of flavonoid content, however, some studies reveal an increase in flavonol content in fresh-cut potatoes and fresh-cut onions (Tudela et al., 2002; Perez-Gregorio et al., 2011; Ioannou et al., 2012). It has also been reported that a freezing process decreases the total phenolic content by 4-20% in 4 cultivars of raspberries (Ancos, 2000).

Storage. Some studies show an increase in the concentration of polyphenols during storage of fruits and vegetables, although a few reports state constant or decreasing levels (Leja et al., 2001; Gennaro et al., 2002; Kevers et al., 2007). It appears that the effect of storage depends on many factors including light, temperature and humidity (Tudela et al., 2002).

2.4.2.4 Analysis of polyphenols: state of the art

The quantification of polyphenolic compounds can be carried out by spectrophotometric analysis. Generally, the visible region of the spectrum is used to quantify polyphenols, flavonoids and tannins, among other substances. The most common and widespread methodology used to quantify the total phenolic compounds in foodstuffs originated from the methodology developed in 1927 by Otto Folin and Vintila Ciocalteu for the measurement of tyrosine (Folin and Ciocalteu, 1927; Everette et al., 2010), and in 1965 it was adapted by Vernon Singleton and Joseph Rossi for the evaluation of the total phenolic content in wine (Singleton and Rossi, 1965). This methodology is based on chemical reduction by a mixture of tungsten and molybdenum oxides (Waterhouse, 2001). Upon reaction with phenols, a blue colour is produced, which absorbs light at 765 nm (Everette et al., 2010). The intensity of light absorption at this wavelength is proportional to the concentration of phenols (Waterhouse, 2001).

Reversed-phase high-performance liquid chromatography (RP-HPLC) is the main method used for the separation of polyphenolic compounds in plant-food material, in

which the stationary phase is less polar than the mobile phase. HPLC systems can be equipped with a wide range of detectors (refractive index, fluorescence, electrochemical, light scattering, mass spectrometric and UV/Vis), which can be used to detect and quantify polyphenols with and without chromophore groups, depending on the methodology used (Thompson and LoBrutto, 2007). Sophisticated systems of liquid chromatography coupled with modern detectors such as UPLC-DAD-MS/MS (ultra-performance liquid chromatography-DAD-tandem mass spectrometry), UPLC-DAD/ESI-MS (ultra-performance liquid chromatography-DAD and electrospray ionisation-mass spectrometry), HPLC-PDA-MS/ELSD (HPLC-photodiode array-mass spectrometry and evaporative light-scattering detector) and HPLC-ESI-TOF/MS (HPLC-electrospray ionisation-time of flight-mass spectrometry) are currently available, which are able to determine the chemical structure of a wide range of compounds (Haminiuk et al., 2012). Recently, the combination of Orbitrap^{TM} technology with a linear ion trap, known as LTQ-Orbitrap-MS, was introduced. LTQ-Orbitrap-MS delivers single-stage mass analysis providing molecular mass information, 2-stage mass analysis (MS/MS) and multi-stage mass analysis (MS^n) delivering structural information (Vallverdu-Queralt et al., 2010, Przybylski et al., 2010). Accurate mass measurement of product ions, formed in MS^n experiments facilitates the elucidation of the structures of unknown compounds (Vallverdu-Queralt et al., 2010).

2.4.2.5 Polyphenols and health

In vitro tests have shown that polyphenolic compounds inhibit cancer cell proliferation, protect neurons, improve insulin secretion, reduce vascularisation and stimulate vasodilation (Ferguson et al., 2004; Silva et al., 2008; George et al., 2009; Del Rio et al., 2010; Haminiuk et al., 2012). Quercetin, kaempferol and isorhamnetin, 3 main flavonoid aglycones, have been shown to have inflammatory effect on activated macrophages (Hamalainen et al., 2007). In addition, quercetin and kaempferol show chemopreventive properties in brain tumours and synergistically suppress cell proliferation in human gut cancer lines (Ackland et al., 2005; Labbe et al., 2009). Higher intakes of kaempferol resulted in a lower risk of coronary heart disease (Lin et al., 2007).

It is generally accepted that the bioavailability of polyphenols is rather low and the values of the relative urinary excretion of the intake range from 0.3% for anthocyanins to 43% for isoflavones such as daidzin, demonstrating the great variability in the bioavailability of the different polyphenols (Manach et al., 2005). This bioavailability can be even lower when the food polyphenols have a large molecular weight, as is the case of hydrolysable and condensed tannins and complex flavonoid conjugates with several sugars and acylated with hydroxycinnamic acids (Selma et al., 2009).

When flavonols are present in the diet as aglycones, they are partially absorbed in the stomach, whereas the glycosidic forms of these flavonols are not (Crespy et al., 2002). Most polyphenols in their native form (polymeric, glycosylated or esterified) must be enzymatically hydrolysed before absorption (Walle, 2004). The rate and extent of intestinal absorption of flavonol glycosides depends largely on species as well as degree of glycosylation and type of sugar linked to a polyphenol (Hollman et al., 1999; Arts et al., 2004; Chang et al., 2005; D'Archivio et al., 2010). Likewise, it has been shown that the absorption of quercetin from onions is up to 3 times higher than that of apples and is 52% and 17.5%, respectively. This is due to the fact that the onion contains easily absorbable glucosides quercetin compounds, while in apples quercetin occurs as a mixture, *i.e.* quercetin 3-galactoside, -rhamnoside, -arabinoside, -xyloside and -glucoside (Olthof et al., 2000). In addition, the peak concentration of quercetin in plasma was much higher and was reached much faster after intake of quercetin 3-O-glucoside than after the intake of equal amount of quercetin 3-O-rutinoside by man. Quercetin glucoside was likely to be actively absorbed from the small intestine, whereas quercetin rutinoside was absorbed only from the colon after deglycosylation (Hollman et al., 1999). There is also evidence indicating that quercetin 3-O-glucoside may be more readily absorbed than quercetin 3-O-galactoside (Chang et al., 2005).

In addition, their interactions with different macromolecules such as proteins and dietary fiber affect their assimilation and metabolic fate *in vivo* (Faulks and Southon, 2005; Parada and Aguilera, 2007; Yang et al., 2008; Palafox-Carlos et al., 2011). In general, polyphenols associated with dietary fiber can be partially bioavailable, although the bioavailability of polyphenols is usually delayed by a high content of dietary fiber (Perez-Jimenez et al., 2009).

Acylation, conjugation, molecular size and solubility also determine the absorption and metabolism of plant polyphenols (Scalbert and Williamson, 2000; Yang et al., 2008; Koli et al., 2010).

Supplements. Supplements of quercetin are present on the market, but quercetin supplements extracted from *Allium* species are limited (Figure 2.10, extracted from seeds of *Dimorphandra mollis*). It is said to prevent oxidants from attacking nearby molecules. It alleviates allergies by stabilizing the membranes of certain immune cells to prevent them from releasing histamines. It also blocks enzymes that are responsible for producing inflammatory molecules that sensitize the body's pain receptors. Quercetin is a beneficial factor in cardiovascular health. It has been shown to lower mortality rates and incidences of heart attack, possibly by decreasing the formation of plaque building substances like LDL cholesterol. It is also able to inhibit a main enzyme in the pathway

that leads to complications associated with diabetes, such as glaucoma, cataracts, and neuropathy (Aor, 2012).

Figure 2.10 Quercetin supplement (Aor, 2012)

2.4.3 L-ascorbic acid (vitamin C)

Vitamin C is the most important vitamin in fruits and vegetables for human nutrition. More than 90% of the vitamin C in human diets is supplied by fruits and vegetables (including potatoes). Some crops accumulate very high levels, *e.g.* the fruit of acerola (*Malpighia glabra* L.) contains over 1% of its fresh weight in vitamin C (Loewus and Loewus, 1987). Citrus fruits and potatoes are known to be the most important sources of vitamin C in the Western diet because of the large quantities consumed (Aditi and Graham, 2012). *Allium* species are also a source of vitamin C, as indicated in Table 2.7. A high recommendation of 100–200 mg day^{-1} has been suggested, because the high stress typical of modern life is known to increase the requirement for vitamin C.

Vitamin C is defined as the generic term for all compounds exhibiting the biological activity of L-ascorbic acid (AA, Figure 2.11). AA is the principal biologically active form but L-dehydroascorbic acid (DHA), an oxidation product, also exhibits biological activity.

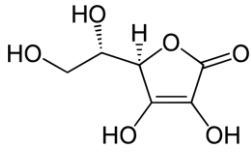


Figure 2.11 General structure of ascorbic acid

As DHA can be easily converted into AA in the human body, it is important to measure both AA and DHA in fruits and vegetables for vitamin C activity. In many horticultural crops DHA represents less than 10% of total vitamin C but DHA tends to increase during storage (Lee and Kader, 2000). AA is easily oxidised, especially in aqueous solutions,

and greatly favoured by the presence of oxygen, heavy metal ions, especially Cu^{2+}, Ag^{+}, and Fe^{3+}, and by alkaline pH and high temperature. DHA can be reduced to AA by reducing agents and also can be irreversibly oxidised to form diketogulonic acid, which has no vitamin C activity (Parviainen and Nyyssonen, 1992). Ascorbate oxidase has been proposed to be the major enzyme responsible for enzymatic degradation of AA to DHA (Saari et al., 1995).

Table 2.7 Vitamin C content in different *Allium* species

***Allium* species**	**Tissue**	**Vitamin C mg g^{-1} dw**	**Ref.**
Leek	white shaft	1.6-2.3	Lundegardh et al. (2008)
		0.1285	Ozgur et al. (2011)
Chives		9.60	Kmiecik and Lisiewska (1999)
Onion		18.89	Mota et al. (2010)

2.4.3.1 Biosynthesis

Figure 2.12 shows the pathway for the biosynthesis of L-ascorbic acid in plants. The enzymes 1–5 convert D-glucose-6-phosphate (D-glucose-6-P) to guanosine diphosphate (GDP) D-mannose and GDP-L-galactose. L-galactose, the first dedicated intermediate, is provided by hydrolysis of GDP-L-galactose; a 2-step hydrolysis (steps 6 and 7) through L-galactose-1-phosphate is shown. L-galactose is oxidised at position C1 by L-galactose dehydrogenase (step 8, L-galactose), forming L-galactono-1,4-lactone. This is oxidised by mitochondrial L-galactono-1,4-dehydrogenase (step 9) to L-ascorbic acid (Wheeler et al., 1998).

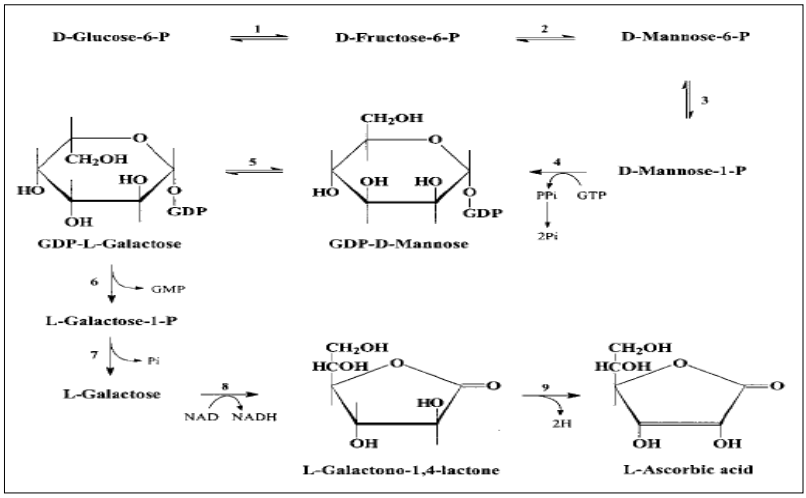


Figure 2.12 Biosynthetic pathway of L-ascorbic acid, with 1, hexose phosphate isomerase; 2, phosphomannose isomerase; 3, phosphomannose mutase; 4, GDP-D-mannose pyrophosphorylase; 5, GDP-D-mannose-3,5-epimerase; 8, L-galactose dehydrogenase; 9, L-galactono-1,4-lactone dehydrogenase (Wheeler et al., 1998)

AA metabolism is evident in the cytosol and in non-photosynthetic organelles including the mitochondria and peroxisomes. The enzyme L-galactono-1,4-lactone dehydrogenase, which is capable of synthesising, is in fact bound to the inner mitochondrial membrane (Pineau et al., 2008).

2.4.3.2 Physiological role in the plant

AA acts as a key factor for photoprotection and reactive oxygen species clearance (Loewus, 1999; Smirnoff, 2000; Muller-Moule et al., 2004). It is also a cofactor for the synthesis of hormones, such as ethylene and gibberellins and is thus involved in flowering, fruit ripening and senescence (Loewus and Loewus, 1987; De Tullio et al., 2004; Barth et al., 2006). AA is further suggested to be important in cell division, expansion and elongation (Arrigoni and De Tullio, 2000; Davey et al., 2000; Smirnoff and Wheeler, 2000; Pastori et al., 2003).

2.4.3.3 Factors influencing the vitamin C content

2.4.3.3.1 Cultivar and tissue type

Vitamin C contents of fruits and vegetables are variable among cultivars (Lee et al., 1995).
Usually skin tissues contain more vitamin C to protect the plant from outside stress caused by light and oxidation (Lee and Kader, 2000).

2.4.3.3.2 Pre-harvest factors

Fertilisation. Nitrogen fertilisers at high rates tend to decrease the vitamin C content in many fruits and vegetables (Lee and Kader, 2000).
Light. Although light is not essential for the synthesis of vitamin C in plants, the amount and the intensity of light during the growing season have a definite influence on the amount of vitamin C formed. The higher the intensity of light during the growing season, the greater the vitamin C content in plant tissues (Zhan et al., 2013).
Temperature. Plants will contain more vitamin C when grown under cool temperatures (Lee and Kader, 2000.).
Irrigation. Vitamin C content of many crops can be increased with less frequent irrigation (Lee and Kader, 2000).
Harvest time. A delay of harvest date from 60 to 120 days after planting of bunching onion (*Allium fistulosum* L.) resulted in a depletion of vitamin C (Kolota et al., 2012).
Vitamin C concentration decreased during maturation of citrus fruits, although the total vitamin C content per fruit tend to increase because the total volume of juice and fruit size increased with advancing maturity (Lee and Kader, 2000).

2.4.3.3.3 Post-harvest factors

Storage. Generally, fruits and vegetables show a gradual decrease in vitamin C content when the storage temperature or duration increases (Barberis et al., 2012).
Processing. Vitamin C is very susceptible to chemical and enzymatic oxidation. Cooking is often responsible for the greatest loss of vitamin C, and the extent of the loss depends upon variation in cooking methods and periods.

2.4.3.4 Analysis of vitamin C: state of the art

Determination of vitamin C can be performed by various methods such as direct titration with iodine or flow injection analyses (Arya et al., 2000; Suntornsuk et al., 2002). The method based on visual equivalent point 2,6-dichloroindophenol titration has been approved by the Association of Official Analytical Chemist for the determination of AA in food products (AOAC International, 2005). However, HPLC has emerged over the last years as a high-resolution, precise, reliable and sensitive method for the analysis of vitamin C in foods (Odriozola-Serrano et al., 2007). In this method, vitamin C is extracted from the sample to be analysed using metaphosphoric acid solution. A reducing solution is used to transform L(+) DHA to L(+)-AA. Total L(+) AA content is determined by HPLC with a UV detection at 265 nm.

2.4.3.5 Vitamin C and health

Vitamin C is required for the prevention of scurvy and maintenance of healthy skin and blood vessels. Vitamin C is also known to have many biological functions in collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of nitrosoamine formation, enhancement of the immune system, and reaction with singlet oxygen and other free radicals. Vitamin C, an antioxidant, reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer and helps to prevent diabetes mellitus, stroke and Parkinson's disease (Harris, 1996; Serra et al., 2008).

2.4.4 Antioxidant capacity

Bioactive compounds from plant origin behave as antioxidants because they can act as free radical scavengers, singlet oxygen quenchers, or metal chelators. Consumption of plant products possessing antioxidant potential protects living organisms from the oxidative damage of reactive oxygen species (ROS). ROS include a variety of oxygen derivatives generated from metabolism, smoking and environmental stress. They are also generated in plant products as a by-product of plant metabolism (Roy et al., 2007). It is established that oxidation processes are involved in various chronic and degenerative diseases and that the intake of antioxidants from plants has beneficial effects on health.

As described in §2.4.1, §2.4.2 and §2.4.3, the antioxidative effect of *Alliums* are related to 3 major groups, including sulfur-containing compounds, polyphenols and vitamins (Kim et al., 1997; Lampe, 1999).

2.4.4.1 *S-Alk(en)yl-L-cysteine sulfoxides*

Sulfur compounds may donate electrons and react with free radicals to convert them to more stable products, and thus terminate the free radical chain reaction. According to previously published work associated with the antioxidant capacity of sulfur compounds in *Allium* species, it seems that the allyl double bond and the length of the S-substituted alk(en)yl group are important in revealing high antioxidant capacity, as well as the number of sulfur atoms in molecules. It was also noted that the double bond associated with the non-bonding electron on sulfur may enhance antioxidant capacity (Duh, 1998; Higuchi et al., 2003).

2.4.4.2 *Polyphenols*

It is suggested that the antioxidant capacity of the flavonoids, and therefore their pathway in oxidative degradation, are linked with their special structural features. First, there is the ortho-dihydroxy structure (often called catechol structure) situated at the B-ring (Figure 2.8). This structure is responsible for donating protons and for the chelation of metal ions. Secondly, there is the 2,3-double bond in combination with the 4-keto function and the 3-hydroxyl group located in the C-ring. This feature is mainly responsible for the formation of a para-quinoic structure and therefore electron delocalisation and stabilisation of the formed radical are possible (Krishnamachari et al., 2002).

The food matrix can induce flavonoid degradation and as a consequence can decrease the antioxidant capacity. Depending on the flavonoid structure, different interactions can occur between flavonoids and the food matrix and thus lead to positive or negative synergies on the antioxidant capacity. For example, kaempferol paired with myricetin resulted in a synergistic interaction, whereas myricetin with quercetin resulted in an antagonistic effect (Hidalgo et al., 2010).

2.4.4.3 *Vitamin C*

As described in §2.4.3, vitamin C has also antioxidant properties (Harris, 1996).

2.4.4.4 *Analysis of the antioxidant capacity*

The measurement of the antioxidant capacity of food products and ingredients is a matter of growing interest. The antioxidative potential of plant extracts and pure compounds can be measured using numerous *in vitro* assays. Each of these assays is based on 1 feature of antioxidant capacity, such as the ability to scavenge free radicals

or to inhibit lipid peroxidation. However, the total antioxidant activities of vegetables cannot be evaluated by any single method, due to the complex nature of phytochemicals (Chu et al., 2000). Two or more methods should always be employed in order to evaluate the total antioxidative effects of vegetables.
Antioxidants can deactivate radicals by 3 major mechanisms: hydrogen atom transfer (HAT), electron transfer (ET) and combination of both HAT and ET (Prior et al., 2005).

- **HAT** measures the ability of an antioxidant to quench free radicals by hydrogen donation. Most HAT-based assays monitor competitive reaction kinetics and the quantification is derived from the kinetic curves. HAT reactions are solvent and pH independent and are usually quite rapid (reaction 2.1).

$$X^\circ + AH \rightarrow XH + A^\circ \qquad (2.1)$$

The oxygen radical absorbance capacity (ORAC) assay and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) are common HAT based competitive assays.
In the ORAC assay, the radical initiator component 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) decomposes spontaneously at 37 °C to form 2 carbon-centered radicals which react with oxygen to generate peroxyl radical, a common radical in human biology. Fluorescent probes used in this assay decompose in a pattern that is consistent with the HAT mechanism of action when exposed to peroxyl radicals (Prior et al., 2005). DPPH is one of a few stable and commercially available organic nitrogen radicals and has an UV-vis absorption maximum at 515 nm. Upon reduction, the solution colour fades (Figure 2.13). The reaction progress is conveniently monitored by a spectrophotometer. The DPPH assay is technical simple, but some disadvantages limits its application. DPPH is a long-lived nitrogen radical, which bears no similarity to the highly reactive and transient peroxyl radicals involved in lipid peroxidation. Many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH (Huang et al., 2005).

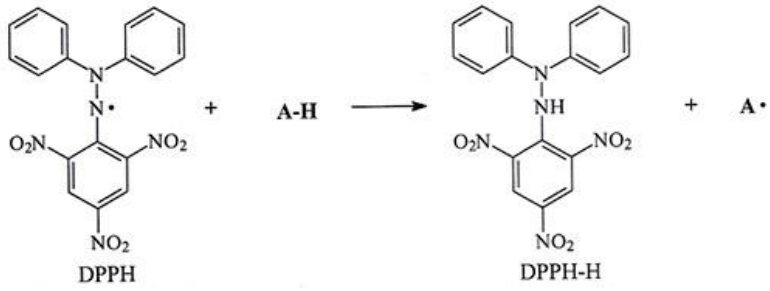


Figure 2.13 Structure of DPPH before and after reaction with antioxidants (A-H)

- **ET** detects the ability of an antioxidant to transfer 1 electron to reduce radicals, metals and carbonyls (Huang et al., 2005). ET reactions are pH dependent (reaction 2.2).

$$X^{\circ} + AH \rightarrow X^{-} + AH^{\circ +} \quad (2.2)$$

The ferric reducing antioxidant power (FRAP) assay is an example of an ET assay.
The FRAP assay takes advantage of electron-transfer reactions. Herein, ferric salt, $Fe(III)(TPTZ)_2Cl_3$ is used as an oxidant, with TPTZ = 2,4,6-tripyridyl-S-triazine (Huang et al., 2005). The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system.

2.4.5 Fructans

In addition to sulfur compounds, polyphenols and vitamin C, fructans are also reported to contribute to the health benefits of *Allium* species. Fructans are oligo- and polysaccharides consisting of short chains of fructose units with a single D-glucosyl unit at the non-reducing end. Fructans with a short chain length of 2-9 units are generally referred to as fructooligosaccharides (FOS) or oligofructose and the longer chain (DP>10) are called inulins (Muir et al., 2007). Most plants store starch or sucrose as reserve carbohydrates, but about 15% of all flowering plant species store fructans, such as cereals (*e.g.* barley, wheat and oat), vegetables (*e.g.* chicory, onion and lettuce), fruits, ornamentals (*e.g.* dahlia and tulip) and forage grasses (*e.g. Lolium* and *Festuca*) (Hendry and Wallace, 1993; Vijn and Smeekens, 1999; Jaime et al., 2001).
In higher plants, 5 major classes of structurally different fructans can be distinguished including inulin, levan, mixed levan, inulin neoseries and levan neoseries. Inulin consists of linear (2-1)-linked β-D-fructosyl units and is usually found in plant species belonging to the order Asterales, such as chicory and Jerusalem artichoke (Bonnett et al., 1994; Koops and Jonker, 1996; Baert, 1997). Levan consists of linear (2-6)-linked β-D-fructosyl units and is found in some grasses (Bonnett et al., 1997). Mixed levan is composed of both (2-1)- and (2-6)-linked β-D-fructosyl units. This type of fructan is found in most plant species belonging to the *Poales*, such as wheat and barley (Carpita et al., 1989; Bonnett et al., 1997). The inulin neoseries are linear (2-1)-linked β-D-fructosyl units linked to both C1 and C6 of the glucose moiety of the sucrose molecule. This results in a fructan polymer with a fructose chain on both ends of the glucose molecule. These fructans are found in plants belonging to the *Alliaceae* and *Asparagaceae* (*e.g.* onion and asparagus; Shiomi, 1989). Table 2.8 presents the content of fructans in different *Allium* species, including leek. The levan neoseries are polymers of predominantly β(2-6)-linked fructosyl

residues on both ends of the glucose moiety of the sucrose molecule. These fructans are found in a few plant species belonging to the *Poales* (*e.g.* oat) (Livingstone et al., 1993; Ernst et al., 1996; Vijn and Smeekens, 1999).

A fructan-rich diet may have health-promoting effects (Roberfroid, 1993). Fructans are a low-calorie food component because they cannot be digested by humans but are instead used efficiently as a carbon source by beneficial bifidobacteria in the colon (Gibson et al., 1995). These bifidobacteria ferment fructans to short-chain fatty acids that have a positive effect on systemic lipid metabolism. Small fructans with DPs of 3 to 6 are sweet tasting and therefore constitute natural low caloric sweeteners. The most agronomically acceptable crop for fructan production is chicory; however, the function of the fructan isolated from chicory is limited because of the degradation of long fructan chains by fructan exohydrolase upon harvesting. High-DP fructans are now being used in alimentary products where they can replace fat. Emulsions of long-chain fructans in water have organoleptic properties similar to fat. High-DP fructans also hold great promise for a variety of non-food applications (Fuchs, 1993; Vijn and Smeekens, 1999).

Table 2.8 Fructan content in different *Allium* species

***Allium* species**	**Tissue**	**Fructan**	**Ref.**
Leek	white shaft	24 g 100 g^{-1} dw	Muir et al. (2007)
		3-10 g 100 g^{-1} fw	Grzelak-Blaszczyk et al. (2011)
	green leaves	nd	Muir et al. (2007)
Onion	bulb	16.1 g 100 g^{-1} dw	Muir et al. (2007)
		9.5 g 100 g^{-1} fw	Grzelak-Blaszczyk et al. (2011)
Garlic		45 g 100 g^{-1} dw	Muir et al. (2007)
		6.4 g 100 g^{-1} fw	Grzelak-Blaszczyk et al. (2011)

nd: not detected

2.4.5.1 Biosynthesis

Fructan is synthesised from sucrose, and like sucrose, fructans are stored in the vacuole (Figure 2.14). Although sucrose is synthesised in the cytoplasm, fructans are produced in the vacuole by the action of specific enzymes (fructosyltransferases, FT) that transfer fructose from sucrose to the growing fructan chain. Fructan synthesis is modulated by light, which changes the availability of sucrose in the cell. The biosynthetic enzymes are evolutionarily related to invertases (I), enzymes that hydrolyse sucrose (Vijn and Smeekens, 1999).

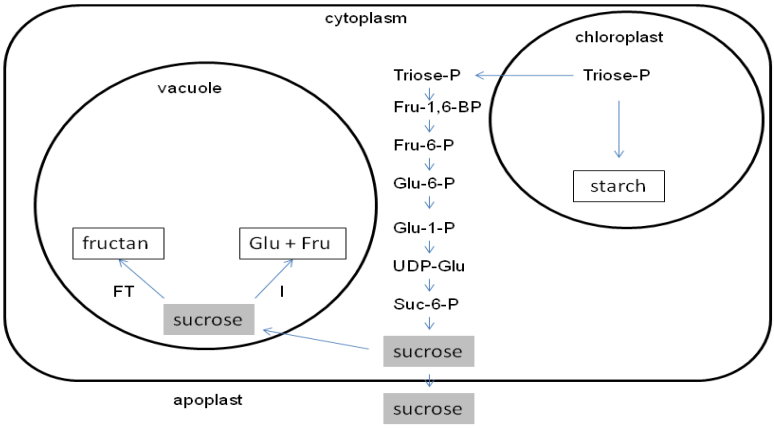


Figure 2.14 Schematic representation of carbohydrate metabolism in a plant cell, with FT: fructosyltrasferase and I: invertase (Vijn and Smeekens, 1999)

Two classes of enzymes, acid invertases and fructan exohydrolases, are involved in the degradation of fructans. Acid invertase is found in the vacuole and normally functions in the irreversible breakdown of sucrose into glucose and fructose, but it also has the ability to breakdown 1-kestose (Ritsema and Smeekens, 2003). Fructan exohydrolases (FEHs) are also localized in the vacuoles and cleave terminal fructose residues. No FEHs have been cloned from onion, but 1-FEH activity during storage has been characterized (Benkeblia et al., 2004).

2.4.5.2 Physiological role of fructans in plants

In plants, fructans may have functions other than carbon storage; they could act as osmoregulators due to their solubility in water inside the vacuole (Sinclair et al., 1995). Fructans have been implicated in protecting plants against water deficit caused by

drought or low temperatures (Hendry and Wallace, 1993; Pilonsmits et al., 1995; Vijn and Smeekens, 1999).

2.4.5.3 Factors influencing the fructan content

2.4.5.3.1 Cultivar and tissue type

There are differences in fructan content of different onion cultivars (Galdon et al., 2009). Fructans are a carbohydrate reserve in stems and underground organs of the *Alliaceae* family. In the *Asteraceae* family, including chicory (*Cichorium intybus* var. *sativum*), fructans serve as a reserve carbohydrate in stems, tubers and taproots (van Arkel et al., 2012).

2.4.5.3.2 Pre-harvest factors

Water stress. The chicory root inulin concentration remained unaffected by water stress (Vandoorne et al., 2012).

Light. As stated in §2.4.5.1, the fructan synthesis is modulated by light (Vijn and Smeekens, 1999).

Harvest time. The content of fructans in chicory roots decreased with harvest time (September, November). This degradation process is faster in cultivars with a low total sugar content than in those with a high total sugar content (Baert, 1997).

2.4.5.3.3 Post-harvest factors

Storage. 26% of fructans in leek (cv. Belton F1) were hydrolysed upon 30 days of storage at 0-1 °C, whereas after 90 days of storage, 80% were subject to hydrolysis. However, fructan hydrolysis depend on cultivar (Grzelak-Blaszczyk et al., 2011).

2.4.5.4 Analysis of fructans: state of the art

A number of methods are applied for the analysis of fructans. While gas chromatography–mass spectrometry (GC-MS), nuclear magnetic resonance (NMR) and matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry have been mainly used to obtain structural information about fructans, thin-layer chromatography (TLC) can be used to assess both the level and the composition of fructans in plant tissues (Ye et al., 2001; Chatterton and Harrison, 2003; Hincha et al., 2007). HPLC, especially high-performance–anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), is the most widely used technique for fructan quantification (Huynh et al., 2008; Hisano et al., 2008). However, direct quantification of fructans by this technique is difficult due to a lack of standards and poor resolution of high DP polymers. The method currently used for total fructan quantification consists of

multiple extractions in water or sequential extractions in ethanol and water, hydrolysis of fructans to fructose and glucose, and multiple HPLC injections to determine the levels of glucose and fructose before and after hydrolysis. The fructan level is then calculated by subtracting the level of free hexoses (including sucrose-derived hexoses) present before hydrolysis from the total level of hexoses after hydrolysis (Clark et al., 2004; Monti et al., 2005).

2.4.5.5 Fructans and health

Fructans stimulate the growth of specific microorganisms in the colon (*e.g.* bifidobacteria, lactobacilli) with generally positive health effects. A minimal dose of 2.5 g of fructans is a condition upon which a bifidogenuous effect takes place (Guigoz et al., 2002). A lot of pharmacological effects are attributed to fructans such as growth inhibition of tumor and microbial cells, reduction of cancer risk, good effects on serum lipids, blood glucose and protection against cardiovascular diseases (Magra et al., 2006; Irkin and Korukluoglu, 2009). Other proposed health benefits of fructans include suppressing the growth of potential pathogens in the colon, increased stool bulking capacity and prevention of constipation, increased calcium absorption (Nyman, 2002; Abrams et al., 2005; Van Loo et al., 2005; Lewis et al., 2005).

CHAPTER 3. PLANT MATERIAL AND ANALYSIS OF BIOACTIVE COMPOUNDS

3.1 Introduction

The aim of this dissertation is to study the effect of different parameters, including leek tissue, leek cultivar, harvest time, related *Allium* species and post-harvest processing (processing on the farm, storage, domestic cooking and valorisation) on the concentration of bioactive compounds.
To evaluate these parameters, with the exception of the processing parameter, different leek cultivars and *Allium* species were grown in 3 repetitions on ILVO-fields in 2 subsequent experimental years. Representative samples were taken and prepared in the laboratory in function of the different analyses that were performed. In order to study the effect of post-harvest processing, leeks were collected in real live situations such as on the farm or from retail. The sample preparation is described more in detail in §3.2.
Different analytical procedures (§3.3) were used to detect and quantify the bioactive compounds in leek, that is the antioxidant capacity, total phenolic, individual polyphenol, ascorbate, ACSO and fructan content. The obtained data was statistically analysed in order to evaluate significant differences.

3.2 Plant material

3.2.1 Plant material to evaluate the influence of leek tissue, leek cultivar, harvest time and *Allium* species (Chapter 4, 5 and 6)

Two experiments were set up during 2 subsequent years, but on different ILVO parcels.
In the **first year (2009)**, 30 leek cultivars were grown in order to evaluate the influence of leek cultivar and leek tissue on the content of bioactive compounds (**Chapter 4**). During this year also 6 related *Allium* species were studied simultaneously (**Chapter 6**). In a **second year (2010),** 9 leek cultivars, also grown in the first year experiment, were grown in order to evaluate the influence of harvest time more in detail (**Chapter 5**).

3.2.1.1 Selection of plant material

In the **first field trial season (2009),** further referring as (I), 30 leek cultivars (Table 3.1) were studied from a selection based on 3 criteria: (1) morphological type (pale green summer leek, dark green winter leek, intermediate autumn leek), (2) manner of breeding and multiplication (F1 hybrids, open pollinated (OP) commercial cultivars, OP farmer selections and OP old landraces) and (3) seed company. The most recently developed

cultivars, *i.e.* the F1 hybrids included in this study are a good representation of which leek material is commercially grown in the European leek producing areas.

Leek seeds were obtained from the collection of the Institute for Agricultural and Fisheries Research (ILVO). Leek breeding at ILVO started in 1994 under the management of ir. Hervé De Clercq as leek breeder. Breeding was initially performed by family selection, in fact negative mass selection. Wendy and Fama are 2 leek cultivars developed at ILVO. Nowadays, hybrid selection is the current breeding technique. Breeding traits on ILVO are pests and disease resistance (rust, thrips, white tip disease), uniformity and erectness.

The ILVO collection is generated from seeds from different farmer selections (not commercial) and seed companies. The seeds are stored in long term storage (LTS, freezer). At the moment, more than 100 leek cultivar accessions are present.

Table 3.1 Overview of the selected leek cultivars of experiment I, cultivars for experiment II are indicated in bold, with OP: open pollinated, F1:first generation progeny of male sterile mother lines

Commercial name	Type	Breeding category	Seed company	Harvest month	Growing days (I)	Growing days (II)
Varna	summer	OP (commercial)	Royal Sluis	October '09	189	
Albana	summer	OP (commercial)	Nunhems	October '09	189	
Nelli	summer	OP (old land-race)	Svalöf Weibull	October '09	190	
Elefant	summer	OP (old land-race)	IPK	October '09	190	
Miracle F1	**summer**	**hybrid**	**Enza**	**October '09**	193	159/211/263/292
Zeus F1	**summer**	**hybrid**	**S&G**	**October '09**	198	159/211/263/292
Striker F1	**summer**	**hybrid**	**Bejo**	**October '09**	200	159/211/263/292
Electra	autumn	OP (commercial)	Clause	November '09	213	
Nebraska	autumn	OP (commercial)	Royal Sluis	November '09	213	
Breugel F1	**autumn**	**hybrid**	**Rijk Zwaan**	**November '09**	221	159/211/263/292
Tadorna	autumn	OP (commercial)	Enza	November '09	227	
Poribleu	autumn	OP (commercial)	Nickerson-Zwaan	December '09	248	
Alcazar	autumn	OP (commercial)	Rijk Zwaan	December '09	248	
Belton F1	**autumn**	**hybrid**	**Nunhems**	**January '10**	291	159/211/263/292
Pretan F1	**autumn**	**hybrid**	**Nickerson-Zwaan**	**January '10**	291	159/211/263/292
Musselburh	winter	OP (old land-race)	D.T. Brown	February '10	305	
Van Limbergen	winter	OP (farmer selection)	Sint Katelijne Waver	February '10	305	
Buelens	winter	OP (farmer selection)	Onze Lieve Vrouw Waver	February '10	305	
Coolidge F1	**winter**	**hybrid**	**Hortiplan**	**February '10**	305	159/211/263/292
Apollo F1	**winter**	**hybrid**	**S&G**	**February '10**	325	159/211/263/292
Artico	winter	OP (old land-race)	IPK	February '10	325	
Farinto	winter	OP (commercial)	Nunhems	February '10	325	
Arkansas	winter	OP (commercial)	Royal Sluis	February '10	326	
Gavia	winter	OP (commercial)	Enza	February '10	326	
Toledo	winter	OP (old land-race)	Thompson & Morgan	February '10	326	
Uyterhoeven	winter	OP (farmer selection)	Onze Lieve Vrouw Waver	February '10	326	
Engels	winter	OP (farmer selection)	Putte	February '10	326	
Vervloet	winter	OP (farmer selection)	Sint Katelijne Waver	February '10	326	
Harston F1	**winter**	**hybrid**	**Nunhems**	**February '10**	326	159/211/263/292
Fahrenheit F1	winter	hybrid	Royal Sluis	March '10	333	

In addition to the 30 leek cultivars, 1 available cultivar of each of the 6 related *Allium* species were also grown in the same field (Table 3.2) in order to make a comparison between leek and some of its related species. Seeds of *A. ampeloprasum* var. *kurrat* (Egyptian leek, CGN18763), *A. schoenoprasum* L. (chive, CGN23459), *A. cepa* L.

(onion, cv. Red Creole, CGN19244) and *A. fistulosum* L. (bunching onion, CGN14764) were obtained from CGN (Wageningen, the Netherlands). Seeds of *A. odorum* L. (Chinese leek) were obtained from Tokita seed (Otone, Japan) and *A. ascalonicum* L. (shallot, cv. Creation F1, nr. 545) and *A. cepa* seeds (onion, cv. Bonus F1) were purchased from Tabernal-Seeds (Venhuizen, the Netherlands).

Table 3.2 Overview of the selected related *Allium* species

Scientific name	Trivial name	Harvest month
Allium ampeloprasum var. *kurrat*	Egyptian leek	March '10
Allium odorum L.	Chinese leek	November '09
Allium schoenoprasum L.	Chive	October '09
Allium cepa L.	Red onion	October '09
	White onion	October '09
Allium fistulosum L.	Bunching onion	October '09
Allium ascalonicum L.	Shallot	September '09

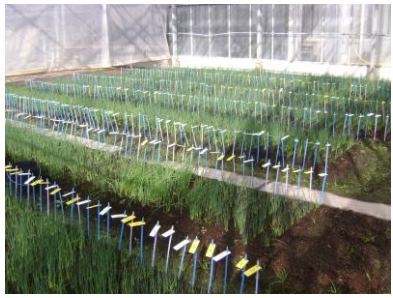
- In a **second field trial (2010),** further referring as (II), the influence of harvest time on 9 leek cultivars, also grown in 2009, was studied. To minimise variation in leek plants, 9 leek hybrids (Table 3.1, indicated in bold) were selected for the analyses as a function of harvest time as within the leek cultivars, hybrids are considered to be the most uniform leek cultivars. Three cultivars were chosen of each type (summer, autumn, winter).

Performing these 2 year experiments, 9 leek cultivars were grown and evaluated in 2 subsequent years, 2009 and 2010. These data can be used to observe differences in antioxidants between the two growing years.

3.2.1.2 Growth of plant material

The 30 leek cultivars (I), 9 cultivars (II) and 6 related *Allium* species (I) were manually sown in triplicate (3 × 75 seeds) on April 6th 2009 (I) and on April 15th 2010 (II) in an unheated greenhouse at ILVO. Trays of 1 m length and 1 cm depth were made in potting soil. Each tray was 10 cm separated from the other. Subsequently, 75 seeds were manually put into the trays, equally distributed along the 1 m length (Figure 3.1 (a)).

(a)



(b)

Figure 3.1 Sowing (a) of the 30 leek cultivars and 6 related species and their growth evolution after 6 weeks (b)

A complete block design was followed, using 3 blocks with 1 block containing 1 repetition of all the leek cultivars and related species, in a specific order. Each cultivar in 1 block represented 75 seeds. Figure 3.1 (b) shows the situation after 6 weeks growth in the green house.

After approximately 12 weeks (June 29th 2009, I and June 30th 2010, II), the little plants were manually harvested in the green house and classified in 3 groups (A, B and C). The A category were plants of good quality and used for planting in the field, while the C category was too small and not further used. The B category was a reserve of the A category. Subsequently, 3 repetitions of 15 leek plants of each cultivar and *Allium* species were planted in the field (I=N 50°58.86, E 3°46.56, II=N 50°58.53, E 3°46.43), using a complete block design (Table 3.3). Each of the three blocks contained 30 leek cultivars and 6 related species in a specific order and were planted in 2 rows. The leek plants were planted on ridges equivalent to a within row plant-to-plant spacing of 18 cm apart, and a depth of 18 cm. Immediately after planting, water was manually added to each plant hole.

Table 3.3 Complete block design of the field plot, each number representing 15 plants of 1 cultivar, with 3 blocks (repetitions) and 30 leek cultivars as treatments

ridge	block																						
1	1	28	29	30	31	32	33	34	75	76	77	78	119	120	121	122	123	169	170	171	172	173	221
2		199	200	201	202	203	204	205	206	207	208	209	210	211	212	214	215	216	217	218	219	220	
3	2	210	211	212	214	215	216	217	218	219	220	28	29	30	31	32	33	34	75	76	77	78	
4		221	119	120	121	122	123	169	170	171	172	173	199	200	201	202	203	204	205	206	207	208	209
5	3	34	75	76	77	78	28	29	30	31	32	33	169	170	171	172	173	221	119	120	121	122	123
6		205	206	207	208	209	199	200	201	202	203	204	216	217	218	219	220	210	211	212	214	215	

The genetic diversity of field trial I is presented in Figure 3.2. The plants were grown next to the candivar testing trial of ILVO and were grown and treated under the same conditions (soil and disease treatment, etc.), which are presented in §3.2.1.5.

Figure 3.2 ILVO field plot on September 25th 2009 for the determination of the influence of leek tissue and leek cultivar in terms of antioxidant properties

Figure 3.3 shows a photograph of the 6 *Allium* species. This picture was taken before harvest, with the exception of *A. ascalonicum.*

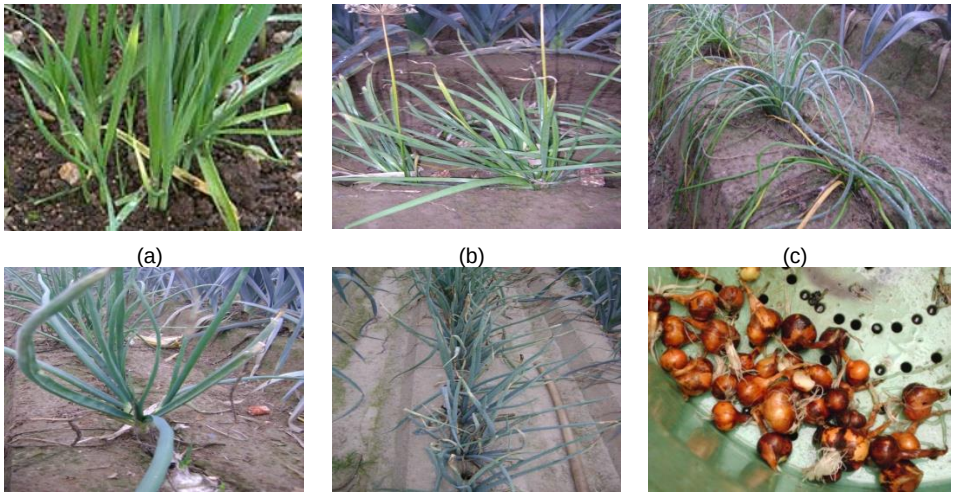


Figure 3.3 *A. kurrat* (a), *A. odorum* (b), *A. schoenoprasum* (c), *A. cepa* (d), *A. fistulosum* (e) *and A. ascalonicum* (f)

3.2.1.3 Harvest and sample preparation

For **experiment I**, 3 × 10 plants (of the 3 × 15) of each cultivar/related species were manually harvested during the optimal harvest period. Growing days (from seed to harvested plant) of the leek cultivars are indicated in Table 3.1. It should be noted that the 30 summer, autumn and winter leek cultivars were grown according to the ILVO practices, which means that harvest took place at their commercially mature stage, but the three types were sown at the same moment in April. In commercial practice, the types are sown at different times and harvested according to their type (as described in §2.2.3.2 and §2.2.3.6). The mean growing days in commercial practice for the summer types is 200 days, for the autumn types 225-300 days and the winter types 350 days (Sanac, 2012). The number of commercial growing days are similar to the growing days of the summer, autumn and winter leek cultivars in our experiment.

For **experiment II**, 3 × 3 plants (of the 3 × 15) of each cultivar (9 in total) were manually harvested on the 8th of September 2010, the 8th of November 2010, the 10th of January 2011 and the 1st of March 2011.

Immediately after harvest, the 3 × 10 (I), respectively 3 × 3 (II) plants were pooled, keeping each replication separately. The leeks were washed and the roots and decayed leaves were removed. The remainder was divided into the white shaft (or bulb) and the leafy section, except for *A. ascalonicum*, where no green leaves were present at the time

of sampling. The intermediate part was not used. The sections were then chopped into ~1 cm^2 pieces. The batches were stored at -80 °C (New Brunswick, Rotselaar, Belgium) prior to freeze-drying (I=CD-Energie, Eke, Belgium, II=FreezyFlor, Menen, Belgium). The freeze-dried samples were milled to pass through a 1 mm sieve (Fritsch, Rotterdam, the Netherlands) and stored in Falcon tubes at 4 °C until the time of analysis.

3.2.1.4 Field properties

Leeks of the two experiments were grown on different soils as shown in Figure 3.4 (picture of August 2009).

Figure 3.4 Plan of the 2 ILVO leek plots (season 2009 and 2010) grown in Lemberge (Belgium)

Some soil properties of the two fields before planting are presented in Table 3.4. For experiment I, soil samples were taken on May 29th 2009, while for experiment II, the samples date from June 1st 2010.

Table 3.4 Soil properties of the two leek fields

	Season 2009 (I)	**Season 2010 (II)**	**Aim value** (Maes et al., 2012)
Total NO_3-N (kg ha^{-1})	89.0	101.7	
Total NH_4-N (kg ha^{-1})	19.1	13.8	
pH	5.88	4.73	6.2 – 6.6
C (%)	0.84	0.85	1.2 – 1.6
P (mg 100 g^{-1} dw)	20.2	16	12 – 18
K (mg 100 g^{-1} dw)	9	5.3	14 – 20
Mg (mg 100 g^{-1} dw)	15.3	9.9	9 – 14
Ca (mg 100 g^{-1} dw)	92.8	67.3	100 – 240
Na (mg 100 g^{-1} dw)	4.5	4	3.1 – 6

A higher mineral content was observed in soil where leek of experiment I were planted.
It is established that a lack of phosphor (P) will result in a dull leaf colour, with purple leaf tops, while a lack of potassium (K) is will manifest in a dull yellow-green colour (personal communication De Clercq). Magnesium increases the colour intensity and the hardness of the leaves.
In addition to the higher mineral content, the pH of the soil of season 2009 (pH 5.88) was near to the optimum pH for leek growth (pH 5.8) as stated in §2.2.3.4.

3.2.1.5 Fertilisation and disease treatment

Table 3.5 summarises the fertilisation practice and the treatment of leek pests and diseases for experiment I and II. In addition, the most important dates were added for both experiments.

Table 3.5 Dates for the application of fertilisation, planting and the addition of herbicides, fungicides and insecticides for experiment I and II

	Season 2009 (I)	Season 2010 (II)
fertilisation	**August 26th 2008** 25 ton manure hectare^{-1} (3.5-1.96 N-P) **January 27th 2009** 30 ton farm yard manure hectare^{-1} (no N-P data available) **June 29th 2009** mineral fertiliser (75 kg N hectare^{-1})	**October 20th 2009** 5 ton lime hectare^{-1} **March 17th 20010** 40 ton manure hectare^{-1} (3.0-1.72 N-P) **June 26th 2010** mineral fertiliser (70 kg N hectare^{-1})
planting	**June 29th 2009**	**June 30th 2010**
herbicide	**July 20th 2009** Butisan, Stomp **September 8th 2009** Totril	**July 16th 2010** Butisan **September 7th 2010** Totril
fungicide	**September 14th 2009** Ortiva	**September 16th 2010** Ortiva
insecticide	**August 26th 2009** Vertimec	*

*, no insectide used

For **experiment I,** the herbicides Butisan (active compounds: metazachloor, 400 g l^{-1} and Quinmerac, 100 g l^{-1}), Stomp (active compound pendimethalin, 400.0 g l^{-1}) and Totril (active compound: ioxanyl, 225 g l^{-1}) were used during leek growth. In addition, the fungicide Ortiva (active compound: azoxystrobin, 250 g l^{-1}) was used against rust, while the insecticide Vertimec (active compound: abamectine, 18 g l^{-1}) was used against thrips. **For experiment II**, the same herbicides and fungicide were used as in experiment I. No insecticide was applied as the abundance of thrips was minimal.
The herbicides, fungicides and insecticides doses were applied as currently used in practice.

3.2.1.6 Meteorological data

Figure 3.5 presents the meteorological data (average month temperature, sunlight hours and precipitation) of growth season 2009 and 2010. The figure also includes the monthly averages from 1981 untill 2010 (KMI, 2012).

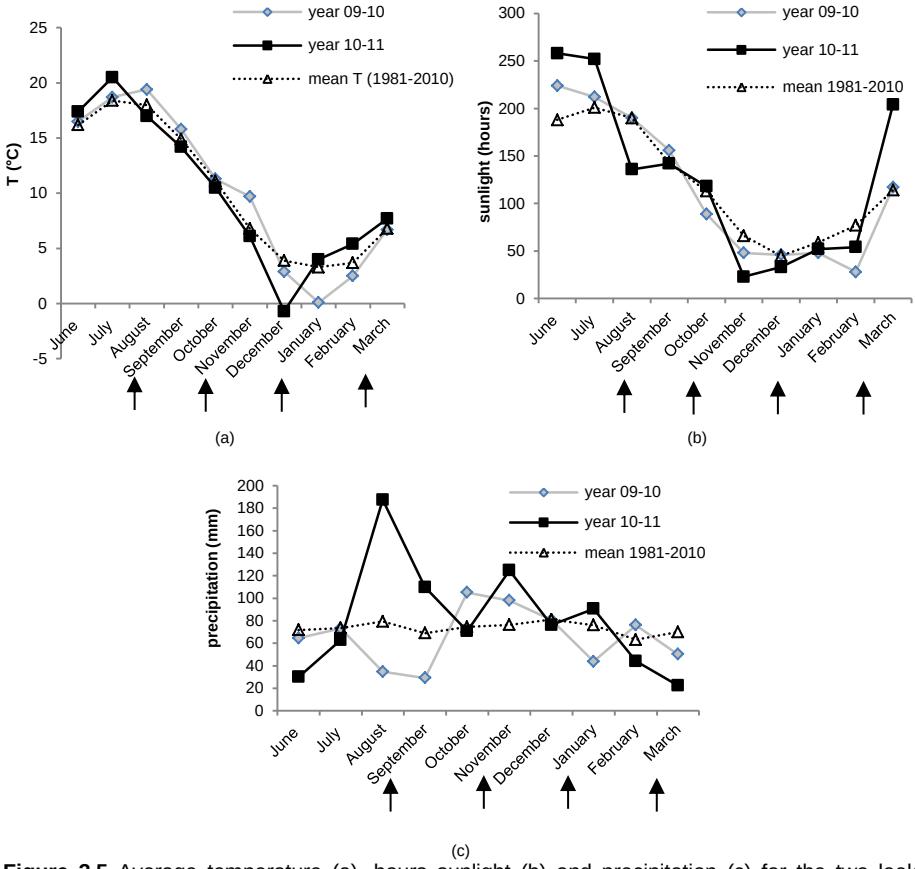


Figure 3.5 Average temperature (a), hours sunlight (b) and precipitation (c) for the two leek growing seasons and the monthly averages from 1981 to 2010. Arrows indicate the harvest time for experiment II (KMI, 2012)

The graphs of Figure 3.5 reveal a lower temperature in December ’10 (II) and January ’09 (I), a lower photoperiod in February ’10 (I) and November ‘10 (II) and a higher amount of rainfall in August ‘10 and September ’10 (II).

3.2.1.7 Statistical analysis

Influence of leek tissue, leek cultivar and Allium species. The results of the bioactive compound analyses are presented as mean ± standard deviation (SD) of 3 measurements (n = 3) for each cultivar and subjected to analysis of variance (ANOVA) by the SPSS V. 17 statistical programme (SPSS Inc., Chicago, USA). ANOVA was accomplished because it allows to determine statistical differences in antioxidant properties (dependent variables) between different groups. A general linear model

ANOVA (univariate) was preferred with 'leek tissue' and 'cultivar' as factors (independent variables). The application of ANOVA is possible when the results of the antioxidant capacity, total phenolic content, ascorbate and ACSO values obtained fulfil the following criteria: (I) behave as a normal distribution, and (II) have homogeneous variances between each group (homocedasticity). Normality was verified by the Kolmogorov-Smirnov test, homocedasticity by the Levene test. When one of the conditions was not fulfilled, a non-parametric test (Kruskal-Wallis) was done. Differences of $p<0.05$ were considered significant. In case of significant differences, multiple comparisons of means was established with the post-hoc Scheffé test in order to analyse the significant differences between the groups ($p<0.05$). The Scheffé test was chosen because the test has the advantage of giving the experimenter the flexibility to test any comparison that appears interesting, although the drawback of this flexibility is that the test has very low statistical power.

A Pearson correlation test was used to determine the correlations between antioxidant capacity (AC) results on the one hand and polyphenolic compounds, ascorbate, ACSO and fructan content on the other hand using Statistica software (version 10).

Principal component analysis (PCA), a multivariate technique, was accomplished to convert a set of observations of variables (antioxidants) into a set of values of linearly uncorrelated variables. Its goal is to extract the important information from the data, to represent it as a set of orthogonal variables called principal components, and to display the pattern of similarity of the observations and of variables as points in maps. PCA is most commonly used to condense the information contained in a large number of original variables into a smaller set of new composite variables or dimensions, at the same time ensuring a minimum loss of information. In addition, PCA can be used to discover important features of a large data set. It often reveals relationships that were previously unsuspected, thereby allowing interpretations of the data that may not ordinarily result from examination of the data. The PCA was based on correlations and the variances were computed as SS/(n-1) using Statistica software (version 10). The PCA score plot was used to assess the effect of 3 aspects (leek tissue, type, breeding origin) on the analysed parameters (ORAC, DPPH, FRAP, polyphenolic compounds, AA, ACSO and fructans). The PCA score plot was also used to assess the differences between leek and some of its related *Allium* species.

Influence of harvest time. The data are presented as mean ± standard deviation (SD) of 3 measurements ($n = 3$) for each cultivar and subject to ANOVA by SPSS with 'plant tissue' and 'harvest time' as factors. Repeated measurements were used in SPSS. A PCA plot was also performed on the results.

3.2.2 Plant material to evaluate the influence of processing (Chapter 7, 8 and 9)

3.2.2.1 Post-harvest processing and storage (Chapter 7)

To study the influence of post-harvest processing and storage (from 'harvest to fridge') on the content of bioactive compounds, leeks were not grown at ILVO, but harvested leeks (cv. Harston) were obtained from a local leek grower (Fracha BVBA, Meulebeke) in January 2012. After harvest, the leeks were processed at the company in order to be sold as whole leek or as packaged trimmed leek, both with specific length requirements (Table 2.2). Therefore, 2 washing lines, 1 trimming line and 1 packaging line were used.
In this study, 2 cases were investigated, **(1)** leek sold as an entire plant and **(2)** leek with a large part of the green leaves removed, where the shafts are sold in a plastic package (Figure 3.6).

(a)

(b)

Figure 3.6 Sampled entire leek (a) and packaged leek (b)

In both cases, leek was harvested on Fracha fields (January 9th 2012), hereby cutting part of the green leaves, which are left on the field directly.
In the **first case**, samples of the harvested leeks (2 × 8 leek plants) were taken after a first washing step at the company. After this preparation step, the leeks are brought to the auction (REO veiling, Roeselare, Belgium). Because we were not able to sample at the auction, we took leeks after washing at the company and stored them for 1 day at the refrigerator of the lab before sampling. Thereafter, sampling was accomplished in the supermarket (Okay, Zingem, Belgium) of leek processed on January 9th 2012 in Fracha. Household storage conditions were simulated in the lab refrigerator. Therefore, we purchased 96 leek plants, harvested and processed at Fracha, at the supermarket and

stored them at 4 °C. Sampling was done on day 2, 5, 6, 7 and 13 of refrigerated storage. Thirteen days of refrigerated storage was considered as the maximum storage time in household practices. In summary, 8 sampling steps in the leek processing and storage process were established.

In the **second case** (plastic package of leek shafts), samples of the white shaft were taken after 2 consecutive washing and cutting steps at the company, after a package step, at the auction, in the supermarket and at day 2, 5, 6, 7 and 13 upon storage under consumer refrigerator conditions (Figure 3.7). In summary, 10 sampling steps in the leek processing and storage process were established.

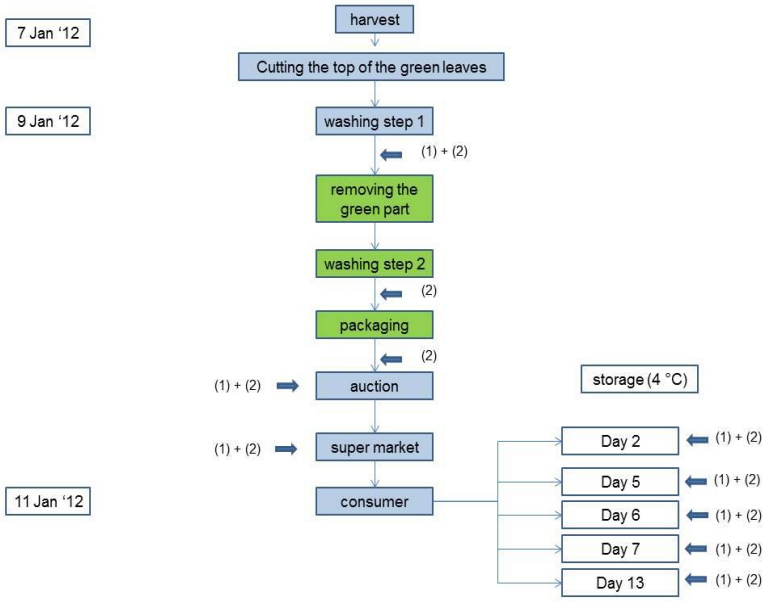


Figure 3.7 Schematic overview of the sampling of the post-harvest storage experiment of entire leek (blue, 1) and packaged white leek stalks (blue + green, 2). Sampling steps are indicated with ⬅

The sampled leeks were transported to the lab, where the roots were removed, and the remainder was divided into white shaft (and leaf sections for the first case). The intermediate part was not used. Each sample comprises a pool of 8 individual plants. The sections were then chopped into ~1 cm^2 pieces. Three portions of 100 g were taken for each sample. Subsequently, the samples were stored in bags at -80 °C (New Brunswick, Rotselaar, Belgium) prior to freeze-drying (Martin Christ, Osterode am Harz, Denmark). The freeze-dried samples were milled to pass through a 1 mm sieve (Fritsch, Rotterdam, the Netherlands) and stored in Falcon tubes at 4 °C until the time of analysis.

3.2.2.2 Domestic food processing (Chapter 8)

To study the influence of domestic cooking on the content of bioactive compounds, leeks were purchased from a Carrefour wholesale distribution centre (Melle, Belgium). The samples were prepared according to the following steps: the leeks were washed, the roots and decayed leaves were removed, and the remainder was divided into white shaft and green leaf sections. The sections were then chopped into ~1 cm^2 pieces before heat treatment.

The leek samples were processed under different heat treatments, *i.e.* blanched (to simulate the processing step before industrial freezing), boiled (to simulate soup preparation) and steamed (to simulate steamed leek in a dish).

Blanching of the leek samples was done in a stainless steel vessel, containing boiling water and covered with a lid. After 90 sec, samples were taken. The **boiling** preparation was similar to blanching, but samples were taken after 10 min, 20 min, 40 min and 60 min of boiling. Leek samples were **steamed** during 10 min, 20 min and 30 min in a pressure cooker (stainless steel, Seb, Fleurus, Belgium) filled with 1 l of water. These protocols are applied in the present study because they are similar to everyday food preparation. For each heat treatment, 3 samples of 100 g were taken. A raw chopped leek sample was taken as a reference sample. Subsequently, the samples were drained and immediately cooled in ice and stored in bags at -80 °C prior to freeze-drying. The freeze-dried samples were milled to pass through a 1 mm sieve (Fritsch, Rotterdam, the Netherlands) and stored in Falcon tubes at 4 °C until the time of analysis.

3.2.2.3 Stabilisation and valorisation of leek: fermentation and drying (Chapter 9)

3.2.2.3.1 Stabilisation by fermentation

Spontaneous fermentation

To determine the influence of a spontaneous leek fermentation process on the content of bioactive compounds, leek was purchased from the Interprovincial Research Centre for Organic Farming (PCBT, Rumbeke, Belgium). Organically cultivated leek of the Kenton F1 cultivar was chosen to eliminate any possible interference in the fermentation process from pesticide residues. The green and white parts of the leek were separated for fermentation; the intermediate part of the leek plant was not used. The green and white leek parts were chopped (Robot Coupe CL 50 Gourmet; Jackson, MS, USA) and thoroughly rinsed with water to remove any soil. Leek fermentations were performed in ceramic 7.5-l jars (BMS Wijndepot, Kuurne, Belgium) in duplicate to investigate their

influence on the antioxidant properties of the leek part (white shaft, green leaves). Therefore, 8 fermentation jars were filled with 4 kg of chopped and washed green or white leek parts. After addition of 2.5% NaCl (w/w), the leek parts were tamped to accelerate brine formation. Thereafter, weights were put on top of the tamped leek parts to keep them submerged in the forming brine. Finally, this mixture was sealed airtight using a lid with a water closure to avoid contact with light and oxygen. All fermentations were carried out in a temperature-controlled room at 18 °C. Throughout fermentation, leek particle samples were withdrawn at specific time points, *i.e.* at the start of the fermentation (chopped and tamped), and after 2 days and 21 days of fermentation. To avoid interruption of the course of the fermentations by sampling (*e.g.* disturbance of the anaerobic conditions by opening the fermentation jars), 2 jars were withdrawn per sampling time point. Leek particle samples were subsequently stored at -80 °C prior to freeze-drying (GAMMA 1-16 LSC Martin Christ, Osterode am Harz, Germany). The freeze-dried samples were milled to pass through a 1-mm sieve (Fritsch, Rotterdam, the Netherlands) and the dry powder obtained was stored in Falcon tubes at 4 °C until analysis.

Starter culture fermentation

The same setup as described above was used for fermentation induced with starter cultures, with the exception of the starting plant material. In this experiment, the whole leek plant was used, while in the natural fermentation both leek parts were fermented separately. The applied starter cultures were *Lactobacillus (Lb.) plantarum* IMDO[4] 788 (further referred to as the plantarum fermentation), a mixture of *Lb. plantarum* IMDO 788 and *Leuconostoc (L.) mesenteroides* IMDO 1347 (further referred to as the mixed fermentation), and *Lb. sakei* IMDO 1358 (further referred to as the sakei fermentation). The fourth fermentation relied on a spontaneous fermentation process and served as a control fermentation. The lyophilized starter culture [± 6.0 log (cfu ml^{-1})] was resuspended in 10 ml of saline (0.85% NaCl, m/v) and pipetted over the leek parts during tamping.

Throughout fermentation, leek particle samples were withdrawn at specific time points, *i.e.* at the start of the fermentation (chopped and tamped), after 3 days, and 21 days of fermentation.

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3.2.2.3.2 Stabilisation by drying

In addition to fermentation, 3 drying techniques were investigated as possible valorisation routes of leek processing by-products, *i.e.* freeze-drying (FD), air-drying (AD) and refractance window drying (RWD). First, freeze-drying was compared with air-drying (performed at ILVO). Secondly, freeze-drying was compared with a relative new drying technique, refractance window drying (performed in MCD-Technologies in the United States).

After buying leek in a local supermarket, the leeks were washed, the roots and decayed leaves were removed, and the remainder was divided into white shaft and green leaf sections. The intermediate part was not used. The sections were then chopped into ~1 cm^2 pieces. For freeze-drying experiments, samples were first put in a freezer of -80 °C.
To compare **freeze-drying with air-drying**, leek was freeze-dried for 5 days (final pressure 130 10^{-6} bar, Virtis, Benchtop K, SP Scientific, Suffolk, UK). Air-drying was accomplished with a vertical air flow at 70 °C during 7 hours (Dörrex 0075, Stöckli, Netstal, Switzerland). In this procedure, chopped leek was put on 4 piled trays with a thickness of 5 cm. Afterwards, the dried samples were milled to pass through a 1 mm sieve (Fritsch, Rotterdam, the Netherlands).
When comparing the **freeze-drying method with refractance window drying**, leek was freeze-dried (2 days, final pressure 46.66 10^{-6} bar) and milled by a milling device at the Washington State University (Pullman, USA), while the refractance window drying (RWD) technique was done by MCD-Technologies (Tacoma, USA). The RWD technology involves applying the product, as a thin layer, to the top surface of a transparent plastic conveyor belt. Under the plastic sheet, hot water circulates that carries thermal energy to the product (Nindo and Tang, 2007). Therefore, leek was first mixed into a puree. The residence time of the white shaft and green leaves on the dryer's heated surface was 2 min 8 s and 2 min 22 s, respectively. The circulating water temperature was 97 °C, while the maximum product temperature was approximately 64 °C. The residual moisture content of the dried white part and green leaves was 4.81% and 4.72%, respectively. The RWD flakes were packed in aluminium-coated polyethylene bags, heat sealed and transported to ILVO.

3.2.2.4 Statistical analysis

The data are presented as mean ± standard deviation (SD) of 3 measurements (n = 3) and subject to ANOVA by the SPSS, with 'cooking times/fermentation time/drying

method' as factor. Differences of $p<0.05$ were considered significant. The Scheffé test was performed to analyse the significant differences between the data ($p<0.05$).

3.3 Analysis of bioactive compounds

Different analyses were accomplished to determine the health-promoting compounds present in leek and some related *Allium* species (Table 3.6). The procedure for these analyses is described below. Some analyses were implemented on ILVO on the basis of literature (ORAC, DPPH, total phenolic content, ACSO and fructans determination), other methods (FRAP, vitamin C and polyphenols) were already established in the James Hutton Institute (JHI). Validation was only performed for the ACSO analysis, as the other methods are well established methods or already validated.

Table 3.6 Chemical analysis for determination of bioactive compounds in leek

Analysis		Method
Antioxidant capacity	ORAC DPPH FRAP	spectrophotometric
Polyphenolic compounds	Total phenolic content	spectrophotometric
	Flavonoids and phenolic acids	U-HPLC-ESI-Orbitrap-MS(/MS)
L-ascorbic acid		HPLC-PDA
S-alk(en)yl-L-cysteine sulfoxides		HPLC-MS/MS
Fructans		HPLC-RI

3.3.1 Chemicals

All reagents were of pro analysis (p.a.) quality.

Antioxidant capacity.

ORAC - Fluorescein sodium salt, 6-Hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid (Trolox), 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and phosphate buffer were purchased from Sigma-Aldrich (Bornem, Belgium). Ethanol was purchased from VWR (Leuven, Belgium). The Trolox stock dilution (1 mM) was made in 10 mM phosphate buffer (pH 7.4) and was stored at -20 °C for 1 month. The fluorescein stock solution (1 µM) was also made in 10 mM phosphate buffer (pH 7.4) and stored at 4 °C for several months. The fluorescein working solution was prepared daily in 10 mM phosphate buffer by diluting the fluorescein stock solution to a final concentration of 1 nM.

DPPH **-** 2,2-diphenyl-1-picrylhydrazyl was purchased from Sigma-Aldrich (Bornem, Belgium).

FRAP - Aceton was purchased from Fisher Scientific (Loughborough, UK), formic acid from Fluka Analytical (Dorset, UK), sodium acetate 3-hydrate from Merck (Darmstadt, Germany), 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) and ferric chloride from Sigma-Aldrich (Dorset, UK).

Total phenolic content.

Hydrochloric acid (37%), Folin-Ciocalteu's phenol reagent, sodium carbonate and gallic acid were purchased from Sigma-Aldrich (Belgium). Methanol and ethanol from Merck (Darmstadt, Germany).

Flavonoids and phenolic acids.

U-HPLC-ESI-Orbitrap-MS/MS (James Hutton Institute, JHI) - Methanol, acetonitrile, formic acid and acetic acid were of analytical grade and were purchased from Fisher Scientific (Loughborough, UK). Water was purified with an Aquatron water purification system (Aquatron A4000D, Staffordshire, UK). Luteolin 4'-methylether (diosmetin), kaempferol 4'-methylether (kaempferide), kaempferol 3-O-glucoside (astragalin), quercetin 3,4'-O-diglucoside, luteolin 7-O-glucoside, luteolin 6-C-glucoside (homoorientin), quercetin 3-O-galactoside (hyperin) and isorhamnetin 3-O-glucoside were purchased from Extrasynthese (Lyon, France). Quercetin 3-glucoside (isoquercitrin) was purchased from Phytolab (Vestenbergsgreuth, Germany). Kaempferol, quercetin, luteolin and galangin were purchased from Sigma-Aldrich (Bornem, Belgium). Standards were dissolved in methanol and stored at -20 °C.

U-HPLC-ESI-Orbitrap-MS (De Paepe et al., 2013) - The same chemicals as described above were used with the addition of analytical standards of the flavonoids were purchased from Phytolab (Vestenbergsgreuth, Germany), *i.e.* apigenin, apigenin 7-O-glucoside (apigetrin), luteolin, luteolin 7-O-glucoside (cynaroside), kaempferol 3-O-glucoside (astragalin), quercetin, quercetin 3-O-glucoside (isoquercitrin), quercetin 3-O-galactoside (hyperin), quercetin 3-O-rutinoside (rutin), quercetin 3-O-arabinoside (avicularin), quercetin 3-O-rhamnoside (quercitrin), phloretin, phloretin O-2'-glucoside (phlorizin), (+)-catechin, (-)-epicathecin, (+)-dihydroquercetin ((+)-taxifolin), (+)-dihydrokaempferol ((+)-aromadendrin), naringenin, naringenin 7-O-neohesperidoside (naringin), isorhamnetin, procyanidin B2, galangin, cyanidin chloride, cyanidin-3-O-glucoside chloride (kuromanin chloride), cyanidin-3-O-galactoside chloride (ideain chloride) and cyanidin-3-O-rutinoside chloride (keracyanin chloride). Analytical standards of the phenolic acids of the highest purity available were bought from Sigma-Aldrich (Bornem, Belgium), *i.e.* salicylic acid, protocatechuic acid, gallic acid, propyl gallate, 4-p-hydroxyphenyl acetic acid, p-coumaric acid, ferulic acid, dihydroferulic acid, caffeic acid,

dihydrocaffeic acid, sinapinic acid and chlorogenic acid. These polyphenolic compounds are commonly found in plants and have been widely investigated.

L-ascorbic acid.

L-Ascorbic acid (>99%) was obtained from Sigma-Aldrich (Dorset, UK). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP, >98%) was purchased from Fluka (Dorset, UK). Potassium dihydrogen orthophosphate (>99%), orthophosphoric acid (85% in water), metaphophoric acid (60% + 40% sodium phosphate stabiliser), dithiothreitol (>98%) and acetonitrile (≥99.9%) were obtained from VWR International (Poole, UK). Ascorbate oxidase (E.C. 1.10.3.3) from *Cucurbitaceae* sp. was purchased from Roche Diagnostics (Lewes, UK).

ACSOs.

Acetonitrile (MeCN, LC-MS) and formic acid (99%, LC-MS) were obtained from Biosolve B.V. (Valkenswaard, the Netherlands). Water was HPLC grade (generated by a Milli-Q Gradient purification system, Millipore, Bedford, MA). The alliin standard (purity ≥ 98%) was purchased from Sigma-Aldrich (Bornem, Belgium) and methiin from Enzo Life Sciences (Antwerp, Belgium). Because isoalliin was not available as a standard, isoalliin was identified based on the mass spectra and quantified as alliin (Lundegardh et al., 2008). O-(carboxymethyl)hydroxylamine hemihydrochloride (OCMHA) was purchased from Sigma-Aldrich (Bornem, Belgium).

Fructans.

Potassium hydroxide solution, sodium hydroxide and hydrogen chloride were obtained from Fluka (Steinheim, Germany). Citric acid monohydrate was purchased from Merck (Darmstadt, Germany). Inulinase from *Aspergillus Niger* and D-(-)-Ribose (99%) were obtained from Sigma-Aldrich (Steinheim, Germany).

3.3.2 Antioxidant capacity

Three distinct antioxidant capacity assays were used for determining the antioxidant capacity in this work: the oxygen radical absorbance capacity (ORAC) assay, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay and the ferric reducing antioxidant power assay (FRAP).

ORAC and DPPH analyses were carried out with a FLUOstar OPTIMA plate reader (BMG LABTECH, Offenburg, Germany) equipped with a temperature-controlled incubation chamber. Filters with a wavelength of 485 nm and 520 nm were purchased from Isogen Life Sciences. The control and evaluation software V2.20 from BMG LABTECH were used for further calculation. The 96 well plates used for the ORAC and DPPH assay were obtained from VWR International. FRAP analyses were carried out with an Ultraspec 2100 pro (Amersham Biosciences, Bath, UK).

3.3.2.1 Oxygen Radical Absorbance Capacity (ORAC) assay

For ORAC analysis, extracts were obtained by adding 10 ml of 70% aqueous ethanol solution (v/v) to a precisely weighed amount (500 mg) of freeze-dried leek powder (FDLP). This mixture was shaken for 2 h on an orbital shaker at 300 rpm (Shaker type 3500, GFL, Burgwedel, Germany). The homogenates were centrifuged at 4500 rpm for 10 min (Heraeus Labofuge 400 R, Thermo, Aalst, Belgium) and the supernatants were used for the ORAC analysis.

The ORAC procedure established by Prior et al. (2003) and the application note of Ganske and Dell (2006) were followed. Briefly, to each microplate well, 25 µl Trolox dilution, 25 µl sample dilution or 25 µl 10 mM phosphate buffer (pH 7.4) was pipetted in triplicate for the calibration curve, sample or blank, respectively. 150 µl of the fluorescein solution (10 nM) was then added to each well. In the next step, the microplate was sealed and incubated at 37 °C for 30 min. The fluorescence (Ex. 485 nm, Em. 520 nm) was subsequently determined every 90 s. After 3 cycles, 25 µl of 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (240 mM) was added manually to each well. Fluorescent measurements were taken up for 90 min. Calculations were based on the area under the fluorescence decay curve (AUC) using formula (3.1), with CT-cycle time in min, y_n-the relative fluorescence unit at cycle n

$$AUC = CT \times \left[\frac{1}{2} + (y_2 + y_3 + y_4 + \cdots y_{60})\right] \quad (3.1)$$

ORAC values were calculated using a regression equation for a linear regression on the range of 12.5-200 µM Trolox standards. The net area under the curve was obtained by subtracting the area under the curve for the blank values from the curves of samples and standards. ORAC values were expressed in µmoles of Trolox Equivalents per gram of dry weight (µmol TE g^{-1} dw).

3.3.2.2 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The extraction method for DPPH analysis was the same as described for the ORAC determination (§3.3.2.1).

The DPPH procedure is essentially as described by Hogan et al. (2009) with some modifications. Briefly, an aliquot (100 µl) of DPPH solution in ethanol (0.4 mM) was added to the sample extract (100 µl) at various concentrations. The absorption at 520 nm was determined immediately after the reaction was initiated. Each well was read once every 60 s for 90 min.

DPPH values were calculated using a regression equation for a linear regression on the range of 12.5-200 µM Trolox standards. The relative scavenging capacity of the leek extracts were expressed in µmoles of Trolox Equivalents per gram of dry weight (µmol TE g^{-1} dw).

3.3.2.3 Ferric Reducing Antioxidant Power (FRAP) assay

Extracts for FRAP analysis were obtained by adding 2 ml of extraction solvent (acetone-water (70:30) with 0.1% formic acid) to an accurately weighed amount (100 mg) of FDLP. The mixture was vortexed for 15 s and agitated for 2 h using a tube rotator (Stuart Scientific blood tube rotator SB1, Staffordshire, UK). The homogenates were centrifuged at 4000 rpm for 15 min (Eppendorf Centrifuge 5810R, Hamburg, Germany). An aliquot (1 ml) of the supernatant was centrifuged at 13 200 rpm for another 5 min.
The ferric reducing power of the leek extracts was determined using the method of Deighton et al. (2000) and employed a 100 µl aliquot of the leek extract (1:2) added to 900 µl of FRAP reagent. Thereafter, the procedure was followed as described. The results were expressed as µmoles of ferric reducing/antioxidant power of 1 g FDLP and were compared with the standard curve prepared using $FeSO_4$ in a range of concentration from 100 to 1000 µM (µmol $FeSO_4$ g^{-1} dw). FRAP analyses were performed in collaboration with the research group 'Enhancing Crop Productivity and Utilisation' of the James Hutton Institute (JHI, Dundee, Scotland).

3.3.3 Polyphenolic compounds

The analysis of polyphenolic compounds was divided into 2 parts. In a first part, the total phenolic content was measured with a spectrophotometric method. In that case, a global polyphenol content of the white shaft and green leaves could be obtained. In a second part, a more specific analysis was executed to identify and, if possible, quantify flavonoids and phenolic acids in leek.

3.3.3.1 Total phenolic content

Extraction for the total phenolic content was as described by Vinson et al. (1998) with some slight modifications. Briefly, a precisely weighed amount (500 mg) of FDLP was diluted with 10 ml 1.2 M HCl in 50% aqueous methanol. This mixture was shaken for 2 h at 80 °C and centrifuged (4500 rpm, 10 min). The supernatant was used for the total phenolic analysis. The TP content was determined according to the Folin–Ciocalteu method (Singleton and Rossi, 1965; Deighton et al., 2000). However, a drawback with

the Folin-Ciocalteu approach is that compounds such as fructose, amino acids and ascorbic acid can contribute to the TP content. Nevertheless, the extraction procedure of Vinson et al. (1998) ensures that the interference of vitamin C with the Folin–Ciocalteu method was not significant. The determination procedure described by Waterman and Mole (1994) was followed with some modifications. An appropriate volume (0.100 ml) of the filtrate was added to 0.5 ml Folin–Ciocalteu reagent (1:10) in a volumetric flask of 10 ml. After 6 min, 20% Na_2CO_3 (w/v, 1.5 ml) was added. Filling up to 10 ml with distilled water, the mixture was shaken and reacted for 2 h at room temperature in the dark. Absorbance readings were taken against a blank at 740 nm. The standard curve was made using gallic acid standard solution (100-500 mg l^{-1}) under the same procedure as above; the same applied to the blank. All determinations were carried out in triplicate and the results were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} dw).
Total phenolic analyses were carried out with a FLUOstar OPTIMA plate reader (BMG LABTECH) equipped with a temperature-controlled incubation chamber.

3.3.3.2 Flavonoids and phenolic acids

Two methods for the analysis of flavonoids and phenolic acids were used in this project.

3.3.3.2.1 U-HPLC-ESI-Orbitrap MS/MS (JHI, Dundee, Scotland, UK)

A U-HPLC-ESI-Orbitrap-MS/MS system was used for analysis of flavonoids and phenolic acids.
Sample preparation and analyses were performed in collaboration with the research group ‘Enhancing Crop Productivity and Utilisation’ of the James Hutton Institute (JHI, Dundee, Scotland).
Sample preparation was as follows; 100 mg of FDLP was extracted with 4 ml of 50% aqueous methanol (1% acetic acid). An internal standard galangin was used in a concentration of 1.25 mg l^{-1}. The mixture was shaken during 60 min at room temperature. The extracts were then centrifuged for 10 min (RC 1022, Jouan) and the supernatant was filtered through a 0.45 µm PTFE membrane filter (Syringeless filter devices 0.45 µm, Whatman) in a vial.
U-HPLC analyses of the leek samples were performed using a Finnigan Surveyor LC system (Thermo Scientific Ltd., Bremen, Germany), which comprised a MS pump, an auto sampler and a photo diode array (PDA) detector. The separation of the analytes was carried out using an U-HPLC system equipped with a reversed-phase C_{18} analytical column of 50 mm x 2.1 mm and 1.9 µm particle size (Thermo Hypersil Gold) protected

with a C_{18} guard column. The column temperature was maintained at 30 °C. The injected sample volume was 5 µl. The mobile phase consisted of a combination of HPLC water (solvent A) and 90% acetonitrile (solvent B), containing 0.2% formic acid. The initial mobile phase composition (95% A and 5% B) was followed by a linear gradient to 100% B in 8.5 min. This composition was held for 30 sec. In the next minute the system was placed in the initial condition, to ensure complete elution of the matrix from the column. The flow rate used was 400 µl min^{-1}. UV spectra recorded were in the range 200-600 nm.

For accurate mass measurements, an LTQ Orbitrap XL™ mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) was used, equipped with an ESI source. The MS was operating in negative electrospray ionization mode in full scan for masses between *m/z* 80 and 2000 at 30 000 resolution at *m/z* 400. Operation parameters were as follows: source voltage, 3 kV; sheath gas flow rate, 45 Arbitrary Units (AU); auxiliary gas flow rate, 5 AU; and capillary temperature 300 °C. Default values were used for most acquisition parameters. Two scan events were performed: full-scan analysis followed by data-dependent MS^2 of the most intense ions. The MS detector was tuned against kaempferol, quercetin, galangin and luteolin.

The U-HPLC system and mass detector were controlled by Xcalibur 2.0.7 software (Thermo Fisher Scientific). Data processing was done by Sieve V 1.2™, LC Quan. Mass accuracy data were collected using ToxID software (version 2.2.1.56, Thermo Fisher Scientific).

For **identification**, accurate masses of the detected compounds were compared with data from literature (accurate mass of $[M-H]^-$ adducts of 234 flavonoids and 95 phenolic acids present in fruits and vegetables), using ToxID (Thermo Fisher Scientific) based on accurate mass scan and the following parameters, RT window: 10 min, exact mass window: 5 ppm. Mass peaks which matched within a 5 ppm error window were assigned putative identities and further identified on the basis of authentic standards or literature data. When no standard was available, identification was executed on the basis of their retention time (RT) and MS^2 fragmentation data. Based on the MS fragmentation data in ESI negative mode, kaempferol mono-, di- and triglycosides are predicted to produce kaempferol aglycone (*m/z* 285) after the loss of glycosyl units, while the quercetin glycosides will produce quercetin aglycone (*m/z* 301) after the loss of glycosyl units. In the case of isorhamnetin glycosides, the aglycone isorhamnetin will be produced (*m/z* 315). Assignment of different sugar substitutions to the flavonoid hydroxyl groups and their interglucosidic linkage was carried out in accordance with previous studies. These studies showed that the first fragmentation of the deprotonated molecular ion $[M-H]^-$ is due to the breakdown of the O-glycosidic bond at the 7-O position and the remaining sugars on the flavonoid molecule should be linked to the hydroxyl group at the 3-O

position of the flavonol skeleton (Vallejo et al., 2004). Ferreres et al. (2004) reported that both flavonol sophorosides (1->2 glycosidic linkage) and flavonol gentiobiosides (1->6 glycosidic linkage) are characterised by the fragment ion $[M-324]^-$ as their base peak in MS^2 experiments. On the other hand, flavonol diglucosides with sugar moieties linked to different hydroxyl positions of the flavonol nucleus have the fragment ion $[M-H-162]^-$ as their base peak. In addition, flavonol sophorosides were defined by the fragment ion $[M-H-180]^-$ and were also able to produce the fragment ions $[M-H-162]^-$ and $[M-H-120]^-$ (Ferreres et al., 2004).
Polyphenols were **quantified** on the basis of a calibration curve obtained from standard solutions of reference compounds.

3.3.3.2.2 U-HPLC-ESI-Orbitrap-MS (Flemish Institute for Technology Research, Mol, Belgium)

In Chapter 9, the validated method to quantify polyphenols, developed by Domien De Paepe at the Flemish Institute for Technological Research (VITO), was applied (De Paepe et al., 2013).
The extraction system consists of a consecutive extraction of 0.5 g FDLP weighted in a BD Falcon™ conical tube (BD, Sunderland, United Kingdom) added with 10 ml MeOH:water (20/80, v/v) in a first step and 100% MeOH in a second step. Each extraction was performed by ultrasound-assisted solid-liquid extraction with 5 ml of the appropriate solvent by using a 2200 R-4 Ultrasonic sonicator (40 kHz, 100 W) (Branson Ultrasonic Corporation, Danbury, USA) for 60 min at room temperature. After adding the solvent to the extraction tube and after 30 min of extraction, the solutions were stirred with an IKA MS2 Minishaker (IKA® Werke GmbH & Co. KG, Staufen, Germany) for 15 min. During sonication, the temperature was kept below 40 °C. Following extraction, a separation between the solid particles and the liquid phase was obtained by centrifuging at 3000 rpm using an Allegra™ Centrifuge (Beckman Coulter Inc., CA, USA). Subsequently, the supernatant was collected and stored in a capped vial at 4 °C. When the two consecutive extraction cycles were performed, the two supernatants were combined in a microtube (50/50, v/v) and centrifuged using a Galaxy 16DH ultracentrifuge (VWR, Leuven, Belgium). Finally, the obtained supernatant was diluted (dilution factor 1/5 (v/v)) in a microvial by adding MeOH:water (60/40, v/v) and stored at 4 °C prior to injection into the U-HPLC-ESI-Orbitrap-MS system. The LC system consisted of an Accela™ quaternary solvent manager, a Hot Pocket column oven (Thermo Fisher Scientific, Bremen, Germany) and a CTC PAL™ autosampler (CTC Analytics, Zwingen, Switzerland). A reversed phase separation was performed on a Waters Acquity UPLC® BEH SHIELD RP18 column, with dimensions 3.0 mm x 150 mm,

1.7 µm (Waters, Milford, USA). To protect the UHPLC column, an Acquity BEH RP18 VanGuard pre-column, with dimensions 1.7 µm, 2.1 x 5 mm (Waters) was coupled with the analytical column. The mobile phase consisted of water + 0.1% formic acid (solvent A) and acetonitrile + 0.1% formic acid (solvent B). The gradient was varied linearly from 0% to 26% B (v/v) in 9.91 min, to 65% B at 18.51 min, and finally set at 100% B at 18.76 min and held at 100% B to 20.76 min. Afterwards, the initial conditions of 100% A were re-equilibrated from 20.88 min to 23 min prior to the next injection. The flow rate was 500 µl min^{-1} and the column temperature was set at 40 °C. Aliquots of 5 µl of the sample extract were injected into the chromatographic system. The U-HPLC system was coupled to an Orbitrap mass spectrometer (Exactive™, Thermo Fisher Scientific) operating with an Ion Max™ ESI source (Thermo Fisher Scientific) in negative ionisation mode (ESI^-) using the following operation parameters: spray voltage -2.5 kV; sheath gas (N_2, > 99.99%) 47 (adimensional); auxiliary gas (N_2, > 99.99%) 15 (adimensional); skimmer voltage -25 V; tube lens voltage -110 V; and capillary temperature 350 °C. The mass spectra were acquired using an acquisition function as follows: resolution, high (equivalent to a mass resolving power of 50 000 FWHM at m/z 200); automatic gain control (AGC), balance (target value of 1×106), and scan speed, 2 Hz. Mass range in the full scan experiments was set at m/z 90-1800. To guarantee high mass accuracy during run-time, the Orbitrap™ was externally calibrated in both positive and negative ionisation mode prior to each measurement. All the analyses were performed using a lock spray with internal lock mass of a solution of (D-Ala)2-leucine enkephalin (5000 ng ml^{-1}, 12C $[M-H]^-$, m/z 568.27767) delivered to the ESI source at 5 µl min^{-1} by using an additional LC pump (Hewlett Packard HP / Agilent 1100 HPLC Pump, Santa Clara, CA, USA). Detection of the targeted polyphenolic compounds was based on theoretical exact mass and on retention time. Data were evaluated by Xcalibur 2.2.1 (Thermo Fisher Scientific).

3.3.4 L-ascorbic acid

L-ascorbic acid (ascorbate, AA) analyses were performed in collaboration with the research group 'Enhancing Crop Productivity and Utilisation' of the James Hutton Institute (JHI, Dundee, Scotland).

Extracts for ascorbic acid content determination were prepared by adding 1 ml of 5% metaphosphoric acid (MPA) buffer containing 5 mM tris(2-carboxyethyl)phosphine (TCEP) to an accurately weighed amount (60 mg) of FDLP. In that case, the dehydroascorbic acid (DHA) form of vitamin C was reduced to ascorbic acid and only the AA form of vitamin C was analysed. The samples were vortexed for 3 min, and subsequently centrifuged for 10 min at 13 200 rpm at 1 °C. An aliquot (400 µl) of the supernatant was transferred directly to miniprep HPLC vials and filtered through 0.22 µm

filters prior to HPLC-analysis. Standard solutions of L-ascorbic acid (1-1000 µg ml^{-1}) were prepared by dilution of a 1 mg ml^{-1} solution in 5% MPA containing 5 mM TCEP.
Total L-ascorbic acid content was determined as described by Hancock et al. (2000) using an HPLC-photodiode array detection approach. Briefly, 20 µl of sample supernatant was injected onto a 300 × 7.8 mm ID Coregel 64H ion exclusion column (Interaction Chromatography, San Jose, CA, USA) with a 4 × 3 mm ID carbo-H^+ guard cartridge (Phenomenex, Macclesfield, UK) maintained at 50 °C. The eluent flow of the mobile phase (8 mM H_2SO_4) was set at 0.6 ml min^{-1} and AA was detected at 245 nm using a Gynkotech UVD 340S diode array detector (Dionex, Camberley, UK). The identification of the peak, corresponding to AA, was deduced by their co-elution with standards. Under the above mentioned conditions, the retention time of AA was 11.70 min.

3.3.5 S-Alk(en)yl-L-cysteine sulfoxides

Extraction of the ACSOs was performed as described by Lundegardh et al. (2008) with some modifications. Briefly, a precisely weighed amount (100 mg) of FDLP was diluted with 10 ml OCMHA (1.1 g l^{-1}). This mixture was shaken on a horizontal shaker for 10 min and centrifuged at 4500 rpm during 10 min. Subsequently, the supernatant was 1:5 diluted by adding HPLC-H_2O containing 0.1% formic acid. This extract was filtered (Millex GV, Millipore, 0.22 µm) and used for the ACSO HPLC-MS/MS determination.
ACSO levels were quantified using a model 2695 Alliance LC system (Waters, Milford, Massachusetts, USA) interfaced to an MS equipment consisting of a Quattro LCZ (Waters) equipped with a Z-spray system.
Separation of the ACSOs was performed on a Hypurity Aquastar C_{18} column (2.1 × 150 mm) with 5 µm particle size protected with a C_{18} guard column (2.1 × 10 mm; 5 µm) (Thermo, Louvain-la-Neuve, Belgium). The HPLC eluent was water with 0.1% formic acid. The isocratic eluent flow was set at 0.15 ml min^{-1} and the injection volume at 20 µl. The column temperature was held at 20 °C for chromatographic separation. The LC effluent was connected to the interface via a divert valve to avoid clogging the cone of the mass spectrometer. The instrument operated in the Selected Reaction Monitoring (SRM) mode with a dwell time of 0.50 s, an interchannel delay of 0.01 s and an interscan delay of 0.10 s. The MS system was controlled by version 4.1 of the MassLynx software (Waters, Zellik, Belgium).
Mass spectrometric characteristics such as cone voltage and collision energy were optimised by continuously infusing pure standards (1 µg ml^{-1}, 10 µl min^{-1}) into the mass spectrometer combined with a flow of 200 µl min^{-1} HPLC-H_2O + 0.1% formic acid using a T-piece. Standard stock solutions of alliin and methiin were prepared in water at a

concentration of 1 mg ml^{-1} and stored at –18 °C. Tuning solutions of 1 µg ml^{-1} were obtained by diluting the working solution of 10 µg ml^{-1} in acetonitrile/water (50/50, v/v) containing 0.1% formic acid. For both compounds, ionisation was performed in the electrospray (ES) positive mode. The precursor ion and the two product ions with the highest signal-to-noise (S/N) value and the highest peak intensity were selected for both analytes. The sum of both ions was used for quantification. The detection parameters of alliin and methiin are listed in Table 3.7.

Table 3.7 Mass spectrometer detector settings for *S*-alk(en)yl-L-cysteine sulfoxide determination

compound	ionisation mode	precursor ion *(m/z)*	cone voltage (V)	product ions *(m/z)*	collision energy (eV)	retention time (min)
alliin	ES+	178.23	20	88.01 / 70.26	10 / 20	4.21
methiin	ES+	152.15	20	88.22 / 70.08	10 / 15	3.67

Nitrogen was used as cone gas and desolvation gas at flow rates of 60 l h^{-1} and 700 l h^{-1}, respectively. The source block and desolvation temperature were set at 120 °C and 300 °C, respectively. Collision gas pressure was 2.5×10^{-3} mbar.

For quantification of individual compounds from peak areas, external calibration of alliin and methiin in a blank leek matrix was used (matrix-matched calibration curves). The blank matrix was a leek mixture which was left to stand during 24 h without adding OCMHA. Alliin and methiin were completely converted into breakdown products during these 24 h (Lundegardh et al., 2008). Results were expressed as mg isoalliin/methiin per gram dry weight (mg g^{-1} dw).

The method was validated before analyses were performed as it was a new developed HPLC method. Therefore, the analyte-dependent characteristics of the ACSO method concerns specificity, linearity, possible matrix effects, apparent recovery (R_A), repeatability (RSD_r), intralaboratory reproducibility (RSD_R), and limits of detection (LOD) and quantification (LOQ). These parameters were validated according to the guidelines of Commission Decision 2002/657/EC. A blank leek sample was used for spiking experiments. Specificity was checked by analysing 1 µg ml^{-1} of each pure liquid standard separately and searching for signal interference among the various Multiple Selection Monitorings (MRM). To evaluate linearity, possible matrix effects, R_A, and RSD_r, 3 series blank samples were spiked with pure ACSO standards. Each series included 1 specific spiking level in 6 replicates. A matrix-matched calibration curve of at least 5 spiking concentrations was also taken into account. Linearity of the matrix-matched calibration

curves was evaluated on the basis of graphical interpretation of R^2 (≥0.99) and the F-statistic (goodness-of-fit). A minimum of 5 data points for each calibration curve was used within the concentration range of 0-5 mg g^{-1}. R_A percentages at the three spiking levels were calculated by using the matrix-matched calibration curves for quantification. Specifically, for each spiking level, the observed concentration levels were calculated by using the peak area and the matrix-matched calibration curve. Subsequently, the apparent recovery was expressed as a percentage by comparing these observed values to the actual spiked levels. The data obtained from these experiments conducted on a single day were used to study the intraday precision repeatability by calculating the relative standard deviation (RSD_r). For interday precision (RSD_R), these experiments were carried out on 3 separate days. For both compounds, the limit of detection (LOD) and quantification (LOQ) were calculated by 3 and 6 times the standard error of the intercept divided by the slope of the calibration curve, respectively. The calibration curves of the spiked extracts were used to determine possible matrix effects by comparing them to the corresponding calibration curves of the pure standards. These effects were expressed in terms of signal suppression/enhancement (SSE) and were calculated as follows: SSE (%) = 100 × $slope_{extract\text{-}matched\ standard}/slope_{pure\ standard}$ (Van Pamel et al., 2011).

Before quantifying the two most abundant ACSOs present in leek (isoalliin and methiin), the method was first validated. Calibration curves of matrix-matched standards were used to evaluate linearity in terms of R^2 values and goodness-of-fit testing. For both compounds, linearity was found to be adequate; R^2 = 0.9920 (alliin) and R^2 = 0.9967 (methiin). The calibration curves of spiked extracts were used to determine SSE by comparing them to the corresponding calibration curves of the pure standards. The results shown in Table 3.8 indicate that both compounds seemed to be subject to signal suppression (SSE<100%). The observed SSE emphasises the need to quantify ACSOs in leek samples by means of matrix-matched calibration curves. Table 3.8 gives an overview of the overall percentage apparent recovery (R_A), repeatability (RSD_r), interday precision over 3 days (RSD_R), and LOD and LOQ (µg/g) for both compounds. The overall R_A was calculated as a mean of the three concentrations extracted and was within the range of 80-110% for both compounds, the strictest limits set in Commission Decision 2002/657/EC. The repeatability and intralaboratory reproducibility were considered to be acceptable according to the guidelines stipulated in the performance criteria of Commission Decision 2002/657/EC. The HPLC-MS/MS method used had an LOD of 31 µg g^{-1} for alliin and 22 µg g^{-1} for methiin, and an LOQ of 62 µg g^{-1} for alliin and 44 µg g^{-1} for methiin.

Table 3.8 Overview of the percentage apparent recovery (R_A), repeatability (RSD_r), and interday precision over 3 days (RSD_R) at the three concentrations Used for validation and limits of detection and quantification (LOD and LOQ) and signal suppression/enhancement (SSE) for alliin and methiin

		Low concentration			Medium concentration			High concentration					
	Concentration (mg/g)	R_A (%)	RSD_r (%)	RSD_R (%)	R_A (%)	RSD_r (%)	RSD_R (%)	R_A (%)	RSD_r (%)	RSD_R (%)	LOD (µg/g)	LOQ (µg/g)	SSE (%)
alliin	1.0-1.5-2.0	98	3	6	98	2	3	96	1	4	31	62	48
methiiin	1.0-1.5-2.0	108	4	11	108	2	3	109	2	3	22	44	13

3.3.6 Fructans

The fructan analysis was based on the method developed by Beneo Orafti (Tienen, Belgium). Extracts for fructan analysis were obtained by adding 50 g of boiling water (MilliQ) to 1 g of FDLP. When the pH was not in the range of 5.5-8.0 (InoLab pH level 1, WTW, Leuven, Belgium), pH was adjusted with KOH (0.05 M) or HCl (0.05 M). The mixture was shaken for 1 h in a shaking warm water bath (130 rpm, GFL Shaking Water Bath Type 1086, GFL, Burgwedel, Germany) at 75 °C. The homogenates were centrifuged at 4000 rpm for 15 min (Eppendorf Centrifuge 5810R). 1 part of the supernatant was used for HPLC-analysis of glucose, fructose and sucrose before enzymatic hydrolysis. Another part of the supernatant was used for enzymatic hydrolysis, therefore, 5 g of the supernatant was added to 5 g citrate buffer. The pH of the solution was adjusted to 4.5 (pH 4.45-4.55) with HCl (0.2 M) or NaOH (0.2 M). After adding 0.40 g inulinase, the solution was shaken for 30 min in a warm water bath of 65 °C and the supernatant was used for HPLC-analysis of glucose and fructose.

An HPLC (Perken Elmer series 2000, Zaventem, Belgium) system was used connected with a refractive index detector (Perken Elmer series 2000) to quantify the content of glucose, fructose and sucrose. Separation of the sugars was accomplished using an Aminex HPX-87P column (Biorad, Nazareth, Belgium). Ribose was added as an internal standard. A calibration curve of glucose, fructose and sucrose was made.

The fructan content was calculated based on formula (3.2)

$$\frac{b\times[[a\times(G_t+F_t)]-(G_0+F_0+1.05\times S_0)]}{11000} = fructans\ (g\ 100\ g^{-1}dw\ \ ) \qquad (3.2)$$

With
G_t = mg glucose after hydrolysis l^{-1} extract
F_t = mg fructose after hydrolysis l^{-1} extract
G_0 = mg free glucose l^{-1} extract
F_0 = mg free fructose l^{-1} extract
S_0 = mg sucrose l^{-1} extract
a = dilution factor
b = dilution factor

The results were afterwards confirmed by the acid hydrolysis method developed by Cosucra (Warcoing). Briefly, 174 g of water was added to 2 g of FDLP. This mixture was placed in a warm water bath (85 °C) for 1 h after the addition of 10 ml HCl (3M). Subsequently, 10 ml NaOH and 6 ml $Al_2(SO_4)_3$ were added, the mixture was filtered and the filtrate was used for analysis of the sugars after hydrolysation. The same procedure (without the addition of HCl) was performed for the determination of the free sugars. Using formula (3.2), the present amount of fructans could be determined.

Because of practical reasons, only 1 analysis could be done for each cultivar.

3.3.7 Overview of the analyses as a function of the different parameters

To summarise, Table 3.9 shows an overview of the performed analyses and the place of analysis for each parameter.

Table 3.9 Performed analyses for each chapter

			Leek tissue and leek cultivar	Harvest time	Leek vs. related *Allium* species	Post-harvest processing and storage	Domestic cooking	Fermentation		Drying
								Spontaneous	Starter cultures	
		Place of analysis	Chapter 4	Chapter 5	Chapter 6	Chapter 7	Chapter 8	Chapter 9	Chapter 9	Chapter 9
Antioxidant capacity	ORAC	ILVO	✓	✓	✓	✓	✓	✓	✓	✓
	DPPH	ILVO	✓	✓	✓	✓	✓	✓	✓	✓
	FRAP	JHI	✓	✓						
Polyphenols	Total phenolic content	ILVO	✓	✓	✓	✓	✓	✓	✓	✓
	Flavonoids and phenolic acids	JHI	✓	✓	✓					✓
		VITO						✓		✓
Vitamin C		JHI	✓	✓						
ACSOs		ILVO	✓	✓	✓	✓	✓	✓	✓	✓
Fructans		ILVO	✓		✓					

CHAPTER 4. ANALYSIS OF BIOACTIVE COMPOUNDS AS A FUNCTION OF LEEK TISSUE AND LEEK CULTIVAR

Redrafted from

Bernaert, N., De Paepe, D., Bouten, C., De Clercq, H., Stewart D., Van Bockstaele, E., De Loose, M. and Van Droogenbroeck, B. (2012). Antioxidant capacity, total phenolic and ascorbate content as a function of the genetic diversity of leek (*Allium ampeloprasum* var. *porrum*). Food Chemistry, 134, 669-677.

Bernaert, N., Goetghebeur, L., De Clercq, H., De Loose, M., Daeseleire, E., Van Pamel, E., Van Bockstaele, E. and Van Droogenbroeck, B. (2012). S-alk(en)yl-L-cysteine sulfoxides as a function of the genetic diversity and maturity of leek (*Allium ampeloprasum* var. *porrum*). Journal of Agricultural and Food Chemistry, 60, 10910-10919

Bernaert, N., De Paepe, D., De Clercq, H., Stewart, D., Van Bockstaele, E., De Loose, M. and Van Droogenbroeck, B. Characterization of flavonoids and phenolic acids in leek (*Allium ampeloprasum* var. *porrum*) using liquid chromatography/electrospray ionization linear ion trap quadrupole Orbitrap mass spectrometry. In preparation

4.1 Introduction

Many papers describe the bioactive compounds in *Allium* species, such as garlic (Gorinstein et al., 2009; Hornickova et al., 2010; Ovesna et al., 2011), onion (Ou et al., 2002; Coolong and Randle, 2003) and bunching onion (Masamura et al., 2011). Moreover, many papers reveal the difference between several *Allium* cultivars on the antioxidant properties (Moon et al., 2010; Perez-Gregorio et al., 2010; Beato et al., 2011).

Although leek is widely distributed and consumed, little information can be found on the composition and the variability of bioactive compounds as a function leek tissue and cultivar. As described in Chapter 2, these parameters can influence the content of bioactive compounds. Notwithstanding the several bioactive compound analyses on 1 leek cultivar (Proteggente et al., 2002; Vandekinderen et al., 2009), little information is available on antioxidant properties of the white shaft and green leaves of leek among the range of commercial available and less commonly leek cultivars. These data could be of interest for all the leek producing countries and can recommend leek growers to use specific cultivars and types to maximise the antioxidant concentration. As a consequence, the consumer will take advantage by consuming leek, containing higher levels of antioxidants. In the long term, implementation of the data in breeding programmes will result in new cultivars with a higher antioxidant potential.

The first aim of this study was to investigate the antioxidant potential of the white shaft and green leaves of 30 leek cultivars from different types (summer, autumn, winter) and (breeding) origins (hybrid, open pollinated). To this end, 3 distinct antioxidant capacity assays were used, the oxygen radical absorbance capacity (ORAC) assay, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay and the ferric reducing antioxidant power assay (FRAP). The second aim of this study was to investigate the major classes of bioactive compounds in leek in more detail such as polyphenols, ascorbate, the two major *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs) present in leek, isoalliin and methiin, and the fructans in the white shaft and green leaves of 30 leek cultivars.

In a last part, correlations were made between the antioxidant capacity assays and the results of the polyphenolic, ascorbate, ACSO and fructan analyses in order to evaluate the antioxidant potential of each class of bioactive compounds. In addition, principal component analysis (PCA) was performed to reduce the amount of data in order to assess and visualise the results.

The results presented in this Chapter have been partly established in collaboration with Prof. Dr. Derek Stewart of the James Hutton Institute (Enhancing Crop Productivity and Utilization Theme) with regard to the FRAP, flavonoids/phenolic acids and ascorbate analyses.

4.2 Plant material

§3.2.1 described the selection of plant material and sample preparation for field trial I. Briefly, the white shaft and green leaves of 30 leek cultivars (Table 3.1) were studied from a selection based on 3 criteria: (1) morphological type (pale green summer leek, dark green winter leek, intermediate autumn leek), (2) manner of breeding and multiplication (F1 hybrids, open pollinated (OP) commercial cultivars, OP farmer selections and OP old landraces) and (3) seed company.
The leek cultivars were grown in 2009 and harvested at their commercially mature stage (from October '09 until March '10).

4.3 Bioactive compound analysis

Table 4.1 gives an overview of the analyses (as described in §3.3) performed on the 30 leek cultivars. Three assays were used in order to determine the antioxidant capacity: the ORAC, DPPH and FRAP assay. The analysis of polyphenolic compounds was divided into 2 parts. In a first part, the total phenolic content was measured with a spectrophotometric method. In that case, a global polyphenol content of the white shaft and green leaves of the 30 leek cultivars could be obtained. In a second part, a more specific analysis was executed to identify and, if possible, quantify flavonoids and phenolic acids in leek. In addition, 3 other important groups of bioactive compounds in *Allium* species were analysed as well, *i.e.* ascorbic acid, the *S*-alk(en)yl-L-cysteine sulfoxides and fructans.

Table 4.1 Overview of the performed analyses as a function of the 30 leek cultivars

Analysis		**Method**
Antioxidant capacity	ORAC DPPH FRAP	Spectrophotometric
Polyphenolic compounds	Total phenolic content	Spectrophotometric
	Flavonoids and phenolic acids	U-HPLC-ESI-Orbitrap-MS/MS
L-ascorbic acid		HPLC-PDA
S-alk(en)yl-L-cysteine sulfoxides		HPLC-MS/MS
Fructans		HPLC-RI

4.4 Results

4.4.1 Antioxidant capacity

4.4.1.1 ORAC

The ORAC values (Figure 4.1) for the white shaft and green leaves of the 30 leek cultivars covered significant ranges; 27-88 and 82-135 µmol TE g^{-1} dw, respectively.
The highest ORAC value in the **white shaft** extracts was observed from the leek cultivars Pretan F1, Uyterhoeven and Van Limbergen and from the **green leaf** extracts of Electra, Tadorna and Engels. Among all cultivars tested, the whole leek plant (approximately 55% white shaft and 45% green leaves) of cultivar Uyterhoeven contained the highest mean ORAC value (97.78 µmol TE g^{-1} dw). In all cases, the ORAC values for the green leaves were significantly higher than those measured in the white shaft of leek, except for the cultivar Pretan F1, where no significant difference between the two distinct plant parts was measured.
In general, the white shaft of the summer, autumn and winter leek types had a mean ORAC value of 35, 56 and 68 µmol TE g^{-1} dw, respectively. The antioxidant capacity of the white shafts of the winter cultivars was significantly higher than the capacity of shafts of the summer and autumn cultivars. The capacity of the white shaft of the autumn cultivars on his turn was significantly higher than the capacity of the summer types. Furthermore, the green leaves of the summer, autumn and winter types contained a mean ORAC value of 97, 107 and 100 µmol TE g^{-1} dw, respectively, but did not show significant differences.

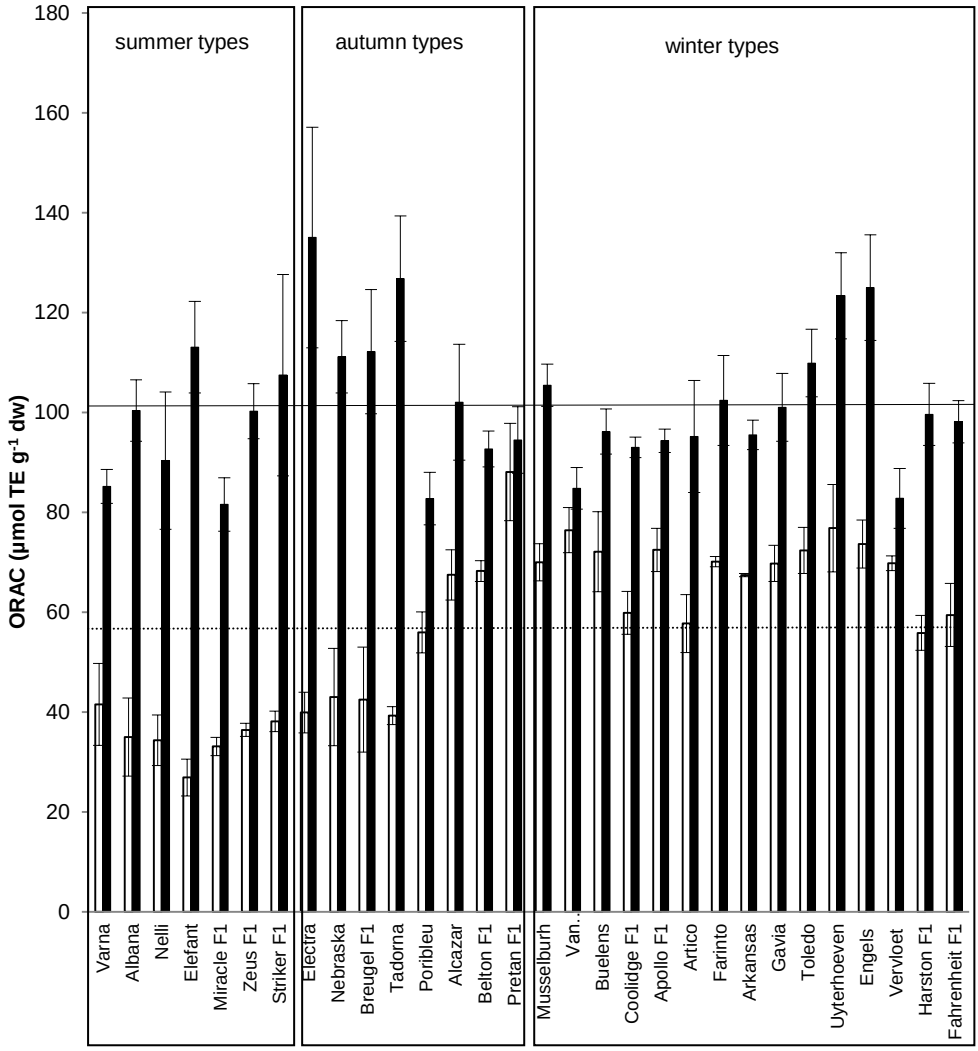


Figure 4.1 The ORAC-derived antioxidant capacities of the white shaft (□) and green leaves (■) of 30 leek cultivars (n=3), with dotted line indicating the mean ORAC value of the white shaft of the 30 cultivars, and the full line indicating the mean ORAC value of the green leaves of the 30 cultivars

4.4.1.2 DPPH

Similarly, the associated DPPH values (Figure 4.2) of these cultivars covered the ranges 2-11 and 5-14 µmol TE g^{-1} dw for the white shaft and green leaves, respectively.

The highest DPPH value from the **white shaft** extracts was observed from Pretan F1, Nebraska and Apollo F1 and from the **green leaf** extracts of Gavia, Artico and Pretan F1.

Among all the cultivars tested, the whole leek plant of the cultivar Pretan F1 contained the highest mean DPPH value (10.88 µmol TE g^{-1} dw). Generally the DPPH values for the green leaves were significantly higher than those measured in the white shaft of leek, except for the cultivars Miracle F1, Breugel F1, Poribleu, Pretan F1, Buelens, Apollo F1, Farinto, Arkansas, Kenton F1 and Fahrenheit F1, where no significant difference was measured.

In general, the white shaft of the summer, autumn and winter leek types had a mean DPPH value of 3.7, 7.5 and 6.6 µmol TE g^{-1} dw, respectively. The DPPH free radical capacity of the white shaft of the autumn and winter cultivars was significantly higher compared with the capacity of the summer types. Furthermore, the green leaves of the summer, autumn and winter types contained a mean DPPH value of 8.11, 8.83 and 9.42 µmol TE g^{-1} dw, respectively, and showed no significant differences.

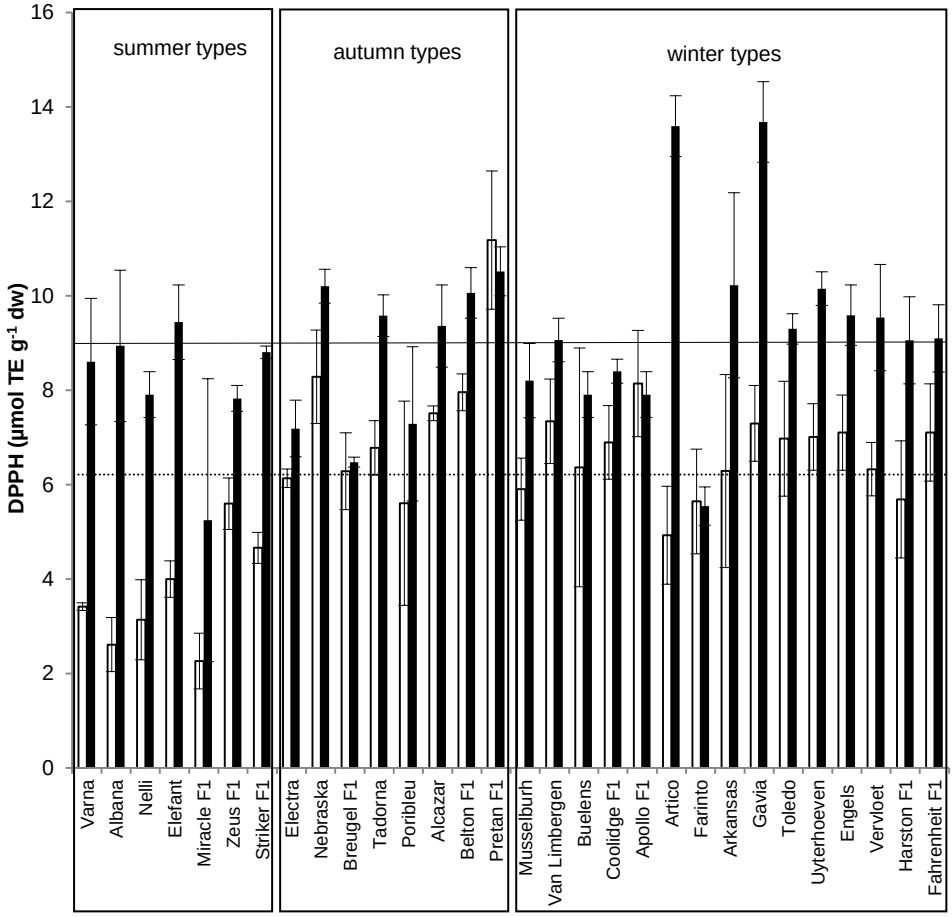


Figure 4.2 The DPPH-derived antioxidant capacities of the white shaft (□) and green leaves (■) of 30 leek cultivars (n=3), with the dotted line, indicating the mean DPPH value of the white shaft of the 30 cultivars, and the full line, indicating the mean DPPH value of the green leaves of the 30 cultivars

4.4.1.3 FRAP

The FRAP values (Figure 4.3) ranged between 3-18 and 14-37 µmol $FeSO_4$ g^{-1} dw for the white stalks and green leaves, respectively.

The highest FRAP value from the **white shaft** extracts was observed from Fahrenheit F1, Pretan F1 and Apollo F1 and from the **green leaf** extracts of Vervloet, Fahrenheit F1 and Zeus F1. In general, the whole leek plant of the cultivar Fahrenheit F1 contained the highest mean FRAP value (26 µmol $FeSO_4$ g^{-1} dw). With the exception of Fahrenheit F1,

the FRAP values for the green leaves were significantly higher than those measured in the white shaft of leek.

In general, the white shaft of the summer, autumn and winter leek types had a mean FRAP value of 5.8, 8.9 and 11.4 µmol $FeSO_4$ g^{-1} dw, respectively. The antioxidant capacity of the white shaft of the winter cultivars was significantly higher compared with the FRAP value of the summer cultivars. Furthermore, the green leaves of the summer, autumn and winter types contained a mean FRAP value of 26, 21 and 28 µmol $FeSO_4$ g^{-1} dw, respectively. The antioxidant capacity of the green leaves of the winter cultivars was significantly higher compared with the FRAP value of the autumn cultivars.

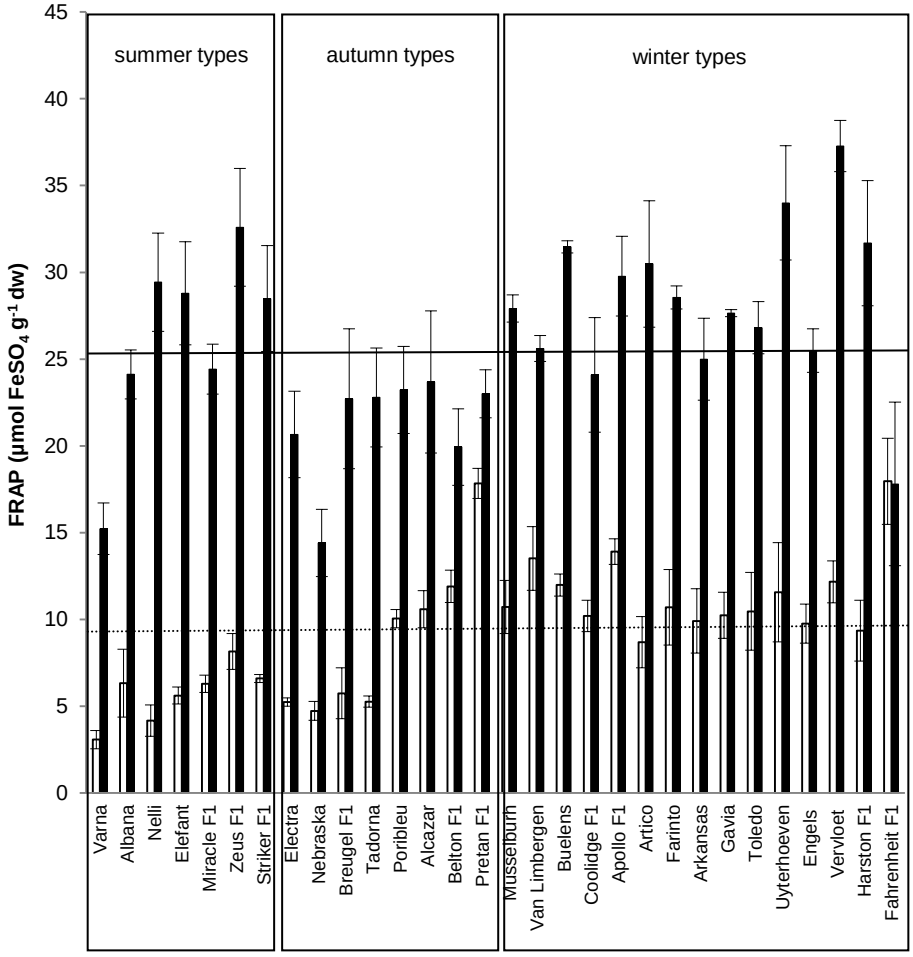


Figure 4.3 The FRAP-derived antioxidant capacities of the white shaft (□) and green leaves (■) of 30 leek cultivars (n=3), with the dotted line, indicating the mean FRAP value of the white shaft of the 30 cultivars, and the full line, indicating the mean FRAP value of the green leaves of the 30 cultivars

4.4.2 Polyphenolic compounds

4.4.2.1 Total phenolic content

The TP content in the white shaft and green leaves of the 30 leek cultivars varied from 5 to 14 mg GAE g^{-1} dw and from 5 to 15 mg GAE g^{-1} dw, respectively (Table 4.2).

Table 4.2 Total phenolic content and L-ascorbic acid content in the white shaft and green leaves of 30 leek cultivars (means of replicates ± standard deviation), the mean ascorbic acid values between brackets are mean values of the quantified levels (n=3; nd; not detected)

	Total Phenolic Content (mg GAE g^{-1} dw)		**L-ascorbic acid (mg L-ascorbic acid g^{-1} dw)**	
Cultivar name	**White shaft**	**Green leaves**	**White shaft**	**Green leaves**
Varna	7.98 ± 0.90	7.87 ± 1.35	0.89 ± 0.21	2.77 ± 0.34
Albana	7.89 ± 0.37	9.57 ± 0.22	2.13 ± 0.49	8.52 ± 1.08
Nelli	7.41 ± 0.62	7.98 ± 0.34	1.26 ± 0.31	6.33 ± 0.76
Elefant	7.57 ± 0.59	8.83 ± 0.30	1.89 ± 0.41	6.97 ± 0.57
Miracle F1	6.98 ± 0.88	7.89 ± 1.11	1.03 ± 0.89	4.15 ± 1.67
Zeus F1	8.67 ± 0.42	11.30 ± 1.78	2.70 ± 0.92	6.74 ± 2.28
Striker F1	7.95 ± 0.11	8.89 ± 0.15	2.04 ± 0.61	6.75 ± 0.27
Mean summer	**7.8**	**8.9**	**1.7**	**6.0**
Electra	8.34 ± 0.50	9.15 ± 0.23	nd	nd
Nebraska	7.57 ± 0.26	8.34 ± 0.25	nd	nd
Breugel F1	7.82 ± 0.31	8.76 ± 0.14	nd	nd
Tadorna	5.83 ± 1.22	6.70 ± 1.26	nd	nd
Poribleu	5.31 ± 0.75	5.47 ± 0.81	1.49 ± 0.33	3.73 ± 0.60
Alcazar	7.60 ± 1.14	7.71 ± 1.54	2.72 ± 0.30	5.42 ± 1.71
Belton F1	8.11 ± 0.15	9.56 ± 0.37	1.27 ± 0.19	3.25 ± 2.82
Pretan F1	7.76 ± 0.24	9.20 ± 1.44	3.25 ± 0.55	3.23 ± 0.59
Mean autumn	**7.3**	**8.1**	**1.1 (2.2)**	**2.0 (3.9)**
Musselburh	8.04 ± 0.29	9.52 ± 0.39	1.44 ± 0.50	4.25 ± 0.64
Van Limbergen	11.29 ± 0.19	11.23 ± 1.22	2.55 ±0.48	5.01 ± 1.75
Buelens	7.41 ± 0.32	8.57 ± 0.36	1.80 ± 0.48	4.71 ± 0.94
Coolidge F1	7.79 ± 0.81	9.08 ± 0.67	2.07 ± 0.83	4.09 ± 0.83
Apollo F1	7.79 ± 0.42	8.87 ± 0.63	1.85 ± 0.51	3.69 ± 0.38
Artico	9.14 ± 0.29	10.56 ± 0.14	1.05 ± 0.27	5.14 ± 0.78
Farinto	9.07 ± 0.69	10.50 ± 1.09	1.29 ± 0.26	3.58 ± 0.64
Arkansas	8.64 ± 0.53	9.95 ± 0.12	1.64 ± 0.23	4.40 ± 0.12
Gavia	8.71 ± 0.12	9.64 ± 0.52	2.13 ± 0.47	6.74 ± 0.25
Toledo	13.96 ± 0.71	15.14 ± 0.23	1.75 ± 0.59	6.37 ± 0.45
Uyterhoeven	7.73 ± 0.09	8.74 ± 0.58	1.64 ± 0.70	7.48 ± 1.05
Engels	8.50 ± 0.45	10.29 ± 0.45	1.51 ± 0.65	4.51 ± 1.13
Vervloet	7.91 ± 1.48	9.67 ± 1.73	1.59 ± 0.15	6.36 ± 1.61
Harston F1	7.33 ± 0.68	8.21 ± 1.05	1.66 ± 0.40	5.29 ± 0.83
Fahrenheit F1	9.58 ± 0.13	9.02 ± 0.69	3.55 ± 0.82	7.96 ± 0.50
Mean winter	**8.9**	**9.9**	**1.8**	**5.3**
Global mean	8.2	9.2	1.6	5.6

The highest TP content was observed from the **white shaft** extracts of the cultivars Toledo, Van Limbergen and Artico, and from the extracts of the **green leaves** of the cultivars Toledo, Zeus F1 and Van Limbergen. The cultivar Toledo showed a significantly higher TP content in both white shaft and green leaves in comparison with the other

cultivars. Among the 30 leek cultivars, the whole leek plant of the cultivar Toledo rated highest for mean TP content (14 mg GAE g^{-1} dw).

The leek cultivars Albana, Elefant, Striker F1, Nebraska, Breugel F1, Belton F1, Musselburh, Buelens, Artico, Arkansas, Gavia, Uyterhoeven, Engels and Kenton F1 showed a significantly higher TP content in the green leaves in comparison with the TP content measured in the white shaft. For the other cultivars, there was no significant difference.

In general, the white shaft of the summer, autumn and winter leek types had a mean TP content of 7.8, 7.3 and 8.9 mg GAE g^{-1} dw, respectively and did not show significant differences. Furthermore, the green leaves of the summer, autumn and winter types contained mean TP levels of 8.9, 8.1 and 9.9 mg GAE g^{-1} dw, respectively. The green leaves of the winter types exhibited a significantly higher phenolic content compared to the autumn types, while it was not significantly higher compared to content of the summer cultivars.

4.4.2.2 Flavonoids and phenolic acids

In addition to the global measurement of the total phenolic content of the leek samples, analyses were performed to identify and quantify flavonoid glycosides and phenolic acids in leek with a U-HPLC-ESI-Orbitrap-MS/MS system (JHI).

4.4.2.2.1 Identification

Based on the MS fragmentation data in ESI negative mode, kaempferol mono-, di- and triglycosides are predicted to produce kaempferol aglycone (*m/z* 285) after the loss of glycosyl units, while the quercetin glycosides will produce quercetin aglycone (*m/z* 301) after the loss of glycosyl units. In the case of isorhamnetin glycosides, the aglycone isorhamnetin will be produced (*m/z* 315). Assignment of different sugar substitutions to the flavonoid hydroxyl groups and their interglucosidic linkage was carried out in accordance with previous studies. These studies showed that the first fragmentation of the deprotonated molecular ion $[M-H]^-$ is due to the breakdown of the O-glycosidic bond at the 7-O position and the remaining sugars on the flavonoid molecule should be linked to the hydroxyl group at the 3-O position of the flavonol skeleton (Vallejo et al., 2004). Ferreres et al. (2004) reported that both flavonol sophorosides (1->2 glycosidic linkage) and flavonol gentiobiosides (1->6 glycosidic linkage) are characterised by the fragment ion $[M-324]^-$ as their base peak in MS^2 experiments. On the other hand, flavonol diglucosides with sugar moieties linked to different hydroxyl positions of the flavonol nucleus have the fragment ion

[M-H-162]$^-$ as their base peak. In addition, flavonol sophorosides were defined by the fragment ion [M-H-180]$^-$ and were also able to produce the fragment ions [M-H-162]$^-$ and [M-H-120]$^-$ (Ferreres et al., 2004). The result of the identification process is shown in Table 4.3.

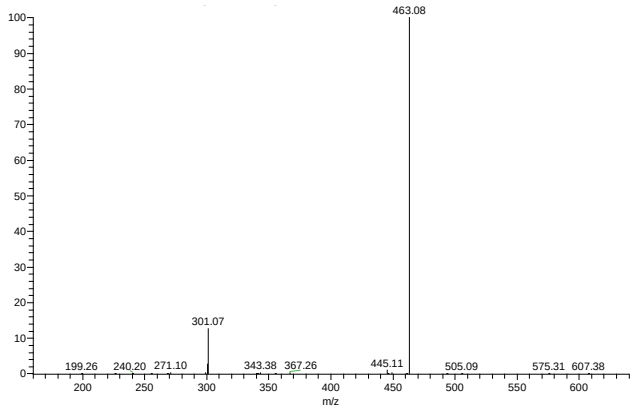
Table 4.3 MS and MS^2 data of identified polyphenol compounds in leek. Compounds indicated in bold are confirmed with authentic standards (FA, flavonoid aglycones; FM, flavonoid monoglucosides; FD, flavonoid diglucosides; PA, phenolic acids)

MS		MS	MS^2												Presence	
Experimental [M-H]$^-$ (*m/z*)	nr	Error (ppm)	[M-H-15]$^-$	[M-H-22]$^-$	[M-H-34]$^-$	[M-H-50]$^-$	[M-H-120]$^-$	[M-H-162]$^-$	[M-H-180]$^-$	[M-H-324]$^-$	[M-H-176]$^-$	RT	Compound	Class	White shaft	Green leaves
301.03448	**1**	**-3.0**		**179 (100)**		**151 (61)**						**3.62**	**Quercetin**			■
285.03946	**2**	**-3.5**			**151 (100)**							**4.13**	**Kaempferol**	**FA**	■	■
315.04959	**3**	**-4.6**	**300 (100)**									**4.17**	**Isorhamnetin**			■
463.08688	**4**	**-2.8**					**343 (2)**	**301 (100)**				**2.72**	**Quercetin 3-*O*-glucoside**			■
447.09183	**5**	**-3.3**					**327 (16)**	**285 (100)**				**2.95**	**Kaempferol 3-*O*-glucoside**	**FM**	■	■
477.10291	**6**	**-2.0**					**357 (19)**	**315 (100)**				**2.97**	**Isorhamnetin 3-*O*-glucoside**			■
625.13934	7	-2.7						463 (6)	445 (4)	301 (100)		2.65	Quercetin 3-*O*-sophoroside			■
609.14441	8	-2.8						447 (2)	429 (1)	285 (100)		2.88	Kaempferol 3-*O*-sophoroside	**FD**		■
609.14441	9	-2.8						447 (66)		285 (100)		2.37	Kaempferol 3-*O*-gentiobioside		■	■
609.14441	10	-2.8						447 (100)		285 (23)		2.03	Kaempferol 3,7-*O*-diglucoside			■
625.13934	**11**	**-2.7**						**463 (100)**		**301 (13)**		**2.36**	**Quercetin 3,4'-O-diglucoside**		■	■
299.05515	**12**	**-3.2**	**284 (100)**									**5.23**	**Kaempferol 4'-methylether**			■
355.10239	13	-3						193 (100)				1.98	Ferulic acid 4-*O*-glucoside	**PA**	■	■

The flavonol aglycones quercetin, kaempferol and isorhamnetin were identified by their deprotonated molecular ions (*m/z* 301 for **quercetin**, *m/z* 285 for **kaempferol** and *m/z* 315 for **isorhamnetin**) and characteristic mass fragment ions (*m/z* 151 and 179 for quercetin. *m/z* 151 for kaempferol and *m/z* 300 for isorhamnetin). They matched with authentic standards, corresponding to compound 1, 2 and 3, respectively (Table 4.3).

The monoglucosides were characterised by the fragment ions $[M-H-162]^-$ in the MS^2 spectra, corresponding to the loss of 1 glucose moiety (162 Da). Peak 4, 5 and 6 matched the retention time and MS/MS spectra of the authentic standards **quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside and isorhamnetin 3-*O* glucoside.** In each MS^2 spectra of these compounds, the cleavage of 120 Da was found.

Furthermore, different structures of diglucosides were elucidated. According to Ferreres et al. (2004), he MS^2 fragmentations of compound 7 and 8 are typical for 3-O-sophorosides. The MS^2 spectra of these compounds revealed the base peak $[M-H-324]^-$, the fragment ions $[M-H-180]^-$ and $[M-H-162]^-$, suggesting a sophoroside (1->2 glycosidic linkage) at the 3-O position, consequently they were identified as **quercetin 3-*O*-sophoroside** and **kaempferol 3-*O*-sophoroside,** and were also confirmed by the data of Vallejo et al. (2004). The absence of fragment ion $[M-H-180]^-$ in the MS^2 data of compound 9 revealed the interglycosidic linkage at the 3-O position as a gentiobioside (1->6 glycosidic linkage) and was identified as **kaempferol 3-*O*-gentiobioside.** Compound 10 lost 1 glucose moiety in MS^2, resulting in the base peak $[M-H-162]^-$ (loss at 7-*O* position) and fragment ion $[M-H-162-162]^-$ (loss at 3-*O* position) and was classified as **kaempferol 3,7-*O*-diglucoside,** also confirmed by the data of Vallejo et al. (2004). Compound 11 lost 1 glucose moiety in MS^2, resulting in the base peak $[M-H-162]^-$ and fragment ion $[M-H-162-162]^-$ and matched with the retention time and MS^2 spectra of an authentic standard **quercetin 3,4'-*O*-diglucoside.** The MS spectrum of compound 11 is shown in Figure 4.4.



Quercetin, kaempferol and isorhamnetin triglycosides are present in the samples, referring their deprotonated molecular ions *m/z* 787, *m/z* 771 and *m/z* 801, respectively, and their typical fragment ions (*m/z* 625, *m/z* 609 and *m/z* 639, data not shown). On the base of the MS^2 data it is possible to exclude the 3-*O*-triglucoside form because the base peak of 463, 447 and 477, respectively is not present. On the other hand, it is not possible to classify them as 3-*O*-sophoroside-7-*O*-glucoside or as 3,7,4'-*O*-triglucoside with only the MS^2 data. The MS spectra of compound 12 showed a deprotonated molecular ion at *m/z* 299 $[M-H]^-$ and fragment ions at *m/z* 284 $[M-H-15]^-$, corresponding to the loss of a methyl group. The peak matched the retention time and MS^2 spectra of an authentic standard of **kaempferol 4'-methylether.** The MS spectra of compound 13 showed a deprotonated molecular ion at *m/z* 355 $[M-H]^-$ and fragment ions at *m/z* 193 $[M-H-162]^-$, corresponding to the loss of 1 molecule of glucose from ferulic acid (Herchi et al., 2011), resulting in **ferulic acid 4-*O*-glucoside.**

Thirteen compounds were identified in leek and are summarised in Table 4.3, with their fragment ions in the MS^2 spectra. Only 5 polyphenolic compounds could be identified in the white shaft, while 13 compounds were present in the green leaves. In addition, Table 4.4 shows the chemical structure of the identified compounds

Table 4.4 Identified flavonols and phenolic acid with position and type of functional groups. OH: hydroxyl. O-Glu: esterified glucoside unit. O-CH_3: esterified methyl group. O-Soph: esterified sophoroside unit. O-Gent: esterified gentiobioside unit

Flavonoid subclass	Trivial name	Carbon position				
		3	**5**	**7**	**3'**	**4'**
flavonol	quercetin	OH	OH	OH	OH	OH
	Quercetin 3-O-glucoside	O-Glu	OH	OH	OH	OH
	Quercetin 3-O-sophoroside	O-Soph	OH	OH	OH	OH
	quercetin 3.4'-O-diglucoside	O-Glu	OH	OH	OH	O-Glu
	kaempferol	OH	OH	OH		OH
	Kaempferol 3-O-glucoside	O-Glu	OH	OH		OH
	Kaempferol 3-O-sophoroside	O-Soph	OH	OH		OH
	Kaempferol 3-O-gentiobioside	O-Gent	OH	OH		OH
	Kaempferol 3.7-diglucoside	O-Glu	OH	O-Glu		OH
	Kaempferol 4'-methylether	OH	OH	OH		O-CH_3
	isorhamnetin	OH	OH	OH	O-CH_3	OH
	Isorhamnetin-3-O-glucoside	O-Glu	OH	OH	O-CH_3	OH

Phenolic acid subclass	Trivial name	Carbon position	
		4	**5**
Hydroxycinnamic acid	Ferulic acid 4-O-glucoside	O-Glu	O-CH_3

4.4.2.3 Quantification

Polyphenols were quantified on the basis of a calibration curve obtained from standard solutions of reference compounds. As a consequence, quantification could only be done

for the identified compounds, of which standards were commercially available, *i.e.* quercetin 3,4'-O-diglucoside (Q34'G), kaempferol 3-O-glucoside (K3G), isorhamnetin 3-O-glucoside (I3G), quercetin 3-O-glucoside (Q3G), quercetin (Q), kaempferol (K), isorhamnetin (I) and kaempferol 4'-methylether (K4'M). Only Q34'G, K3G and K could be quantified in the **white shaft** of some cultivars (data not shown). The results were in the range of nd to 1.08 mg polyphenols 100 g^{-1} dw. The white shaft of cultivar Breugel F1 contained the highest polyphenol content.

The results of the individual polyphenol quantification in the **green leaves** of the 30 cultivars are shown in Figure 4.5. Seven compounds could be quantified in the green leaves of the leek samples, *i.e.* Q34'G, K3G, I3G, Q3G, Q, K and I. A lot of variation in polyphenol content was observed between the green leaves of the 30 leek cultivars. K3G was present in the highest amount, and was in the range of 6.17-104.96 mg 100 g^{-1} dw, followed by Q3G, which was in the range of nd – 61.60 mg 100 g^{-1} dw. The content of Q34'G varied from 0.12 to 25.99 mg 100 g^{-1} dw. The content of I3G and I in the green leaves were in the same range, whilst the aglycones Q and K were present in the lowest concentration. The green leaves of the cultivars Breugel F1, Artico and Pretan contained the highest amount of polyphenol compounds. The average content of polyphenols in the green leaves of the 30 cultivars was 45.70 mg 100 g^{-1} dw.

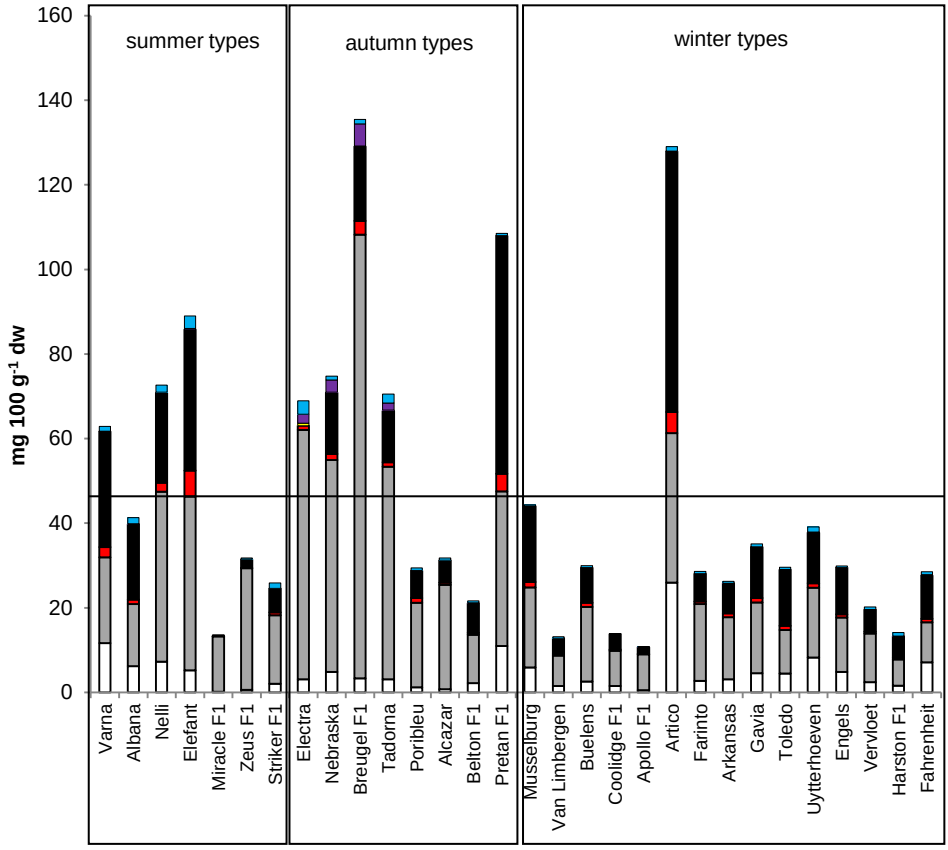


Figure 4.5 Polyphenol content (mg 100 g^{-1} dw) in the green leaves of the analysed leek cultivars, with ■, isorhamnetin, ■, kaempferol, ■ quercetin, ■ quercetin 3-O-glucoside, ■ isorhamnetin 3-O-glucoside, ■ kaempferol 3-O-glucoside and □ quercetin 3,4'-O-glucoside, with the full line, indicating the mean quantified polyphenol content of the green leaves of the 30 cultivars (n=3)

In general, there were no significant differences in individual polyphenol concentrations between the green leaves of the three leek types, except for K3O and kaempferol, where the autumn cultivars had a higher concentration than the winter cultivars.

4.4.3 L-ascorbic acid

The ascorbate content in the white shaft and green leaves varied from 0.89 to 3.55 mg g^{-1} dw and from 2.77 to 8.52 mg AA g^{-1} dw, respectively (Table 4.2). In most cases, the ascorbate levels in the green leaves were significantly higher than those measured in the white shaft, except for the cultivars Alcazar, Belton F1, Pretan F1 and the farmer selection of Van Limbergen, where no significant difference was measured. For some cultivars no

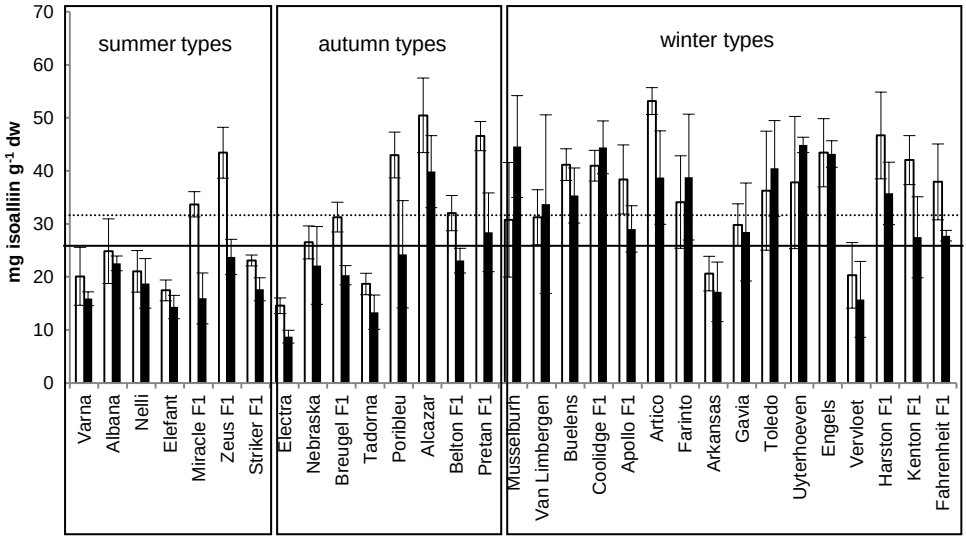
detectable ascorbate even appeared to be present using the method described (Electra, Nebraska, Breugel F1 and Tadorna). The highest total L-ascorbic acid content was observed in the white shaft extracts from Fahrenheit F1, Pretan F1 and Alcazar, and in the green leaf extracts of Albana, Fahrenheit F1 and Uyterhoeven. Of all the cultivars tested, the whole leek plant of the cultivar Fahrenheit F1 contained the highest mean ascorbate content (5.54 mg ascorbate g^{-1} dw).

In general, the white shaft of the summer, autumn and winter leek types had a mean ascorbate content of 1.7, 1.1 and 1.8 mg g^{-1} dw, respectively, but did not change significantly. Furthermore, the green leaves of the summer, autumn and winter types contained mean ascorbate levels of 6.0, 2.0 and 5.3 mg g^{-1} dw, respectively. The winter and summer cultivars had a significantly higher ascorbate concentration compared to the autumn cultivars.

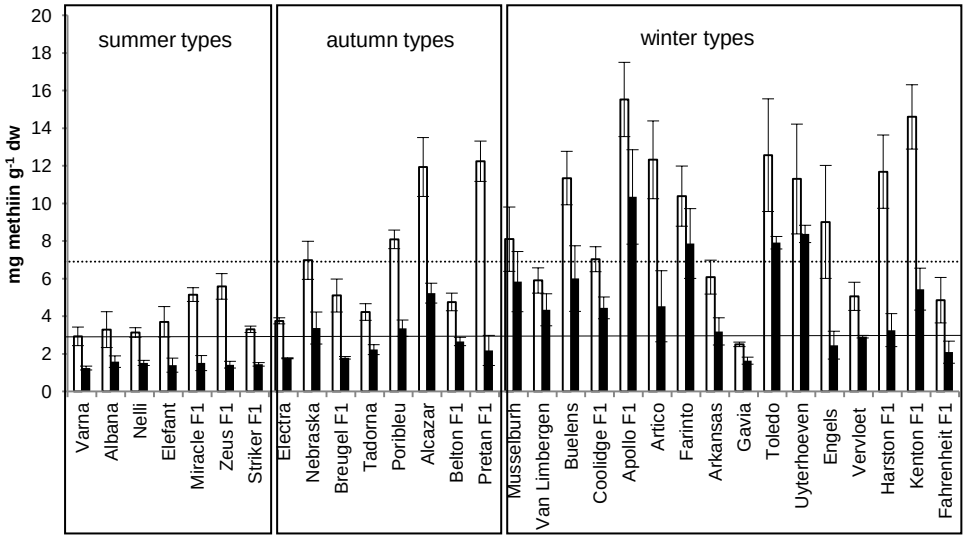
4.4.4 *S*-Alk(en)yl-L-cysteine sulfoxides

The results of the isoalliin and methiin content as a function of leek cultivar are shown in Figure 4.6 (a) and Figure 4.6 (b), respectively.

The isoalliin content of the white shaft and green leaves of the 30 leek cultivars varied from 14.56 to 53.17 mg g^{-1} dw and from 8.73 to 44.90 mg g^{-1} dw, respectively. For half of the leek cultivars, the isoalliin content was significantly higher in the white shaft than the green leaves. The highest isoalliin content was obtained from the white shaft extracts of the cultivars Artico, Alcazar and Harston F1 and from the extracts of the green leaves of the cultivars Uyterhoeven, Musselburh and Coolidge F1. Among the 30 leek cultivars, the whole leek plant of the cultivar Artico rated the highest for the mean isoalliin content (46.69 mg isoalliin g^{-1} dw).



(a)



(b)

Figure 4.6 Isoalliin (a) and methiin (b) content (mg g^{-1} dw) in the white shaft (□) and green leaves (■) of the analysed leek cultivars (n=3), with the dotted line, indicating the mean isoalliin/methiin content of the white shaft of the 30 cultivars, and the full line, indicating the mean isoalliin/methiin content of the green leaves of the 30 cultivars

The methiin content of the white shaft and green leaves of the 30 leek cultivars varied from 2.93 to 15.52 mg methiin g^{-1} dw and from 1.24 to 10.34 mg methiin g^{-1} dw. The white shaft of the 30 cultivars contained significantly higher amounts of methiin than the green leaves, except for cultivar Uyterhoeven. The highest methiin content was observed from the white shaft extracts of the cultivars Apollo F1, Kenton F1 and Toledo, and from the extracts of the green leaves of the cultivars Apollo F1, Uyterhoeven and Toledo. Among the 30 leek cultivars, the whole leek plant of the cultivar Apollo F1 rated highest for mean methiin content (13.19 mg methiin g^{-1} dw).

In general, the white shaft of the summer, autumn and winter leek types had a mean ACSO content (isoalliin + methiin) of 30.11, 40.02 and 45.81 mg g^{-1} dw, respectively. The ACSO content of the white shafts of the winter cultivars was significantly higher than the ACSO content of white shafts of the summer cultivars. Furthermore, the green leaves of the summer, autumn and winter types contained a mean ACSO content of 19.87, 25.35, and 39.15 mg g^{-1} dw, respectively. The green leaves of the winter cultivars contained a significantly higher ACSO content than the green leaves of the summer and autumn cultivars as well. In general, the whole leek plant of the winter cultivars contained a significantly higher amount of ACSOs.

4.4.5 Fructans

Results of the fructan quantification in the white shaft and green leaves are shown in Figure 4.7. However, because of practical reasons, only 1 analysis could be done for each cultivar and as consequence statistical analysis could not be performed.

The fructan content of the white shaft and green leaves of the 30 leek cultivars varied from 7.36 to 83.10 g fructan 100 g^{-1} dw and from 2.45 to 11.01 g fructan 100 g^{-1} dw, respectively. Large variation in the fructan content could be observed between the white shaft and green leaves of the 30 cultivars. In general, the white shaft of the summer, autumn and winter leek types had a mean fructan content of 51, 26 and 30 g 100 g^{-1} dw, respectively. The fructan content of the white shaft of the summer cultivars was higher than the content of shafts of the autumn and winter cultivars. Furthermore, the green leaves of the summer, autumn and winter types contained a mean fructan content of 6, 5, and 8 g 100 g^{-1} dw, respectively. The fructan concentration in the green leaves of the winter cultivars was higher than the concentration in the autumn cultivars.

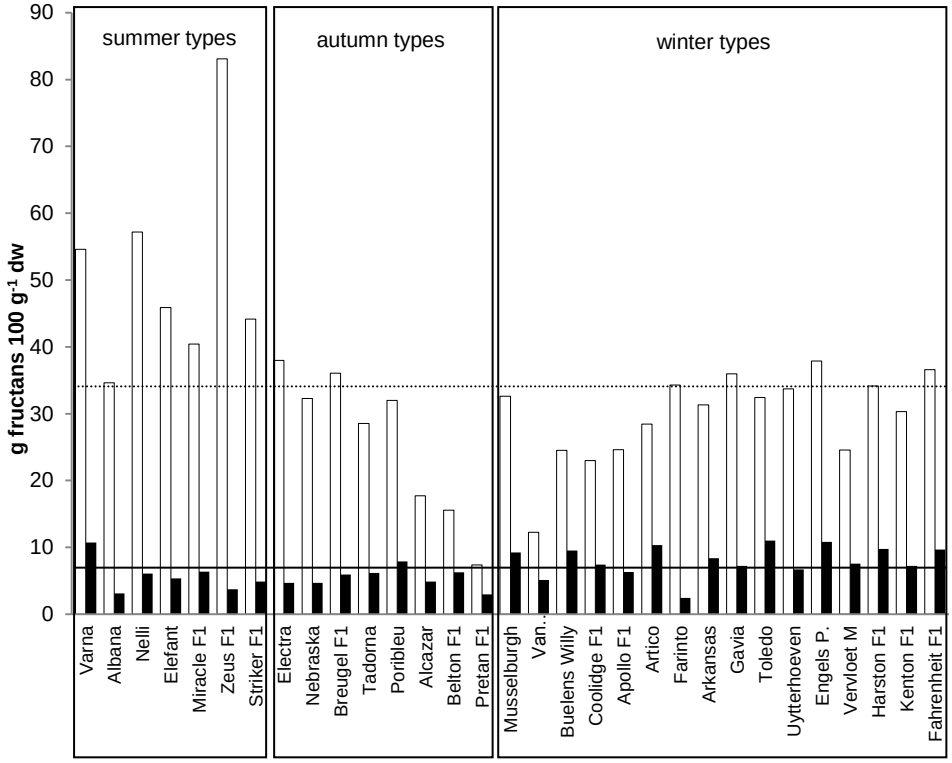


Figure 4.7 Fructan content (g 100 g^{-1} dw) in the white shaft (□) and green leaves (■) of the analysed leek cultivars (n=1), with the dotted line, indicating the mean fructan content of the white shaft of the 30 cultivars, and the full line, indicating the mean fructan content of the green leaves of the 30 cultivars

4.4.6 Correlation

To explore the influence of the major phytochemical constituents on the antioxidant capacity in the leek extracts of the 30 leek cultivars, we determined the correlation between the antioxidant capacity and the different classes of antioxidant substances and fructans. The correlation coefficients for the white shaft and green leaves are shown in Table 4.5.

Results of the ORAC and FRAP assay were positively but weakly correlated to the ascorbate and ACSO content, while the assays were negatively correlated with the fructan content in the **white shaft**. The DPPH results were only correlated with methiin and fructan content. The ORAC value of the **green leaves** was positively correlated with the flavonoids K3G, Q and K, while the FRAP results correlated well with the ascorbate and K

content. The content of Q34'G was positively correlated with the DPPH free radical scavenging activity of the green leaves.

Table 4.5 Pearson's correlation coefficients of the antioxidant activities (ORAC, FRAP and DPPH) with the antioxidant capacity, total phenol (TP), L-ascorbic acid (AA), ACSO, polyphenol and fructan content of the white shaft (first line) and green leaves (second line) of 30 leek cultivars

	TP	AA	isoalliin	methiin	Q34'G	K3G	I3G	Q3G	Q	K	I	fructans
ORAC	0.34	**0.40***	**0.48***	**0.63***	-0.33	-0.30	-	-	-	-0.31	-	**-0.66***
	0.09	-0.26	0.01	0.03	0.00	**0.44***	0.10	-0.04	**0.61***	**0.44***	**0.57***	-0.10
FRAP	0.28	**0.70***	**0.52***	**0.50***	-0.36	**-**	-	-	-	**-**	-	**-0.56***
	0.24	**0.52***	0.28	0.34	-0.03	**0.39***	-0.05	-0.05	-0.32	**0.37***	-0.11	0.04
DPPH	0.17	0.24	0.35	**0.49***	-0.31	0.09	-	-	-	0.12	-	**-0.60***
	0.19	0.25	0.18	-0.07	**0.56***	-0.13	0.32	0.50*	-0.16	-0.20	0.03	0.25

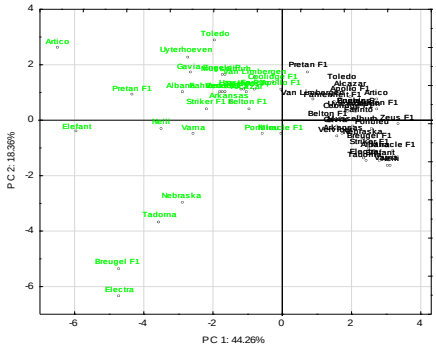
With Q34'G, quercetin 3,4'-O-diglucoside, K3G, kaempferol 3-O-glucoside, I3G, isorhamnetin 3-O-glucoside, Q3G, quercetin 3-O-glucoside, Q, quercetin, K, kaempferol and I, isorhamnetin

4.4.7 Principal component analysis

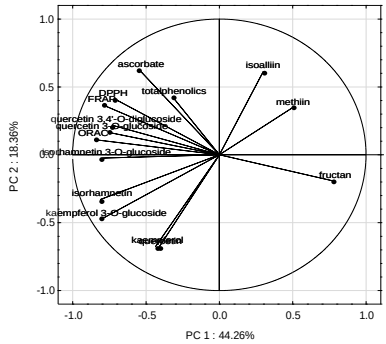
To reveal the internal structure of the results of the antioxidant capacity assays, polyphenol, ascorbate, ACSO and fructan analyses, principal component analysis (PCA) was applied on the whole data set of the white shaft and green leaves of the 30 leek cultivars. The dimensionality of the data was reduced from 15 partially correlated variables to 2 uncorrelated principal components, PC1 and PC2, accounting for 62.62% of the variation. The PCA plot (Figure 4.8 (a) and (b)) convincingly segregated the white shaft and the green leaves on the basis of the different parameters. More specifically, the white shaft of the 30 leek cultivars contained a higher isoalliin, methiin and fructan level, whilst the green leaves gave the best value for the other analysed parameters. It is clear that the green leaves of the 30 leek cultivars possessed stronger antioxidant properties than the white shaft.

A second PCA was applied on the data set of the results of the **white shaft** of the 30 leek cultivars. PC1 and PC2 accounted for 62.56% of the variation. The PCA plot (Figure 4.8 (c) a (d)) showed a clear segregation between the white shaft of the summer, autumn and winter types on the basis of the different parameters as well. More specifically, the summer cultivars were the cultivars with the highest content of fructan and quercetin 3,4'-O-diglucoside, while half of the autumn cultivars (Electra, Breugel F1, Tadorna and Nebraska) were highest in the kaempferol and kaempferol 3-O-glucoside. The winter cultivars and the other half of the autumn cultivars contained the highest amount of ACSOs, ascorbate, total phenolic content and possess the highest antioxidant capacity.

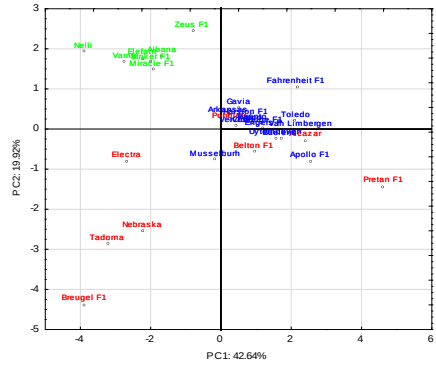
A third PCA was applied on the data set of the results of the **green leaves** of the 30 leek cultivars, where PC1 and PC2, accounted for 51.79% of the variation. The PCA plot (Figure 4.8 (e) and (f)) showed a clear segregation between the green leaves of the summer, autumn and winter types on the basis of the different parameters. More specifically, the winter cultivars contained the highest amount of ACSOs, fructan, total phenolic content, ascorbate and FRAP, while half of the autumn cultivars (Electra, Breugel F1, Tadorna and Nebraska) were highest in the three flavonoid aglycones quercetin, kaempferol and isorhamnetin, kaempferol 3-O-glucoside and ORAC value. The cultivars Elefant, Pretan F1 and Artico contained the highest amount of the other quantified polyphenolic compounds and DPPH free radical scavenging activity.



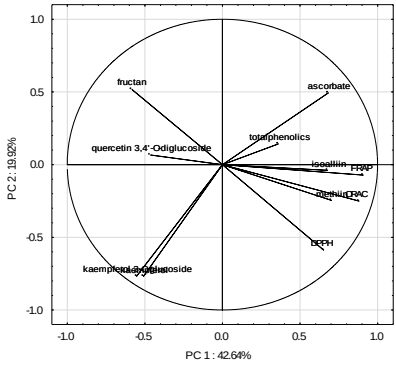
(a)



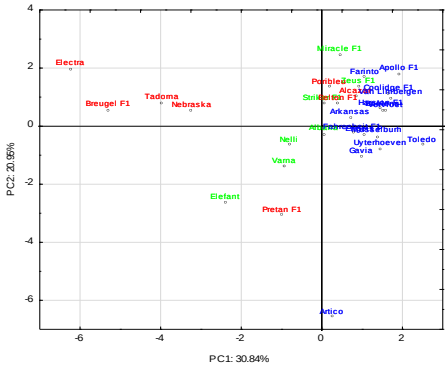
(b)



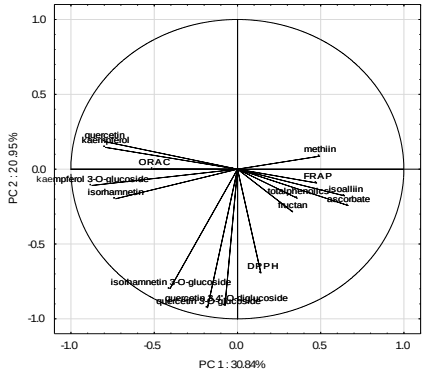
(c)



(d)



(e)



(f)

Figure 4.8 PCA plot of the scores (a, c, e) and loadings (b, d, f) of the whole dataset (a,b), white shaft (c,d) and green leaves (e,f) of 30 leek cultivars with summer (-), autumn (-) and winter types (-)

As stated in §3.2.1.1, leek cultivars were selected on the basis of morphological type (summer, autumn, winter) and breeding origin (hybrid, open pollination). In order to evaluate the influence of plant tissue (white shaft, green leaves), type of cultivar and breeding origin on the antioxidant properties, ANOVA was performed. The results of the tests are shown in Table 4.6.

Table 4.6 Analysis of variance of the antioxidant properties in 30 leek cultivars of different types and breeding origin

		ORAC	DPPH	FRAP	TP	AA	methiin	isoalliin
Plant tissue		*	*	*	*	*	*	*
type of cultivar	white shaft	*	*	*	*	*	*	*
	green leaves			*	*	*	*	*
breeding origin	white shaft			*				
	green leaves			*			*	

With * p<0.05

Table 4.6 indicated that the antioxidant properties of the leek extracts were significantly influenced by leek tissue. Type of cultivar had a significant influence on the antioxidant properties, except on the ORAC and DPPH activity of the green leaves, while breeding origin had a poor influence on the antioxidants, except for the FRAP assay and the methiin content.

4.4.8 Summary

To summarise the results, the average antioxidant values of the white shaft and green leaves of 30 leek cultivars are presented in Table 4.7.

Table 4.7 Antioxidant properties (mean ± standard deviation) of the white shaft and green leaves of 30 leek cultivars

	White shaft		Green leaves
ORAC (µmol TE g^{-1} dw)	57.14 ± 16.68	<	101.41 ± 13.64
DPPH (µmol TE g^{-1} dw)	6.15 ± 1.85	<	8.96 ± 1.83
FRAP (µmol $FeSO_4$ g^{-1} dw)	9.43 ± 3.69	<	25.90 ± 5.28
total phenolic content (mg GAE g^{-1} dw)	8.19 ± 1.52	<	9.21 ± 1.66
Q34'G (mg 100 g^{-1} dw)	0 - 0.32	<	4.65 ± 4.98
K3G (mg 100 g^{-1} dw)	0 - 0.84	<	24.78 ± 20.78
I3G (mg 100 g^{-1} dw)	nd	<	1.35 ± 1.46
Q3G (mg 100 g^{-1} dw)	nd	<	13.51 ± 14.59
quercetin (mg 100 g^{-1} dw)	nd	<	0.04 ± 0.12

kaempferol (mg 100 g^{-1} dw)	nd	<	0.48 ± 1.13
Isorhamnetin (mg 100 g^{-1} dw)	nd	<	0.88 ± 0.75
ascorbate (mg g^{-1} dw)	1.61 ± 0.89	<	4.59 ± 2.34
isoalliin (mg g^{-1} dw)	32.99 ± 10.60	>	27.52 ± 11.01
methiin (mg g^{-1} dw)	7.26 ± 3.68	>	3.59 ± 2.45
fructan (g 100 g^{-1} dw)	33.79 ± 14.34	>	6.84 ± 2.47

nd: not detected

Table 4.7 indicates the higher antioxidant capacity, the higher quantity of polyphenols and ascorbate in the green leek part compared to the white shaft. The white shaft was more rich in ACSOs and fructans.

4.5 Discussion

4.5.1 Antioxidant capacity

Antioxidant capacity methods differ in terms of their assay principles and experimental conditions. Because multiple reaction characteristics and mechanisms are usually involved in the assays, no single assay will accurately reflect all antioxidants in a mixed or complex system. Thus, to fully elucidate a full profile of antioxidant capacity, different antioxidant capacity assays may be needed (Ma et al., 2011). Therefore, the antioxidant capacity of the white shaft and green leaves of 30 leek cultivars was determined using 3 antioxidant capacity assays, including ORAC, DPPH and FRAP.

For the ORAC assay, the white shaft and green leaves of the 30 leek cultivars covered ranges of 27-88 and 82-135 µmol TE g^{-1} dw, respectively. The U.S. Department of Agriculture[5] (2010) measured the ORAC value of 277 selected foods and reported a total ORAC value of 569 µmol TE 100 g^{-1} fresh weight (fw) in the bulb and lower leaves of leek. Our results are expressed in g^{-1} dw, however a precise recalculation to 100 g^{-1} fw is not possible, because the water content was not determined for all the leek samples. For the samples that were analysed for their water content, the mean water content was 87% in the white shaft and 85% in the green leaves, resulting in a mean water content of 86% in the whole leek plant. Using this conversion factor to convert our results to g^{-1} fw, the ORAC values reported by the USDA are in the same range as those reported in this study, 379 to 1242 µmol TE 100 g^{-1} fw in the white shaft and 1150 to 1904 mg TE 100 g^{-1} fw in the green leaves. In the study of Proteggente et al. (2002) leek exhibited an ORAC value of 413 µmol TE 100 g^{-1} fw, which is in the range of our ORAC value of the white

[5] USDA

shaft of leek.
Comparing with other vegetables, the ORAC value for leek as reported by the USDA was in the same range as raw cabbage (529 µmol TE 100 g^{-1} fw), higher than the ORAC of raw tomatoes (387 µmol TE 100 g^{-1} fw) but lower than the ORAC in raw broccoli (1510 µmol TE 100 g^{-1} fw), raw garlic (5708 µmol TE 100 g^{-1} fw) and onion (913 µmol TE 100 g^{-1} fw).

DPPH results of the white shaft and green leaves of the 30 leek cultivars, covered ranges 2-11 and 5-14 µmol TE g^{-1} dw, respectively. Similarly, Mladenovic et al. (2011) reported a higher DPPH free radical capacity in the green leaves of leek (cv. Varna) compared to the shaft. Comparing with other *Allium* species, Gorinstein et al. (2009) investigated the antiradical activity against the DPPH radical for the *Allium* species garlic (7 µM TE g^{-1} dw), red onion (22 µM TE g^{-1} dw), white onion (21 µM TE g^{-1} dw) and yellow onion (20 µM TE g^{-1} dw), values which were higher than found in our study of leek, especially for onion.

The FRAP values ranged between 3-18 and 14-37 µmol $FeSO_4$ g^{-1} dw for the white shaft and green leaves of the 30 leek cultivars, respectively. This concurs with Nencini et al. (2011) who reported a higher antioxidant capacity in the leaves of 4 *Allium* species (*A. neopolitanum* Cyr., *A. roseum* L., *A. subhirsutum* L. and *A. sativum* L.) compared to the the bulb. Halvorsen et al. (2002) analysed the total antioxidant capacity in a variety of dietary plants, including leek, by the reduction of Fe^{3+} to Fe^{2+}. Leek contained a higher antioxidant capacity than tomato, cauliflower and cucumber, but a lower FRAP value than spinach, broccoli and red cabbage. In the study of Proteggente et al. (2002), leek exhibited a FRAP value of 160 µmol $FeSO_4$ 100 g^{-1} fw, which is in the range of our results using the conversion factor described above.

Each antioxidant component in a complex sample has a different activity pattern in each antioxidant capacity assay, resulting in different data for each assay (Prior et al., 2005; Serrano et al., 2007; Perez-Jimenez et al., 2008; Tafulo et al., 2010). Therefore, to ensure that a specific sample exhibits significantly higher antioxidant capacity than the other samples, several methods were used. The ORAC, DPPH and FRAP assays are the most widely used methods for determining antioxidant capacity *in vitro* and were applied in this study. The ORAC assay is the only method that takes free radical action to completion and uses the Area Under the Curve (AUC) technique for quantification. It combines both inhibition percentage and the length of inhibition time of the free radical action by antioxidants into a single value (Prior and Cao, 1999). The ORAC assay is considered to be a preferable method because of its biological relevance to the *in vivo* antioxidant efficacy (Gu et al., 2006). However, the ORAC only measures water soluble antioxidants. Although, there is convincing evidence showing that the water-soluble part of commonly

consumed foods contain the majority of the phytochemicals and antioxidants compared with the lipophilic fraction (Chu et al., 2000; Wu et al., 2004). For example, the study of Wu et al. (2004) showed that the hydrophilic part of onion contributes to over 98% of its total antioxidant capacity.

The DPPH assay is simple to use, but has some disadvantages that limits its application. Many antioxidants that react rapidly with peroxide radicals may have a very slow reaction to DPPH or may even be inert to it (Kurechi et al., 1980; Huang et al., 2005).

FRAP is the only assay that directly measures antioxidants in a sample. The other assays are indirect because they measure the inhibition of reactive species (free radicals) generated in the reaction mixture, and these results also depend strongly on the type of reactive species used. The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric reaction. Furthermore, the other assays use a lag phase type of measurement (Halvorsen et al., 2002). One disadvantage with the FRAP assay is that this assay does not react with thiols, because the reduction potential for thiols are generally below that of Fe^{3+}/Fe^{2+} half-reaction. However, vegetables from the *Alliaceae* family contain a high number of sulfur-containing compounds. In the study of Cao et al. (1993) garlic had the highest antioxidant capacity, analysed by the ORAC assay, while Halvorsen et al. (2002) showed that it had a very low ferric reducing potential.

The combination of the results of the three antioxidant capacity analyses gave an insight in the variation observed in function of leek tissue and leek cultivar. As these antioxidant capacities are due to the presence of antioxidants, specific bioactive compounds were also determined in the white shaft and green leaves of 30 leek cultivars and are described in the next sections.

4.5.2 Polyphenolic compounds

All of the leek cultivars tested, contained significant levels of total phenolic content (white part 5.31-13.96 mg GAE g^{-1} dw and green leaves 5.47-15.14 mg GAE g^{-1} dw) and compare favourably against those of the sister species such as onion (2-30 mg GAE g^{-1} dw) and garlic (20 mg GAE g^{-1} dw) (Kahkonen et al., 1999; Gorinstein et al., 2009).

Turkmen et al. (2005) determined 3 mg GAE g^{-1} dw in leek, which was less than found in our study. Proteggente et al. (2002) reported a TP content in the whole leek plant of 22 mg GAE 100 g^{-1} of fw, while Vandekinderen et al. (2009) found a content of 38-56 mg GAE 100 g^{-1} fw. Assuming a water content of 86% in our leek samples, the TP contents reported by Proteggente et al. (2002) and Vandekinderen et al. (2009) are much lower than found for the cultivars tested here (75 to 197 mg GAE 100 g^{-1} fw in the white shaft and 77 to 213 mg GAE 100 g^{-1} fw in the green leaves). These variations could be due to differences among cultivars, growing seasons, agricultural practices and variations in

applied total phenolic determination assays.

The U.S. Department of Agriculture (2010), reported a TP content of 47 mg GAE 100 g^{-1} fw in the bulb and lower leaves of leek. Again, this TP content is lower than our results. The TP content of leek reported by the USDA was in the same range as iceberg lettuce (50 mg GAE 100 g^{-1} fw), higher than the TP content of raw carrots (35 mg GAE 100 g^{-1} fw), raw cucumber (22 mg GAE 100 g^{-1} fw) and raw onions (23 mg GAE 100 g^{-1} fw), but lower than the TP content in raw broccoli (316 mg GAE 100 g^{-1} fw) and raw cabbage (202 mg GAE 100 g^{-1} fw).

Half of the leek cultivars analysed in this study contained a significantly higher total phenolic content in the green leaves compared to the white shaft, while this was not the case for the other half. These results are in contrast with other studies and the expectation that the green leaves in all cases would contain a significantly higher amount of total phenolic content in comparison with the white part (Mladenovic et al., 2011). It is reported that polyphenol synthesis is stimulated by light, and as a result polyphenols will accumulate in the outer and aerial plant tissue, in case of leek the green leaves (Cortell and Kennedy, 2006). Accordingly, several reports identified a correlation between enhanced polyphenol production and exposure to UV-B radiation (sunlight) in St. John's wort (Germ et al., 2010), barley (Kaspar et al., 2010) and Arabidopsis (Jordan et al., 1998). The explanation for why this is only true for half of our analysed leek cultivars could be attributed to other environmental factors and stress conditions that the white shaft can experience (Michalak, 2006). Besides UV light exposure, insect and microorganism pressure, low temperatures and low nutrient conditions correlate with the synthesis of polyphenols and can be responsible for a similar value of both leek tissues (Duval et al., 1999; Michalak, 2006). Environmental factors are not the only possible explanation for this phenomenon; the method employed to analyse the polyphenols can also result in varying results. The Folin-Ciocalteu method may also determine other reducing compounds such as reducing sugars, which are present in leek. These sugars can interfere with the Folin method if they are present in high concentrations (Vinson et al., 2001). Muir et al. (2007) reported 13.07 g and 6.43 g fructose 100 g^{-1} dw in the white shaft and green leaves of leek, respectively, while 9 g glucose 100 g^{-1} dw was present in both parts (own results). Because the white shaft of leek contains higher levels of sugars, it might result in a higher contribution to the interference with Folin-Ciocalteu method. In addition, the Folin-Ciocalteu reagent also reacts with some nitrogen compounds such as amino acids (*e.g.* tyrosine, tryptophan), vitamin C and amines (Peterson, 1979; Ikawa et al., 2003). Nevertheless, the applied extraction procedure of Vinson et al. (1998) ensures that the interference of vitamin C with the Folin–Ciocalteu method was not significant.

In addition to the general assay which determines the total phenolic content, a U-HPLC-

ESI-Orbitrap-MS/MS method was used to identify and quantify a set of flavonoids and phenolic acids in leek. Thirteen compounds could be **identified** based on RT, MS, MS^2 data and authentic standards. Seven of the 13 identified compounds were already reported in *Allium* species. For example, Q, Q3G and Q34'G were found in significant amounts in shallot and onion (Wiczkowski et al., 2008; Bonaccorsi et al., 2008; Lee and Mitchell, 2011a). K, I and I3G were present in onion cultivars (Bonaccorsi et al., 2005; Galdon et al., 2008; Lee et al., 2012) while K3G was identified both in onion (Muminova et al., 2006) and in leek (Fattorusso et al., 2001). Kaempferol/quercetin 3-O-sophoroside-7-O-glucuronide could be identified in guard cells of onions, but not the compounds kaempferol/quercetin 3-O-sophoroside (Urushibara et al., 1992). The remaining 6 identified polyphenols in leek, including kaempferol/quercetin 3-O-sophoroside, kaempferol 3-O-gentiobioside, kaempferol 3,7-O-diglucoside, kaempferol 4'-methylether and ferulic acid 4-O-glucoside are, to our knowledge, not yet identified in *Allium* species. However, these compounds have been identified in other species. Quercetin 3-O-sophoroside and kaempferol 3,7-O-diglucoside were found in broccoli florets by Price et al. (1998). Quercetin 3-O-sophoroside and kaempferol 3-O-sophoroside were also found in cowpea seeds. In addition kaempferol 3-O-gentiobioside was also identified in Senna leaves (Demirezer et al., 2011). Singh et al. (2006) isolated kaempferol 4'-methylether from *Echinops echinatus* and ferulic acid 4'-O-glucoside could also be detected in different berries (0.27-0.55 mg 100 g^{-1} dw) (Phenol-Explorer[6], 2011).

Quantification of flavonoids studied in leek revealed that the green leaves contained the highest amount, with an average of 48.70 mg polyphenols 100 g^{-1} dw.
Only Q34'G, K3G and K could be quantified in the white shaft of some cultivars (nd to 1.08 mg polyphenols 100 g^{-1} dw), while seven compounds could be quantified in the green leaves of the leek samples, *i.e.* Q34'G, K3G, I3G, Q3G, Q, K and I.
K3G was the most prominent flavonoid compound in leek (6.17-104.96 mg 100 g^{-1} dw). Analysis of leek by the method of Justesen et al. (1998), which was based on an acid hydrolysis, resulted in a K content of 3.1 mg 100 g^{-1} fw. The study of Hertog et al. (1992b) also quantified flavonoids in leek on the base of acid hydrolysis, and reported 2 mg Q 100 g^{-1} dw and 29.5 mg K 100 g^{-1} dw. Lugasi and Hovari (2000) also found trace amounts of Q (0.50 mg 100 g^{-1} fw) in leek. Comparing with other *Allium* species, the level of Q34'G was the highest in red onion (556 mg 100 g^{-1} dw) compared with the content in white onion (11 mg 100 g^{-1} dw). Q3G was not detected in the white onion, while 5 mg 100 g^{-1} dw was present in red onion (Zill-e-Huma et al., 2011). Lee et al. (2012) revealed that onion contained 73 mg Q34'G kg^{-1} fw and 0.7 mg Q3G kg^{-1} fw, while quercetin and isorhamnetin

[6] The first comprehensive database on polyphenol content in foods

could not be detected.
Some compounds were identified in leek, but could not be quantified, while other studies succeed in quantifying these compounds. Similarly, quercetin 3-O-sophoroside (6.50 mg 100 g^{-1} dw) and kaempferol 3,7-O-diglucoside (1.50 mg 100 g^{-1} dw) were quantified in broccoli florets in the study of Price et al. (1998). Ferulic acid 4'-O-glucoside was also quantified in different berries (0.27-0.55 mg 100 g^{-1} dw) (Phenol-Explorer, 2011).

4.5.3 L-ascorbic acid

Our reported L-ascorbic acid levels of the **white shaft** of leek (nd - 3.55 mg ascorbate g^{-1} dw) were in the same range as reported by Lundegardh et al. (2008) (1.6-2.3 mg ascorbate g^{-1} dw). Proteggente et al. (2002) reported a total vitamin C content in leek of 16 mg 100 g^{-1} fw and Vandekinderen et al. (2009) reported a content of 8.26-15.78 mg ascorbate 100 g^{-1} fw, which is in the same range as our results using the dry weight conversion factor (12-50 mg ascorbate 100 g^{-1} fw in the white shaft and 39-120 mg ascorbate 100 g^{-1} fw in the green leaves). However, the AA concentration in the edible part of leek (cv. Inegol-92), reported by Ozgur et al. (2011) (0.1285 mg g^{-1} dw) was lower than the average concentration in our analysed leek samples.
In general, higher ascorbate levels were found in the **green leaves** (nd – 8.52 mg ascorbate g^{-1} dw) of the leek cultivars compared to the white part. The higher levels in the sunexposed plant tissue is because AA is essential for protection against harmful side-effects of light during photosynthesis. AA is important for the detoxification of superoxide and hydrogen peroxide in chloroplasts (Hancock and Viola, 2005). Additionally, based on various experiments, Lester (2006) has reported that vegetable and fruit size majorly affects the concentration of available phytonutrients such as ascorbic acid. In general, ascorbic acid decreases with increasing size. Indeed, in our results, we found a significant but weak negative correlation for the green leaves ($r = -0.44$, $p < 0.05$) between the average weight of the leek plant for each cultivar and its ascorbate content (data not shown).

4.5.4 *S*-Alk(en)yl-L-cysteine sulfoxides

The most prominent ACSOs in leek, that is isoalliin and methiin, were analysed in this study. The isoalliin content of the white shaft and green leaves of the 30 leek cultivars varied from 14.56 to 53.17 mg g^{-1} dw and from 8.73 to 44.90 mg g^{-1} dw, respectively, while the methiin content of the white shaft and green leaves of the 30 leek cultivars varied from 2.93 to 15.52 mg methiin g^{-1} dw and from 1.24 to 10.34 mg methiin g^{-1} dw. Lundegardh et al. (2008) reported an ACSO content in the edible leek plant of ± 23.0 mg isoalliin g^{-1} dw

and ± 1.5 mg methiin g^{-1} dw. These values are in the range of our results, but less than the average amount of ACSOs quantified in our study. These variations could be due to differences among cultivars, growing seasons, agricultural practices and variations in the analytical method. Yamazaki et al. (2011) on the other hand, reported a mean isoalliin and methiin content of 37.4 mg g^{-1} dw and 7.7 mg g^{-1} dw, respectively, analysing 3 leek pools from 3 regions (USA, Belgium and Australia). These results are in the same range of our results.

Although quantified, the absolute content of these ACSOs is less important for their olfactory properties than their relative composition (Fritsch and Keusgen, 2006). In this study, we found that isoalliin predominated in leek. It constituted 87% of the total ACSOs analysed in the white shaft of the summer cultivars, 82% in the autumn cultivars and 80% in the winter cultivars. The same relative decreasing trend of isoalliin can be observed in the green leaves, 93% isoalliin in the summer cultivars, 88% in the autumn cultivars and 87% in the winter cultivars. In the study of Lundegardh et al. (2008), isoalliin constituted 92 to 96% of the ACSOs present in leek. In the study of Yamazaki et al. (2011), the molar ratio of isoalliin/methiin in the edible portion of leek was 81/19 (%), while Yoo and Pike (1998) found a ratio of 91.9/8.1 (%) in the leek leaves. Fritsch and Keusgen (2006) reported an isoalliin/methiin ratio in leek of 79/13 and also found 7% propiin. Yamazaki et al. (2011) concluded that onion, Welsh onion and leek generate similar flavours and result in an isoalliin/methiin/alliin ratio of 81-89/11-19/0 (%). Based on their research, Yoo and Pike (1998) identified 3 distinctive groups: the isoalliin, the methiin and the alliin dominant groups. Leek belongs, along with onion, shallot and bunching onion, to the isoalliin group. Species in this group contain no alliin or an undetectable amount of alliin. Garlic belongs to the alliin-dominant group, with an isoalliin/methiin/alliin ratio of 1/16/83 (%) (Hornickova et al., 2010).

4.5.5 Fructans

In a last test, fructans were determined in the range of 30 leek cultivars. The data set is based on 1 experimental analysis, therefore the conclusions should be restricted to reporting tendencies.

An average amount of 33.79 g fructans 100 g^{-1} dw and 6.84 mg 100 g^{-1} dw could be quantified in the white shaft and green leek leaves, respectively. The content of fructans is variable and depends on the cultivar. In the study of Grzelak-Blaszcyk et al. (2011), the highest content of fructans was observed in the shaft of leek cultivar Parton F1 (5.8 g 100 g fw^{-1}), whereas the least content in cultivar Belton F1 (3.8 g 100 g fw^{-1}), values which were in the range of our results.

Muir et al. (2007) determined the fructan content in leek, using the megazyme fructan

assay, and reported a value of 24 g fructans 100 g^{-1} dw in the white bulb of leek, while they did not detect fructans in the green leaves. Our reported values are higher compared to the results of Muir et al. (2007). To compare with related species, the same authors determined 16.1 g fructans 100 g^{-1} dw in the bulb of onion and 45 g in garlic. The highest content was quantified in Jerusalem artichoke (48.8 g fructans 100 g^{-1} dw).
The high fructan concentrations found in the present study in leek were also confirmed by the standardised method used in Cosucra (Warcoing) to quantify fructans in chicory roots (data not shown).

4.5.6 Correlation between the antioxidant capacity and the bioactive compounds of leek

A large number of different types of antioxidant compounds might contribute to the total antioxidant capacity. Therefore, to explore the influence of the analysed phytochemical constituents on antioxidant capacity in leek, we determined the correlation between the antioxidant capacity and main antioxidant substances (total phenolic content, flavonoids, ascorbate and ACSOs) and fructans.
The correlation study elucidated the correlation of the antioxidant capacity assays with the kaempferol content in the green leaves. Additionally, the ORAC antioxidant capacity correlated strongly with quercetin in the green leaves.
In general, the antioxidant capacity of polyphenolic compounds depends on several factors as described in §2.4.4.2 such as chemical structure of the individual component, synergistic interaction among them and specific conditions applied in different assays (Huang et al., 2005). The antiradical potential of polyphenolic compounds is due to their ability to donate hydrogen, which closely depends on the number of hydroxyl groups, the substitution pattern of hydroxyl groups and the chemical structure of the compound. It is reported that glycosylation of polyphenolic compounds reduces its activity when compared to their aglycone forms (Shahidi et al., 1992). Similarly, studies report the high *in vitro* antioxidant capacity of quercetin when compared with other polyphenolic compounds (RiceEvans et al., 1996).
Moreover, a significant correlation was observed between the antioxidant capacity assays and the ACSOs of the white shaft, indicating the antioxidative potential of the ACSOs. Similarly, the ORAC and FRAP assays were positively correlated with the AA content. Interestingly, the fructans in the white shaft were negatively correlated with the results of the three antioxidant capacity assays.
Moreover, some compounds correlated well with 1 antioxidant capacity assay, while other correlated with 2 or 3 antioxidant capacity assays. Some authors have reported that each polyphenol has different accessibility to the radical centre of DPPH, which may influence

the antioxidant power measured by this method (Sanchez-Moreno et al., 1998). It was found that quercetin can reduce more than 4 molecules of DPPH whereas ascorbic acid reduces nearly 2 DPPH molecules (Davalos et al., 2004).

4.5.7 Factors influencing the bioactive compounds of leek

All the results together showed that the antioxidant properties of the leek extracts were influenced by leek tissue (white shaft/green leaves) and type of cultivar (summer/autumn/winter) to a large extent, whilst the breeding origin (hybrid/open pollinated) had no influence on the antioxidant properties.

- In general, the white shaft of the 30 leek cultivars contained a higher isoalliin, methiin and fructan level, whilst the green leaves gave the best value for the other analysed parameters. It is clear that the green leaves of the 30 leek cultivars possess stronger antioxidant properties than the white shaft.
- In addition to leek part, leek type had a significant influence on the antioxidants as well. The white shaft of the summer cultivars had the highest content of fructan and quercetin 3,4'-O-diglucoside, while half of the autumn cultivars (Electra, Breugel F1, Tadorna and Nebraska) were highest in the kaempferol and kaempferol 3-O-glucoside. The winter cultivars and the other half of the autumn cultivars contained the highest amount of ACSOs, ascorbate, total phenolic content and possess the highest antioxidant capacity. The green leaves of the winter cultivars contained the highest amount of ACSOs, fructan, total phenolic content, ascorbate and FRAP, while half of the autumn cultivars (Electra, Breugel F1, Tadorna and Nebraska) were highest in the three flavonoid aglycones quercetin, kaempferol and isorhamnetin, kaempferol 3-O-glucoside and ORAC value. The cultivars Elefant, Pretan F1 and Artico contained the highest amount of the other quantified polyphenolic compounds and DPPH free radical scavenging activity.

The lower fructan content in the winter leek cultivars can be attributed to the hydrolysis of fructans as also observed in Jerusalem artichoke bulbs during winter seasoning (Grzelak-Blaszczyk et al., 2011).

The higher ascorbate content in the green leaves of the summer and winter cultivars may be due to climatic conditions, including light and average temperature during growth and development of plant tissues, which have a strong influence on the ascorbic acid content of horticultural crops. Although light is not essential for the synthesis of AA in plants, the amount and intensity of light during the growing season have a definite influence on the amount of AA formed (Lee and Kader, 2000). In general, the lower the light intensity during growth, the lower the AA content of plant tissues (Harris, 1975), which can explain the high values in the summer cultivars. Average growth temperature also influences the composition of plant tissues during growth and development. It is stated that plants will

contain more vitamin C when grown under cool temperatures, which can be an explanation for the higher ascorbate levels in winter cultivars (Lee and Kader, 2000).
The observed increase of the ACSO content towards the winter cultivars can be attributed to the role of these sulfur compounds in plants (defence against pests and predation, particularly in the overwintering bulb) and carbon, nitrogen, and sulfur storage and transport (Lancaster and Boland, 1990). These stress conditions can result in the conversion of the corresponding γ-glutamyl dipeptides to sulfoxides (Hornickova et al., 2010). The different accumulation pattern of ACSOs between the summer, autumn and winter leek types can also be explained by the genetic background and other environmental stress factors. Light radiation and water stress is reported to affect the biosynthesis of organosulfur compounds in onion (Freeman and Mossadeg, 1973). In addition, the average growing temperature as well as the root zone temperature (RZT) could strongly affect the flavour composition of onion (Coolong and Randle, 2006). However, a detailed understanding of the influence of environmental factors and their interactions with agricultural practices in relation to ACSOs present in leek is still lacking.

- Breeding origin had a poor influence on the antioxidants, except for the FRAP assay and methiin content

This study revealed the variability in antioxidants between a range of leek cultivars. This variability is necessary in breeding experiments, because it allows to distinguish possible cross parents (cultivars), rich in a specific bioactive compounds. Similarly, phenolic content in asparagus spears is considered an important characteristic in selecting breeding lines that show high antioxidant capacity and its determination might make the screening process relatively easy (Maeda et al., 2005).

4.6 Conclusion

To our knowledge, this is the first study that reveals the antioxidant properties among the range of commercially available and less commonly leek cultivars.
There were significant differences between the 30 leek cultivars with regard to the metabolites analysed including antioxidant capacity, total phenolic content, ascorbate and ACSOs. Dedicating from our results, cultivars Uyterhoeven, Pretan and Fahrenheit F1 gave the highest ORAC, DPPH and FRAP value, respectively, while Toledo and Breugel F1 had the highest polyphenol levels. Fahrenheit F1 contained the highest ascorbic acid levels, while Apollo and Artico were rich in ACSO levels. Zeus F1 was the cultivar with the highest fructan content. Based on our results, it is difficult to recommend a specific cultivar to leek growers, because it depends on the antioxidant compounds preferred, as shown in Figure 4.8. For example, some cultivars will have high kaempferol 3-O-glucoside levels,

but a low fructan content. Therefore, a choice has to be made between the desired properties.

In addition, this study reported the identification of 13 individual polyphenols in the white shaft and green leaves of leek. Six polyphenols identified in this study, including kaempferol/quercetin 3-O-sophoroside, kaempferol 3-O-gentiobioside, kaempferol 3,7-O-diglucoside, kaempferol 4'-methylether and ferulic acid 4-O-glucoside are, to our knowledge, not yet identified in *Allium* species.

The antioxidant properties of the leek extracts were influenced by part and type of cultivar to a large extent, whilst the manner of breeding had no influence on the antioxidant properties. The distinction between the three types can be explained by their genetic background, but due to different harvest times, stress factors such as temperature, solar radiation, pathogens etc. may also partly explain the different accumulation patterns of health-related compounds between the summer, autumn and winter leek. However, an understanding of the influence of environmental factors and their interactions with agricultural practices in relation to antioxidants present in leek is still lacking.

The cultivars of the 3 types were harvested in their commercial harvest period for each of the types. Therefore, it is not clear whether the difference in type is attributed to the genetic background or harvest time. In Chapter 5, the influence of harvest time on the antioxidant capacities will help to elucidate the differences between cultivars and may lead to recommended practises to maximise the antioxidant properties of leek.

CHAPTER 5. ANALYSIS OF ANTIOXIDANTS AS A FUNCTION OF HARVEST TIME

Redrafted from

Bernaert, N., Goetghebeur, L., De Paepe, D., De Clercq, H., De Loose, M, Daeseleire, E., Van Bockstaele, E. and Van Droogenbroeck, B. (2012). *S*-Alk(en)yl-L-cysteine sulfoxides as a function of the genetic diversity and maturity of leek (*Allium ampeloprasum* var. *porrum*). Journal of Agricultural and Food Chemistry, 60, 10910-10919.

5.1 Introduction

The results obtained from Chapter 4 revealed that leek tissue (white shaft, green leaves) and type of cultivar (summer, autumn, winter) mainly affects the antioxidant properties of leek whilst the manner of breeding (hybrid, open pollinated) did not have a large influence. As mentioned in Chapter 3, the different leek types studied in Chapter 4 were all sown at the same moment in April, but harvested in their respective commercial season (I). In commercial practice the different leek types are sown and harvested at different times. Because of the different times of harvest, it is not clear whether the observed difference between the leek types is attributed to the time of harvest or to the genetic background of the cultivars. As reported in other studies, this distinction could be due to the environmental factors, such as climatic (sun exposure, temperature, rainfall) or biotic factors (Torelli, 2000; D'Archivio et al., 2007). To elucidate the role of genetic background and harvest time, further detailed analyses were accomplished in growing season 2010 (II) and are described in this Chapter. Therefore, nine leek hybrids (3 cultivars from each type) were harvested at 4 subsequent time points along the growth season, whereupon the antioxidant properties were evaluated. Nine hybrids, also grown in season 2009, were chosen because of the uniformity of each object compared to open pollinated cultivars.
The results presented in this Chapter have been partly established in collaboration with Prof. Dr. Derek Stewart of the James Hutton Institute (Enhancing Crop Productivity and Utilization Theme) with regard to the flavonoid and phenolic acid analyses.

5.2 Plant material

§3.2.1 described the selection of plant material and sample preparation for field trial II. Briefly, 9 F1 leek hybrids (Table 5.1) were grown in 2010 and harvested at 4 subsequent time points along the growth season, that is September '10, November '10, January '11 and March '11.

Table 5.1 Nine leek hybrids grown in field trial II

Commercial name	Type	Breeding category	Seed company	Growing days
Miracle F1	summer	hybrid	Enza	159/211/263/292
Zeus F1	summer	hybrid	S&G	159/211/263/292
Striker F1	summer	hybrid	Bejo	159/211/263/292
Breugel F1	autumn	hybrid	Rijk Zwaan	159/211/263/292
Belton F1	autumn	hybrid	Nunhems	159/211/263/292
Pretan F1	autumn	hybrid	Nickerson-Zwaan	159/211/263/292
Coolidge F1	winter	hybrid	Hortiplan	159/211/263/292
Apollo F1	winter	hybrid	S&G	159/211/263/292
Harston F1	winter	hybrid	Nunhems	159/211/263/292

5.3 Bioactive compound analysis

A summary of the analyses (as described in §3.3) performed on the leek samples harvested as a function of harvest time is given in Table 5.2.

Table 5.2 Overview of the performed analyses as a function of harvest time

Analysis		Method
Antioxidant capacity	ORAC	spectrophotometric
	DPPH	spectrophotometric
Polyphenolic compounds	Total phenolic content	U-HPLC-ESI-Orbitrap-MS/MS
	Flavonoids and phenolic acids	HPLC-MS/MS
S-alk(en)yl-L-cysteine sulfoxides		

Because of practical reasons, FRAP, ascorbate and fructan analysis were not performed on these samples.

5.4 Results

As shown in Chapter 3 (Table 3.1), the nine leek hybrids were harvested after 159, 211, 263 en 292 growing days (from sowing to harvesting), corresponding with harvest in September ‘10, November ‘10, January ‘11 and March ‘11, respectively.

5.4.1 Antioxidant capacity

5.4.1.1 ORAC

The ORAC values (Figure 5.1) of the white shaft and green leaves covered significant ranges; ranging from 24.69-73.08 and 86.91-142.87 µmol TE g^{-1} dw, respectively.

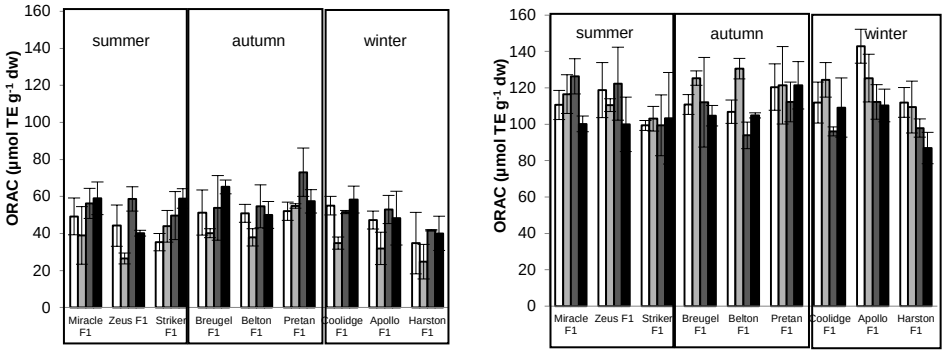


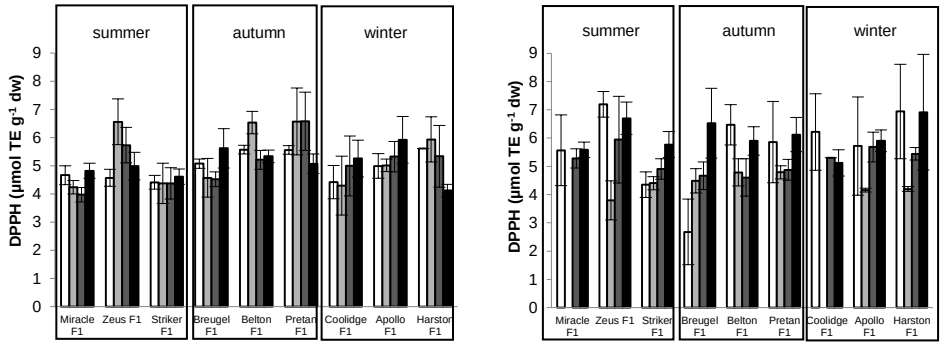
Figure 5.1 ORAC value of the white shaft (a) and green leaves (b) of 9 leek cultivars harvested in September '10 (□) , November '10 (■), January '11 (■) and March '11 (■), n=3

The white shaft of cultivars Zeus F1, Belton F1 and Pretan F1 contained significantly higher antioxidant capacities when harvest took place in January, compared with harvest in November. Harvest of Miracle F1, Striker F1 and Breugel F1 in March resulted in a significantly higher ORAC value compared with harvest in November. November harvest of Zeus F1, Belton F1 and the three winter cultivars resulted in the lowest antioxidant capacity of the white shaft.

Time of harvest did not have an influence on the ORAC value of the green leaves of Striker F1 and Pretan F1. When Miracle F1, Zeus F1, Breugel F1, Pretan F1, Apollo F1 and Harston F1 were harvested in March, the antioxidant capacity in the green leaves was the lowest. Harvest of Belton F1 and Coolidge F1 in November resulted in the highest ORAC value.

5.4.1.2 DPPH

Similarly, the associated DPPH values of these cultivars covered the ranges 3.91-6.57 and 2.67-7.20 µmol TE g^{-1} dw for the white shaft and green leaves, respectively (Figure 5.2).



(a) (b)

Figure 5.2 DPPH value of the white shaft (a) and green leaves (b) of 9 leek cultivars harvested in September '10 (□) , November '10 (■), January '11 (■) and March '11(■), n=3

Harvest time did not influence the antioxidant capacity of the white shaft of Striker F1, Coolidge F1 and Apollo F1, measured with the DPPH assay. Harvest of the white shaft of Zeus F1, Belton F1, Pretan F1 and Harston F1 in November resulted in the highest antioxidant capacity in comparison with harvest in March. When Miracle F1 and Breugel F1 were harvested in March, the antioxidant capacity was significantly higher compared with harvest in November and January.

Harvest time did not influence the antioxidant capacity of the green leaves of Miracle F1 and Coolidge F1, measured with the DPPH assay. The DPPH value of the green leaves of the cultivars Striker F1, Breugel F1 and Pretan F1, harvested in March was significantly higher compared with harvest in November and January. When Zeus F1, Belton F1, Apollo F1 and Harston F1 were harvested in November the DPPH value was the lowest compared with harvest in January and March.

5.4.2 Polyphenolic compounds

5.4.2.1 Total phenolic content

Figure 5.3 shows the results of the phenolic content evolution in the white shaft and green leaves of 9 leek cultivars, harvested at 4 different time points.

The TP content of the white shaft and green leaves of the nine leek cultivars varied from 2.89 to 7.69 mg GAE g^{-1} dw and from 5.56 to 8.91 mg GAE g^{-1} dw, respectively.

The white shaft of cultivars Miracle F1, Striker F1, Breugel F1, Belton F1 and Coolidge F1 contained a significant higher phenolic content when harvest took place in January or March compared with harvest in September. For the white shaft of cultivar Zeus F1, the highest polyphenol content was observed when harvest took place in November and January. Harvest of Pretan F1 in March resulted in the highest polyphenol levels, whilst January was the optimal month for Apollo F1. Harvest of Harston F1 in November resulted in the lowest level of total phenolic content.

The total phenolic content in the green leaves of cultivars Zeus F1, Belton F1 and Coolidge F1 did not change significantly during the growth season. Harvest in March, resulted in the highest polyphenol content in the green leaves of Breugel F1, whilst harvest in January ends in higher phenol content in the green leaves of Apollo F1. When Miracle F1, Striker F1 and Harston F1 were harvested in November, the polyphenol content was the lowest.

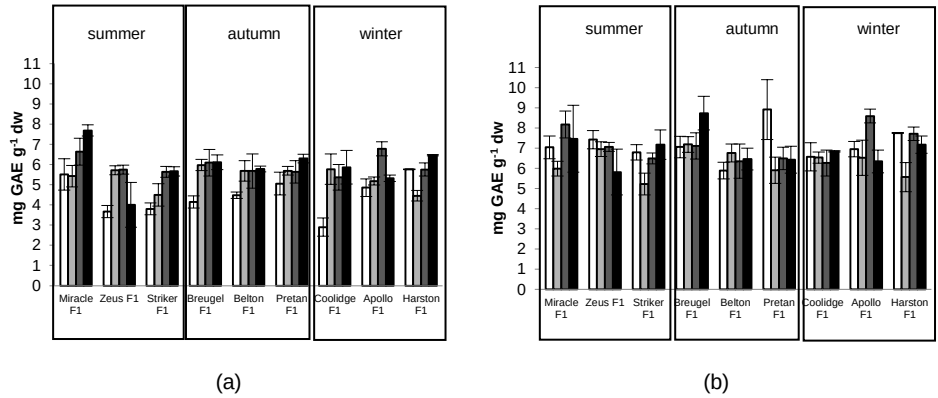


Figure 5.3 Total phenolic content of the white shaft (a) and green leaves (b) of 9 leek cultivars harvested in September '10 (□) , November '10 (■), January '11 (■) and March '11 (■), n=3

5.4.2.2 Flavonoids and phenolic acids

In Chapter 4 (§ 4.3.2.3), we quantified 7 polyphenolic compounds among a range of leek cultivars, that is quercetin 3,4'-O-diglucoside (Q34'G), kaempferol 3-O-glucoside (K3G), quercetin 3-O-glucoside (Q3G), isorhamnetin 3-O-glucoside (I3G), quercetin (Q), kaempferol (K) and isorhamnetin (I). In the current chapter, the same method (U-HPLC-ESI-Orbitrap-MS/MS) was used to quantify polyphenols as a function of harvest period. However, Q and Q34'G could not be quantified in these samples. Kaempferol 4'-methylether (K4'M) was not quantified in the samples of Chapter 4, but quantification was performed in the samples as a function of harvest period.

Figure 5.4 shows the content of the quantified flavonoids in green leaves of the nine cultivars harvested at 4 different moments. Only K3G, I and K4'M could be quantified in the white shaft of the samples (data not shown). K3G and Q3G were present in the highest amount in the green leaves of the 9 cultivars, followed by I3G (Figure 5.4). A great deal of variation was noticed in individual polyphenol content of the green leaves. For example, cultivars Breugel F1, Pretan F1 and Apollo F1 contained higher K3G levels compared to the other cultivars. Pretan F1 contained the highest level of Q3G, I3G and I. Q3G and I3G could not be quantified in the green leaves of Zeus F1. I3G was not present when Belton F1, Apollo F1 and Harston F1 was harvested in September and November, but could be quantified when harvested in January.

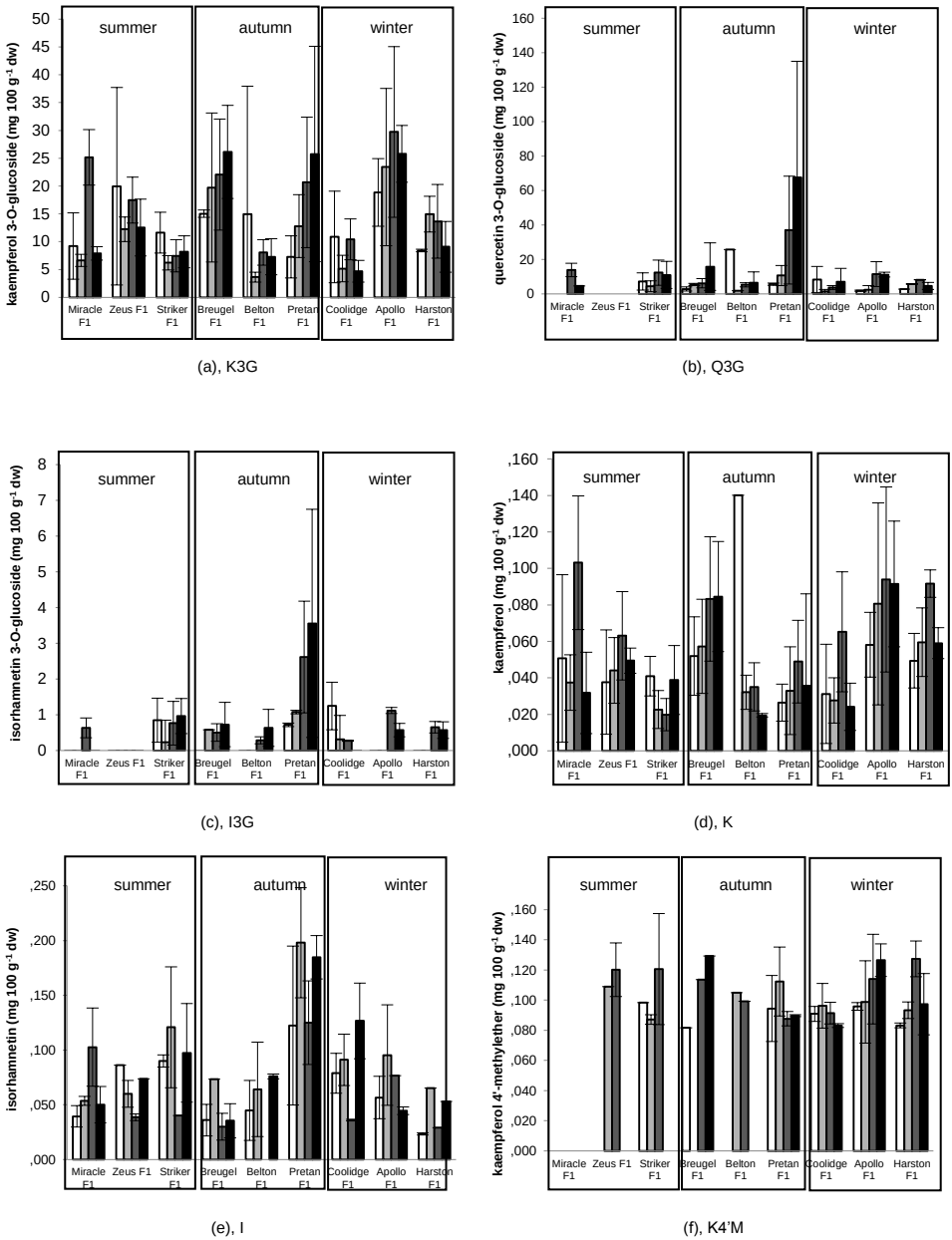


Figure 5.4 Kaempferol 3-O-glucoside (a), quercetin 3-O-glucoside, isorhamnetin 3-O-glucoside (c), kaempferol (d), isorhamnetin (e) and kaempferol 4' methylether (f) content of the green leaves of 9 leek cultivars harvested in September '10 (□) , November '10 (■), January '11 (■) and March '11(■), n=3

5.4.3 *S*-Alk(en)yl-L-cysteine sulfoxides

To determine whether differences in antioxidant properties between the leek types (summer, autumn, winter) were attributed to the genetic background or time of harvest, ACSOs were also quantified in 9 leek hybrids at 4 different periods during their growth. The results of the isoalliin and methiin content in the white shaft are shown in Figure 5.5. The ACSO content in the white shaft of the nine leek cultivars varied from 14.38 to 44.85 mg isoalliin g^{-1} dw and from 2.68 to 15.94 mg methiin g^{-1} dw, respectively.

The isoalliin content in the white shaft was significantly higher for all cultivars (except Pretan F1) when leek was harvested in September as compared by November harvest. For some of the cultivars (Miracle F1, Breugel F1, Apollo F1 and Harston F1), the isoalliin content was significantly higher when harvested in September in comparison with the other 3 harvest periods.

For most of the cultivars, November harvest resulted in a significant lower isoalliin content in the white shaft as compared with the plant material harvested at the other 3 harvest periods. The extracts of the white shaft of the autumn cultivars contained in general the highest amount of isoalliin, irrespective of the time of harvest.

Next to the isoalliin content, also the methiin content in the white shaft was significantly higher for all cultivars (except for Pretan F1, Harston F1, Zeus F1 and Apollo F1) harvested in September in comparison with November. For some of the cultivars (Miracle F1, Breugel F1 and Coolidge F1), the methiin content was significantly higher when harvested in September in comparison with the other 3 harvest periods. November harvest resulted in a significant lower methiin content in the white shaft of most of the cultivars in comparison with harvest at the other 3 periods. The methiin content of the summer cultivars Miracle F1 and Striker F1 was significantly lower than the autumn and winter cultivars, irrespective of the date of harvest. The autumn cultivars contained the highest amount of methiin, followed by the winter cultivars.

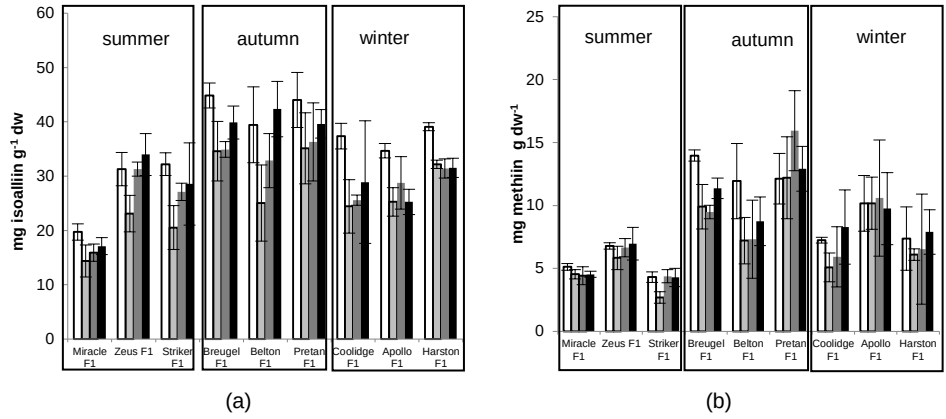
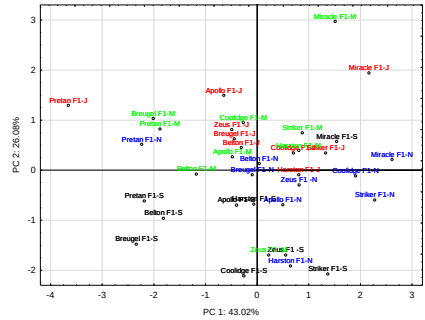


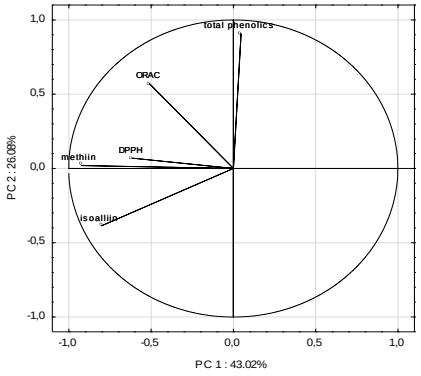
Figure 5.5 Isoalliin (a) and methiin (b) content of the white shaft of 9 leek cultivars harvested in September '10 (□) , November '10 (▫), January '11 (▪) and March '11 (■), n=3

5.4.4 Principal component analysis

To visualise the results of the antioxidant capacity assays, polyphenol content and ACSO levels, PCA was applied on the data set of the white part of the nine cultivars harvested on 4 time points. The dimensionality of the data was reduced from 5 partially correlated variables to 2 uncorrelated principal components, PC1 and PC2, accounting for 69.10% of the variation. The PCA plot (Figure 5.6) segregated the samples which were harvested in September and November from the other samples on the basis of the different antioxidant parameters. The white shaft of the cultivars harvested in November contained lower levels of antioxidants compared with harvest at the other 3 time points. The cultivars harvested in January and March are a good source of polyphenolic content and possess a high ORAC value, while harvest in September resulted in a higher isoalliin content. Cultivar Miracle F1 was clearly high in polyphenols, while Pretan F1 gave a high value for the other antioxidants, irrespective the time of harvest.



(a)



(b)

Figure 5.6 PCA plot of the scores (a) and loadings (b) of the white part of 9 leek cultivars harvested in September (-), November (-), January (-) and March (-)

5.4.5 Influence of harvest time

Analysis of variance was accomplished in order to evaluate the influence of harvest time on the antioxidant properties. Table 5.3 summarises the effect of cultivar and harvest time on the antioxidant properties of the white shaft of 9 leek cultivars. Cultivar and harvest time had a significant effect on the antioxidants, with the exception of the non-significant influence of harvest time on the DPPH free radical scavenging activity.

Table 5.3 The analysis of variance results for the variables: ORAC, DPPH, total phenolic content, isoalliin and methiin for the white shaft

	ORAC	DPPH	TP	isoalliin	methiin
cultivar	*	*	*	*	*
harvest time	*		*	*	*
cultivar × harvest time	*	*	*	*	*

* p<0.05

The interaction term was significant for each parameter, indicating the importance of the choice to harvest a certain cultivar at a specific time on the content of antioxidants. The results indicate that harvest time obviously plays a role in the antioxidant content of leek.

5.4.6 Influence of year

As described in Chapter 3, 9 leek F1 cultivars were grown both in 2009 and 2010. As a result, samples of the two years were analysed for their antioxidant properties, and the mutual data for the two years consist of ORAC, DPPH, TP, isoalliin and methiin data.

Table 5.4 presents the ranges of the antioxidant properties of the nine cultivars grown in 2009 and 2010. For the cultivars grown in 2010, the mean value and range was calculated on the basis of the harvest moment closest to the harvest moment of 2009.

Table 5.4 Antioxidant ranges in the white shaft and green leaves of the 9 leek cultivars grown in 2009 and 2010

	2009		2010	
	White shaft	**Green leaves**	**White shaft**	**Green leaves**
ORAC (µmol TE g^{-1} dw)	33.13-88.07	81.57-112.18	24.69-73.09	86.92-142.88
DPPH (µmol TE g^{-1} dw)	2.27-11.18	5.25-10.52	3.97-6.57	2.33-7.20
Total phenolic content (mg GAE g^{-1} dw)	6.98-8.67	7.89-11.30	2.89-7.69	5.22-8.91
Isoalliin (mg g^{-1} dw)	23.13-46.68	15.96-44.43	14.38-44.85	12.93-38.11
Methiin (mg g^{-1} dw)	3.31-15.52	1.42-10.34	2.68-15.93	1.15-8.63

Table 5.5 shows the results of the analysis of variance in order to evaluate the influence of year. Significant differences were found among cultivars within years, but also between the two years (2009 and 2010), except for the methiin content. The methiin content in the 9 cultivars did not differ between the two years.

Table 5.5 The analysis of variance results for variables: ORAC, DPPH, TP, isoalliin and methiin

		ORAC		DPPH		TP		Isoalliin		Methiin	
	DF	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Cultivar	8	19.9	<0.0001	24.3	<0.0001	3.2	0.008	13.6	<0.0001	32.5	<0.0001
Year	1	24.8	<0.0001	56.8	<0.0001	256.1	<0.0001	38.5	<0.0001	0.3	0.619
Cultivar × year	8	4.5	0.001	15.6	<0.0001	4.0	0.002	7.6	<0.0001	8.8	<0.0001

5.5 Discussion

To elucidate the role of harvest time on the antioxidant properties (as potentially observed in Chapter 4), further detailed analyses were accomplished in growing season 2010 and were described in this Chapter. Therefore, nine leek hybrids (3 cultivars from each type) were harvested at 4 subsequent time points along the growth season, whereupon the antioxidant properties were evaluated.

In general, the results of the **antioxidant capacity** demonstrate the significant effect of harvest period on the antioxidant capacity of the nine cultivars. The ORAC results of the white shaft revealed the lowest antioxidant capacity of the samples harvested in November. Harvest in January or March resulted, in most of the cultivars and in both parts, in a higher DPPH antioxidant capacity. Between the nine cultivars, few differences were noticed.

The results of the **TP** analysis demonstrated the effect of harvest time as well. Harvest in January or March resulted, in most of the cultivars and in both parts, in a higher TP content. The differences in harvest period were not significant for the individual polyphenols. Few differences could be seen in the TP content between the nine cultivars, while the individual polyphenol content varied significantly, with the exception of kaempferol 4'-methylether. In general both parameters, *i.e.* harvest time and cultivar, play a role in the content of polyphenolic compounds. These results are in accordance with Yang et al. (2009), who concluded that the content and profile of flavonol glycosides in sea buckthorn berries depended highly on the origins, subspecies and cultivars and were strongly influenced by the harvesting dates and annual variations. In addition, the flavonoid concentration in the baby spinach leaves also varied as a result of the influence of several factors, such as plant age and seasonal variation in environmental conditions (Bergquist et al., 2007). In contrast, the phenolic content of asparagus spears was largely influenced by the genetic background, while harvest time did not have an effect (Papoulias et al., 2009). Similarly, Patil et al. (1995) studied the variation of the flavonoid quercetin in onion bulbs in 5 growth stages. The quercetin content varied slightly, but concentrations at the final growth stage were similar to the

first growth stage. The same study revealed that meteorological factors (including temperature and rainfall patterns) have a stronger influence on quercetin concentration in onion cultivars than soil factors or plant maturity. Higher flavonoid levels in onion could be related to a higher global radiation and lower rainfall during the growing season. These meteorological conditions can enhance secondary metabolism in onions, favouring the synthesis of flavonoids (Rodrigues et al., 2011). Similarly, extreme climatic conditions in terms of salinity, low rainfall and high radiation are likely related to the increase of the Tunisian halophytes antioxidant potentialities as a response to the oxidative stress generated by the formation of reactive oxygen species in these hostile environments (Ksouri et al., 2008). Significant relationships were also revealed among meteorological (rainfall, sunshine incidence and temperature) and antioxidant variables for strawberries (Wang and Zheng, 2001).
The meteorological conditions during leek growth from September 2010 to March 2011 were monitored (KMI, 2012). Figure 3.5 presents the average month temperature, hours sunlight and precipitation during the leek harvest season 2010. These graphs revealed the lower temperature in December and January, the limited photoperiod in November, December, January and February, the high rainfall in November and the low precipitation in February and March. These different conditions between the harvest months of 2010 can explain the distinction in polyphenol content at 4 harvest times. For example, the lower temperature in January can explain the higher total phenolic content. Similarly, Mori et al. (2005) demonstrated that higher temperature can result in less accumulation of anthocyanins in berries and variation in polyphenolic composition. While, Pineli et al. (2011) found the lowest amount of anthocyanins in berries in the month with the lowest average temperature and highest photoperiod.

Little has been written on the variation of **ACSOs** related to harvest time or environmental parameters. Our results demonstrated that harvest of leek in September, corresponding with a high average temperature (Figure 3.5) resulted in the highest amount of ACSOs for each cultivar. These findings are in accordance with Coolong and Randle (2003), who reported a significantly higher total ACSO content in onions grown at high temperatures in comparison with ACSO content of onions grown at lower temperatures. However, our results do not agree the results of the study of Hornickova et al. (2010), indicating that the content of ACSOs in 52 garlic genotypes primarily depends on various genetic factors and post-harvest storage conditions, whereas the climatic conditions (*e.g.* temperature, irrigation) during the growth influence their levels to a lesser extent.

In general, we can observe significant differences between the **four harvest times**,

based on the different parameters, except for the DPPH radical scavenging capacity. Based on the principal component analysis, there is a clear distinction between harvest in September and harvest in November, and between September/November and the other 2 months. Harvest in January or March resulted, in most of the cultivars and both parts, in a higher antioxidant capacity and polyphenol levels. Harvest in September had a positive influence on the ACSO content.
In addition to the influence of harvest time, leek cultivar has also an influence on the antioxidant properties. More specifically, leek cultivar Miracle F1 was clearly high in polyphenols, while Pretan F1 gave a high value for the other antioxidants, irrespective the time of harvest. Although, these trends were not observed in the results of Chapter 4.

The significant differences in antioxidant levels (except for methiin) for the 9 cultivars between the **two subsequent years** (higher levels in 2009 plant material) could be attributed to different meteorological conditions (Figure 3.5) between the two years, but also to the different soil properties (mineral content, pH) and fertilisation and disease treatment (Table 3.4 and Table 3.5).
Figure 3.5 gives the differences in average month temperature, sunlight hours and precipitation for the region between the two growing seasons (KMI, 2012). The mean month temperature in November and December was much lower in year 2010 in comparison with year 2009. In addition there was higher rainfall in August ’10 and September ’10. These stress conditions can be a reason why the antioxidants of the leek cultivars show significant differences.
The soil data indicate that the soil of season 2009 had a higher mineral content (P, K, Mg, Ca, Na) compared to the soil of 2010. A higher mineral content can be responsible for higher antioxidant levels in the plant as some studies report a correlation between minerals and antioxidants. For example, an enhanced K-fertilisation increased the level of phenolic compounds and the corresponding antioxidant capacity in sweet potatoes leaves (Redovnikovic et al., 2012).
The difference in N-fertilisation and disease treatment (no insecticide in 2010 because no thrips) could be another explanation for the difference between the two years. For example, soaking plants in a fungicide (phosphite) solution prior planting was effective in activating strawberry defence mechanisms, since fruit ascorbic acid and anthocyanin content increased (Moor et al., 2009). Similarly, different levels of ACSOs were found in white rot (*Sclerotium cepivorum*) resistant onions compared to susceptible cultivars.
In addition, the pH of the soil of 2009 was in the range of the optimum pH for leek, while this was not the case for the soil of 2010.

5.6 Conclusion

In Chapter 4, we revealed that leek tissue and type of cultivar (summer, autumn, winter) mainly affects its antioxidant properties, rather than breeding origin. In that experimental set-up, the leek cultivars were collected at the time the particular leek cultivar is harvested in normal commercial agricultural practices. In this chapter, 9 leek F1 hybrid cultivars were harvested at 4 time points during the leek growth season and antioxidant properties were determined. In general, we could observe differences between the cultivars and between the four harvest times, based on the different antioxidant parameters, except for the DPPH antioxidant capacity. There is a clear distinction between harvest in September and harvest in November, and between September/November and the other 2 months. Harvest in January or March resulted, in most of the cultivars and both parts, in a higher antioxidant capacity and polyphenol levels. Harvest in September had a positive influence on the ACSO content.
Based on these results, we can conclude that the difference in antioxidants between the leek types, as observed in Chapter 4, is attributed both to harvest time and the genetic background of the cultivar.

In addition, some dissimilarities were found between the antioxidants in leek grown in 2009 and 2010. These differences in antioxidant levels could be attributed different meteorological conditions or to different soil properties (mineral content, pH) and disease treatments between the two years.

CHAPTER 6. COMPARISON OF THE BIOACTIVE COMPOUNDS IN LEEK AND SOME OF ITS RELATED *ALLIUM* SPECIES

6.1 Introduction

Chapter 4 gave an overview of the antioxidant properties of 30 leek (*Allium ampeloprasum* var. *porrum*) cultivars. We could observe significant differences in health-promoting compounds between white shaft and green leaves and type of cultivar (summer, autumn, winter), while manner of breeding (hybrid, open pollination) did not have a large effect.

It would be of interest to position the obtained data for leek in comparison with some members of the same genus as several studies report high antioxidant levels in onion (*Allium cepa* L.), shallot (*Allium ascalonicum* L.), bunching or Welsh onion (*Allium fistulosum* L.), chives (*Allium schoenoprasum* L.) and garlic (*Allium sativum* L.), but studies on leek are limited (Bianchini and Vainio, 2001; Griffiths et al., 2002; Benkeblia et al., 2004; Bloem et al., 2004; Fritsch and Keusgen, 2006; Aoyama and Yamamoto, 2007; Lu et al., 2011). Moreover, garlic and onion extracts have been recently reported to be effective in prevention of cardiovascular disease, because of their hypocholesterolemic, hypolipidemic, anti-hypertensive, anti-diabetic, antithrombotic and anti-hyperhomocysteinemia effects, and many other biological activities including antimicrobial, antioxidant, anticarcinogenic, antimutagenic, antiasthmatic, immunomodulatory and prebiotic activities (Corzo-Martinez et al., 2007).

Therefore, it is relevant to perform the same antioxidant analyses on species related to leek, grown under the conditions as described in Chapter 3. The results of these analyses can realise a better comparison of leek and its family members. However, we have to take into account that the consumption quantity of different *Allium* species used in human diet differ significantly. Onion, garlic and chives are universally used spice plants and are extensively used for food flavouring (Augusti, 1990), while leek and bunching onion are more important as vegetables with additional flavouring properties (Fritsch and Keusgen, 2006).

In order to compare leek with some of its related *Alliums*, the same bioactive compound analyses as described in Chapter 4 were performed on the different plant parts of 6 related species.

The results presented in this Chapter have been partly established in collaboration with Prof. Dr. Derek Stewart of the James Hutton Institute (Enhancing Crop Productivity and Utilization Theme) with regard to the FRAP, flavonoids/phenolic acids and ascorbate analyses.

6.2 Plant material

§3.2.1 described the selection of plant material and sample preparation for the *Allium* species (Table 6.1) grown in field trial I. The *Allium* species were grown in 2009 and harvested from September '09 until March '10.

Table 6.1 Overview of the selected *Allium* species

Scientific name	Trivial name	Harvest month
Allium ampeloprasum var. *kurrat*	Egyptian leek	March '10
Allium odorum L.	Chinese leek	November '09
Allium schoenoprasum L.	Chive	October '09
Allium cepa L.	Red onion	October '09
	White onion	October '09
Allium fistulosum L.	Bunching onion	October '09
Allium ascalonicum L.	Shallot	September '09

6.3 Bioactive compound analysis

A summary of the analyses (as described in §3.3) performed on the *Allium* species is given in Table 6.2.

Table 6.2 Overview of the performed analyses on leek and some of its related *Allium* species

Analysis		Method
Antioxidant capacity	ORAC DPPH FRAP	Spectrophotometric
Polyphenolic compounds	Total phenolic content	Spectrophotometric
	Flavonoids and phenolic acids	U-HPLC-ESI-Orbitrap-MS/MS
L-ascorbic acid		HPLC-PDA
S-alk(en)yl-L-cysteine sulfoxides		HPLC-MS/MS
Fructans		HPLC-RI

6.4 Results

The overall average antioxidant properties of the 30 leek cultivars (7 summer, 8 autumn and 15 winter cultivars) described in Chapter 4, were used to make the comparison between leek and its related species and are indicated in Figure 6.1, 6.2, 6.4, 6.5 and 6.6.

6.4.1 Antioxidant capacity

The results of the three antioxidant capacity assays (ORAC, DPPH and FRAP), are shown in Figure 6.1 (a), (b) and (c), respectively. The mean value for the white shaft and green leaves of the 30 leek cultivars, and the summer, autumn and winter cultivars is given in the left-hand side of the graph as well, as a reference.

6.4.1.1 ORAC

The antioxidant capacity (as measured with the ORAC assay) of the **white part** of the different species ranged from 31 to 230 µmol TE g^{-1} dw (Figure 6.1 a). The lowest ORAC value was found in the white bulb of *A. cepa, A. schoenoprasum* and *A. odorum*. The ORAC value of white part of *A. kurrat*, *A. cepa* (cv. Red Creole), *A. fistulosum* and *A. ascalonicum* was significantly higher than the ORAC of *A. odorum* and *A. schoenoprasum.* The ORAC results of the **green leaves** ranged from 81 to 271 µmol TE g^{-1} dw. In general, *A. cepa* (cv. Red Creole) gave the highest results. In all cases, the ORAC value of the green part was significantly higher than the white part, except for the plant material of *A. fistulosum.*

The antioxidant capacity of the white shaft of the leek cultivars was in the same range as the capacity of the bulb of *A. odorum, A. schoenoprasum* and *A. cepa*, but was significantly lower than *A. kurrat*, *A. cepa* (cv. Red Creole), *A. fistulosum* and *A. ascalonicum.* The ORAC values of the green part of the related species were significantly higher than the green leaves of the leek cultivars, except for the ORAC of the green leaves of *A. fistulosum* which was in the same range of leek.

6.4.1.2 DPPH

The DPPH results of the **white bulb** ranged from 2 to 14 µmol TE g^{-1} dw (Figure 6.1 b). The DPPH value of the bulb of *A. cepa* (cv. Red Creole) was higher than the other related species. The DPPH results of the **green leaves** ranged from 5 to 14 µmol TE g^{-1} dw. The DPPH value of the green leaves of *A. kurrat* and *A. cepa* (cv. Red Creole) were

significantly higher than the green part of the other species. In all cases, the DPPH value of the green part was significantly higher than the white part, except for plant material of *A. cepa* (cv. Red Creole) and *A. fistulosum.*

The DPPH results of the white shaft of leek were in the same range as the DPPH value of the white bulb of the other related species, except for *A. cepa* (cv. Red Creole). Only the green leaves of *A. kurrat* and *A. cepa* (cv. Red Creole) contained significantly higher DPPH levels than leek and the other related species.

6.4.1.3 FRAP

The FRAP results of the **white bulb** ranged from 5 to 28 µmol $FeSO_4$ g^{-1} dw (Figure 6.1 c). The antioxidant capacity of the white bulb of *A. cepa* (cv. Red Creole) and *A. fistulosum* were significantly higher than the other species. The FRAP results of the **green leaves** ranged from 17 to 100 µmol $FeSO_4$ g^{-1} dw. The green leaves of *A. odorum* and *A. cepa* (cv. Red Creole) had significantly higher FRAP values than the other species.
The results of the white shaft of the leek cultivars were in the same range of *A. kurrat*, *A. odorum, A. schoenoprasum*, *A. cepa and A. ascalonicum*. The FRAP value of the green part of the leek cultivars was significantly lower than the green leaves of the related species, except for *A. kurrat*.

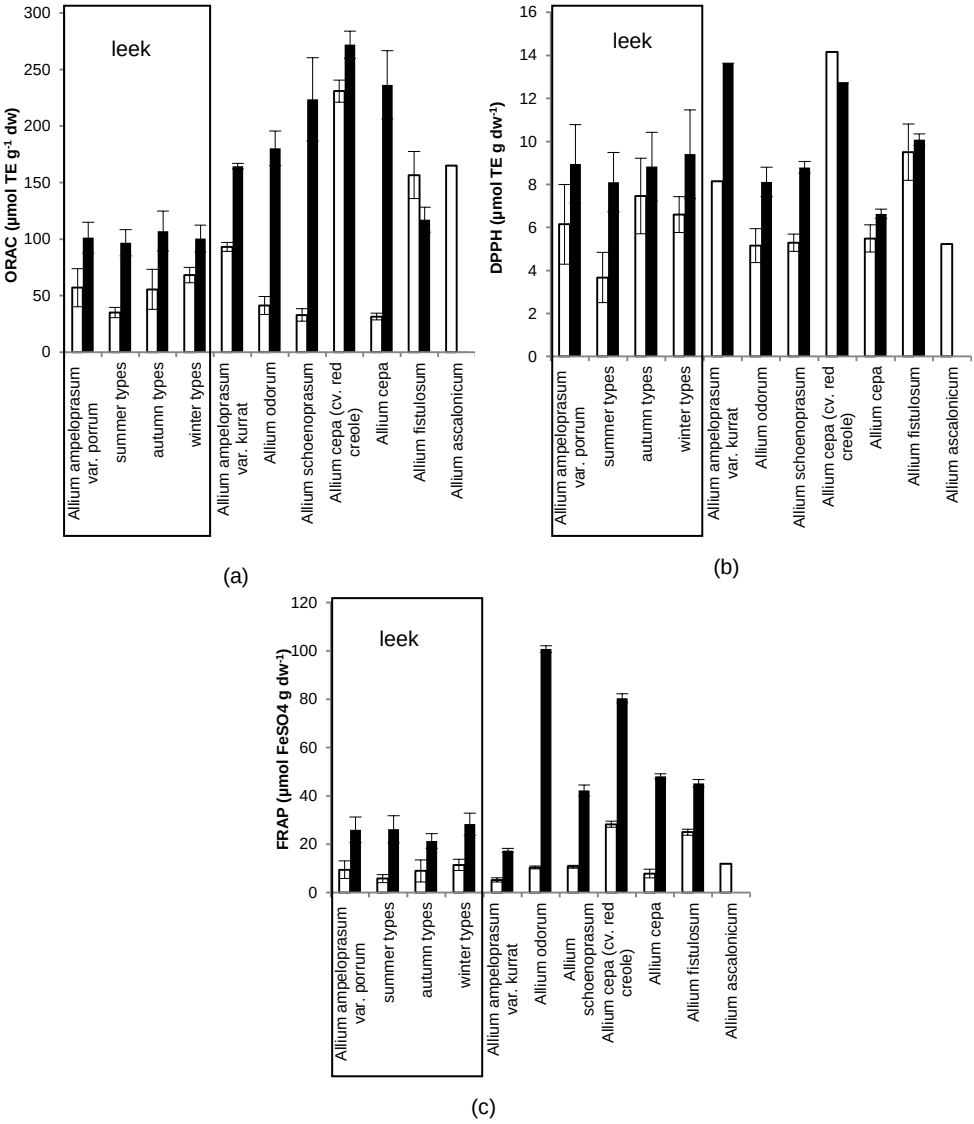


Figure 6.1 Results of the ORAC (a), DPPH (b) and FRAP (c) assay of the white part (□) and green leaves (■) of leek and related species (n=3)

6.4.2 Polyphenolic compounds

6.4.2.1 Total phenolic content

The total phenolic (TP) content of the related species is shown in Figure 6.2. The mean value of the white shaft and green leaves of the 30 leek cultivars, as well as the mean value of the summer, autumn and winter cultivars is given in the left-hand side of the graph as well as a reference. The graph lacks data of the TP content in the green leaves of kurrat and *A. odorum*.

The total phenolic content of the **white bulb** ranged from 7.43 to 11.89 mg GAE g^{-1} dw. The phenolic level of the white bulb of *A. schoenoprasum* and *A. cepa* was significantly lower than the content in the bulb of *A. fistulosum* and *A. odorum*. The TP content of the **green leaves** ranged from 9.02 to 11.57 mg GAE g^{-1} dw. In the case of *A. schoenoprasum* and *A. cepa*, the TP content of the green part was significantly higher than the white part. The bulb of the *A. cepa* (cv. Red Creole) contained a significantly higher TP level (9.44 mg GAE g^{-1} dw) compared with the white *A. cep*a (7.58 mg GAE g^{-1} dw).

The levels of the total phenolic content in the white bulb of the related species were in the same range as the white shaft of leek, except for the bulb of *A. odorum* and *A. fistulosum* which were higher than the concentration in leek. Only the TP content of the green leaves of *A. cepa* was significantly higher than the green part of leek.

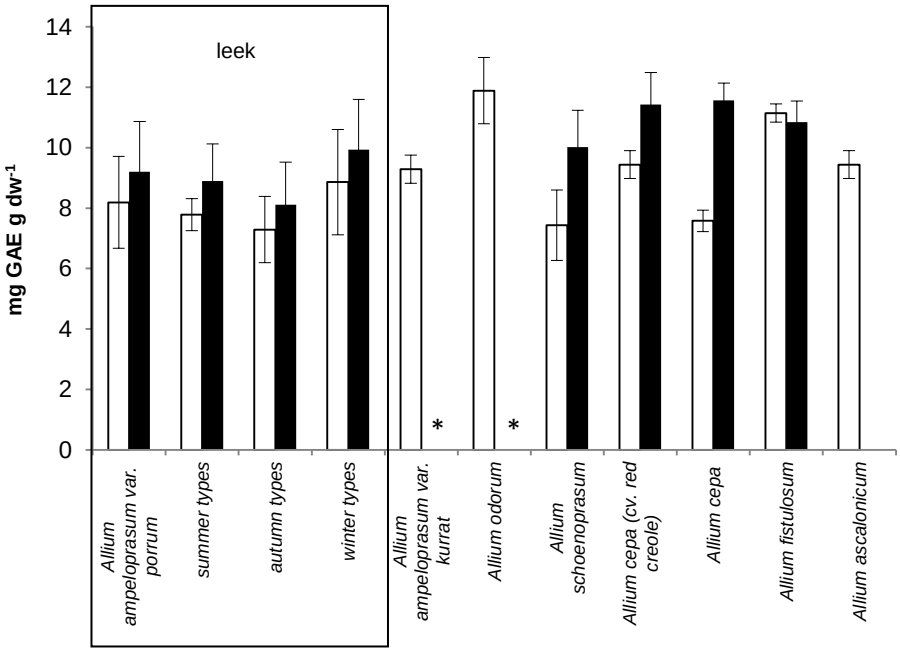


Figure 6.2 Total phenolic content in the white part (□) and green leaves (■) of leek and related species, with *, missing results, n=3

6.4.2.2 Flavonoids and phenolic acids

6.4.2.2.1 Identification

In Chapter 4, 13 polyphenolic compounds could be identified in leek. The same U-HPLC-ESI-Orbitrap-MS/MS (JHI) method was also used to identify polyphenolic compounds in the six related *Allium* species. In addition to the thirteen identified compounds in leek, 5 other polyphenolic compounds, which were not present in leek, could be identified in the related *Allium* species, namely quercetin 7-O-glucoside, quercetin 4'-O-glucoside, isorhamnetin 4'-O-glucoside, quercetin 3,7-O-diglucoside and isorhamnetin 3,4'-O-diglucoside. The presence of the 18 identified compounds in leek and related species is given in Table 6.3.

Table 6.3 Presence of phenolic compounds in leek and *Allium* species; with grey colour: new identified compounds

Compound	*A. porrum*		*A. kurrat*		*A. odorum*		*A. schoenoprasum*		*A. cepa*		*A. fistulosum*		*A. ascalonicum*
	White shaft	Green leaves	White bulb	Green leaves	White bulb	Green leaves	White bulb	Green leaves	White bulb	Green leaves	White bulb	Green leaves	White bulb
Quercetin		■		■					■	■	■		■
Kaempferol	■	■	■	■		■	■	■		■	■	■	■
Isorhamnetin		■		■				■	■	■	■	■	■
Quercetin 7-*O*-glucoside									■	■	■		■
Quercetin 3-*O*-glucoside		■		■		■		■	■	■	■	■	■
Kaempferol 3-*O*-glucoside	■	■	■	■	■	■	■	■	■	■	■	■	■
Isorhamnetin 3-*O*-glucoside		■		■			■	■	■	■	■	■	■
Quercetin 4'-*O*-glucoside									■	■	■	■	
Isorhamnetin 4'-*O*-glucoside									■	■	■		■
Quercetin 3-*O*-sophoroside		■		■		■		■		■	■	■	
Kaempferol 3-*O*-sophoroside		■	■	■				■	■	■	■	■	■
Kaempferol 3-*O*-gentiobioside	■	■	■	■		■		■	■	■	■	■	■
Kaempferol 3,7-*O*-diglucoside		■		■		■		■	■	■	■	■	
Quercetin 3,7-*O*-diglucoside				■		■		■	■		■	■	
Quercetin 3,4'-*O*-diglucoside	■	■		■		■		■	■	■	■	■	■
Isorhamnetin 3,4'-*O*-diglucoside						■		■	■	■	■		■
Kaempferol 4'-methylether		■	■	■	■	■	■	■	■	■	■	■	■
Ferulic acid 4'-*O*-glucoside	■	■		■			■	■	■	■		■	

The bulb as well as the green part of *A. cepa* and *A. fistulosum* contained almost all of the 18 identified polyphenolic compounds. The white bulb of *A. kurrat*, *A. odorum* and *A. schoenoprasum* contained fewer different polyphenolic compounds than their green part, while 12 compounds were detected in the white bulb of *A. ascalonicum*. All the *Allium* species contained significant amounts of kaempferol 3-*O*-glucoside in the white shaft/bulb as well as in the green leaves.

6.4.2.2.2 Quantification

Polyphenols were quantified on the basis of a calibration curve obtained from standard solutions of reference compounds. As a consequence, quantification could only be done for the identified compounds, where standards were available, that is quercetin 3,4'-O-diglucoside (Q34'G), kaempferol 3-O-glucoside (K3G), isorhamnetin 3-O-glucoside (I3G), quercetin 3-O-glucoside (Q3G), quercetin (Q), kaempferol (K), isorhamnetin (I) and kaempferol 4'-methylether (K4'M). Figure 6.3 shows the results of the quantification of 7 identified flavonoids in the white part (a) and green leaves (b) in leek (mean value of the 30 cultivars) and related *Allium* species. K4'M could not be quantified in these samples.

The red cultivar of *A. cepa* contained the highest flavonoid content in the **bulb** (6210 mg 100 g^{-1} dw), while the white bulb of *A. fistulosum* and *A. ascalonicum* had a flavonoid content of 2942 mg 100 g^{-1} dw and 2270 mg 100 g^{-1} dw, respectively. The main compounds in the bulb of these related species were Q3G and Q34'G. The seven flavonoids could not be detected in the white bulb of *A. odorum*, while 9.14, 9.04 and 6.50 mg flavonoids 100 g^{-1} dw were present in the bulb of *A. cepa*, *A. kurrat* and *A. schoenoprasum.*

The **green leaves** of the red cultivar of *A. cepa* contained the highest flavonoid content (2176 mg 100 g^{-1} dw), while the green leaves of *A. schoenoprasum* and *A. kurrat* had a flavonoid content of 720 mg 100 g^{-1} dw and 556 mg 100 g^{-1} dw, respectively. The leaves of *A. cepa*, *A. fistulosum* and *A. kurrat* contained 222, 105 and 67 mg flavonoids 100 g^{-1} dw. The main compounds in the leaves of these related species were K3G, Q3G and Q34'G.

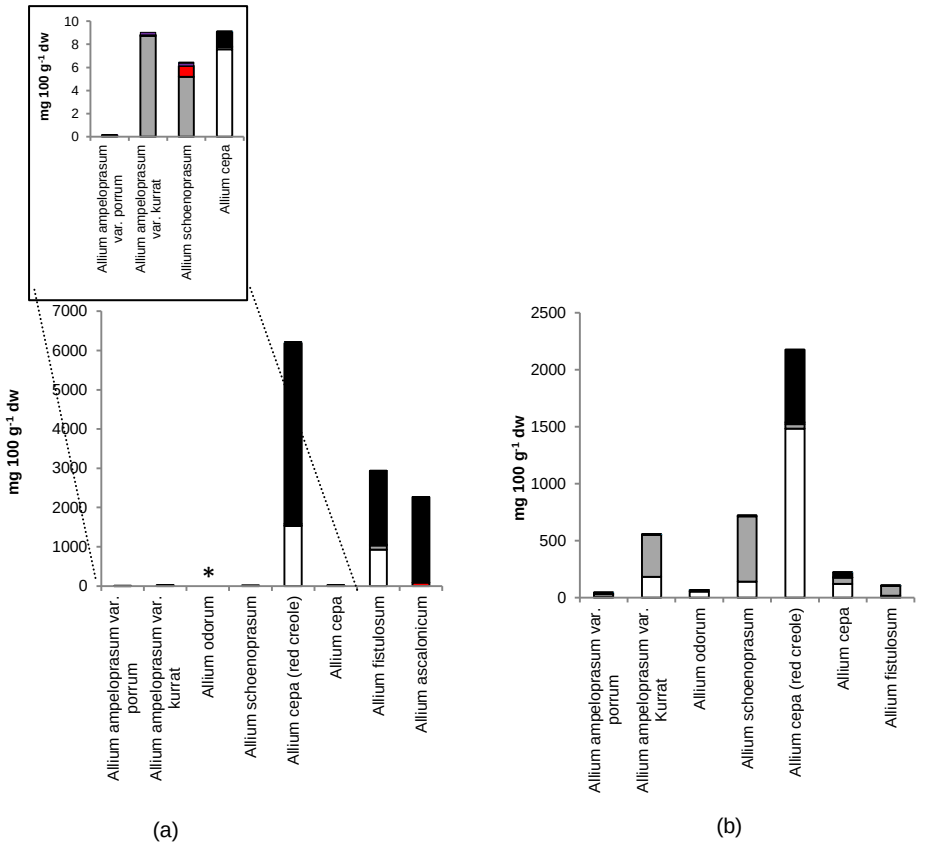


Figure 6.3 Individual polyphenol content of the white part (a) and green leaves (b) of leek and related species, with ■, isorhamnetin, ■, kaempferol, ■ quercetin, ■ quercetin 3-O-glucoside, ■ isorhamnetin 3-O-glucoside, ■ kaempferol 3-O-glucoside and □ quercetin 3,4'-O-glucoside, n=3, with *, no detected levels

The **Q34'G** content ranged from not detected levels to 926 mg 100 g^{-1} dw in the white bulb of the *Allium* species, and from 5 to 1481 mg 100 g^{-1} dw in the green leaves. The bulb of *A. fistulosum* and the leaves of *A. cepa* (cv. Red Creole) contained the highest Q34'G amount. The **Q3G** content ranged from nd to 4594 mg 100 g^{-1} dw in the white bulb, and from not detected levels to 636 mg 100 g^{-1} dw in the green leaves of the *Allium* species. The bulb and the leaves of *A. cepa* (cv. Red Creole) contained the highest Q3G amount.

K3G ranged from not detected levels to 108 mg 100 g^{-1} dw in the bulb of *Allium* species, while the content in the green leaves varied from 14 to 572 mg 100 g^{-1} dw. **Kaempferol** was present in relative low amounts, except for the white part (6.10 mg 100 g^{-1} dw) and green leaves (1.48 mg 100 g^{-1} dw) of *A. schoenoprasum*.

The **isorhamnetin** content ranged from not detected levels to 1.95 mg 100 g^{-1} dw in the white bulb, and from 0.04 to 0.66 mg 100 g^{-1} dw in the green leaves. **Quercetin** could only be quantified in the white bulb of red *A. cepa* (26 mg 100 g^{-1} dw), *A. fistulosum* (5 mg 100 g^{-1} dw) and *A. ascalonicum* (3 mg 100 g^{-1} dw), while quercetin was also quantified in the green leaves of red (1.71 mg 100 g^{-1} dw) and white *A. cepa* (0.11 mg 100 g^{-1} dw) and *A. odorum (*0.02 mg 100 g^{-1} dw). The level of **isorhamnetin 3-O-glucoside** (I3G) was the highest in white bulb of *A. ascalonicum* (88.79 mg 100 g^{-1} dw).

The polyphenol levels in leek were remarkably lower compared to the content in the related species.

6.4.3 L-ascorbic acid

The results of the ascorbate determination in the bulb and the green leaves of the related *Allium* species are shown in Figure 6.4. A higher ascorbate level could be quantified in the green leaves of the *Allium* species, compared with the content in the white part. The ascorbate content ranged from not detected levels to 3.55 mg g^{-1} dw in the **white bulb**, and from not detected levels to 9.40 mg g^{-1} dw in the **green leaves**. No ascorbate could be detected in the white bulb and green leaves of *A. schoenoprasum*, *A. cepa* and *A. ascalonicum*.

The ascorbate content of *A. odorum* and *A. cepa* was in the same range as the content in the white shaft of leek, while the content in the white shaft of leek was significantly higher than the content in *A. kurrat*. The ascorbate content in the green leaves of *A. fistulosum* was significantly lower than the content in the green leaves of leek, while the content in *A. odorum* was significantly higher.

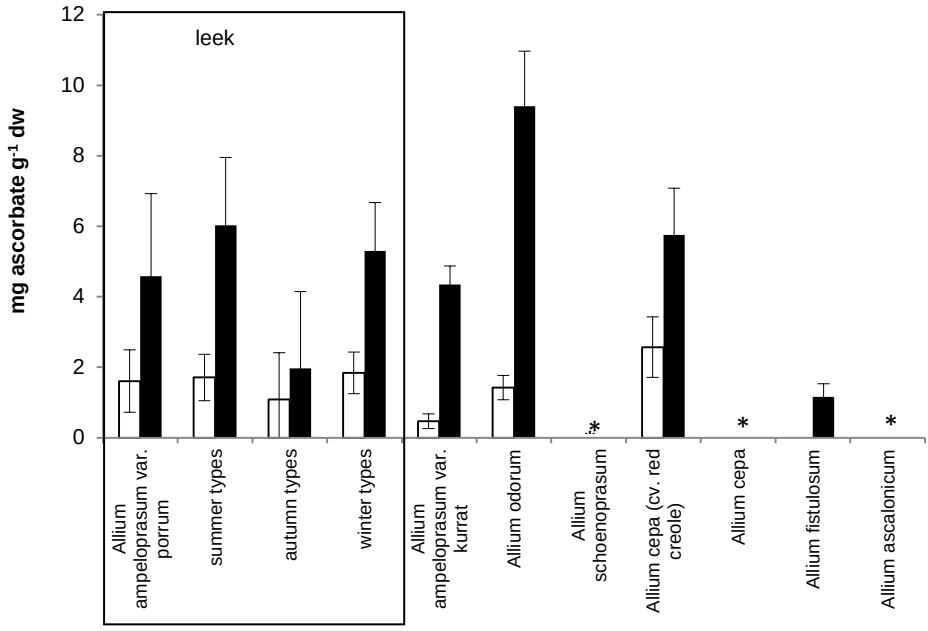


Figure 6.4 Ascorbate content in the white part (□) and green leaves (■) of leek and related species, with *, no detected levels, n=3

6.4.4 *S*-alk(en)yl-L-cysteine sulfoxides

Figure 6.5 shows the results of the isoalliin and methiin content in the white part and green leaves of leek and related species. The isoalliin content ranged from 10.24 to 36.01 mg isoalliin g^{-1} dw and from 7.86 to 53.46 mg isoalliin g^{-1} dw in the white part and green leaves, respectively. The methiin content of the white part and green leaves ranged from 3.41 to 83.26 mg methiin g^{-1} dw and from 1.86 to 59.87 mg methiin g^{-1} dw, respectively.

The green leaves of *A. kurrat* and *A. cepa* (cv. Red Creole) contained a higher methiin and isoallin content in the green leaves compared with their white bulb. The white bulb of *A. odorum* contained the highest ACSO (sum isoalliin and methiin) amount (97 mg g^{-1} dw), while *A. kurrat* had highest level in the green leaves (75 mg g^{-1} dw).

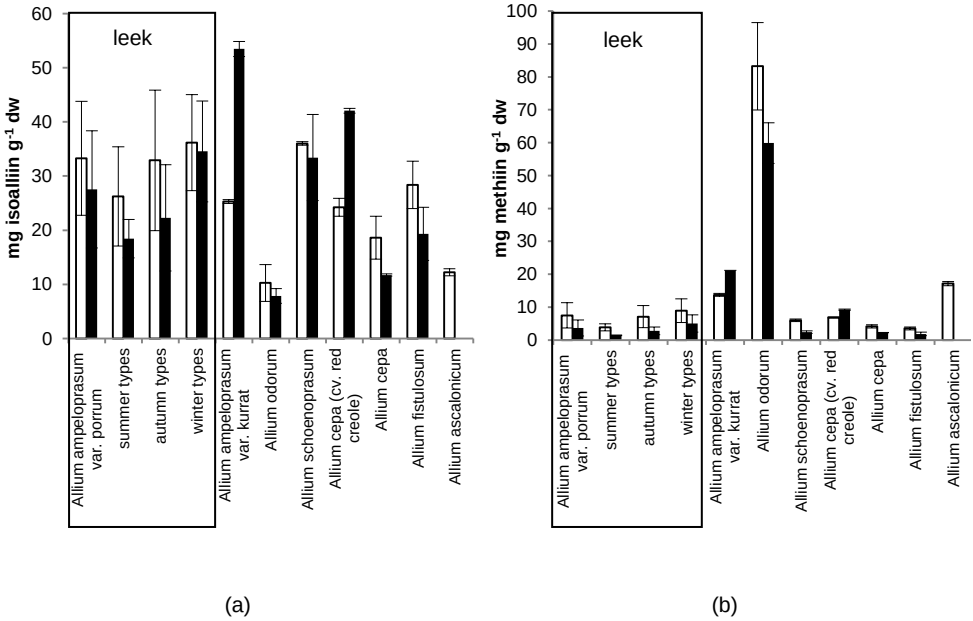


Figure 6.5 Isoalliin (a) and methiin (b) content of the white part (□) and green leaves (■) of leek and related species, n=3

Interestingly, *A. odorum* also contained 3.94 ± 0.34 mg alliin g^{-1} dw in the white part, and 1.28 ± 0.18 mg alliin g^{-1} dw in the green leaves.

The methiin content of *A. odorum, A. kurrat* and *A. ascalonicum* was significantly higher than the methiin content of leek. The content in the other related species was in the same range as leek. The isoalliin content of *A. odorum*, *A. cepa* and *A. ascalonicum* was significantly lower than the content in leek. The green leaves of kurrat contain a significantly higher isoalliin content in comparison with the green leaves of leek.

6.4.5 Fructans

Figure 6.6 shows the results of the fructan analysis of the white part and green leaves of leek and related species. However, only 1 analysis could be performed as described in Chapter 4. As consequence statistical analysis could not be performed.

The fructan content ranged from 5.98 to 52.82 g 100 g^{-1} dw in the white bulb, while the fructan content in the green leaves ranged from 3.67 to 7.54 g 100 g^{-1} dw.

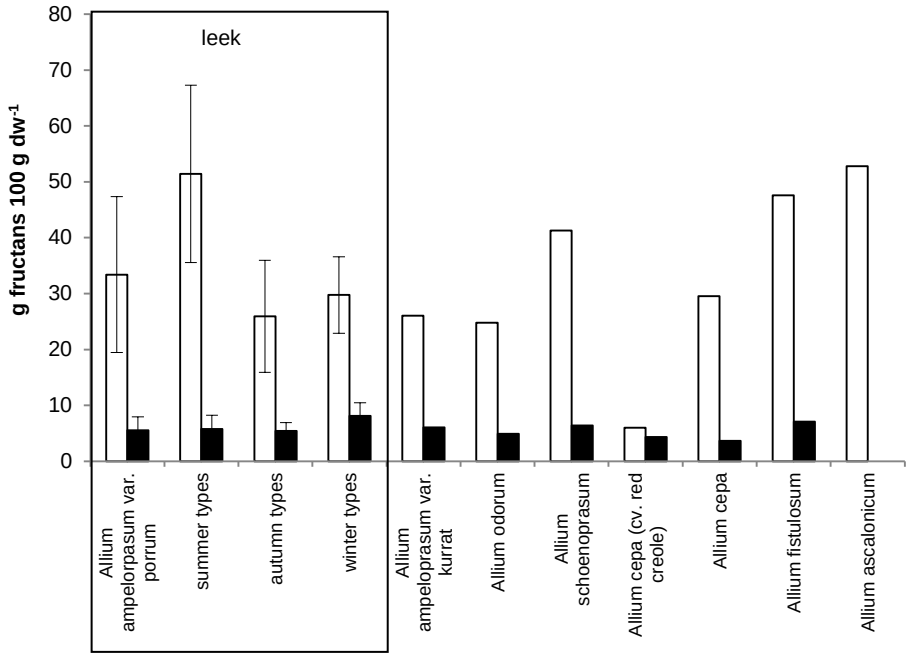


Figure 6.6 Fructan content of the white part (□) and green leaves (■) of leek and related species, n=1

The fructan content of the bulb of *A. schoenoprasum, A. fistulosum* and *A. ascalonicum* was higher than the content in the white shaft of leek. The fructan content in the green part of the related species was in the same range as the content of leek.

6.4.6 Principal component analysis

In order to visualise the results of the antioxidant capacity assays, polyphenolic, ACSOs, ascorbate and fructan content, PCA was performed on the whole data set of the white part and green leaves of leek and some of its related species. The dimensionality of the data was reduced from 15 partially correlated variables to 2 uncorrelated principal components, PC1 and PC2, accounting for 53.93% of the variation. The PCA plot (Figure 6.7 a and b) convincingly segregated the white part and the green leaves on the basis of the different antioxidant parameters. More specifically, the green leaves possessed a higher antioxidant capacity and contained a higher isoallin, kaempferol 3-O-glucoside and ascorbate content. The bulb of *A. cepa* (cv. Red Creole) is situated in the left bottom quadrant, indicating a high level of polyphenolic compounds except for kaempferol.

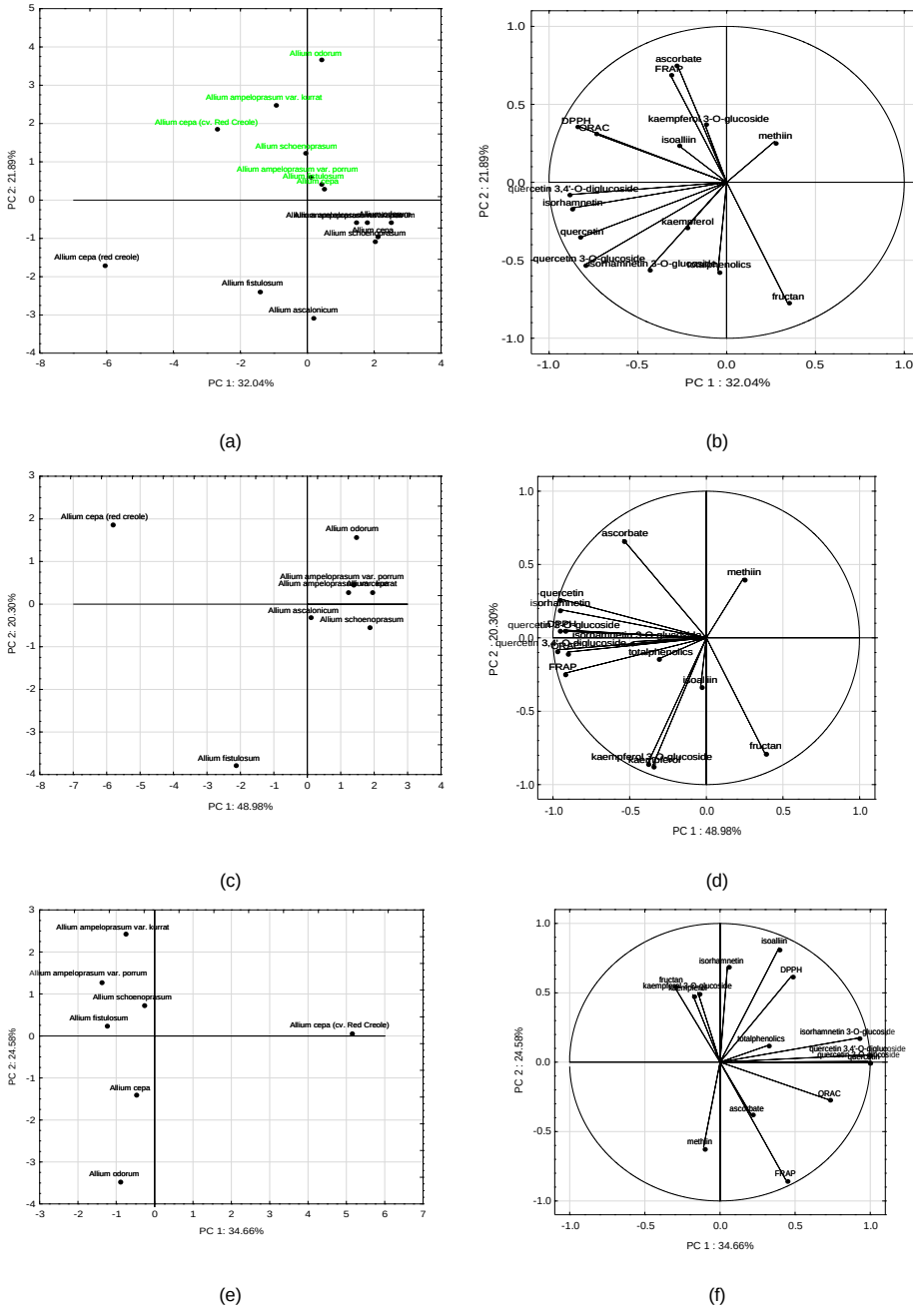


Figure 6.7 PCA plots of the scores (a,c,e) and loadings (b,d,f) of the entire data set (white-, green-), the white shaft and the green leaves of leek and 6 related species

In a second PCA, the data set of the white part of leek and its related species was used. The dimensionality of the data was reduced to PC1 and PC2, accounting for 69.28% of the variation. The PCA plot (Figure 6.7 c and d) convincingly segregated the bioactive compound content of the white part from the different species. *A. cepa* (cv. Red Creole) and *A. fistulosum* were dissimilar from the other *Allium* species. The bulb of *A. fistulosum* was rich in kaempferol and kaempferol 3-O-glucoside, while the bulb of red onion contained high amounts of the other quantified flavonoids.
PCA was then applied on the data set of the green leaves of leek and its related species. PC1 and PC2 accounted for 58.24% of the variation. The PCA plot (Figure 6.7 e and f) convincingly segregated the bioactive compound content of the green leaves from the different species. The leaves of *A. cepa* (cv. Red Creole) were rich in flavonoid glycosides.

6.5 Discussion

The present chapter compared the antioxidant properties of leek with some related family members, including *A. kurrat, A. odorum, A. schoenoprasum, A. cepa, A. fistulosum* and *A. ascalonicum*. The same analyses as performed in Chapter 4 were accomplished.
Firstly, the **antioxidant capacity** was measured using 3 assays, ORAC, DPPH and FRAP. The green parts had the highest antioxidant capacity in most of the cases, with the exception of *A. fistulosum* (ORAC, DPPH). Similarly, Stajner et al. (2011) reported a significantly higher FRAP value in the green leaves of *A. schoenoprasum* (5266 µmol dm^{-3} Fe^{2+}), compared to the white stalk (2566 µmol dm^{-3} Fe^{2+}). In general, our results showed that *A. cepa* (cv. Red Creole) possessed the highest antioxidant capacity.
A lot of studies already reported the antioxidant capacity of different *Allium* species, including brown (278 µmol TE g^{-1} dw), red (201 µmol TE g^{-1} dw), yellow (121 µmol TE g^{-1} dw) and white *A. cepa* (76 µmol TE g^{-1} dw) (Rautenbach and Venter, 2010; Zill-e-Huma et al., 2011). These values are all in the range of our results. Moreover, Ou et al. (2002) reported an ORAC of 85 and 143 µmol TE g^{-1} dw in white onion and purple onion, respectively, which were lower than our results. Halvorsen et al. (2002) have found that the FRAP value for red onion was about 54-57 µmol Fe^{2+} g^{-1} dw, while our results of the bulb of red onion were much lower.
Some studies made the comparison of different *Allium* species. Like that, Lu et al. (2011) determined the antioxidant capacity of *A. cepa* (white and red) and *A. ascalonicum* using the FRAP and DPPH assay, and concluded that *A. ascalonicum* contained the highest antioxidant capacity, whilst in our research red *A. cepa* gave the best results. Aoyama

and Yamamoto (2007) concluded that the antioxidant capacity of green Welsh onion (*A. fistulosum*), measured with the FRAP and TEAC assay, was at the same level of yellow *A. cepa*, but lower than red *A. cepa* as also observed in our results. However, there was no study yet who compared leek with some *Allium* species.

It is clear that the three antioxidant assays, *i.e.* ORAC, DPPH and FRAP, gave different antioxidant capacity trends. Ou et al. (2002) explained that the discrepancy in the results is based on the chemistry principles upon which these methods are built and is yet explained in Chapter 4.

In addition to the antioxidant capacity assays, the **total phenolic content** was measured. In the case of *A. schoenoprasum* and *A. cepa*, the TP content of the green part was significantly higher than the white part. This is in accordance with Stajner et al. (2011) who report a higher total phenolic content in the green leaves of *A. schoenoprasum* (52.65 mg GAE 100 g^{-1} fw) compared with the TP content of the white shaft (33.16 mg GAE 100 g^{-1} fw). Similar to our results, Gokce et al. (2010) and Gorinstein et al. (2009) found that red *A. cepa* had the greatest TP content (2.2 mg GAE g^{-1} dw; 15.56 mg GAE g^{-1} dw) compared with the white group (1.1 mg GAE g^{-1} dw; 11.92 mg GAE g^{-1} dw).

Some studies on *Allium* species (Stratil et al., 2006) report a similar total phenolic content as our results while others found values which are twice as high as our results (Gorinstein et al., 2009) or much lower (Nuutila et al., 2003; Gokce et al., 2010). This is most likely due to the use of different cultivars, the part analysed, extraction methods, etc. In the study of stratil et al. (2006), the same extraction procedure as our method, based on Vinson et al. (1998) was used, while Nuutila et al. (2003) only used methanol as extraction solvent.

Eighteen **polyphenolic compounds** could be *identified* in the bulb and leaves of the related *Allium* species. Ten of these were already reported in previous studies to be present in *Allium* species. Similarly, Q34'G, Q3G, Q, I4'G and I34'G were found in *A. ascalonicum* (Wiczkowski et al., 2008; Bonaccorsi et al., 2008). Significant amounts of Q34'G, Q4'G, Q3G, I4'G, I3G, I34'G, K3G, Q, I and K were found in *A. cepa (*Muminova et al., 2006; Bonaccorsi et al., 2008; Lee and Mitchell, 2011a; Perez-Gregorio et al., 2011). The remaining 8 identified polyphenols in the *Allium* species (Table 6.2), including quercetin 7-O-glucoside, kaempferol/quercetin 3-O-sophoroside, kaempferol 3-O-gentiobioside, kaempferol/quercetin 3,7-O-diglucoside, kaempferol 4'-methylether and ferulic acid 4-O-glucoside are, to our knowledge, not yet identified in *Allium* species. However, these compounds have been identified in other species. Similarly, quercetin 7-O-glucoside was detected in hops (*Humulus Lupulus L*.) (Hubacek, 1970), while quercetin 3-O-sophoroside and kaempferol 3,7-O-diglucoside were also found in broccoli florets (*Brassica olearacea*) (Price et al., 1998). Quercetin 3-O-sophoroside and

kaempferol 3-O-sophoroside were also found in cowpea seeds. In addition, kaempferol 3-O-gentiobioside was also identified in Senna leaves (Demirezer et al., 2011). Singh et al. (2006) isolated kaempferol 4'-methylether from *Echinops echinatus*, while ferulic acid 4'-O-glucoside has been detected in different berries, including blackcurrant, gooseberry, highbush blueberry and jostaberry (0.27-0.55 mg 100 g^{-1} dw) (Phenol-Explorer, 2011).

Of the eighteen identified polyphenols, 7 polyphenols could be *quantified* in the related *Allium* species, including Q34'G, K3G, I3G, Q3G; Q, K and I. The white bulb of the related species contained more I3O, Q3G, Q and K than their green leaves, while the green leaves were rich in Q34'G, K3O and I. The white bulb and green leaves of *A. cepa* (cv. Red Creole) contained the highest amount of polyphenols, while the concentration in *A. odorum* and leek was the lowest.

The main compounds in the bulb of the related species were **Q3G** (nd-4594 mg 100 g dw^{-1}) and **Q34'G** (nd-926 mg 100 g dw^{-1}). Similar as our results, Bonaccorsi et al. (2008) revealed a higher Q34'G and Q3G content in *A. ascalonicum* compared to the content in white *A. cepa*. As observed in our results, number of studies report high polyphenol levels in red *A. cepa* (Prakash et al., 2007). In the study of Zill-e-Huma et al. (2011), the level of Q34'G was the highest in red *A. cepa* (556 mg 100 g^{-1} dw) compared with the content in white *A. cepa* (11 mg 100 g^{-1} dw). However, these values are lower than the concentrations found in this study. In the same study, Q3G was not detected in the white *A. cepa*, while 5 mg 100 g^{-1} dw was present in red *A. cepa*. However, in our study, Q3G was present in both red (4594 mg 100 g^{-1} dw) and white ones (1 mg 100 g^{-1} dw). Differences in quantities with our results could be due to the genetic diversity, growth conditions (climate, soil, fertiliser) and analytical method. Moreover, different parts and scales of the *A. cepa* can contain different concentrations of quercetin (Slimestad and Vagen, 2009; Soininen et al., 2012).

K3G ranged from not detected levels to 108 mg 100 g^{-1} dw in the bulb of *Allium* species, while the content in the green leaves varied from 14 to 572 mg 100 g^{-1} dw. Our results show that K3G is the major flavonoid in the green leaves of *A. fistulosum* and in the whole plant of *A. schoenoprasum* and *A. kurrat*. **Kaempferol** was present in relative low amounts, except for the white part (6.10 mg 100 g^{-1} dw) and green leaves (1.48 mg 100 g^{-1} dw) of *A. schoenoprasum*. Our results are in accordance with Nuutila et al. (2003), who reported a higher kaempferol content (based on hydrolysis) in the leaves of *A. schoenoprasum*, *A. cepa* (cv. Giant) and yellow *A. fistulosum* compared to quercetin, while quercetin was predominant in yellow and red onion.

The **isorhamnetin** content ranged from not detected levels to 1.95 mg 100 g^{-1} dw in the white bulb, and from 0.04 to 0.66 mg 100 g^{-1} dw in the green leaves. **Quercetin** could only be quantified in the white bulb of red *A. cepa* (26 mg 100 g^{-1} dw), *A. fistulosum* (5 mg 100 g^{-1} dw) and *A. ascalonicum* (3 mg 100 g^{-1} dw), while quercetin was also

quantified in the green leaves of red (1.71 mg 100 g^{-1} dw) and white *A. cepa* (0.11 mg 100 g^{-1} dw) and *A. odorum* (0.02 mg 100 g^{-1} dw). Isorhamnetin and quercetin could also be quantified in *A. cepa* in the study of Marotti and Piccaglia (2002). The level of **isorhamnetin 3-O-glucoside** (I3G) was the highest in white bulb of *A. ascalonicum* (88.79 mg 100 g^{-1} dw).

In general, quercetin glucosides largely prevail in the edible portion of most of the *Allium* species, however, the sugar moieties strongly lower the antioxidant capacity of the aglycone due to the conjugation of the OH groups at C3 and C4', which are critical to the H-donating activity (Zill-e-Huma et al., 2011). Based on their research, it can be assumed that the quercetin glucosides only make a marginal contribution to the overall antioxidant capacity of *Allium* extracts. Other compounds with antioxidant properties will be responsible for the antioxidant capacity of the *Allium* samples.

Differences in content of polyphenols between different *Allium* species can be explained by the enzymes that catalyze the subsequent steps of flavonoid pathway. These enzymes vary from one plant species to another, giving rise to different flavones, flavonols, anthocyanins and/or proanthocyanidins (Hanhineva, 2008).

The *Allium* species were also analysed for their **ascorbate** content. A higher ascorbate level could be quantified in the green leaves, compared with the content in the white part. It is found that, among the *Allium* vegetables, *A. schoenoprasum* held the highest level of vitamin C (9.60 mg g^{-1} dw) (Kmiecik and Lisiewska, 1999). This finding does not endorse our results, where we could not detect ascorbate in *A. schoenoprasum*. Stajner et al. (2006) determined the vitamin C content of different *Allium* species, and found a content of 0.122, 0.005 and 0.161 mg g^{-1} fw in the fresh leaves of *A. schoenoprasum*, *A. cepa* and *A. fistulosum*, respectively. The vitamin C content of *A. cepa* (18.89 mg g^{-1} dw) reported by Mota et al. (2010) was higher than found in leek, which is not consistent with our results.

Most of the *Allium* species, contained more **ACSOs** in the white part, compared to the green leaves, while this was not the case for *A. kurrat* and *A. cepa* (cv. Red Creole). The white bulb of *A. odorum* and the green leaves of *A. kurrat* contained the highest ACSO (sum isoalliin-methiin) amount (97 mg g^{-1} dw; 75 mg g^{-1} dw).

To compare, Yamazaki et al. (2011) reported a mean methiin and isoalliin concentration in onion bulbs of 3.5 mg g^{-1} dw and 21.1 mg g^{-1} dw, respectively, which are in the same range as our results. Moreover, the study of Yamazaki et al. (2011) revealed that the leaves of *A. tuberosum (*also referred as *A. odorum*), contained 35.5 and 3.4 mg g^{-1} dw methiin and isoalliin, respectively, while the authors found a mean content in the leaves of *A. fistulosum* of 7.1 and 34.8 mg g^{-1} dw. In addition, 5 and 48.8 mg g^{-1} dw was found in the bulbs and roots of *A. schoenoprasum* (Yamazaki et al., 2011). These values are

again in the same range as the concentration found in our study, with the exception of the levels of *A. odorum*.

Interestingly, *A. odorum* also contained significant amounts of alliin, another ACSO found in *Allium* species. In general, the isoalliin/methiin/alliin ratio in the related species ranged from 10-89/11-85/0-4 (%) in the white part and from 11-93/7-87/0-2 (%) in the green leaves. Fritsch and Keusgen (2006) reported a codomination of alliin and isoalliin in *A. odorum,* while our study found that methiin dominated in *A. odorum* (11/85-87/2-4). Growth conditions can affect the dissimilarity in the data. In addition, the content of cysteine sulfoxides is naturally rather variable, which results in high coefficients of variation (Fritsch and Keusgen, 2006). In addition, methiin dominated in *A. ascalonicum (*42/58/0), while isoalliin dominated in the other *Allium* species.

Several authors have suggested (Keusgen, 1999) that the content of the four major cysteine sulfoxides (alliin, isoalliin, methiin and propiin) underlies the different tastes of common onion, garlic and leek. Isoalliin is responsible for the typical onion-smell, while methiin is associated with an unpleasant and "hard" taste and smell (Fritsch and Keusgen, 2006). As stated in Chapter 4, Yoo and Pike (1998) identified 3 distinctive groups: the isoalliin, the methiin and the alliin dominant groups. Leek belongs, along with onion, shallot and bunching onion, to the isoalliin group. Species in this group contained no or an undetectable amount of alliin. Also Yamazaki et al. (2011) found that onion, Welsh onion (*A. fistulosum)* and leek generate similar flavours and result in an isoalliin/methiin/alliin ratio of 81-89/11-19/0 (%). Garlic belongs to the alliin-dominant group, with an isoalliin/methiin/alliin ratio of 1/16/83 (%) (Hornickova et al., 2010). In addition, alliin dominates in wild leek (*A. obliquum*) and sand leek (*A. scorodoprasum*) (Fritsch and Keusgen, 2006).

The bulb of all related *Allium* species contained much more **fructans** compared to the green leaves. Muir et al. (2007) measured the fructan content in 43 fruits and 60 vegetables, including species from the *Alliaceae* family and reported a content of 11.5 g fructans 100 g^{-1} dw in the bulb of white *A. cepa,* 16.1 g 100 g^{-1} dw in *A. fistulosum* bulbs, 33 g 100 g^{-1} dw in *A. ascalonicum* and 45 g 100 g^{-1} dw in *A. sativum.* Our values are higher compared with the data reported in the literature. There may be several reasons for this difference, as many factors affect fructan levels in foods including storage time, storage temperature, food variety, seasonal variation and climate (Benkeblia et al., 2004; Monti et al., 2005). Muir et al. (2007) found that the onion family is a good source of fructans, in addition to the members of the *Compositae* family (*e.g*, Jerusalem Artichoke), even when serving size was taken into consideration (see below).

Principal component analysis of the data revealed that the white shaft of leek was closely related to the white bulb of *A. kurrat* and *A. cepa* with regard to the antioxidant

properties. This can be explained by the fact that leek *(A. porrum*) and kurrat *(A. kurrat)* are cultigens of wild forms of *A. ampeloprasum.* The green leaves of leek on the other hand, were more related to green part of *A. schoenoprasum* and *A. fistulosum.*

To evaluate a possible link between the content of antioxidants in *Allium* species and their genetic background, a comparison of Figure 6.7 was made with Figure 6.8. The data presented in Figure 6.8 is based on data from the AFLP (Amplified Fragment Length Polymorphism) analysis of 16 leek accessions and related species (Filjushin et al., 2011). In the study of Filjushin et al. (2011) the largest genetic distances were detected between the accessions of *A. porrum* and the section of *A. cepa*, which was also observed in the antioxidant data (in case of *A. cepa* cv. Red Creole).

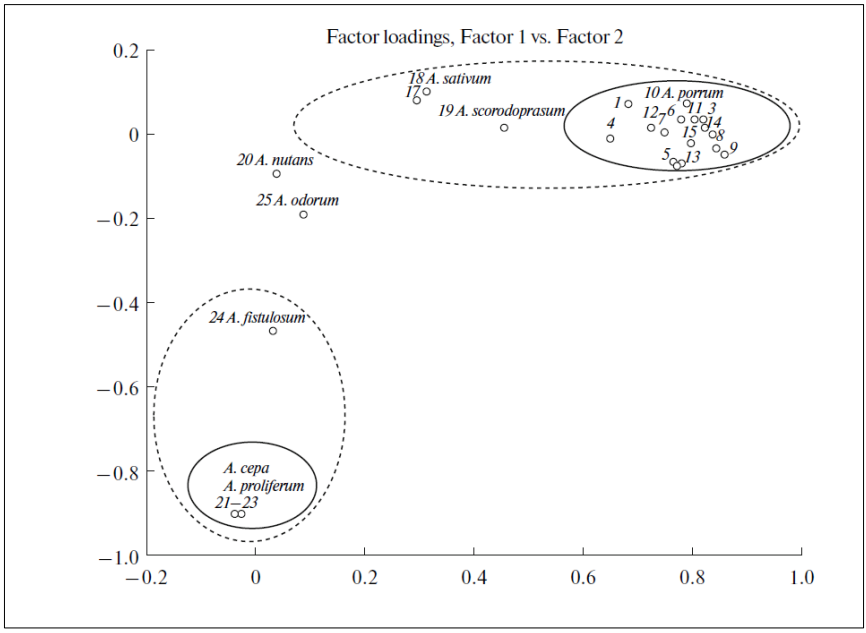


Figure 6.8 Analysis of the main components PCoA – a plot of genetic diversity of the studied accessions of A. porrum and related Allium species according to the results of the AFLP analysis (Filjushin et al., 2011)

The present chapter compared the antioxidant properties of leek to a number of its famous family members with regard to antioxidant properties. However, these data are based on the comparison of 30 leek cultivars with only 1 cultivar of the related species. Variation will exist between the cultivars of 1 species, as observed for leek (Chapter 4) but also in some other studies on garlic (Beato et al., 2011) and onion (Leon et al., 2009; Moon et al., 2010).

When comparing the bioactive compound profile of different *Allium* species, however, the consumption and use of the *Allium* species can be totally different and must be taken into account. Onion, garlic and chives are universally used spice plants and are extensively used for food flavouring (Augusti, 1990), while leek and bunching onion are more important as vegetables with additional flavouring properties (Fritsch and Keusgen, 2006). Chives are usually served in small amounts and never as the main dish (Stajner et al., 2011). Table 6.4 shows data concerning serving size for each *Allium* species, derived from Foodworks Version 4 (Muir et al., 2007). However, the table lacks data for kurrat, Chinese leek and chives.

Table 6.4 Average serving size and antioxidant properties of *Allium* species (Muir et al., 2007)

Allium species	Average serving size (g fw)	ORAC (µmol TE serve^{-1})	DPPH (µmol TE serve^{-1})	FRAP (µmol $FeSO_4$ serve^{-1})	TP (mg GAE serve^{-1})	AA (mg serve^{-1})	Isoalliin (mg serve^{-1})	Methiin (mg serve^{-1})	Fructans (g serve^{-1})
Leek, white shaft	83	616.49	66.35	101.73	88.36	17.34	359.08	80.90	3.60
Leek, green leaves	83	1262.51	111.51	322.51	114.62	57.09	342.62	45.43	0.69
Bunching onion	16	325.70	19.77	51.90	23.18	0	58.98	7.37	0.99
Onion	16	65.47	11.41	16.27	15.76	0	38.70	8.67	0.61
Shallot	12	257.23	8.15	18.48	14.72	0	19.08	26.79	0.82

From Table 6.4, we can conclude that when leek is consumed in a meal, it is 5 times the quantity of that of (bunching) onion and 7 times the proportion of shallot. Taking this data into account, there will be a higher intake of antioxidants coming from leek, compared to the related species (based on these serving sizes).

However, not only data regarding serving size has to be evaluated, also statistics about the consumption per capita per year should be considered. We obtained data concerning the purchased amount of leek and onion per capita per year in Belgium; 3.10 kg leeks per capita were bought in Belgium in 2010, while each Belgian purchased 6.60 kg onion, more than twice the amount of leek (VLAM, 2012). However, based on the serving sizes of Table 6.4, the data of purchase and the antioxidant properties of leek and onion, still more antioxidants will come from the consumption of leek compared to onion.

6.6 Conclusion

This chapter compared the antioxidant properties of leek to a number of its famous family members with regard to antioxidant properties. Based on the results, the antioxidant properties of the white shaft of leek are closely related to the antioxidant potential of the bulb of *A. kurrat* and *A. cepa*, while the green leaves of leek resemble the

antioxidant profile of *A. schoenoprasum* and *A. fistulosum*. *A. odorum* and *A. cepa* (cv. Red Creole) differ from leek in terms of antioxidant properties. These species are higher in methiin and flavonoid content, respectively.

In addition to the 13 polyphenols identified in leek, 5 additional compounds could be identified in the related *Allium* species, including quercetin 7-O-glucoside, quercetin 4'-O-glucoside, isorhamnetin 4'-O-glucoside, quercetin 3,7-O-diglucoside and isorhamnetin 3,4'-O-diglucoside.

CHAPTER 7. INFLUENCE OF POST-HARVEST PROCESSING AND STORAGE ON THE ANTIOXIDANTS OF LEEK – FROM 'HARVEST TO FRIDGE'

Redrafted from

Bernaert, N., De Clercq, H., Van Bockstaele, E., De Loose, M. and Van Droogenbroeck, B. Antioxidant changes during post-harvest processing and storage of leek (*Allium ampeloprasum* var. *porrum*). Accepted with revisions in Postharvest Biology and Technology

7.1 Introduction

After *harvest* of leek, several days can elapse before leeks reach the market. During this period, the nutritional and physiological quality of the vegetable can deteriorate. In addition, on-farm processing, including cutting the leaves, packaging, etc. can change the antioxidant profile, including the content of polyphenols, organosulfur compounds and vitamins. For example, mechanical actions such as cutting and slicing increase oxidation and can lead to a decrease of the polyphenol content. On the other hand, wounding enhances polyphenol biosynthesis through the induction of phenylalanine ammonia-lyase enzyme which is related to the wound-healing process in order to fight pathogen attack after tissue wounding (Tudela et al., 2002). As a result, some studies reveal an increase in flavonol content in fresh-cut potatoes (*Solanum tuberosum* L.) and fresh-cut onions (*Allium cepa* L.) (Tudela et al., 2002; Perez-Gregorio et al., 2011; Ioannou et al., 2012).

After *purchase* of leek in the market, leek is prepared in the consumer's kitchen. However, time can elapse between purchase and cooking. During that time, leek is normally stored at cool temperatures (4 °C). Refrigerated storage is known to extend the shelf life of fresh fruits and vegetables by delaying the biochemical and microbial changes. However, during storage, vegetables undergo various physiological changes such as weight loss due to water evaporation, decay, internal shoot growth and compositional changes (Yoo et al., 2012). Some studies indicate an increase in antioxidant capacity and concentration of polyphenols during storage of vegetables (Leja et al., 2001; Kevers et al., 2007), although a few reports report constant or decreasing levels (Gennaro et al., 2002; Kevers et al., 2007). It appears that the effect of storage on the antioxidants depends on many factors including light and temperature. Light can act as a stress signal and can induce flavonoid synthesis. For example, storage of potato strips at 4 °C under light exposure is reported to induce a higher flavonol accumulation rate than in darkness (Tudela et al., 2002). Storage temperature is also reported to have an influence on the antioxidants. For example, strawberry fruits (*Fragaria x ananassa* cv. Chandler) stored at 5 °C and 10 °C showed a higher antioxidant capacity and contained higher levels of polyphenols and anthocyanins than those stored at 0 °C (Ayala-Zavala et al., 2004). Moreover, when garlic *(Allium sativum* L.) was stored at 4 °C for 150 days, a marked conversion of the γ-glutamyl peptides, γ-L-glutamyl-*S*-allyl-L-cysteine and γ-L-glutamyl-*S*-(trans-1-propenyl)-L-cysteine (GSPC), to sulfoxides, alliin and isoalliin, was observed. Interestingly, however, when garlic was stored at 23 °C, a decrease in GSPC and a marked increase in cycloalliin, rather than isoalliin, occurred (Ichikawa et al., 2006).

In general, post-harvest processing and storage of vegetables can influence the antioxidant properties. In some cases, these factors can induce the formation of compounds with novel antioxidant properties, which can maintain or even enhance the overall antioxidant potential of foods (Nicoli et al., 1997). However, post-harvest processing and storage can cause loss of antioxidants or formation of compounds with pro-oxidant action which may lower the antioxidant capacity.
The evaluation of processing at the farm and the impact of subsequent storage on the antioxidants of vegetables is of great practical importance. However, reports on their effect on the antioxidant capacity, polyphenol and ACSO content of the vegetable leek are limited. The objective of this study was to determine the evolution of the antioxidant capacity, total phenolic and ACSO content in leek from harvest until purchase by the consumer and their subsequent refrigerated storage after purchase or in summary the determination of the antioxidant properties in leek from 'harvest to fridge'.

7.2 Plant material

The selection of plant material and sample preparation are described in §3.2.2.1. Briefly, 2 cases were investigated, (1) leek sold as an entire plant and (2) leek with a large part of the green leaves removed, where the shafts are sold in a plastic package. Sampling was performed during post-harvest processing at the farm and upon 13 days of refrigerated storage (~maximum domestic storage time), and is illustrated in Figure 7.1 (blue arrows).

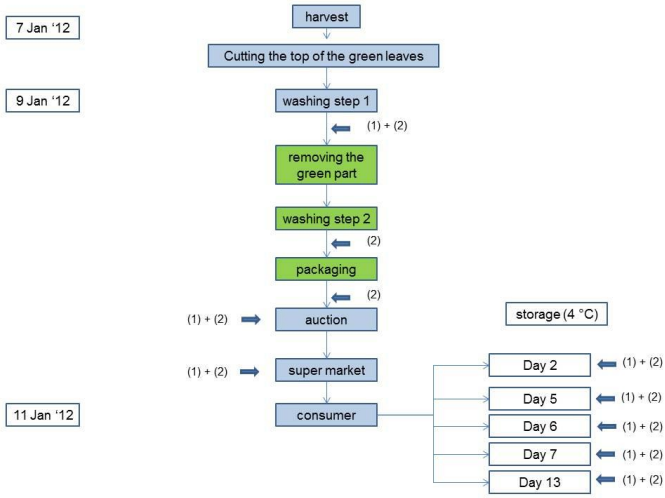


Figure 7.1 Schematic overview of the sampling of the post-harvest storage experiment of entire leek (blue, 1) and packaged white leek shafts (blue + green, 2). Sampling steps are indicated with

7.3 Bioactive compound analysis

A summary of the analyses (as described in §3.3) performed on the samples is given in Table 7.1.

Table 7.1 Overview of the analyses performed

Analysis		Method
Antioxidant capacity	ORAC DPPH	Spectrophotometric
Polyphenolic compounds	Total phenolic content	Spectrophotometric
S-alk(en)yl-L-cysteine sulfoxides		HPLC-MS/MS

Because of practical reasons, FRAP, individual polyphenols, ascorbate and fructan analysis were not performed on these samples.

7.4 Results

7.4.1 Antioxidant capacity

7.4.1.1 ORAC

Figure 7.2 shows the evolution of the ORAC value during post-harvest processing of leek at the farm and upon 13 days of refrigerated storage of the white shaft and green leaves of the entire leek plant (EL) and the white shaft of packaged leek (PL).

The ORAC value of the white shaft **(EL)** did not change significantly during processing and cool storage for 13 days. The antioxidant capacity of the green leaves in the market was significantly lower compared to the washed samples and leek sampled at the auction, but increased again starting at 2 days storage in the refrigerator.

The ORAC value of the **packaged** white shaft stored for 6 days was significantly higher compared with leek after the first washing step as well as leek after 7 days of storage. Storage for 13 days resulted again in a higher ORAC value compared to storage for 7 days, but not compared to the initial antioxidant capacity.

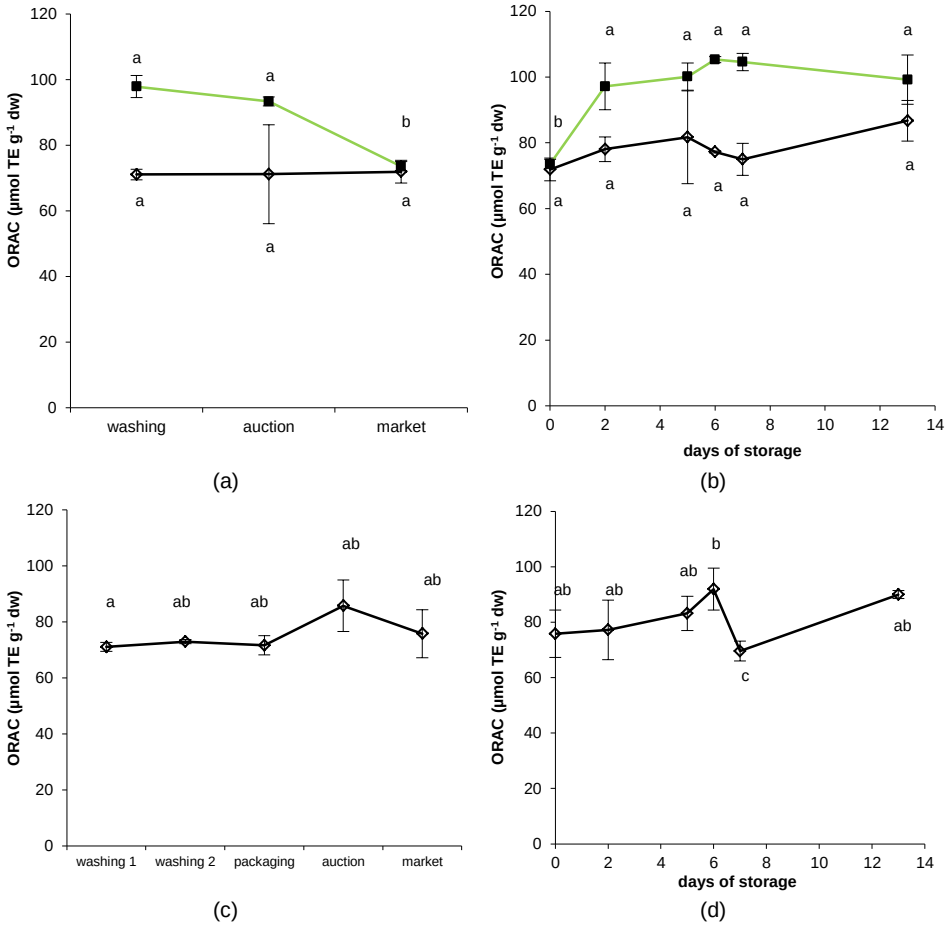


Figure 7.2 ORAC results of the white shaft (-) and green leaves (-) during post-harvest processing of the entire leek (a) and packaged leek (c), and during post-harvest storage of the entire leek (b) and packaged leek (d), a, b, c: points with a different subscript show statistical significance (n=3)

7.4.1.2 DPPH

Figure 7.3 shows the evolution of the DPPH value during post-harvest processing and storage of the entire leek plant and packaged leek.

The white shaft of leek **(EL)** had a significantly higher antioxidant capacity at the auction, and after 5 and 6 days of storage compared with the DPPH value of the washed white shaft. The green leaves (EL) of samples taken at day 2 in the refrigerator possessed a significantly higher antioxidant capacity compared with the other samples, but at the end of the storage experiment no significant difference was observed compared to the initial antioxidant capacity.

The DPPH radical scavenging activity of the **packaged** leek was significantly lower after the first washing step compared with washing step 2, however the end DPPH value did not differ from the antioxidant capacity of the start of the experiment.

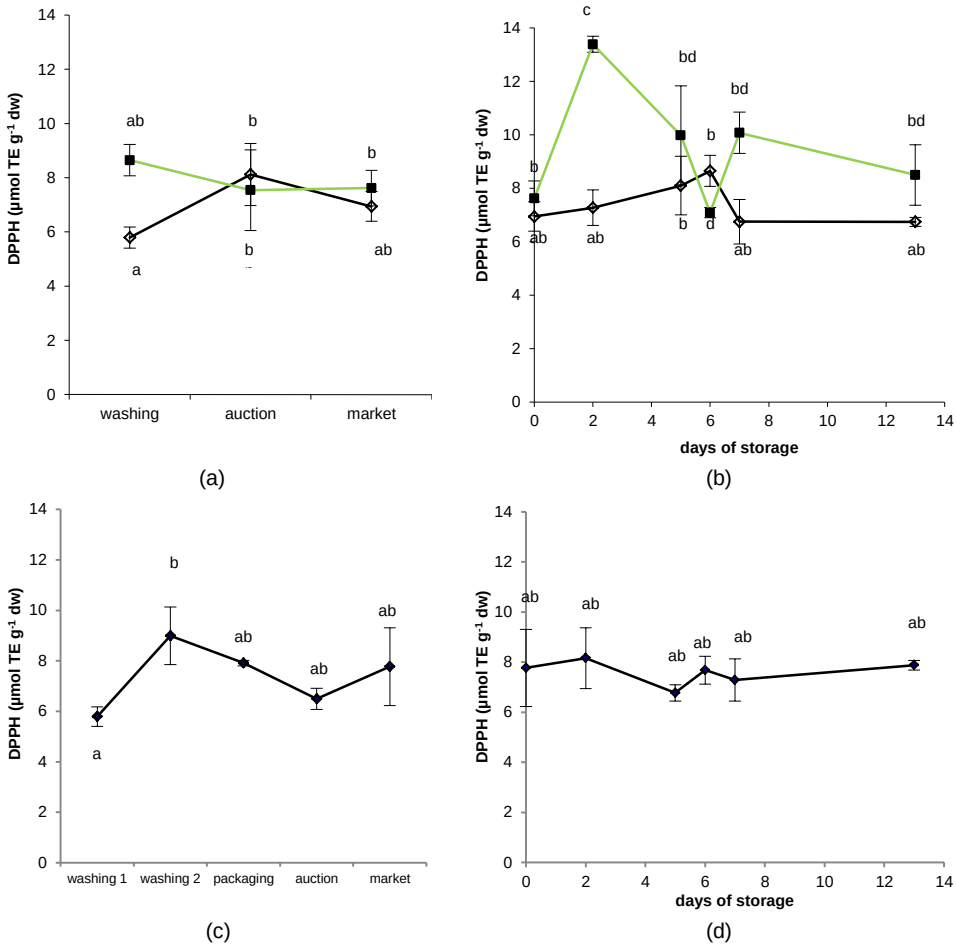


Figure 7.3 DPPH results of the white shaft (-) and green leaves (-) during post-harvest processing of the entire leek (a) and packaged leek (c), and during post-harvest storage of the entire leek (b) and packaged leek (d), a, b, c: points with a different subscript show statistical significance (n=3)

7.4.2 Polyphenolic compounds

7.4.2.1 Total phenolic content

Figure 7.4 shows the evolution of the total phenolic content during post-harvest processing and storage of the entire leek plant and packaged leek.

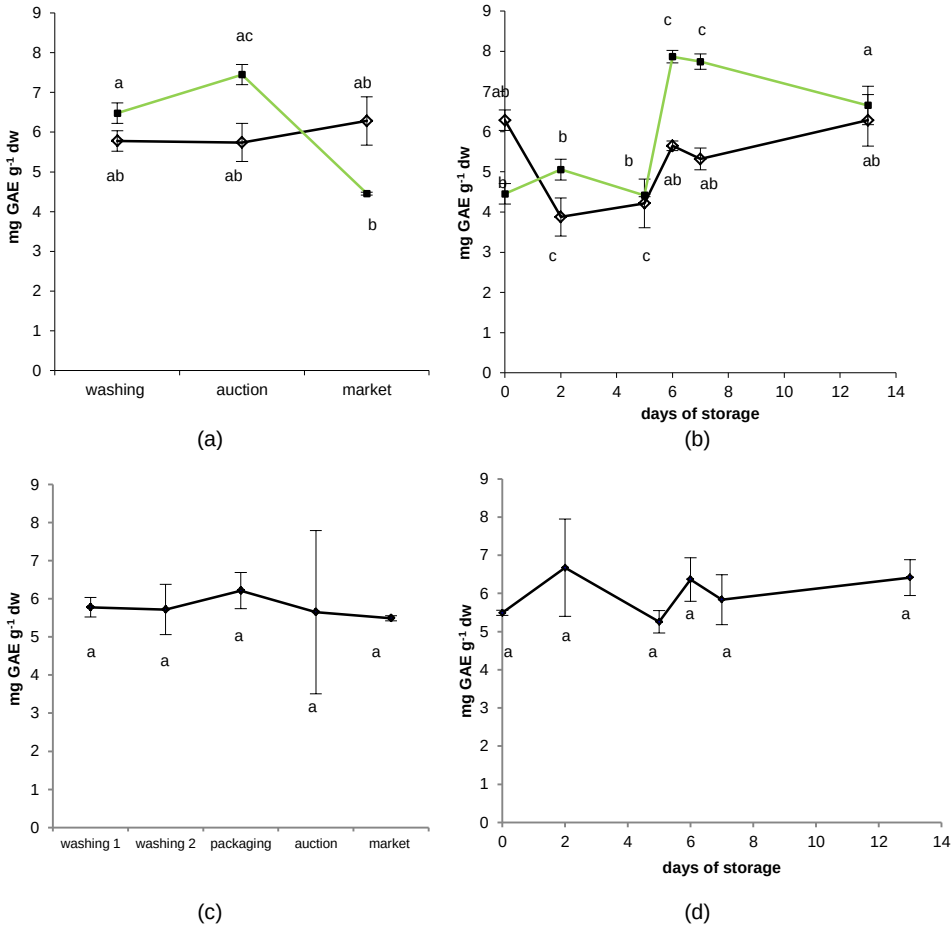


Figure 7.4 Total phenolic contents of the white shaft (-) and green leaves (-) during post-harvest processing of the entire leek (a) and packaged leek (c), and during post-harvest storage of the entire leek (b) and packaged leek (d), a, b, c: points with a different subscript show statistical significance (n=3)

The TP content of the white shaft **(EL)** decreased significantly after 2 and 5 days of storage, but increased again after a longer storage period to a value which was not

significantly different from the initial TP content. The same phenomenon was seen in the green leaves. A significant decrease in TP content of the green leaves was observed when the leek was sold in the market and after a storage of 5 days, but the TP content increased again upon 7 days of storage.
The total content of polyphenolic compounds in the white shaft of **packaged** leek was stable during processing at the farm and 13 days of storage.

7.4.3 *S*-Alk(en)yl-L-cysteine sulfoxides

Figures 7.5 and 7.6 show the evolution of the isoalliin and methiin content, respectively, during post-harvest processing and storage of entire leek and packaged leek.
A significantly higher isoalliin content of the white shaft **(EL)** was seen after 5 days of storage compared with the isoalliin content of the washed and auction samples (Fig. 7.5). In addition, the isoalliin content after 13 days of storage was significantly higher compared to the initial content. The isoalliin content in the green part did not change during processing and subsequent storage, however. No remarkable loss of isoalliin during the storage of **packaged** leek was observed.
Storage of the white shaft of **EL** for 5 days resulted in a significantly higher methiin level compared with the content immediately after harvest at the first washing step (Fig. 7.6). The methiin content in the green leaves of the whole leek did not change significantly after a refrigerated storage during 13 days. At the auction, a significantly lower methiin level was observed compared with the methiin content when the leaves were stored for 6 days.
After 5, 6, 7 and 13 days of storage, the methiin content in the white shaft of **packaged** leek increased significantly compared with the samples taken after washing step 2, packaging and in the market. The end methiin content, however, was not significantly higher compared to the methiin content after harvest.

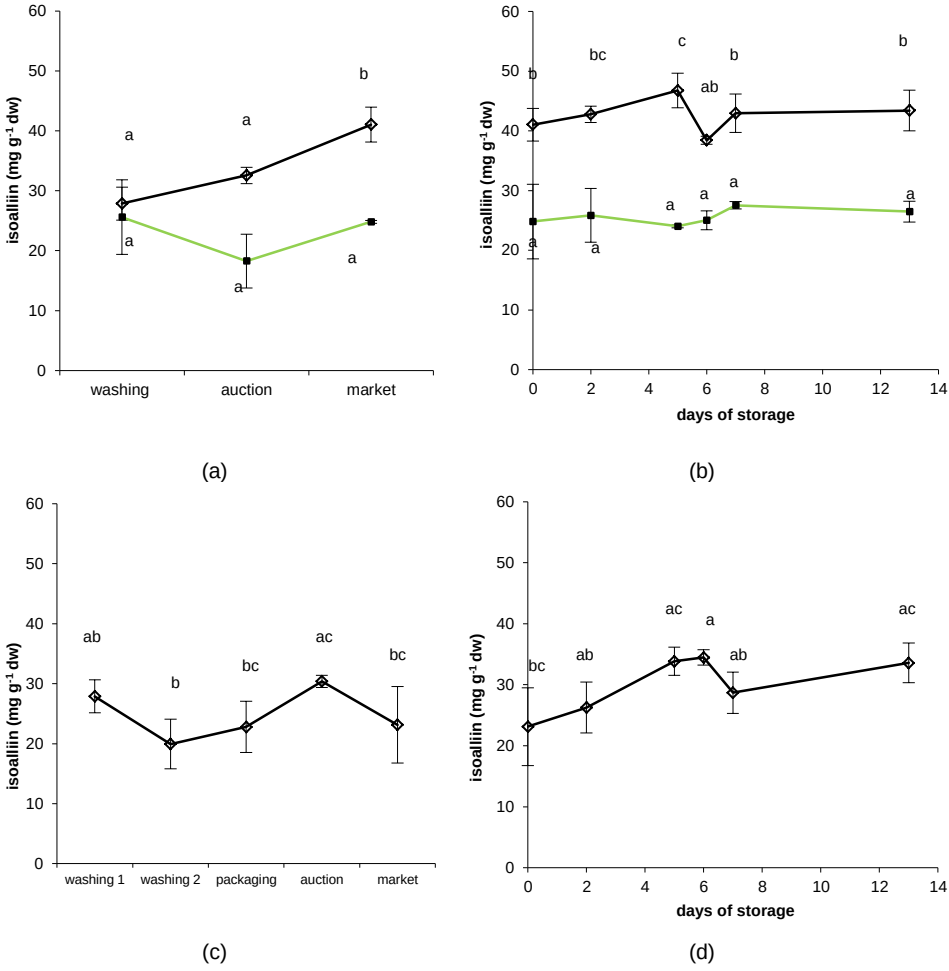


Figure 7.5 Isoalliin content of the white shaft (-) and green leaves (-) during post-harvest processing of the entire leek (a) and packaged leek (c), and during post-harvest storage of the entire leek (b) and packaged leek (d) a, b, c: points with a different subscript show statistical significance (n=3)

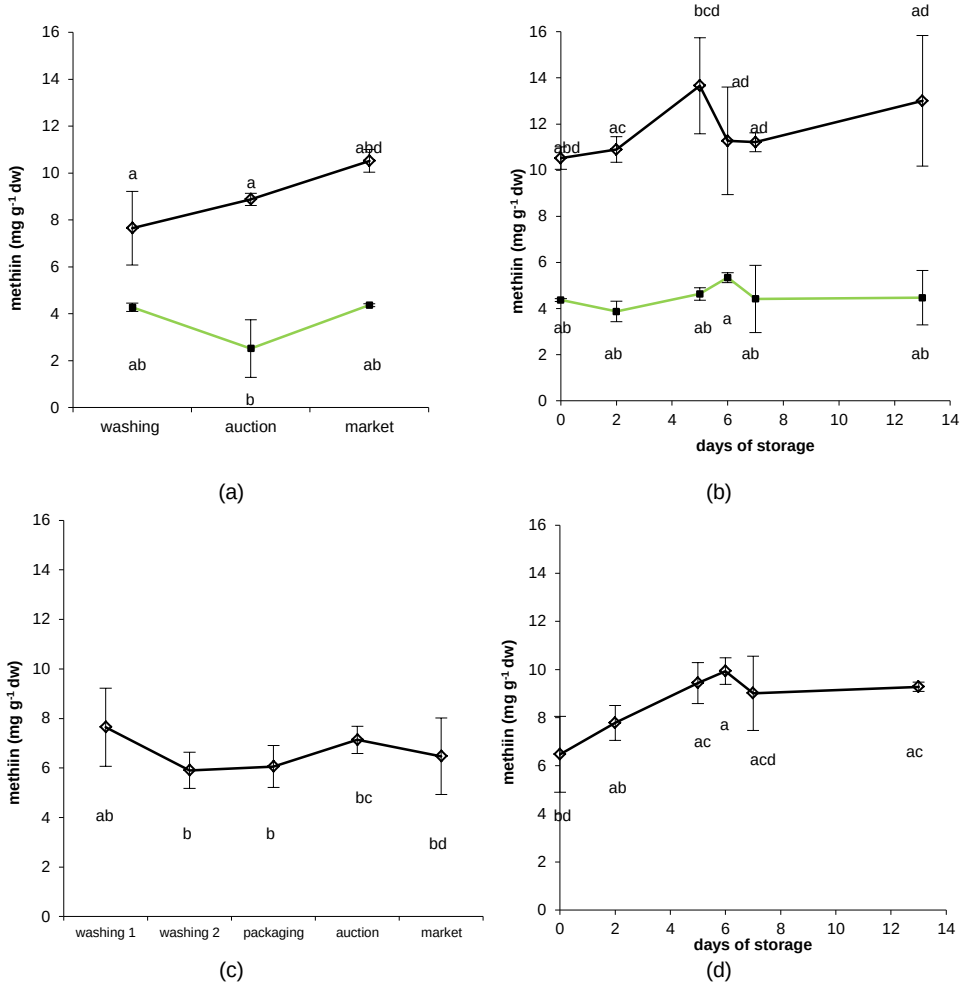


Figure 7.6 Methiin content of the white shaft (-) and green leaves (-) during post-harvest processing of the entire leek (a) and packaged leek (c), and during post-harvest storage of the entire leek (b) and packaged leek (d), a, b, c, d: points with a different subscript show statistical significance (n=3)

7.5 Discussion

This chapter discussed the influence of leek processing at the farm and the impact of subsequent refrigerated storage on the health-promoting compounds, including antioxidant capacity, polyphenols and organosulfur compounds.

Despite the slight change in **antioxidant capacity** during storage, at the end of a 13 day-storage, the ORAC and DPPH value of leek did not show any decrease or increase. This stable antioxidant capacity result is in contrast with Kevers et al. (2007), who

studied the white shaft of leek during storage of 23 days, 10 days longer than our experiment, and concluded that the antioxidant capacity, measured with the ORAC and DPPH assay, decreased. However, the stable DPPH value during our storage-experiment agrees with the study of Tsouvaltzis et al. (2007), who reported no significant change in DPPH free radical scavenging activity upon storage of leek for 7 days at 10 °C. Similarly, the total antioxidant capacity of leek upon 7 days of storage, using the Trolox Equivalent Antioxidant Capacity (TEAC), decreased by 47.3%. The variance observed between the reported data can be explained by various factors as post-harvest losses in nutritional quality are enhanced by physical damage, extended storage duration, high temperatures, low relative humidity and chilling injury of chilling-sensitive commodities (Lee and Kader, 2000; Navarro et al., 2006). In addition to the difference due to methods used, the variance observed between reported data can be explained by various factors such as extraction procedure, cultivar and weather conditions of the production season (Michiels et al., 2012).
Comparing with other vegetable matrices, storage of onions during 6 weeks in different conditions, all of them mimicking home storage habits, resulted in a decrease of 29% to 36% of the total antioxidant capacity (Gennaro et al., 2002). However, these studies cover a longer storage than our study, and can result in higher antioxidant losses. Similarly as our results, storage of white asparagus for 6 days (2 °C) did not affect the DPPH free radical scavenging activity (Papoulias et al., 2009). However, by contrast, Leja et al. (2001) reported an increase in antioxidant capacity of broccoli (*Brassica oleracea* var. *italica*, cv. Lord) flower buds during short-term storage at 20 °C and at 5 °C for 3 and 7 days, respectively, when they were either unpackaged or packaged in polymeric films.

The stable **total phenolic** content in the post-harvest processed samples do partly confirm the findings of Kevers et al. (2007), who concluded that the total phenolic content in leek increased during the first days of storage at 4 °C but stabilised afterwards. Comparing with other vegetables, storage (2 °C) of asparagus spears for 6 days significantly affected the total flavonoid content resulting in an increase from 0.179 to 0.292 mg rutin equivalents g^{-1} fw (Papoulias et al., 2009). When onion was kept under refrigeration (4 °C) in darkness for 6 months, no effect was noticed in the quercetin conjugate content (Price et al., 1997). In contrast, the total flavonols in onion increased up to 64% after 6 or 7 months of storage, with an increase of 58% during the first 3 months (Rodrigues et al., 2010). However, the study of Rodrigues et al. (2010) and Leja et al. (2003) observed that the increase of total phenols during storage in most cases was accompanied by a decrease of anthocyanins.

It has been demonstrated that polyphenolic compounds undergo oxidative polymerisation during food processing or storage increasing the chain-breaking activity as well as the antioxidant capacity (Manzocco et al., 1998). Monitoring specific classes of polyphenolic compounds, has indicated that not all polyphenols are affected in the same manner (Kevers et al., 2007). Interestingly, the concentrations of different quercetin glucosides react differentially during storage. Quercetin 4-glucoside levels have been demonstrated not to change during storage, whereas the levels of quercetin 3,4-O-diglucoside increased by 30–50% (Olsson et al., 2010). These different behaviours can finally result in a stable total phenolic content, as observed in our results.

It is also observed that the effect of storage on the polyphenolic content depends on many factors including temperature and light as stated in the introduction (§7.1). Regarding the effect of storage temperature on the biosynthesis of polyphenolic compounds, several reports have shown that low temperatures can increase the susceptibility to induce flavonoid accumulation (especially anthocyanins) in apple (Marais et al., 2001) or strawberries (Cisneros-Zevallos, 2003). These results differ from those reported by Kalt et al. (1999), who suggests that storage at ambient or above ambient temperatures will positively affect polyphenolic metabolism to enhance the antioxidant capacity. Similarly, it is reported that strawberry fruits stored at 5 °C and 10 °C show higher antioxidant capacity, polyphenols and anthocyanins than those stored at 0 °C (Ayala-Zavala et al., 2004). However, the post-harvest life based on overall quality was longer at 0 °C than at 5 °C and 10 °C.

In addition to the role of temperature, storage of potato strips at 4 °C under light exposure is reported to induce a higher flavonol accumulation rate than in darkness (Tudela et al., 2002). Light keeps flavonoids synthesis active even if the temperature is low, 4 °C. In this study, leeks were stored in darkness, resulting in a stable total phenol content.

Relating to the **ACSOs**, a significant increase of the isoalliin content was observed after 13 days of refrigerated storage of the white shaft (EL), while a stable ACSO concentration was observed for the other cases. To our knowledge, this is the first study who determined ACSOs in leek as a function of post-harvest processing and refrigerated storage. Although, studies were performed who describe the evolution of sulfur compounds during storage of other *Allium* species. Storage of onion for 6 months at 5 °C resulted in a significant increase in isoalliin content, while methiin levels remained unchanged (Yoo et al., 2012). When garlic *(Allium sativum* L.) was stored at 4 °C for 150 days, marked conversion of the γ-glutamyl peptides, γ-L-glutamyl-*S*-allyl-L-cysteine and γ-L-glutamyl-*S*-(trans-1-propenyl)-L-cysteine (GSPC), to sulfoxides, alliin and isoalliin, was observed. Interestingly, however, when garlic was stored at 23 °C, a decrease in GSPC and a marked increase in cycloalliin, rather than isoalliin, occurred (Ichikawa et

al., 2006). Similarly, from the study of Beato et al. (2012), it can be concluded that the three γ-glutamyl peptides in pickled blanched garlic were significantly degraded during storage at room temperature (49% loss after 1 year of storage). However, Hughes et al. (2006) did not find a variation in the alliin content when garlic was stored for 6 months at 4 °C, but isoalliin increased significantly from 0.6 mM to 7.1 mM in the outer cloves. It is reasonable to argue that the γ-glutamyl peptides were slowly degraded by an increased activity of a transpeptidase during storage to yield the corresponding *S*-alk(en)yl-L-cysteine, isoalliin, which increased during storage as also observed in our study.

Comparing the two cases, the entire leek and processed/packaged leek, the antioxidant activities and TP content were similar. However, it is reported that pre-cut on foods, as in the second case, can increase oxidation and as a consequence decrease the flavonoid content and antioxidant capacity. On the other hand, wounding enhances flavonol biosynthesis through the induction of phenylalanine ammonia-lyase enzyme which is related to the wound-healing process in order to fight pathogen attack after tissue wounding (Tudela et al., 2002). As our results indicate a stable antioxidant value, these 2 reactions were not performed or in balance.
The ACSO content (sum of isoalliin and methiin) in the white shaft of packaged leek, on the other hand, was much lower than the content in the white part of the entire leek. Therefore, the minimal processing step, that is, cutting the green leaves and roots, had a negative influence on the ACSO levels. Similarly, packaging steps negatively affect the levels of the ACSOs in garlic (Beato et al., 2012). Similarly, in the study of Tsouvaltzis et al. (2007), thiosulfinate levels of base removed leeks decreased significantly upon storage for 7 days (10 °C).

7.6 Conclusion

The evaluation of the effect of post-harvest processing and storage on the health-promoting compounds of vegetables is of great practical importance. Reports on the effect of storage on the antioxidant capacity, polyphenol and ACSO content of the white shaft and green leaves of leek are limited.
In general, the results indicated that refrigerated storage during 13 days did not affect negatively the antioxidant capacity and the total phenolic content in the white shaft and green leaves of the entire and packaged leek. Leek will visually spoil before any significant antioxidant capacity loss.

A slight increase in isoalliin level could be observed after a cool storage period. The difference between the antioxidant properties of the white shaft of the entire leek and the packaged leek was minimal, except for the lower ACSO values in packaged leek.

CHAPTER 8. INFLUENCE OF DOMESTIC FOOD PROCESSING ON THE ANTIOXIDANTS OF LEEK – 'FROM FRIDGE TO FORK'

Redrafted from

Bernaert, N., Van Bockstaele, E., De Loose, M. and Van Droogenbroeck, B. Antioxidant changes during domestic food processing of leek (*Allium ampeloprasum* var. *porrum*). Submitted in Journal of the Science of Food and Agriculture

8.1 Introduction

Vegetables are usually processed (boiling or steaming) before consumption and/or storage (blanching, freezing or canning), which improves flavour and palatability of foods (Delchier et al., 2012).
The *Allium* species are usually consumed after a heat treatment, including blanching, boiling, steaming and stewing (Chau and Cheung, 1997). The vegetable leek can be consumed in raw form in salads, however it is usually consumed after a cooking process, such as in soups, oven dishes, stewed with béchamel sauce, etc. (Compernol and De Ryck, 2011).

Although, it is widely considered that vegetables exposed to a thermal treatment, show a reduced content of thermolabile compounds such as vitamins C, E and A and some polyphenols. For example, processing can drastically affect the polyphenol content and behaviour and can, by consequence, influence the antioxidant capacity (Seruga et al., 2011; Ioannou et al., 2012). A number of studies report the effect of heat treatment on the antioxidant capacity of foods, but their conclusions do not all agree. In general, processing often results in either depletion or increase of the antioxidant properties of foods. As stated in §7.1, processing can induce the formation of compounds with novel antioxidant properties, which can maintain or even enhance the overall antioxidant potential of foods (Nicoli et al., 1997). However, during processing, loss of antioxidants or formation of compounds with pro-oxidant action may lower the antioxidant capacity.
In previous studies, leek extracts lost 20% of its total phenolic content when subjected to a thermal treatment (100 °C, 60 min ~ soup preparation). However, the same heat treatment increased its antioxidant capacity (Roy et al., 2007). The same authors suggest that heating *Alliums* may generate or modify some components which are more antiradical than their status in raw vegetables. Moreover, they reported, in addition to an increase in the total antioxidant capacity, a reduction in the pro-oxidant elements upon thermal treatment (Gazzani et al., 2000). This observation indicates that pro-oxidant molecules in *Alliums* are thermolabile. However, it is not clear to what extent the pro-oxidant elements interfere with the measurement of the total antioxidant capacity (Roy et al., 2007). Interestingly, Wangcharoen and Morasuk (2009) reported a decrease in antioxidant capacity of heated garlic by the decomposition of some polyphenolic and sulfur-containing compounds. But, when browning pigments developed, the antioxidant capacity of the heated brown garlic increased with the degree of browning.
The degree to which antioxidants change during processing depends on the sensitivity of the compound to modification or degradation, and the length of exposure to a processing technique (Breene, 1994). But losses (or gains) of antioxidants can also vary with

cooking or processing method (Ewald et al., 1999; Ioku et al., 2001; Lee et al., 2008). In case of onion and broccoli, highest losses have been observed in boiled products compared to frying and microwave cooking (Masrizal et al., 1997; Price et al., 1998a, b; Ioku et al., 2001).

For an understanding of antioxidants before absorption and digestion, it is essential to determine the loss (or gain) of these compounds in cooking processes. Therefore, the evaluation of the impact of domestic food processing on the antioxidants of vegetables is of great practical importance. However, reports on their effect on the antioxidant capacity, polyphenol and ACSO content of the leek matrix are limited as lot of studies have essentially focused on raw foods. Because leek is usually consumed after a heat treatment, the aim of this study was to evaluate the antioxidant properties during common domestic processes (blanching, cooking and steaming) in order to mimic the consumer's food processing habits – 'from fridge to fork'.

8.2 Plant material

The plant material and sample preparation is described in §3.2.2.2. Briefly, the leek samples were processed under different heat treatments, *i.e.* blanched (to simulate the processing step before industrial freezing), boiled (to simulate soup preparation) and steamed (to simulate steamed leek in a dish) with different duration times.

8.3 Bioactive compound analysis

A summary of the analyses (as described in §3.3), performed on the processed samples is given in Table 8.1.

Table 8.1 Overview of the analyses performed on cooked leek samples

Analysis		Method
Antioxidant capacity	ORAC DPPH	Spectrophotometric
Polyphenolic compounds	Total phenolic content	Spectrophotometric
S-alk(en)yl-L-cysteine sulfoxides		HPLC-MS/MS

Because of practical reasons, FRAP, individual polyphenols, ascorbate and fructan analysis were not performed on these samples.

8.4 Results

8.4.1 Antioxidant capacity

8.4.1.1 ORAC

Figure 8.1 shows the results of the ORAC assay of blanched/boiled (a) and steamed (b) leek samples.

Blanching and **boiling** did not influence the antioxidant capacity of the white shaft of leek, as measured with the ORAC assay. Blanching of the green leaves resulted in a 19% higher antioxidant capacity compared with the raw samples. When the green leaves were boiled for 40 min or 60 min, the ORAC values were significantly higher compared with raw leek, corresponding with an increase of 12% and 21%, respectively.

Steaming the white shaft during 20 min resulted in a significantly lower ORAC value compared to 10 min of steaming, although the steamed samples showed no significant difference with the raw samples. When the green leaves were steamed, a significant increase in the antioxidant capacity was measured compared with the raw samples. Steaming during 10 min resulted in an increase of 38% of the initial capacity of the green leaves, and decreased gradually after a longer period of steaming, but still with a higher end antioxidant capacity.

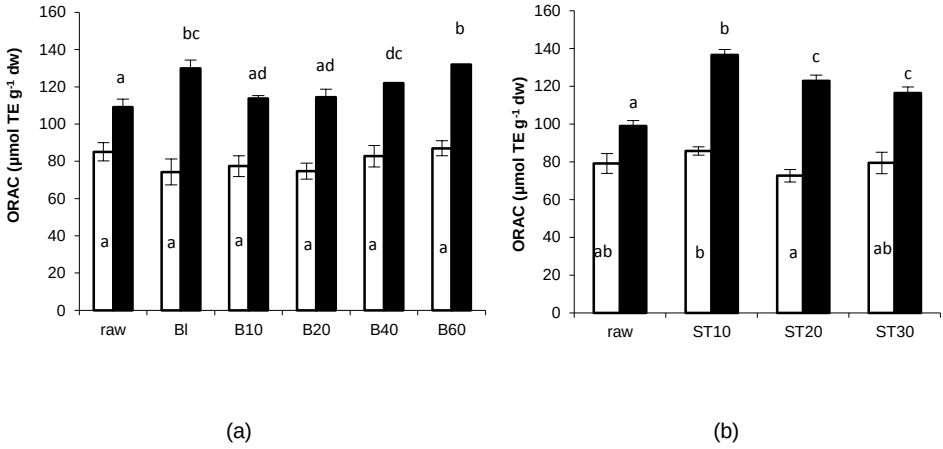


Figure 8.1 ORAC value of the white shaft (□) and green leaves (■) after boiling (a) and steaming (b) with Bl: blanched, B10, 20, 40, 60: boiled for 10, 20, 40 and 60 minutes respectively and ST10, 20, 30: steamed for 10, 20 and 30 minutes respectively; a, b, c, d: bars with a different subscript show statistical significance (n=3)

8.4.1.2 DPPH

Figure 8.2 shows the results of the DPPH assay of blanched/boiled (a) and steamed (b) leek samples.

The DPPH value of the **boiled** (60 min) white leek shaft decreased significantly (39%) compared to the capacity before boiling, while the DPPH value of the green leek leaves decreased with 46%.

Steaming did not influence the DPPH value of the white shaft. When the green leaves were steamed for 20 or 30 min, a significant increase of 41% and 51% of the initial antioxidant capacity in DPPH value was observed.

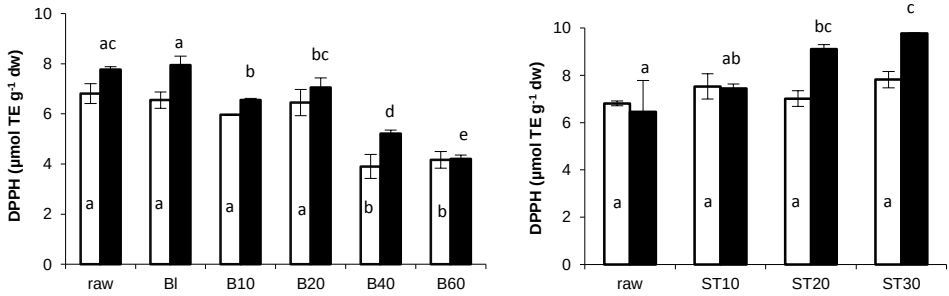


Figure 8.2 DPPH value of the white shaft (□) and green leaves (■) after boiling (a) and steaming (b) with Bl: blanched, B10, 20, 40, 60: boiled for 10, 20, 40 and 60 minutes respectively and ST10, 20, 30: steamed for 10, 20 and 30 minutes respectively; a, b, c, d, e: bars with a different subscript show statistical significance (n=3)

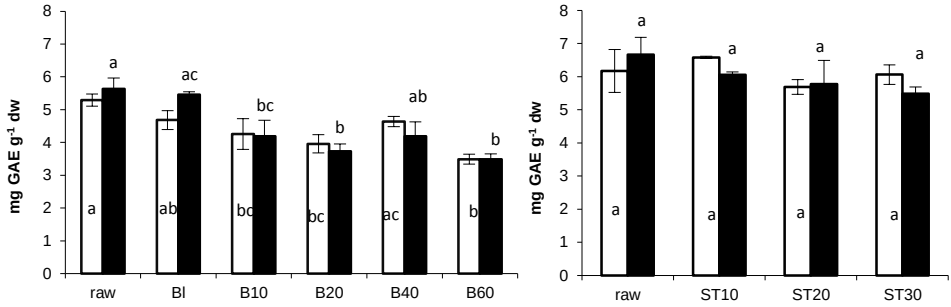
8.4.2 Polyphenolic compounds

8.4.2.1 Total phenolic content

Figure 8.3 shows the results of the total phenolic content in the white shaft and green leaves after a blanching/boiling (a) and steaming (b) process.

The total phenolic content of the white shaft was significantly lower when leek was **boiled** for 10 and 20 min compared with the raw samples. When the white shaft of leek was boiled for 60 min, the content decreased with 34% compared with the raw samples. When the green leaves of leek were boiled for 20 min and 60 min, a significant decrease of 34% and 38% respectively, of its initial TP content was observed.

In contrast with boiling, **steaming** did not have a significant influence on the total phenolic content.



(a) (b)

Figure 8.3 Total phenolic content of the white shaft (□) and green leaves (■) after boiling (a) and steaming (b) with Bl: blanched, B10, 20, 40, 60: boiled for 10, 20, 40 and 60 minutes respectively and ST10, 20, 30: steamed for 10, 20 and 30 minutes respectively; a, b, c: bars with a different subscript show statistical significance (n=3)

8.4.3 S-Alk(en)yl-L-cysteine sulfoxides

Figure 8.4 shows the results of the isoalliin (1) and methiin (2) content in the white shaft and green leaves after a blanching/boiling (a) and steaming (b) process.

The isoalliin content of the **blanched** white shaft was significantly higher compared to the raw samples. However, after a **boiling** time of 10 min, the isoalliin content decreased with 41%. After 40 min of boiling, isoalliin was already depleted. The isoalliin content in the green leaves decreased with 40% after 10 min of cooking, while 60 min of boiling resulted in a decrease of 92%. The isoalliin content declined with 80% and 75% in the white shaft and green leaves, respectively, after a **steaming** process of 10 min, but did not change significantly after a longer period of steaming.

The methiin content in the white shaft and green leaves increased significantly after a **blanching** process, by 26% and 62% respectively. A longer **boiling** time resulted in a gradual decrease of the methiin content. Steaming of the white shaft of leek did not influence the methiin content. **Steaming** of the green part, on the other hand, resulted in a decrease of the initial methiin content, although there was no significant difference in methiin content of the green leaves between 10 min, 20 min and 30 min of steaming.

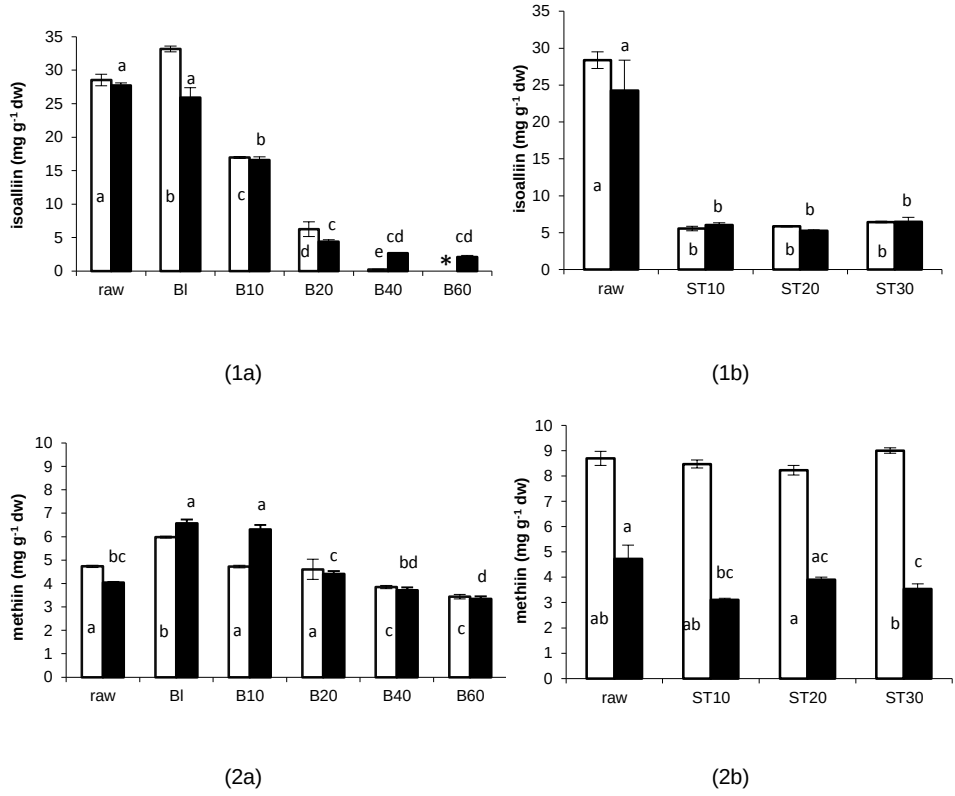


Figure 8.4 Isoalliin (1) and methiin (2) content of the white shaft (□) and green leaves (■) after cooking (a) and steaming (b) with Bl: blanched, B10, 20, 40, 60: boiled for 10, 20, 40 and 60 minutes respectively and ST10, 20, 30: steamed for 10, 20 and 30 minutes respectively; with *, not detected; a, b, c, d: bars with a different subscript show statistical significance (n=3)

8.5 Discussion

As leek is usually consumed after applying a heat treatment, the present chapter elucidated the influence of domestic food processing, including blanching, boiling and steaming on the antioxidant properties of the white shaft and green leaves of leek.

With respect to the influence of **antioxidant capacity**, the decreasing trend of the DPPH radical scavenging activities of leek after boiling, agrees with a lot of studies. Turkmen et al. (2005) noticed a 20% decrease of the DPPH capacity after boiling leek for 5 min. Our results are also consistent with Jastrzebski et al. (2007), who reported a significant decrease in DPPH value of garlic boiled at 100 °C for 40 min and 60 min. A study on onion juice showed a decrease in antioxidant capacity which was 63% of the capacity before boiling. Some studies, however, reported a significantly higher DPPH value upon

thermal treatment of leek (Roy et al., 2007), onion (Woo et al., 2007) and garlic ((Wangcharoen and Morasuk, 2009). However, the above results may not be comparable because of the different food matrix. This food matrix can act as a barrier to imposed heat effects or induce degradation (Ioannou et al., 2012). Accordingly, Aoyama and Yamamoto (2007) found that during thermal treatment, the antioxidant capacity of white Welsh onion (*Allium fistulosum L.*), yellow and red onions (*Allium cepa* L.) decreased as measured with the TEAC and FRAP assay, while the antioxidant capacity of green Welsh onion increased.

The results of the ORAC and DPPH assay for the leek boiling processes are dissimilar. The ORAC assay indicates an increase in antioxidant capacity of the green leaves, while the DPPH assay shows a decrease. These opposite findings are in contrast with the study of Xu and Chang (2008), who observed a reduction in the DPPH free radical scavenging capacity of boiled legumes with 60-70% in green pea (*Pisum sativum* L. cv. Stratus) and 9-30% in lentils (*Lens culinaris* cv. CDC Richlea), as well as in the ORAC value, *i.e.* 58-77% in green pea and 54-62% in lentil. The two assays can differ for many reasons, including differences in experimental methodology used, *e.g.* in the free radical that was applied, different reaction times, different absorption wavelength, etc. The different trends in antioxidant capacity could also be attributed to the increase or the formation of specific compounds, which could provide more or less hydrogen atoms during oxidation-reduction reaction. Different classes of compounds may have different contributions to ORAC and DPPH values.

The decrease of DPPH free radical scavenging activity of boiled leek samples can be due to the decomposition of some polyphenolic compounds and also some sulfur containing compounds such as ACSOs, as seen in Figures 8.3 and 8.4, but also diallyl sulfide, S-ethyl cysteine, N-acetyl cysteine, which would be lost when the temperature reaches 65 °C (Yin et al., 2002). The decrease in DPPH value can also be the result of a decrease in ascorbic acid content, an antioxidant which is known to decompose during a thermal treatment and is measured in the antioxidant capacity assays (Rumm-Kreuter and Demmel, 1990).

The increase in ORAC value can be the result of the fact that thermal treatments can break the glucosides of flavonoids to form aglycones which possess higher antioxidant properties (Buchner et al., 2006). As stated in the introduction (§ 8.1), it is assumed that, heating *Alliums* for a longer period may generate or modify some components which are more antiradical than their status in raw vegetables (Roy et al., 2007). The authors proposed 2 possible mechanisms: (1) formation of brown products; (2) destruction of pro-oxidant substances. Numerous reports have shown that thermal treatment enhances non-enzymatic browning that includes the Maillard reaction, chemical oxidation of phenols and caramelisation. The brown products formed upon thermal treatment have

been shown to be associated with high antioxidant capacity (Payet et al., 2005; Papetti et al., 2006). In addition, the decrease of 2',7'-dichlorofluorescein (DCF), a pro-oxidant, in thermally treated *Allium* products showed that destruction of pro-oxidant molecules may be another factor in the increased antiradical activity of the *Allium* samples (Roy et al., 2007).

In contrast with boiling, our study demonstrated an obvious increase in the antioxidant capacity of the green leek leaves after steaming. The difference between steaming and boiling can be attributed to the higher contact of the leek particles with water in case of boiling, resulting in leachate of water soluble antioxidants in the boiling water.
The two applied antioxidant capacity assays executed on the steamed samples revealed the same trend. This last observation is not in accordance with Xu and Chang (2008), who found that the ORAC value of pressure steamed legumes increased with 69-175% in green pea and 5-13% in lentil, while the DPPH value decreased (Xu and Chang, 2008).
Similarly, boiling was responsible for a significant decrease in total phenolic content of leek, while steaming did not have an influence. The lower total phenolic content upon boiling agree with the results of Roy et al. (2007), who showed that garlic, leek and onion lost about 35%, 20% and 7%, respectively, of their respective total phenolic content when the raw extracts were subjected to thermal treatment (100 °C, 60 min). Similarly, Turkmen et al. (2005) noticed a decrease of 36% TP after boiling leek for 5 min, while steamed leek (7.5 min) lost 15% of its initial TP content.
The losses in the total phenol content of processed leek upon boiling are not generally attributed to a chemical breakdown of flavonoid conjugates or formation of new compounds, but rather leaching of phenolic compounds into the cook water (Price et al., 1997; Crozier et al., 1997; Hirota et al., 1998; Makris and Rossiter, 2001; Xu and Chang, 2008). Therefore, soups are a good source of flavonoids. Some studies, however, report an increase in phenols after a heat treatment on onion and tomatoes (Stewart et al., 2000; Woo et al., 2007). This can be explained by the fact that processing could increase the flavonoid extractability from the matrix in subsequent assays resulting in a higher apparent content.
As mentioned in the introduction (§8.1), the degree to which phytochemicals change during processing depends on the sensitivity of the compound to modification or degradation, processing method and the length of exposure to a processing technique (Breene, 1994). Studies who examined the effect of heat treatment on flavonoids in aqueous solutions show different sensitivity to degradation depending on the flavonoid structure. For quercetin 3-O-rutinoside (rutin), a higher stability compared to its aglycone form (quercetin) is observed (Buchner et al., 2006). Essential for the degradation is the

3-hydroxy-function at the C-ring of the flavonoid. Due to the blocking of this position by a sugar moiety in the case of rutin, the degradation of rutin differs from that of quercetin where this position is unoccupied (Makris and Rossiter, 2000). However, whatever their structure, a significant degradation is observed for temperatures above 100 °C.

Losses (or gains) of polyphenols can also vary with cooking or processing method (Ewald et al., 1999; Ioku et al., 2001; Lee et al., 2008). Based on our results, we can conclude that steaming is a better method to maintain the polyphenolic compounds present in leek than boiling. This result is partly consistent with the study of Lee et al. (2008), who compared different thermal processes and reported the following losses of flavonoids in onion: frying, 33%; sautéing, 21%; boiling, 14-20%; steaming, 14%; microwaving, 4% and baking, 0%.

The degradation of polyphenols is not only a function of sensitivity of the compounds and magnitude of heating; it may also depend on other parameters such as pH, the presence of oxygen and the presence of other phytochemicals in the medium. For pH, Buchner et al. (2006) and Friedman (1997) showed that degradation of rutin and quercetin is higher under weakly alkaline and neutral reaction conditions. For oxygen, Buchner et al. (2006) and Makris and Rossiter (2001) observed that the presence of oxygen highly induces quercetin and rutin degradations while the absence of oxygen has the opposite effect. From these results it is obvious that the presence of oxygen accelerates the degradation of quercetin and rutin. The decay is caused by the so-called reactive oxygen species (ROS). These radicals (*e.g.* superoxide anion radical, hydroperoxide radical and hydroxyl radical) are often formed in aqueous solution and can be captured by the flavonols.

Moreover, it has been shown that the presence of other phytochemicals in the medium like chlorogenic acid plays a protective role in the maintenance of polyphenols (Murakami et al., 2004). In contrast, additional ferrous ions accelerate the loss of flavonoids (Ioku et al., 2001).

With concern to the sulfur compounds, it is remarkable that blanching resulted in a slight increase in the ACSO content. This finding contradicts with the study of Beato et al. (2012), who observed that blanching of garlic (90 °C, 5 min) did not significantly affect the individual organosulfur compound contents. It is also notable that the time of steaming does not play a role in ACSO losses, while this is at least the case for boiling.

Applying a thermal treatment (> 90'') to leek samples negatively influenced the content of isoalliin and methiin. Steaming seems to be responsible for better retention of the ACSOs compared with boiling, however. Methiin was less susceptible to cooking compared to isoalliin. To explain this, it is found that alliinase acts quickly, but differently on the individual ACSOs, such that some of the flavour precursors are more completely degraded than others (Lancaster et al., 1998). The decrease in ACSOs during cooking will result in the formation of volatile sulfur compounds. In a study of Kubec et al. (1998)

methiin was heated in closed model systems at different temperatures (from 80 to 200 °C) in the presence of variable amounts of water (0-98%) for 1-60 min. It was found that thermally generated breakdown products of methiin can significantly contribute to the typical aroma of culinary processed *Allium* vegetables. Dimethyl disulfide was identified as the predominant volatile compound generated by thermal degradation of methiin. Dimethyl trisulfide, dimethyl thiosulfinate, dimethyl thiosulfonate, and alkyl- and alkylthio-substituted pyridines were identified as minor volatile breakdown products arising from methiin.

With relation to the health aspects, an important pharmacological aspect attributed to the *Allium* species is the ability to inhibit platelet aggregation which is mainly attributed to the synergistic action of organosulfur compounds and flavonoids present. Platelet aggregation plays a key role in the development of atherosclerosis, a systemic proliferative and inflammatory disease of the vascular wall of arteries (Nitin, 2004). Raw onions inhibit platelet aggregation, however antiplatelet activity was destroyed between 3 and 6 min of steaming, and at 10 min of steaming, onions stimulated platelet activity, while steaming did not affect the polyphenolic concentration (Hansen et al., 2012). It is possible that the generation of oils or polysulfides from thiosulfinates are partially responsible for this effect. This phenomenon is a concern, however studies on leek, relating to the antiplatelet activity, are to our knowledge, not performed yet.

8.6 Conclusion

Several studies have focused on the determination of antioxidants in raw foods. However, the evaluation of domestic food processing on the health benefits of vegetables is also of great practical importance.

The antioxidant capacity of leek was highly influenced by cooking. However, the antioxidant capacity of the boiled green leaves gave contradictory results. ORAC values indicated an increase, while DPPH results show a decrease. An obvious increase (more than 30%) could be observed in the antioxidant capacity of the steamed green leaves. Boiling had a negative effect on total phenolic content in the white shaft and green leaves. In contrast with boiling, steaming did not have an influence on the polyphenolic content. It is remarkable that blanching results in a slight increase in the ACSO content. Applying a longer thermal treatment turned out to have a negatively influence on the content of isoalliin and methiin. In general, methiin was less susceptible to cooking. Steaming seems to be responsible for a better retention of the bioactive compounds present in leek.

CHAPTER 9. STABILISATION AND VALORISATION OF LEEK

Redrafted from

Bernaert, N., Wouters, D., De Vuyst, L., De Paepe, D., De Clercq, H., Van Bockstaele, E. De Loose, M. and Van Droogenbroeck, B. (2013). Antioxidant changes during spontaneous fermentation of the white shaft and green leaves of leek (*Allium ampeloprasum* var. *porrum*). Journal of the Science of Food and Agriculture, doi: 10.1002/jsfa.6020

Wouters, D., Bernaert, N., Anno, N., Van Droogenbroeck, B., De Loose, M., Van Bockstaele, E., and De Vuyst, L. Application and validation of autochthonous lactic acid bacteria starter cultures for controlled leek fermentations and their influence on the antioxidant properties of leek (*Allium ampeloprasum* var. *porrum*). Submitted for publication in Food Microbiology

9.1 Introduction

Leek is grown in Belgium for its thickened cylindrical white shaft made up of long leaf bases. Despite its interesting bioactive compound profile as described in Chapter 4, a large part of the green leek leaves remains unused because preparations of this part are restricted compared to the white shaft. Two fractions of the unused green leaves can be established, a first fraction is immediately cut off on the field during harvesting (industry), while a second fraction is removed during processing at the farm. To be able to use this large quantity of valuable plant biomass, a way to stabilise the green leaves is needed. Therefore, the application of some alternative value-adding processing and preservation methods such as fermentation and drying was investigated in this chapter.

Preservation of food, including fermentation of perishable raw materials has been used since ages (Prajapati and Nair, 2003). Nowadays, cabbages, cucumbers and olives are fermented on industrial scale (Rodriguez et al., 2009). Sauerkraut, the result of lactic acid fermentation of shredded and brined white cabbage, is an important dietary ingredient in Central Europe (Martinez-Villaluenga et al., 2012). Efforts have been made to enhance the shelf-life of *Allium* species, such as onions and garlic, by fermentation (Desai and Sheth, 1997; de Castro et al., 1998; Roberts and Kidd, 2005; Bisakowski et al., 2007), but studies investigating the fermentation of leek are limited. Leek has only been included in a vegetable fermentation as flavouring ingredient in addition to other vegetables in kimchi, which is probably the most indispensable food for Koreans (de Castro et al., 1998; Roberts and Kidd, 2005). Recently, fermentation of leek has been investigated in collaboration with prof. dr. ir. Luc De Vuyst and dr. ir. Dorrit Wouters from VUB (IMDO[7]) as a potential processing method to valorise the green leaves (Wouters et al., 2012). In the latter study, a thorough characterisation of the spontaneous fermentation of the green and white leek parts revealed that *Leuconostoc mesenteroides* (initial fermentation phase), *Lactobacillus (Lb.) sakei* (middle and final fermentation phase) and *Lb. plantarum*, *Lb. brevis* and *Lb. parabrevis* (final fermentation phase) are the main isolated lactic acid bacteria (LAB) species involved in spontaneous leek fermentation. The presence of *Lb. sakei* was more explicit in fermentations of the green leek parts, whereas the opposite was observed for the presence of *Lb*. *plantarum*.
In addition to preserving food, fermentation improves the food safety and influences the organoleptic quality of food (Adams and Mitchell, 2002; Sicard and Legras, 2011). Additionally, fermentation can improve the nutritional value of food (van Boekel et al.,

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2010). Accordingly, Kusznierewicz et al. (2008) reported an increase of the antioxidant capacity of white cabbage upon fermentation. Other studies have demonstrated an increase in DPPH free radical-scavenging activity during fermentation of carrot juice by *Lb. bulgaricus* and *Lb. rhamnosus* (Nazzaro et al., 2008). In addition to an increase in the antioxidant capacity, the total phenol content increased upon fermentation of buckwheat, barley and rice, while it decreased in fermented sorghum (Dlamini et al., 2007; Dordevic et al., 2010). In addition, lactic acid fermentation may lead to a qualitative modification of proteins, often resulting in an increase of water soluble proteins and amino acids in fermented sorghum products (Dlamini et al., 2007).

Another possible way to stabilise and valorise the green leaves is to apply a drying step. Fresh leek contains 85-87% water, and water levels need to be lowered to less than 15% for their preservation (Diaz-Maroto et al., 2002). Drying is one of the most efficient and ancient ways to preserve foods. It reduces the product's water activity, which inhibits microbial growth and decreases degrading reactions, resulting in enhanced stability. Therefore, various methods can be used. Freeze-drying (FD) is a multi-step process that employs freezing, sublimation and desorption. Freeze-dried products are known to have high quality, since the technique preserves bioactive compounds, colour, texture and flavour. The solid state of water during freeze-drying protects the primary structure and the shape of the products with minimal reduction of volume. However, due to the use of vacuum, sub-zero temperatures and long drying time, freeze-drying is one of the most expensive drying technologies (Hammami and Rene, 1997; Khalloufi and Ratti, 2003). Studies have demonstrated that FD is the best drying method to retain antioxidants (Desobry et al., 1997; Abonyi et al., 2002; Nindo et al., 2003); however, some antioxidants may still be lost because of the long drying time (Kaspar et al., 2012). Other possible drying methods include air-drying (AD) and the recent Refractance Window® (RW^{TM}) drying (RWD). Air-drying is the most extensively used drying method, which involves blowing hot air through the plant material to remove the water from the surface. This creates a diffusion gradient in the food that moves the water from the interior to the outer surface (Gowen et al., 2006). Generally, air-drying is favoured due to processing cost and speed (Katsube et al., 2009). Although very common, the method involves high temperatures and long processing times, which causes losses in nutritional values and sensory properties of foods (Ratti, 2001). Refractance window dehydration method is a relatively new drying method introduced by MCD Technologies, Inc. (Tacoma, Washington, USA). The technology involves applying the product, as a thin layer, to the top surface of a transparent plastic conveyor belt. Under the plastic sheet, hot water circulates that carries thermal energy to the product (Nindo and Tang, 2007). The method uses moderate temperatures and short drying times, which has been reported to

result in low energy costs and high quality products (Abonyi et al., 2002; Nindo et al., 2003; Caparino et al., 2012).
However, the effect of a particular drying method on the quality is not predictable and depends on the involved compounds and the specific plant concerned.
Although a lot papers describe the influence of fermentation and drying on the antioxidant properties of vegetables, very little has been published on the influence of fermentation and drying on health-promoting compounds of *Allium* species, especially for leek. As the lactic acid bacteria naturally present on leek are capable of initiating fermentation, the first part of this chapter aims to investigate the effect of spontaneous fermentation of the green leaves and white shaft in terms of the antioxidant capacity, polyphenol profile (flavonoids and phenolic acids) and ACSO content. In addition to natural fermentation, the effect of starter culture induced fermentation was investigated on the antioxidant properties of a leek mix (whole leek plant). In addition, 3 different drying methods were applied to determine their influence on the antioxidant properties of white shaft and green leaves of leek. As such, the use of these potential valorisation routes were evaluated.
The results presented in this Chapter have been partly established in collaboration with prof. dr. Derek Stewart of the James Hutton Institute (Enhancing Crop Productivity and Utilization Theme) with regard to the flavonoids/phenolic acids analyses of the air-dried and freeze-dried samples. The analysis of flavonoids/phenolic acids in the fermented and RWD samples are performed in collaboration with ir. Domien De Paepe (ILVO/VITO).

9.2 Plant material

Selection of plant material and sample preparation are described in §3.2.2.3. Briefly, spontaneous and starter culture fermentation (21 days) of leek was investigated. In addition to fermentation, 3 drying techniques were investigated as possible valorisation routes of leek processing by-products, *i.e.* freeze-drying (FD), air-drying (AD) and refractance window drying (RWD). First, freeze-drying was compared with air-drying (performed at ILVO). Secondly, freeze-drying was compared with a relative new drying technique, refractance window drying (performed in MCD-Technologies in the United States).

9.3 Bioactive compound analysis

A summary of the analyses (as described in §3.3) performed on the fermented and dried leek samples is given in Table 9.1.

Table 9.1 Overview of the analyses performed as a function of processing techniques

Analysis			Method
Antioxidant capacity	ORAC DPPH		spectrophotometric
Polyphenolic compounds	Total phenolic content		spectrophotometric
	Flavonoids and phenolic acids	Spont. fermentation	U-HPLC-ESI-Orbitrap-MS
		Freeze-drying vs. air-drying	U-HPLC-ESI-Orbitrap-MS/MS
		Freeze-drying vs. Refractance Window Drying	U-HPLC-ESI-Orbitrap-MS
S-alk(en)yl-L-cysteine sulfoxides			HPLC-MS/MS

FRAP, ascorbate and fructan analysis were not performed on these samples. Individual polyphenols were determined in all the samples, with the exception of starter culture fermented leek samples.

9.4 Results

9.4.1 Stabilisation by fermentation

The application of a potential alternative value-adding processing and preservation method of leek, namely fermentation, was investigated in terms of antioxidant changes (antioxidant capacity, polyphenols and organosulfur compounds).

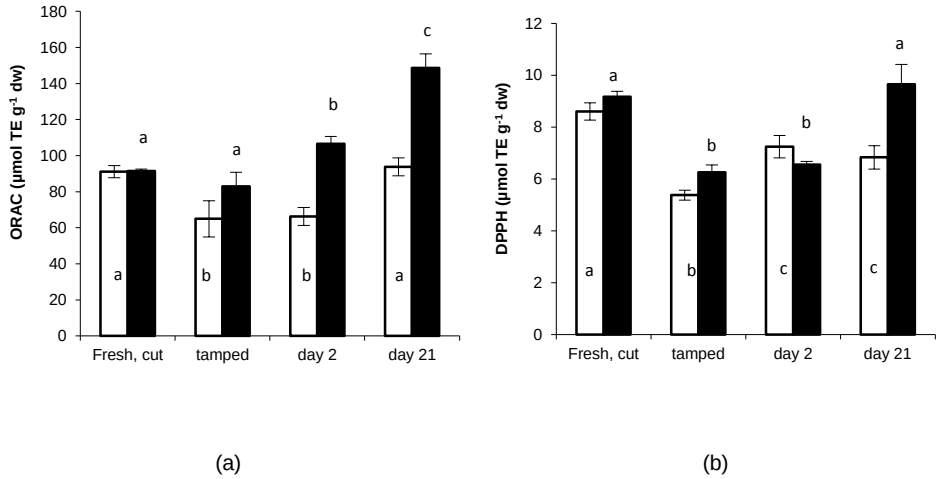
9.4.1.1 Spontaneous fermentation

9.4.1.1.1 Antioxidant capacity

Figures 9.1 (a) and (b) illustrate the evolution of the antioxidant capacity during fermentation of the green leaves and white shaft as determined using the ORAC and DPPH assay, respectively.

The antioxidant capacity of the green leaves, measured using the ORAC assay, was significantly higher after a 2 day-fermentation compared to the antioxidant capacity of fresh, cut and tamped leek leaves. Fermentation of the green leaves for 21 days resulted in an increase of 62% and 79% in antioxidant capacity compared to the fresh and tamped samples, respectively. The antioxidant capacity of the white shaft, measured

using the ORAC assay, did not change significantly after 21 days of fermentation compared to the capacity of fresh white leek, but was significantly higher than the tamped samples (increase of 44%). Tamping the white shaft resulted in a 29% reduction of its antioxidant capacity of the white shaft compared to its initial antioxidant capacity.
In addition to the ORAC assay, the results of the DPPH test after tamping indicated a significant decrease (32% and 38%) of the antioxidant capacity in the green leaves and white shaft, respectively. The DPPH free radical scavenging activity of the green leek leaves did not change significantly after a 21-day spontaneous fermentation compared to its initial condition, while it was significantly higher than tamped (54%) and 2-day fermented (47%) samples. The end DPPH free radical scavenging activity of the white shaft did not differ from the initial activity, but was significantly higher than the tamped samples (27%).
Both assays revealed an increase in antioxidant capacity upon fermentation of both leek parts compared to the tamped leek samples. In addition, the increase was also significant compared to the fresh samples for the green leaves (ORAC) and white part (DPPH).



(a) (b)

Figure 9.1 Antioxidant capacity, measured using the ORAC (a) and DPPH (b) assay, of the green leaves (■) and the white shaft (□); a, b and c: bars with a different subscript show statistical significance, n=3

9.4.1.1.2 Polyphenolic compounds

Total phenolic content

The total phenolic content in the green leaves at the end of the fermentation was significantly higher compared with the tamped samples. The TP content of the white shaft, on the other hand, did not change significantly after a fermentation process, when compared with the tamped samples, but was significantly lower compared to the TP content in the fresh white shaft (Figure 9.2).

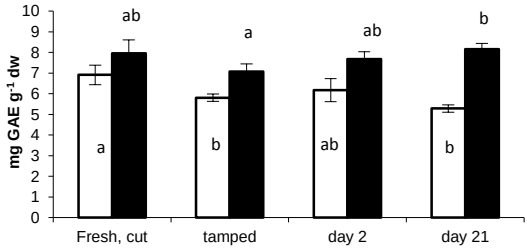


Figure 9.2 Total phenolic content of the green leaves (■) and white shaft (□); a, b and c: bars with a different subscript show statistical significance, n=3

Flavonoids and phenolic acids

The effect of fermentation on the polyphenolic composition and the content of the green leaves and white shaft of leek is represented in Table 9.2. Of the selected polyphenol standards, 14 polyphenolic compounds could be quantified, including 5 phenolic acids and 9 flavonoids.

The main compounds found in the freshly, cut green leaves were (in decreasing order of importance) kaempferol 3-O-glucoside (3.192 mg 100 g^{-1} dw), ferulic acid (2.707 mg 100 g^{-1} dw), quercetin 3-O-galactoside (2.173 mg 100 g^{-1} dw), sinapenic acid (0.376 mg 100 g^{-1} dw), quercetin (0.267 mg 100 g^{-1} dw) and propyl gallate (0.209 mg 100 g^{-1} dw). After 21 days of fermentation, new polyphenolic compounds, such as hydroferulic acid, quercetin 3-O-rutinoside, quercetin 3-O-arabinoside, naringenin and dihydroquercetin were found in the green leaves. Sinapinic acid disappeared after a fermentation of the green leaves. The contents of ferulic acid, astragalin and luteolin increased significantly by 39%, 57% and 13%, respectively, after a leek fermentation process of 21 days compared with the initial concentration. Tamping was responsible for significant losses of

polyphenols in the green leaves; for example the kaempferol 3-O-glucoside content decreased with 55% after tamping.

The main flavonoids and phenolic acids found in the freshly, cut white shaft of leek before fermentation were (in decreasing order of importance) ferulic acid (2.971 mg 100 g^{-1} dw), quercetin 3-O-galactoside (2.323 mg 100 g^{-1} dw), kaempferol 3-O-glucoside (0.350 mg 100 g^{-1} dw), quercetin (0.263 mg 100 g^{-1} dw) and propyl gallate (0.211 mg 100 g^{-1} dw). Sinapenic acid was present in the fresh green leaves, but was not found in the white shaft. The concentration of kaempferol 3-O-glucoside was 10 times lower in the white shaft compared with the content of the green leaves. After fermenting the white shaft, new polyphenolic compounds, such as hydroferulic acid, were found, whereas the contents of ferulic acid, kaempferol 3-O-glucoside and naringenin increased by 51%, 113% and 50%, respectively, compared with the initial concentration. The caffeic acid content, decreased by 19%. Again, tamping was responsible for great losses in polyphenol content.

Table 9.2 Polyphenol content (mg 100 g^{-1} dw) of the green leaves and white shaft before and after fermentation, means ± standard deviation, nd: not detected (a, b and c: values with a different subscript show statistical significance) (n=3)

	Green leaves			White shaft		
Polyphenol	**Fresh, cut**	**Tamped**	**Day 21**	**Fresh, cut**	**Tamped**	**Day 21**
Propyl gallate	0.209 ± 0.002^{a}	0.207 ± 0.001^{a}	0.210 ± 0.003^{a}	0.211 ± 0.001^{a}	0.206 ± 0.002^{b}	0.207 ± 0.002^{ab}
Ferulic acid	2.707 ± 0.143^{a}	1.334 ± 0.097^{b}	3.770 ± 0.071^{c}	2.971 ± 0.039^{a}	2.584 ± 0.319^{a}	4.505 ± 0.456^{b}
Hydroferulic acid	nd	nd	2.230 ± 0.130	nd	nd	2.079 ± 0.018
Caffeic acid	0.093 ± 0.003^{a}	0.088 ± 0.005^{ab}	0.083 ± 0.001^{b}	0.103 ± 0.006^{a}	0.096 ± 0.005^{ab}	0.083 ± 0.001^{b}
Sinapenic acid	0.376 ± 0.020^{a}	0.161 ± 0.012^{b}	nd	nd	nd	nd
Luteolin	0.074 ± 0.002^{a}	0.071 ± 0.001^{a}	0.084 ± 0.002^{b}	0.071 ± 0.001^{a}	0.070 ± 0.001^{a}	0.071 ± 0.002^{a}
Kaempferol	0.085 ± 0.005^{a}	0.073 ± 0.003^{b}	0.079 ± 0.000^{ab}	0.077 ± 0.001^{a}	0.070 ± 0.001^{b}	0.071 ± 0.002^{b}
Kaempferol 3-O-glucoside	3.192 ± 0.266^{a}	1.451 ± 0.054^{b}	5.025 ± 0.261^{c}	0.350 ± 0.012^{a}	0.199 ± 0.010^{b}	0.747 ± 0.069^{c}
Quercetin	0.267 ± 0.004^{ab}	0.258 ± 0.002^{a}	0.271 ± 0.006^{b}	0.263 ± 0.002^{a}	0.258 ± 0.003^{a}	0.262 ± 0.003^{a}
Quercetin 3-O-galactoside	2.173 ± 0.012^{a}	2.227 ± 0.122^{a}	2.256 ± 0.043^{a}	2.323 ± 0.013^{a}	2.296 ± 0.024^{a}	2.299 ± 0.030^{a}
Quercetin 3-O-rutinoside	nd	nd	0.160 ± 0.009	nd	nd	nd
Quercetin 3-O-arabinoside	nd	nd	0.055 ± 0.008	nd	nd	nd
Naringenin	nd	nd	0.023 ± 0.000	0.016 ± 0.000^{a}	0.016 ± 0.001^{a}	0.023 ± 0.002^{b}
Dihydroquercetin	nd	nd	0.073 ± 0.10	nd	nd	nd
sum	**9.193**	**5.871**	**14.320**	**6.383**	**5.795**	**10.348**

9.4.1.1.3 *S*-Alk(en)yl-L-cysteine sulfoxides

Figures 9.3 (a) and (b) show the evolution of the isoalliin and methiin contents of the leek samples throughout fermentation. The tamping process is responsible for a significant decrease of the isoalliin and methiin content in the green leaves (32% and 62%, respectively) and for a decrease of 32% and 48% in the white shaft, respectively, in the white shaft. Moreover, 3 weeks of fermentation resulted in a complete loss of isoalliin and 93% decrease of the methiin content in the green leaves, compared with the fresh samples, while a total loss of isoalliin and a decrease of 91% of methiin was determined in the white shaft.

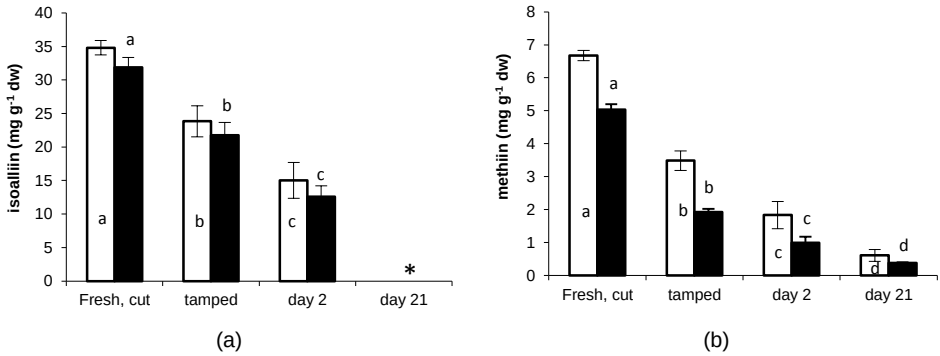


Figure 9.3. Isoalliin (a) and methiin (b) content during fermentation of the green leaves (■) and white shaft (□); with *, not detected; a, b, c and d: bars with a different subscript show statistical significance, n=3

In this part, we described the influence of a spontaneous fermentation process of leek on its antioxidant properties. The second part elucidates the influence of LAB starter cultures on the health-promoting compounds in leek.

9.4.1.2 Starter culture fermentation

9.4.1.2.1 Antioxidant capacity

Figure 9.4 (a) presents the results of the ORAC values of the leek samples (mix of white shaft and green leaves) during starter culture fermentation. Tamping of leek did not change the antioxidant capacity significantly, while 3 day-fermented samples, except for those of the sakei fermentations, exhibited a significant increase. After 3 weeks of fermentation, the antioxidant capacity of all leek fermentation was significantly higher compared to the cut, tamped and 3 day-fermented leek samples. This increase was most

pronounced for the spontaneous fermentations (51%), followed by the fermentations with the mixed (42%), sakei (33%) and plantarum (17%) starter cultures. The antioxidant capacity of plantarum fermented leek was significantly weaker than that of the naturally fermented leek but did not vary from the other starter culture-induced fermentations. In contrast with the ORAC values, a significant decrease of 26% in DPPH values was found after tamping the leek particles (Figure 9.4 (b)). Afterwards, DPPH values of the spontaneous, plantarum and mixed fermentations exhibited again a significant decrease, both after 3 days and 3 weeks of fermentation. This decrease was most pronounced for the plantarum fermentations (76%). In the case of the sakei fermentations, the antioxidant capacity did not change significantly during the fermentation process compared to the capacity of fresh samples, but increased significantly (44%) compared to the tamped samples.

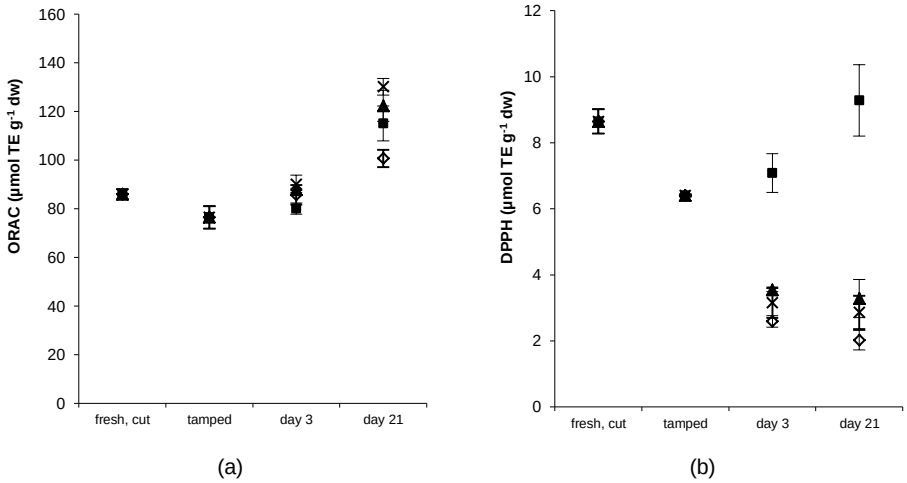


Figure 9.4 Antioxidant capacity determined with the ORAC (a) and DPPH (b) assay of leek samples of the spontaneous (×); plantarum (◊) ; mix (▲); and sakei (■) fermentations, n=3

9.4.1.2.2 Polyphenolic compounds

Total phenolic content

Figure 9.5 presents the evolution of the TP content of the leek samples during fermentation. During fermentation, the total phenolic content of the different leek samples, except for the mixed fermented leek samples, did not change significantly. At the end of the mixed fermentations, the total phenolic content was significantly higher compared to 3 day-fermented leek samples. However, no significant differences were

found between the TP content at the end of the mixed fermentation and that of the cut and tamped leek samples. The total phenolic content of the end-products of the different fermentations did not differ significantly.

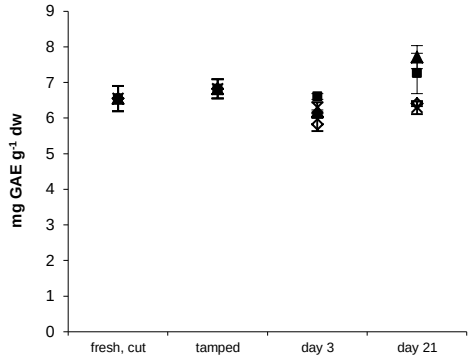


Figure 9.5 total phenolic content of leek samples of the spontaneous (×); plantarum (◊) ; mix (▲); and sakei (■) fermentations, n=3

9.4.1.2.3 *S*-Alk(en)yl-L-cysteine sulfoxides

The evolution of isoalliin and methiin concentrations during fermentation is shown in Figure 9.6 (a) and 9.6 (b), respectively. The isoalliin content of leek reduced significantly with 34% after the tamping process. The subsequent 3 day-fermentation process resulted in an additional significant decrease of the isoalliin content for all fermentations. However, this decrease was more pronounced for the spontaneous and plantarum fermented leek samples (51% and 58%, respectively), while that of the mixed and sakei fermented leek samples only scarcely decreased (3% and 11%, respectively). Upon 3 weeks of fermentation, isoalliin was depleted in all fermentations studied.

For all fermentations, the methiin content decreased (47%) significantly after the tamping process. After 3 days of fermentation, methiin levels of the sakei and mixed fermentations dropped again (62% and 70%, respectively) again significantly, while upon further fermentation no significant change of the methiin content was noticed. In the case of spontaneous and plantarum fermentation, the methiin level did not change significantly after 3 days of fermentation, whereas a 3 week-fermentation resulted in a significant decrease of 70% and 73%, respectively, in comparison with the initial methiin content.

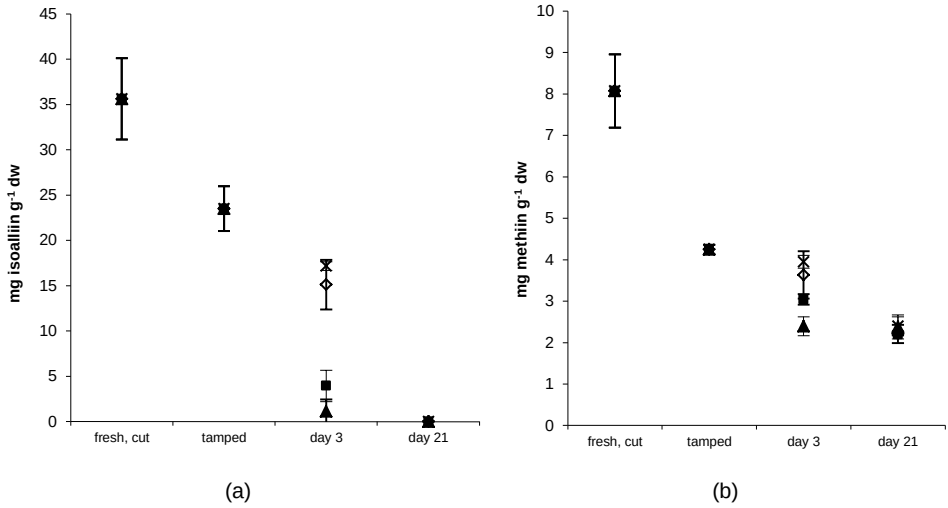


Figure 9.6 Isoalliin (a) and methiin (b) content of leek samples of the spontaneous (×); plantarum (◊) ; mix (▲); and sakei (■) fermentations, n=3

9.4.2 Stabilisation by drying

The application of a second potential alternative value-adding processing and preservation method of leek, namely drying, was also investigated in terms of antioxidant changes (antioxidant capacity, polyphenols and organosulfur compounds). Three drying techniques were examined, that is freeze-drying (FD), air-drying (AD) and Refractance Window Drying (RWD).

9.4.2.1 Antioxidant capacity

9.4.2.1.1 ORAC

Figures 9.7 (a) and (b) show the ORAC values of the comparison between FD and AD leek samples and between FD and RWD leek samples.

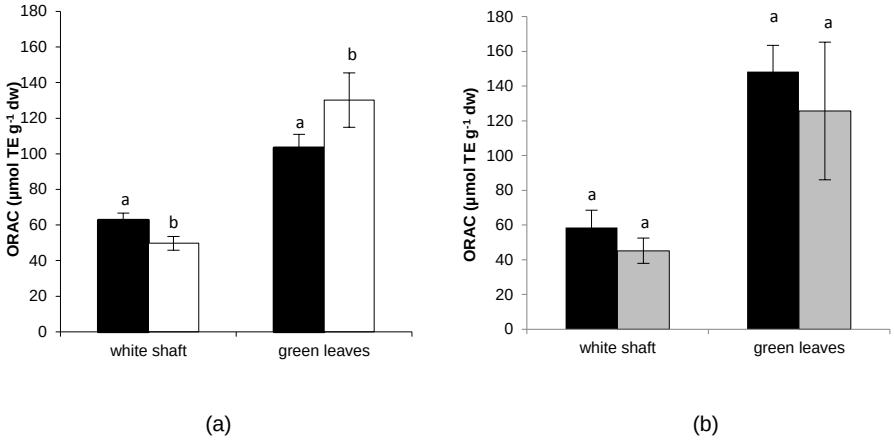


Figure 9.7 Difference in antioxidant capacity (ORAC) between freeze-dried (■), air-dried (□) and refractance window dried (■) white shaft and green leaves; a,b: bars with a different subscript show statistical significance, n=3

The antioxidant capacity of the AD white shaft was significantly weaker than the FD shaft, whereas the green leaves of AD powder possessed a stronger antioxidant capacity. FD and RWD samples possessed comparable ORAC antioxidant capacity.

9.4.2.1.2 DPPH

Figure 9.8 (a) and (b) show the DPPH values of the comparison between FD and AD leek samples and between FD and RWD leek samples.

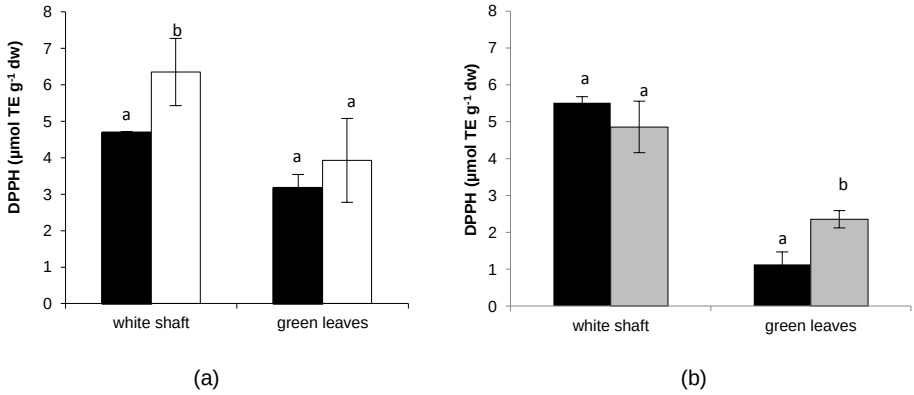


Figure 9.8 Difference in antioxidant capacity (DPPH) between freeze-dried (■), air-dried (□) and refractance window dried (■) white shaft and green leaves**;** a,b: bars with a different subscript show statistical significance, n=3

The AD white shaft had a significantly higher DPPH free radical scavenging activity than FD samples, while this was not the case for the green leaves. There was no difference between FD and RWD white shaft samples in terms of antioxidant capacity, while the RWD green leaves had a higher DPPH value.

9.4.2.2 Polyphenolic compounds

9.4.2.2.1 Total phenolic content

Figure 9.9 shows the total phenol content of the FD, AD and RWD leek samples.

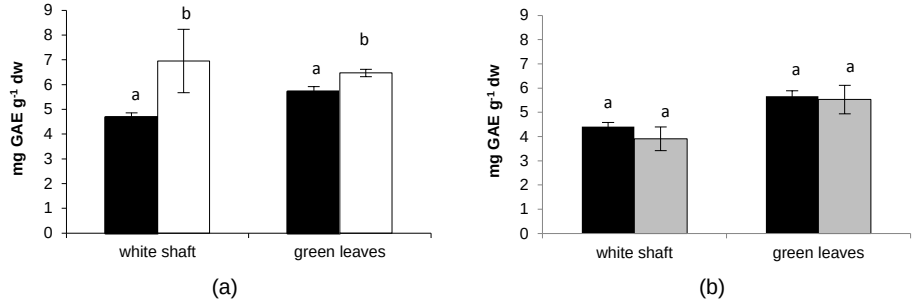


Figure 9.9 Difference in total phenol content between freeze-dried (■), air-dried (□) and refractance window dried (■) white shaft and green leaves; a,b: bars with a different subscript show statistical significance, n=3

The AD samples contained a significantly higher TP content compared to the freeze-dried samples. RWD and FD retained the same amount of total phenolic content for both leek parts.

9.4.2.2.2 Flavonoids and phenolic acids

Figure 9.10 presents the comparison of FD and AD samples in terms of individual polyphenol content. In Chapter 4, we discussed the identification of 13 polyphenolic compounds in 30 leek cultivars using the U-HPLC-ESI-Orbitrap-MS/MS technique (JHI). Eight identified compounds were available as a standard, and could be quantified with the exception of kaempferol 4'-methylether. In this experiment, **freeze-dried** vs. **air-dried**, 5 polyphenol compounds could be quantified in the dried leek samples.

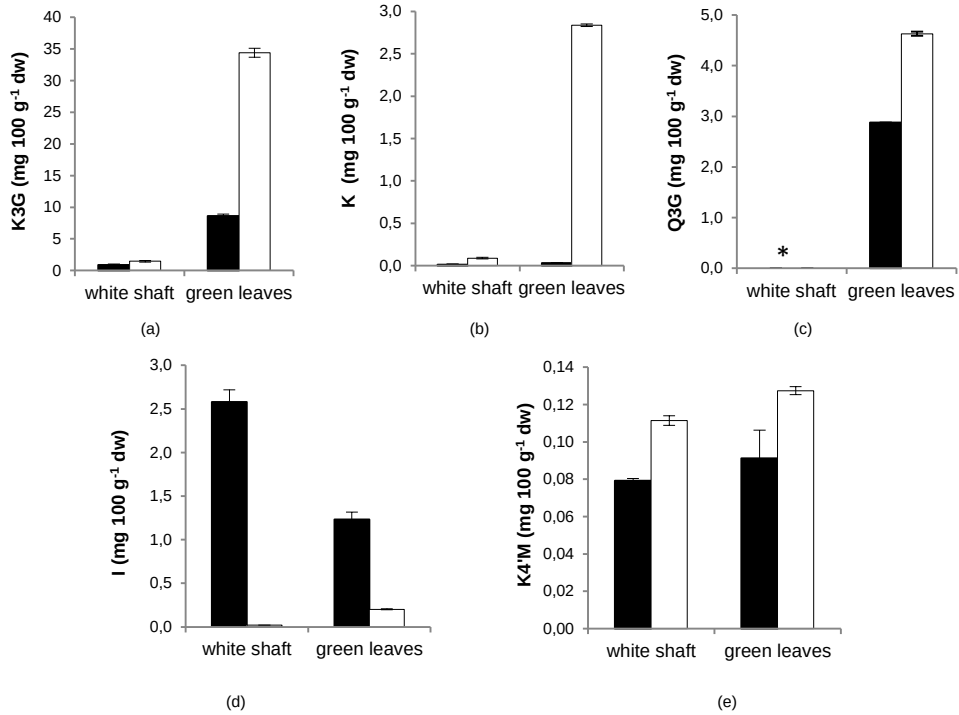


Figure 9.10 Concentration of kaempferol 3-O-glucoside (K3G, a), kaempferol (b), quercetin 3-O-glucoside (Q3G, c), isorhamnetin (I, d) and kaempferol 4'-methylether (K4'M, e) in freeze-dried (■) and air-dried (□) white shaft and green leaves; with *, not detected, n=3

Kaempferol 3-O-glucoside (K3G), kaempferol (K), quercetin 3-O-glucoside (Q3G), isorhamnetin (I) and kaempferol 4'-methylether (K4'M) were present in significant amounts. On the basis of these data, we could observe some remarkable differences between the two drying techniques. The AD leek samples were obviously rich in K3G, K, Q3G and K4'M, while I was more abundant in the FD samples.

Q3G could only be quantified in the dried green leaves. Moreover, the green leaves contained significantly higher amounts of K and K3G, while the white shaft was rich in I. K4'M, on the other hand, was equally distributed in the two leek parts.

The levels of K3G, K and Q3G in the freeze-dried and air-dried leeks are in the same range as the concentration determined in the green leaves of the 30 leek cultivars (§4.3.2.3).

Figure 9.11 shows the results of the polyphenol composition of the **freeze-dried** and **refractance window dried** leek samples.

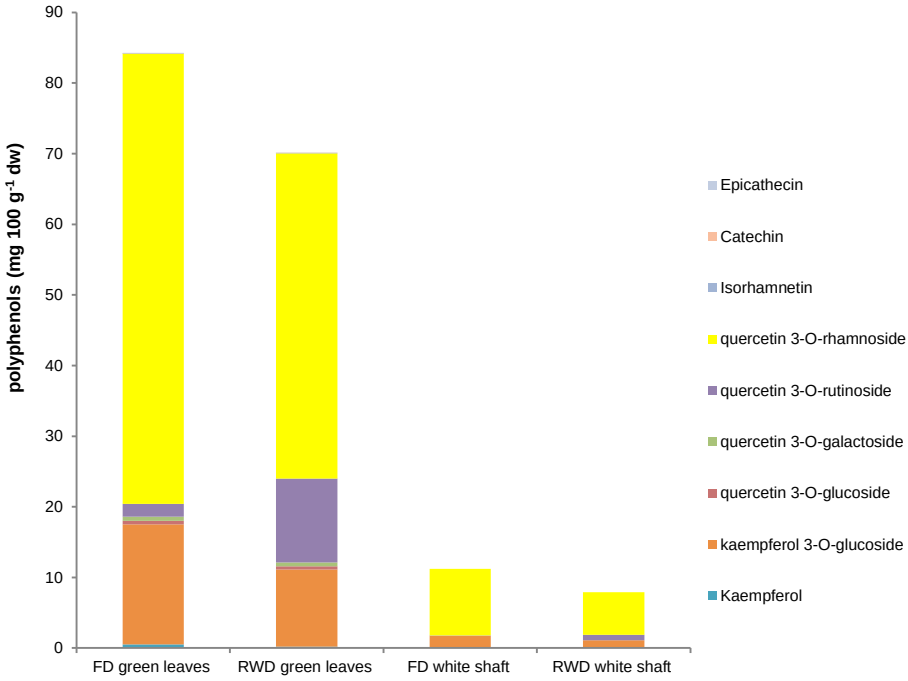


Figure 9.11 Polyphenol profile of freeze-dried and refractance window dried leek samples, n=3

Analyses were accomplished using a U-HPLC-ESI-Orbitrap-MS method, developed at VITO (De Paepe et al., 2013). Using the U-HPLC-ESI-Orbitrap-MS method, 9 compounds could be quantified in the dried green leaves, while only 3 polyphenolic compounds, *i.e.* kaempferol 3-O-glucoside (K3G), quercetin 3-O-rutinoside (Q3R) and quercetin 3-O-rhamnoside (Q3Rh), were present in significant amounts in the white shaft. Q3Rh and K3G were the two most abundant compounds in the dried leek samples. Q3Rh, K3G and K were more present in the FD green leaves, while RWD green leaves were rich in Q3R, Q3G; Q3Ga and I were present in the same amount.

The FD white shaft contained higher amounts of Q3Rh and K3G, while Q3R was higher in the RWD white shaft. Epicatechin and catechin, 2 flavanols, were found in the dried green leaves samples in similar amounts

Based on the quantification of these individual polyphenols, freeze-drying retained more polyphenols compared with refractance window drying.

Comparing Figure 9.10 with 9.11, 4 mutual compounds were quantified, *i.e.* K3G, K, Q3G and I. Air-drying resulted in the highest levels of K3G, K and Q3G compared to FD, while FD gave better results for these compounds than RWD. Freeze-drying revealed a higher I content compared to AD, while FD and RWD samples gave similar amounts of I.

9.4.2.3 S-Alk(en)yl-L-cysteine sulfoxides

Figure 9.12 shows the results of the isoalliin and methiin content of the FD, AD and RWD leek samples.

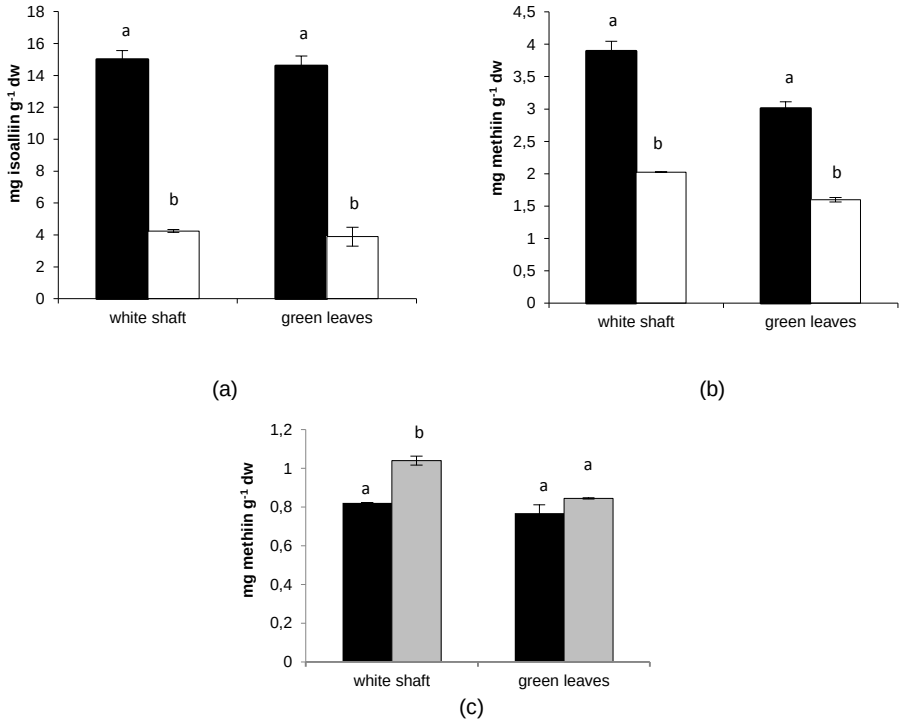


Figure 9.12 Difference in isoalliin (a) and methiin (b,c) content between freeze-dried (■), air-dried (□) and refractance window dried (▫) white shaft and green leaves, n=3

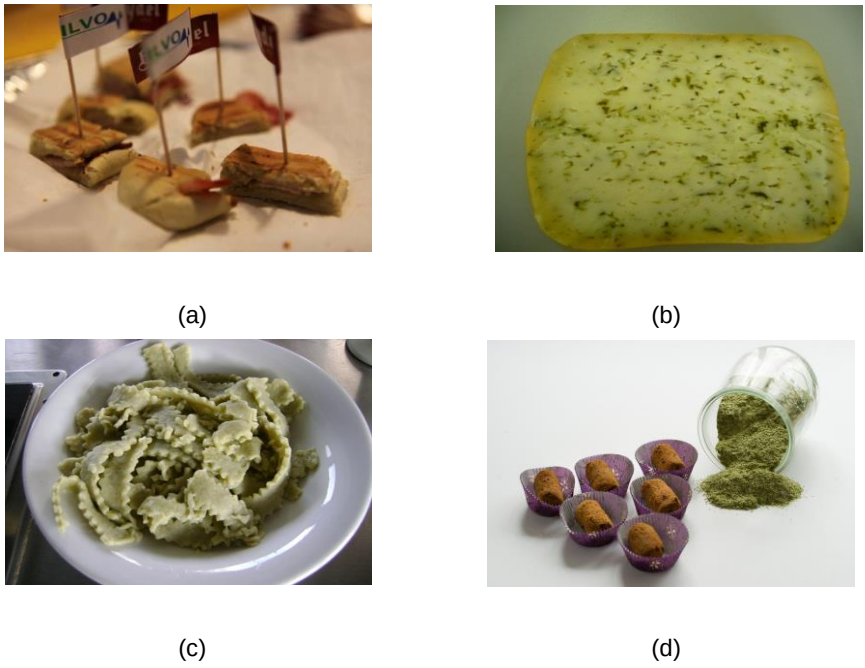
AD leek samples contained remarkably lower amounts of isoalliin and methiin compared to FD samples. Isoalliin could not be quantified in the FD vs. RWD samples, probably because of their loss during transport or because of their loss during processing. The methiin content in the RWD samples, on the other hand, was significantly higher than the content in FD leek samples.

9.4.3 Innovative leek products

As stated in the introduction (§9.1), a large part of the green leaves remains unused, despite its interesting nutritional profile. A first fraction is left behind on the field during harvesting and a second fraction is removed during processing because of specific requirements (Figure 9.13). Groentenhof (Bornem, Belgium), growing 50 ha of leek, obtained a waste of green leaves ranging from 400 to 600 ton a year. This amount of waste corresponds with 8 to 12 ton per hectare, resulting in 38 400 to 57 600 ton in Belgium in 2012 (4800 ha). This biomass is usually brought back to the field, however, this large quantity of plant biomass could be better valorised in food and feed given an adequate stabilisation method.

Figure 9.13 Losses of green leaves upon harvest and processing of leek during the preparation for the fresh market (Fracha, Meulebeke)

Dried leek powder (investigated in §9.3.2) can create opportunities for applications in different food products, as it is a source of a range of bioactive compounds. Therefore, the development of different food products, fortified with different concentrations of leek powder (green leaves) made by applying different drying methods (FD and AD), were preliminary investigated in collaboration with HoGent, Beverse Kaasmakerij, KaHoSint-Lieven. Leek bread, cheese, pasta and croquettes are promising products (Figure 9.14), though the commercial implementation is a complex process depending on several parameters that should be considered.



(a) (b)

(c) (d)

Figure 9.14 Innovative products based on dried leek powder, (a) leek bread, (b) leek cheese, (c) leek pasta and (d) leek croquettes

9.5 Discussion

In the current chapter, we present an investigation of the application of 2 alternative value-added processing and preservation methods including fermentation and drying. Fermentation is a preservation technique used throughout the ages, which includes the conversion of sugars to lactic acid by the presence of lactic acid bacteria. Besides inhibiting growth of non-desirable bacteria, lactic acid fermentation also influences the sensory quality (colour, flavour, aroma and texture) and nutritional properties of the

fermented vegetables (Demir et al., 2006; Kusznierewicz et al., 2008; van Boekel et al., 2010).

Both spontaneous and starter culture induced fermentation were evaluated in this study.

The two antioxidant assays (ORAC and DPPH) revealed an increase in antioxidant capacity upon **spontaneous fermentation** of both leek parts compared to the tamped leek samples, while the increase was not significant compared to the fresh samples (except ORAC results for green leaves and DPPH results of the white shaft). The obtained ORAC results are in accordance with the study of Martinez-Villaluenga et al. (2012), who revealed an increase of the ORAC value in white cabbage upon fermentation (up to 2-fold). Our increase in DPPH partly agrees with Kusznierewicz et al. (2008), who reported an increase of the initial DPPH free radical scavenging activity of white cabbage upon a 2-week fermentation process. Similarly, Pyo et al. (2005) observed an increase in DPPH radical scavenging in soybeans fermented by LAB, essentially due to the presence of compounds, such as mineral salts and polyphenols. However, Nazzaro et al. (2008) observed an increase in the DPPH radical scavenging ability of carrot juice after 2 days of fermentation with *Lb. delbrueckii* subsp*. bulgaricus*, while it decreased after 4 weeks of fermentation.

To account for the increase in antioxidant capacity, the results from the study of Kusznierewicz et al. (2008) suggests that some compounds with antioxidant properties are released during cabbage fermentation. More in detail, during fermentation of white cabbage, glucosinolates undergo complete hydrolysis to form an array of health-promoting products such as ascorbigen, indol-3-carbinol, sulforaphane, allyl thiocyanate, butyl isothiocyanate and phenyethyl isothyocyanate (Tolonen et al., 2002). In addition to the formation of new compounds with antioxidant capacity, the increased antioxidant capacity measured in the green leek leaves in this study can also be attributed to the chemical structure of polyphenols present as the formation of free hydroxyl group(s) by linkage cleavages of flavonoids has been reported to result in a higher antioxidant capacity (Harbaum et al., 2008).

An increase in total phenolic content of the green leek leaves was observed after 21 days of fermentation compared to tamped leek samples. Similarly, Dordevic et al. (2010), reported a significantly higher total phenol content of buckwheat, barley and rice after fermentation. A higher value in TP content can be attributed to the release of bound sugar moieties as reducing sugars have been reported to interfere with the determination of polyphenols through the Folin-Ciocalteu assay (Prior et al., 2005; Harbaum et al., 2008).

In addition to the total phenolic assay, a U-HPLC-ESI-Orbitrap-MS analysis was performed to quantify the individual polyphenols in leek before fermentation, after tamping and after 21 days of fermentation. The increase in individual polyphenol

concentrations upon fermentation was partly in accordance with other studies. Fermentation of sorghum resulted in an increase of naringenin and taxifolin as well (Svensson et al., 2010). The increase of naringenin can be the result of the deconjugation of naringenin-7-O-glucoside as observed in the study of Svensson et al. (2010). The increase of the ferulic acid (in fact *trans*-ferulic acid) content after fermentation was in accordance with the findings of Duenas et al. (2005), who noticed a remarkable increase in *trans*-ferulic acid content upon fermentation of cowpeas. Ferulic acid and hydroferulic acid were also substantially higher in grass silage inoculated with LAB strains than in control silos without added LAB, indicating that LAB facilitate the production of these two phenolic acids (Broberg et al., 2007). Although, some LAB are capable of metabolizing ferulic acid, such as *Lb. plantarum* and *Lb. fermentum*, while neither *Lb. casei* and *Lb. reuteri* degraded ferulic acid (Svensson et al., 2010). Ferulic acid is also the most abundant phenolic acid found in cereal grains (80-200 mg 100 g^{-1} dw) (Lempereur et al., 1997). Ferulic acid in natural resources has some interesting properties, as it can be transformed by microorganisms to biovanillin, a bioflavour (Zamzuri and Abd-Aziz, 2012). Production is already accomplished from ferulic acid of wheat bran, sugar beet pulp and rice bran oil (Walton et al., 2000; Mathew and Abraham, 2008).

The decrease in the content of caffeic acid, as observed in both white shaft and green leaves, can be the result of the decarboxylation to vinyl catechol or the reduction of dihydrocaffeic acid (Svensson et al., 2010). In contrast with our results, Ariffin et al. (2011), reported a decrease in the kaempferol content upon fermentation of *Centella asiatica* herbal teas. More specifically, tamped leek samples exhibited a lower level of kaempferol compared with the fresh samples, while the kaempferol content increased again upon leek fermentation.

Some of our findings contradict similar reports for other plant species, although these plant species are different in metabolite composition, enzymes etc. Some studies revealed a decrease in the quercetin 3-O-rutinoside, luteolin and quercetin content upon fermentation of *Centella asiatica* herbal teas (Martin and Matar, 2005; Ariffin et al., 2011) which was not the case for these compounds in leek. Furthermore, Duenas et al. (2005) reported a decrease in the quercetin 3-O-galactoside and quercetin 3-O-glucoside content upon fermentation of cowpeas. Harbaum et al. (2008) concluded from their results that in addition to a degradation of flavonoids in pak choi and Chinese leaf mustard by fermentation, the flavonoid contents also changed by the cleavage of sugar moieties. During the fermentation process of these *Brassica* vegetables, more flavonoid derivatives with a lower molecular mass (di- and triglucosides) and aglycones of flavonoids and hydroxycinnamic acids are detected compared to non-fermented vegetables (tri- and tetraglycosides of flavonoids and hydroxycinnamic acid derivatives)

(Harbaum et al., 2008). This evolution could not be seen for our results, but the increase of kaempferol 3-O-glucoside, the aglycones kaempferol and quercetin, and (hydro)ferulic acid after fermentation can indicate such an evolution. Similarly, during fermentation of onion, quercetin 3,4'-O-diglucoside and quercetin 4'-O-glucoside were converted into quercetin 3-O-glucoside and quercetin, respectively (Yang et al., 2012). Bisakowski et al. (2007) also noticed a substantial increase in the proportion of quercetin monoglucoside upon fermentation of onion. Moreover, fermentation of *Hamamelis virginiana* leaf extracts resulted in a conversion of flavonol glycosides into their aglycones quercetin and kaempferol (Duckstein et al., 2012). These qualitative changes in polyphenols during fermentation could indicate that LAB are capable of producing β-glucosidase, which catalyses the cleavage of sugar linkages during fermentation (Tsangalis et al., 2002). Moreover, as several forms of glycosides conjugated to quercetin in onion have showed lower antioxidant capacity than that of the quercetin aglycone (Manach et al., 1998), the conversion of quercetin glucoside into quercetin by fermentation is a promising strategy to enhance the bioavailability and bioactivity of onion (Yang et al., 2012).

In contrast with fermentation, tamping was responsible for great losses in polyphenols. Harbaum et al. (2008) also observed losses of polyphenols in the kneading step of cabbage before fermentation. These losses are mainly due to leaching from cut or bruised tissue surfaces and enzymatic reactions as upon cellular fragmentation, enzymes are free to react with polyphenols leading to significant losses.

The losses in isoalliin and methiin upon leek fermentation, can be explained by damaging of the leek tissues during tamping, which results in a cleaving of the ACSOs by the endogenous enzymes alliinase and lachrymatory factor synthase (Lancaster and Kelly, 1983). Upon tissue damage, the first chemical compounds that are formed are sulfenic acids and thiosulfinates, which are intermediates in the formation of the majority of sulfur volatiles, such as dipropyl disulfide and 1-propanethiol (Rose et al., 2005). Next to the decomposition of the ACSOs, leaching could also be the main mechanism causing the losses. Microbial degradation of ACSOs by the LAB has not been investigated, although Beato et al. (2012) deduced from their data that losses of ACSOs in garlic due to microbial action are negligible.

In addition to the analysed components, fermentation is reported to be responsible for a decrease in the antioxidant vitamin C. The study of Martinez-Villaluenga et al. (2009) revealed that cabbage fermentation leads to a decrease in the ascorbic acid content of 34-48%, resulting in a product with decreased antioxidant properties. Moreover, reduction in vitamin C levels was found after the fermentation of carrots, green beans and marrows as well (Di Cagno et al., 2008).

Relating to the **starter culture induced fermentation** experiment, the influence of fermentation on the antioxidant capacity of the leek particles differed according to the applied test, ORAC analyses revealed a positive influence of fermentation, especially natural fermentation, on the antioxidant capacity, whereas DPPH analyses, except for the sakei fermentation, showed a rather negative influence of fermentation on the antioxidant capacity. In general, leek fermented with *Lb. plantarum* IMDO 788 resulted in the lowest antioxidant capacity, for both antioxidant capacity assays, while natural fermented and sakei fermented leek pointed out to have highest ORAC and DPPH value, respectively. Accordingly, Chen et al. (2009) showed that the antioxidant capacity is influenced by the starter culture used. Our findings are partly consistent with other studies in which the influence of fermentation on the antioxidant capacity was determined (Nazzaro et al. 2008; Kusznierewicz et al. 2008; Martinez-Villaluenga et al. 2012). ACSO levels decreased significantly during starter culture induced fermentation, regardless the use of starter cultures, which was also observed upon spontaneous fermentation.

Despite its nutritional value, the introduction of fermented leek as a new food product onto the market will require its acceptance by the consumer, a key factor for its potential marketing success (Sivakumar et al., 2010). In the study of Wouters et al. (2013) the acceptability of fermented leek was evaluated through consumer tasting sessions. Therefore, different preparations of fermented leek samples were judged for appearance, odour, taste, texture and overall appreciation. The sensory analysis of this study revealed that fermented white leek samples are generally more appreciated than fermented green leek samples, independent the preparation method. Fermented green leaves were less pleasant because of toughness and dryness. Nevertheless, acceptable end-products were obtained through the fermentation of white and green leek parts together, which masked the odour and flavour intensity of the fermented green parts. In addition, the application of a mixed starter culture of *Lb. Plantarum* and *L. mesenteroides* delivered end-products of a good flavour and quality, at the same time improving the controllability of the fermentation process. However, more work is needed to introduce fermented leek as a new product on the market, which might be achieved through the addition of herbs and/or other flavour ingredients as well as by the use of other starter culture mixtures.

In addition, the application of a second alternative value-adding processing and preservation method, that is **drying,** was investigated. Drying brings a substantial reduction in weight and volume, which minimises packaging, storage and transportation costs (Sobukola et al., 2007). Moreover, products with low moisture content can be stored at ambient temperature for longer periods of time due to a considerable decrease

in the water activity of the material, reduced microbiological activity and minimised physical and chemical changes (Ozgur et al., 2011). However, food products are sensitive to drying temperature, which can induce degradation (*e.g.* oxidation, loss of colour, shrinkage or loss in texture) and nutritional/functional properties (Attanasio et al., 2004). Three drying techniques, including freeze-drying (FD), air-drying (AD) and Refractance Window Drying (RWD) were compared in terms of antioxidant changes.

We could conclude that leek samples, subjected to the three drying methods, retain almost the same antioxidant capacity, with the exception of the higher ORAC value of AD green leaves, FD white shaft and the higher DPPH value of the AD white shaft. The good results of the AD leek samples are in accordance with the results of Katsube et al., 2009). They found that the radical scavenging activity and levels of polyphenolic compounds in air-dried mulberry leaves (60 °C or below) were not different from those of freeze-dried leaves, whereas the activity in mulberry leaves air-dried at 70 °C decreased significantly. Mejia-Meza et al. (2010) also reported a comparable antioxidant capacity in air-dried and freeze-dried raspberries. Some studies show better antioxidant properties of air-dried samples as well. For example, air-dried *Rabdosia serra* samples exhibited a higher ORAC antioxidant capacity than freeze-dried samples (Lin et al., 2012). Air-dried *Lamiaceae* herbs had a higher antioxidant capacity than freeze-dried samples. Similarly, the air-dried *Rabdosia serra* samples exhibited a higher DPPH antioxidant capacity than freeze-dried samples (Lin et al., 2012).

The similar results between FD and RWD leek samples are consistent with the comparable antioxidant activities of FD and RWD asparagus and potatoes (Abonyi et al., 2002). Moreover, Kaspar et al. (2012) determined the total antioxidant capacity of FD and RWD potato cultivars and did not observe a difference between the two drying methods, but the antioxidant capacity of the RWD dried material of the white cultivar was significantly lower than the FD samples.

The higher level of total phenolic content in AD leek is in accordance with the results of Mejia-Meza et al. (2010). They reported a higher TP content in air-dried raspberries compared to FD samples. The high retention of polyphenols by air-drying was also verified by Hossain et al. (2010). However, the higher TP levels in air-dried leek, are in contrast with Katsube et al. (2009), who found that air-drying at 60 °C resulted in significantly greater loss of polyphenol compounds in mulberry leaves than freeze-drying. Additionally, Asami et al. (2003) noted that freeze-drying preserved higher levels of TPs in berries in comparison with air-drying. Similar to our results, Kaspar et al. (2012) found equal amounts of total phenolic content present in FD and RWD potatoes. The same study demonstrated that FD retained as much anthocyanins as RWD. Nevertheless, a significant higher total phenol content was observed in RWD potato flakes compared to FD flakes (Nayak et al., 2011).

In our experiment, 5 polyphenol compounds could be quantified in the FD vs. AD dried leek samples. The AD leek samples were obviously rich in K3G, K, Q3G and K4'M, while I was more abundant in the FD samples.

Nine individual polyphenols could be identified in the RWD vs. FD samples (this is in contrast with the fermented samples, where 14 individual polyphenols were quantified in the green leaves and 10 in the white shaft). Q3Rh, K3G and K were more present in the FD green leaves, while RWD green leaves were rich in Q3R, Q3G; Q3Ga and I were present in the same amount. Based on the quantification of these individual polyphenols, FD retained more polyphenols compared with RWD.

In general, there is a tendency for glycosides to be more sensitive to dehydration compared to aglycones suggesting that deglycosylated polyphenols may have higher thermal resistance (Mejia-Meza et al., 2010). As air-drying operates at higher temperatures compared to freeze-drying, we expect higher amounts of glycosylated polyphenols in freeze-dried samples, which was not the case for our results.

The observed higher polyphenol levels in AD leek samples are in accordance with the lower amounts of polyphenols including rosmarinic acid, salicylic acid, rutin and pedalitin in FD *Rabdosia serra* leaves compared with AD leaves (Lin et al., 2012). However, our results generally contrast with other studies. A higher polyphenol level was quantified in FD raspberries, kale leaves, mulberry leaves and purple willow leaves compared with the AD plant material (Julkunen-Tiitto and Sorsa, 2001; Asami et al., 2003; Katsube et al., 2009; Mejia-Meza et al., 2010; Korus, 2011).

Air-drying at temperatures higher than 60 °C is regarded unfavourable due to the possibility of inducing oxidative condensation or decomposition of thermolabile compounds. Freeze-drying, on the other hand, is reported to lead to higher extraction efficiency of polyphenols because freeze-drying can lead to the development of ice crystals within the plant matrix. Ice crystals can result in a greater rupturing of plant cell structure, which may allow for better solvent access and extraction (Keinanen and JulkunenTiitto, 1996). However, it has also been reported that freeze-drying showed a less pronounced damaging effect on the tissue structure than other drying methods (Yousif et al., 1999). Intact tissue structure might be very useful for a good appearance but, in relation to the antioxidant capacity of the extract, it acts as a barrier for the release of phenolic compounds in the extracts and results in lower antioxidant capacity, which can clarify our results. With air-drying there is little or no cell rupture and there is the added effect of heat, which can cause losses in polyphenols (Asami et al., 2003). In the study of Caparino et al. (2012), the microstructure of RWD mango powder was smooth, and flaky with uniform thickness as, during RWD drying, the thinly spread puree on the surface of the plastic film conveyor is undisturbed, except for the removal of moisture. The two sides of a single particle are smooth indicating more flow-ability and less

susceptibility to oxidation because of lesser surface area. FD mango powder showed a skeletal-like structure and was more porous than RWD powder. This is because the ice in the material during freeze-drying helps prevent shrinkage and collapse of the structure and shape resulting in an insignificant change in volume (Ratti, 2001).

Based on the sum of the quantified individual polyphenols, air-drying pointed out to be the best technique in retaining polyphenols, followed by freeze-drying and refractance window drying. Similarly, Topuz et al. (2011) compared the influence of refractance window drying, freeze drying, oven drying and natural convective drying on carotenoids of pepper. The natural convective dried samples retained the highest amount of carotenoids. The losses of carotenes in carrots under freeze-drying (5.4%) and RW drying (9.9%) were low and of comparable magnitude, although freeze-drying consistently showed the highest retention (Abonyi et al., 2002).
The lower ACSO levels in AD leek samples can be explained by the high temperature (70 °C) of the air-flow applied during air-drying in this study. Due to this, ACSOs will convert into volatile compounds, as discussed earlier (Chapter 8). The short drying period when applying RWD could be the reason for the higher retention of methiin compared to FD.
Despite the studies in literature which prove the poor retention of antioxidants in air-dried plant material, our results revealed the antioxidative quality of air-dried leek.
Moreover, this dried leek powder can create opportunities for applications in food/feed applications, as it is a source of a range of bioactive compounds. Similarly, studies were accomplished to investigate the effect on the antioxidant properties and sensory value of bread upon adding ground onion skin (OS). The antioxidant potential of bread with 2-3% OS was significantly higher than the activity noted in the control (Gawlik-Dziki et al., 2013).

9.6 Conclusion

Despite the interesting antioxiant profile of the green leaves of leek, a large part remains unused. This large quantity of plant biomass could be valorised given the availability of an adequate stabilisation method. The application of 2 alternative value-adding processing and preservation methods (fermentation and drying) were investigated and described above. Our results demonstrate that application of natural fermentation of leek results in a higher antioxidant capacity and polyphenol content especially in the green leaves. These results indicate the nutritional relevance of fermentation, which can be a promising stabilisation technique for leek. Fermentation does not require extensive materials and is an inexpensive way to stabilise and preserve plant biomass such as

leek. In addition to fermentation, a second stabilisation route was investigated, namely drying. Many papers describe the poor quality of conventional dried products, compared to freeze-dried samples. Our results, however, are in contradiction with these studies, and suggest that this statement should not be generalised. Derived from the results of the antioxidant capacity assays, we can conclude that the leek samples analysed in this study, subjected to 3 drying methods, retained the same antioxidant capacity, with the exception of the higher ORAC value of air-dried green leaves and the higher DPPH value of the air-dried white shaft. Similarly, air-drying resulted in the highest total phenolic content compared with freeze-drying, while freeze-dried and refractance window dried samples exhibited equal amount of polyphenols. The analysis of individual polyphenols revealed again that, air-dried samples contained higher quantities of polyphenols than freeze-dried leek, while freeze-dried leek exhibited higher levels of polyphenols compared to refractance window dried samples. Although air-drying was the best drying technique in terms of retaining the antioxidant capacity and polyphenols, air-drying resulted in high losses of the ACSOs compared to freeze-drying. In fact, refractance window drying was the best drying technique to retain methiin. In conclusion, when applying these methods under the described conditions, air-drying was evaluated to be good and inexpensive technique for the retention of antioxidants, such as polyphenols, but it was poor for retaining ACSOs. Freeze-drying and refractance window drying were comparable in retaining the antioxidants present in leek.

In addition, this dried leek powder can create opportunities for applications in food/feed applications, as it is a source of a range of bioactive compounds but merits a more profound investigation.

CHAPTER 10. GENERAL DISCUSSION

10.1 General discussion

Leek (*Allium ampeloprasum* var. *porrum*) is one of the most important vegetables cultivated outdoors in Belgium, where it is cultivated on 4800 ha (Eurostat, 2012). It is grown for its cylindrical pseudo stem, which is blanched white from growing underground and is made up of long leaf bases. The main area of leek production in Belgium is situated in the region of West Flanders, which houses more than 75% of the leek production. Around 80% of harvested leek is sold for direct consumption, while the remainder is used in the agri-food processing industry. In 2010, 3.10 kg leek was purchased per capita in Belgium, which was higher than the purchase of courgette (1.78 kg), cauliflower (1.92 kg) and sweet pepper (2.29 kg) (VLAM, 2011). The white shaft is used in many culinary preparations, whereas the green leaves are considered inferior and are, therefore, usually only used in soups or discarded during harvesting and processing.

Relating to health aspects, epidemiologic studies elucidated the reduction of the risk of colorectal, stomach, gastric and breast cancer upon the consumption of leek (Bianchini and Vainio, 2001; Zhou et al., 2011). Moreover, a population case-control study (238 case subjects and 471 control subjects) conducted in Shanghai, revealed a significantly lower risk of prostate cancer upon a consumption of leek of more than 10 g day^{-1} compared to a consumption of less than 2.2 g day^{-1} (Bianchini and Vainio, 2001; Hsing et al., 2002). These health benefits are attributed to a range of bioactive compounds present in *Allium* species, *i.e.* (1) sulfur-containing compounds, (2) polyphenols, (3) vitamins and (4) fructans (Havey et al., 2004).

Although leek is a popular vegetable in many European countries, the limited scientific knowledge concerning its health-promoting compounds is in sharp contrast with the well-documented health aspects of its related *Allium* species, *i.e*. onion, shallot, garlic. The objective of this PhD thesis was to develop knowledge on the presence of health-promoting compounds in leek in order to complement the many reports focusing on these compounds in related *Allium* species. On the basis of novel scientific knowledge on health-promoting compounds in leek obtained in the present study, the dissertation aims to stimulate innovation in leek breeding, production, marketing and the consumption pattern of leek.

To fulfil the objectives, the most important bioactive compounds present in *Allium* species, *i.e. S*-alk(en)yl-L-cysteine sulfoxides (ACSOs), polyphenols, vitamin C (ascorbate, AA) and fructans were analysed in leek as a function of different parameters. The specific research questions connected to this PhD research project were: **(1)** Which bioactive compounds are present in the white shaft and green leaves of leek? **(2)** Is there a difference in bioactive compound concentration among the range of current,

commercial and old leek cultivars? **(3)** Is there a difference in leek type (summer, autumn and winter types) with regard to its antioxidant properties, when harvested in their respective harvest season? **(4)** Does harvest time have an influence on the antioxidant concentration? **(5)** Where is leek, based on its content of health-promoting compounds, situated in the *Allium* genus? **(6)** Can we see a change in antioxidants upon post-harvest processing at the farm and subsequent refrigerated storage? **(7)** What is the amount of remaining antioxidants after domestic cooking processes? **(8)** How can we stabilise and valorise the amount of leek by-products generated during the currently used harvesting and processing methods? And finally, **(9)** what is the influence of these stabilisation processes on the content of antioxidants?
These questions were answered in the present study.

(1) Leek comprises a compact white shaft, growing underground and green leaves, which are more exposed to biotic and abiotic stress factors. The **white shaft** of the analysed leek cultivars was rich in ACSOs and fructans, while the **green leaves** possessed higher antioxidant capacities and higher amounts of AA and polyphenols. It is reported that polyphenol synthesis is stimulated by light, and as a result polyphenols will accumulate in the outer and aerial plant tissue, in case of leek the green leaves (Cortell and Kennedy, 2006). The higher AA levels in the sunexposed plant tissue is because AA is essential for protection against harmful side-effects of light during photosynthesis (Hancock and Viola, 2005). The lower ACSO content in the green leaves can be explained by the fact that sulfur is taken up from the soil by the roots as sulfate and therefore mature upper leek leaves will contain lower levels of ACSOs (Doran et al., 2007). In addition, fructans are a carbohydrate reserve in stems and underground organs and will be more prominent in the white shaft of leek.

(2) Statistically significant differences were observed among the 30 leek cultivars in terms of antioxidant capacity, total phenolic content, AA, ACSO and fructan content. Our results show that cultivars Uyterhoeven, Pretan and Fahrenheit F1 gave the highest oxygen radical absorbance capacity (ORAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and ferric reducing antioxidant potential (FRAP) value, respectively, while cultivars Toledo and Breugel F1 had the highest polyphenol levels. Fahrenheit F1 contained the highest AA levels, while Apollo F1 and Artico were especially rich in ACSOs. Zeus F1 was the cultivar with the highest fructan content. Based on these results, it is difficult to recommend a specific cultivar to leek growers, because it depends on the antioxidant compounds preferred. For example, some cultivars will have high kaempferol 3-O-glucoside levels, but a low fructan content. Therefore, the data generated from the present study can offer information on which

cultivar has high levels of specific antioxidants. Cultivars with complementary antioxidant profiles can serve as parent cultivars for crosses, resulting in a mapping population.

(3) Concerning leek type, the white shaft of the **summer cultivars** had the highest content of fructans and quercetin 3,4'-O-diglucoside, while the white shaft of half of the **autumn cultivars** (Electra, Nebraska, Breugel F1 and Tadorn) was rich in kaempferol and kaempferol 3-O-glucoside. The white shaft of the **winter cultivars** and the other half of the **autumn cultivars** (Poribleu, Alcazar, Belton F1 and Pretan F1) contained the highest amount of ACSOs, AA, total phenolic content and possessed the highest antioxidant capacity. The green leaves of the **winter cultivars** contained the highest amount of ACSOs, fructans, total phenolic content, AA and FRAP, while the green part of half of the **autumn cultivars** (Electra, Breugel F1, Tadorna and Nebraska) was rich in 3 flavonoid aglycones, *i.e.* quercetin, kaempferol and isorhamnetin and were also rich in kaempferol 3-O-glucoside and had a high ORAC value. The distinction between the three types can be explained by their genetic background, but as they were harvested on different moments, stress factors such as temperature, solar radiation, pathogens etc. to which the plants are subjected during their corresponding growth period, may also partly explain the different accumulation patterns of health-related compounds between the summer, autumn and winter leek.

The lower fructan content in the winter leek cultivars can be attributed to the hydrolysis of fructans as also observed in Jerusalem artichoke bulbs during winter seasoning (Grzelak-Blaszczyk et al., 2011).

The higher AA content in the green leaves of the summer and winter cultivars may be due to climatic conditions, including light and average temperature during growth and development of plant tissues, which have a strong influence on the AA content of horticultural crops. Although light is not essential for the synthesis of AA in plants, the amount and intensity of light during the growing season have a definite influence on the amount of AA formed (Lee and Kader, 2000). In general, the lower the light intensity during growth, the lower the AA content of plant tissues (Harris, 1975), which can explain the high values in the summer cultivars. In addition; average growth temperature also influences the composition of plant tissues during growth and development. It is stated that plants will contain more vitamin C when grown under cool temperatures, which can be an explanation for the higher AA levels in winter cultivars (Lee and Kader, 2000).

The observed increase of the ACSO content towards the winter cultivars can be attributed to the role of these sulfur compounds in plants, that is defence against pests and predation, particularly in the overwintering bulb) and carbon, nitrogen, and sulfur storage and transport (Lancaster and Boland, 1990). These stress conditions can result in the conversion of the corresponding γ-glutamyl dipeptides to sulfoxides (Hornickova et

al., 2010). The different accumulation pattern of ACSOs between the summer, autumn and winter leek types can also be explained by the genetic background and other environmental stress factors. Light radiation and water stress is reported to affect the biosynthesis of organosulfur compounds in onion (Freeman and Mossadeg, 1973). In addition, the average growing temperature as well as the root zone temperature (RZT) could strongly affect the flavour composition of onion (Coolong and Randle, 2006). However, a detailed understanding of the influence of environmental factors and their interactions with agricultural practices in relation to ACSOs present in leek is still lacking.

(4) In order to investigate the influence of harvest time on the antioxidant levels of leek in more detail, 9 leek hybrids were harvested on 4 harvest times along the next growing season (2010). In general, we could observe a significant difference between the four harvest times (Sept '10, Nov '10, Jan '11, Mar '11), based on the different antioxidant parameters. More specifically, there was a clear distinction in antioxidant levels between harvest in September and harvest in November, and between harvest in September/November and the other 2 months. Harvest in January or March resulted, for most of the cultivars and both leek parts, in a higher antioxidant capacity and polyphenol levels, while harvest in September had a positive influence on the ACSO content.
In addition, there were dissimilarities between the 9 leek hybrids grown in 2009 and 2010 based on the antioxidants, except for methiin. The cultivars from 2009 showed higher levels compared with those of 2010. These differences in antioxidant levels could be attributed to the different meteorological conditions or different soil properties (mineral content, pH) and disease treatments, affecting the results obtained during these two growing seasons. The average month temperature in November and December was much lower in year 2010 in comparison with year 2009, which can be a reason for the year differences. The soil analyses indicated that the soil of season 2009 had a higher mineral content (P, K, Mg, Ca, Na) compared to the soil of 2010. A higher mineral content can be responsible for higher antioxidant levels in the plant as some studies report a correlation between minerals and antioxidants. For example, an enhanced K-fertilisation increased the level of phenolic compounds and the corresponding antioxidant capacity in sweet potatoes leaves (Redovnikovic et al., 2012). The difference in N-fertilisation and disease treatment between the two years (no insecticide in 2010 because no thrips appearance) could be another explanation. For example, soaking plants in a fungicide (phosphite) solution prior planting was effective in activating strawberry defence mechanisms, since fruit ascorbic acid and anthocyanin content increased (Moor et al., 2009). Similarly, lower levels of isoalliin were found in the roots and bulbs of white rot (*Sclerotium cepivorum*) resistant onions compared to susceptible cultivars (Hovius and Goldman, 2005).

(5) Because it is relevant to have knowledge on the (dis)similarities between leek and its related *Allium* species with regard to the antioxidant properties, analyses were performed on 6 related *Allium* species, including *A. kurrat*, *A. odorum* (Chinese leek), *A. schoenoprasum (*chives), *A. cepa* (white and red onion), *A. fistulosum* (bunching onion) and *A. ascalonicum* (shallot). Based on the results, the antioxidant properties of the white shaft of leek was closely related to the antioxidant potential of the bulb of *A. kurrat* and *A. cepa (*white onion*)*, while the green leaves of leek resemble the antioxidant profile of *A. schoenoprasum* and *A. fistulosum*. *A. odorum* and *A. cepa* (cv. Red Creole) were the species with largest antioxidant differences compared to leek. These species were higher in methiin and flavonoid content, respectively.
Our analyses proved the bioactive compound value of leek and the intake of antioxidants coming from *Allium* species. In addition to the difference in antioxidant properties of *Allium* species, consumption and way of use of the *Allium* species are different as well. Onion, garlic and chives are universally used spice plants and are extensively used for food flavouring (Augusti, 1990), while leek and bunching onion are more important as vegetables with additional flavouring properties (Fritsch and Keusgen, 2006). Chives are usually served in small amounts and never as the main dish (Stajner et al., 2011). In conclusion, taking serving sizes and consumption habits into account, daily intake of antioxidants coming from leek is of larger importance than that from other *Allium* species.

(6,7) Chapters 4, 5 and 6 have focused on the determination of antioxidants in raw and freshly harvested *Alliums*. However, the evaluation of the impact of post-harvest storage and domestic food processing on the health benefits of vegetables is also of great practical importance.
Our results showed that cooking highly influenced the antioxidant capacity of leek, while storage did not have an impact on the antioxidants. Similarly, the antioxidant capacity and total phenolic content of asparagus spears were also highly influenced by domestic preparation practices (peeling and cooking) but not by storage (Papoulias et al., 2009).
In general, the antioxidant capacity and the total phenolic content in the white shaft and green leaves of the entire and packaged leek was stable during 13 days of refrigerated storage. However, a slight increase in isoalliin level could be observed after a period of cool storage. It is reasonable to argue that the γ-glutamyl peptides were slowly degraded by an increased activity of a transpeptidase during storage to yield the corresponding ACSOs. The difference between the antioxidant properties of the white shaft of the entire leek and the packaged leek was minimal, except for the lower ACSO values in packaged leek.

In contrast with boiling, our study demonstrated an obvious increase in the antioxidant capacity of the green leek leaves after steaming. Roy et al. (2007) assumed that, heating *Alliums* for a longer period may generate or modify some components which are more antiradical than their status in raw vegetables caused by 2 possible mechanisms: (1) formation of brown products and (2) destruction of pro-oxidant substances.
Boiling had a negative effect on total phenolic content in the white shaft and green leaves. Moreover, the losses in the total phenolic content of processed leek upon boiling are generally not attributed to a chemical breakdown of flavonoid conjugates or formation of new compounds, but rather to the leaching of phenolic compounds into the cooking water (Price et al., 1997; Crozier et al., 1997; Hirota et al., 1998; Makris and Rossiter, 2001; Xu and Chang, 2008). Therefore, soups are a good source of polyphenols. Some studies, however, report an increase in polyphenols after a heat treatment on onion and tomatoes (Stewart et al., 2000; Woo et al., 2007). This can be explained by the fact that processing could increase the flavonoid extractability from the matrix in subsequent assays resulting in a higher apparent content.
In contrast with boiling, steaming did not have an influence on the polyphenolic content.
In general, the degradation of polyphenols is a function of the sensitivity of the compounds, processing method, magnitude of heating, pH, the presence of oxygen and other phytochemicals in the medium (Friedman, 1997; Buchner et al., 2006).
It is remarkable that blanching resulted in a slight increase in the ACSO content. But, applying a longer thermal treatment on leek samples had a negative influence on the content of methiin and isoalliin. Methiin was less susceptible to cooking compared to isoalliin. To explain this, it is found that alliinase acts quickly, but differently on the individual ACSOs, such that some of the flavour precursors are more completely degraded than others (Lancaster et al., 1998).
In general, steaming seemed to be responsible for a better retention of the bioactive compounds present in leek. The difference between steaming and boiling can be attributed to the higher contact of the leek particles with water in case of boiling, resulting in leachate of water soluble antioxidants in the boiling water.

(**8,9**) Despite the interesting bioactive compound profile of the green leek leaves, it is often only the white shaft that is used in many culinary applications, whereas the green leaves are considered inferior and are therefore, usually only used as ingredient in soups. A large part of the green biomass of leek is left behind on the fields during harvest (leek for processing industry) or removed during product preparation for the fresh market and processing. This large quantity of leek biomass in Belgium, *i.e.* 38 400 – 57 600 ton year^{-1}, could be valorised given the availability of an adequate stabilisation

method, as there is an increasing demand towards the agri-food sector for recovery, bioconversion and maximal utilisation of valuable constituents from food wastes.

The application of 2 alternative value-adding processing and preservation methods, including lactic acid fermentation and drying, were therefore investigated with regard to the retention of the antioxidant properties. **Fermentation** does not require extensive materials and is an inexpensive way to stabilise and preserve plant material. Fermentation of leek resulted in a higher antioxidant capacity and polyphenol content especially for the green leaves. After 21 days of fermentation, new polyphenolic compounds were found such as hydroferulic acid, quercetin 3-O-rutinoside, quercetin 3-O-arabinoside, naringenin and dihydroquercetin, while sinapinic acid disappeared. The contents of ferulic acid, kaempferol 3-O-glucoside, luteolin and naringenin increased significantly after a leek fermentation process of 21 days compared with the initial concentration, while the caffeic acid content decreased.

The qualitative changes in polyphenols during fermentation could indicate that lactic acid bacteria are capable of producing β-glucosidase, which catalyses the cleavage of sugar linkages during fermentation (Tsangalis et al., 2002). Moreover, as several forms of glycosides conjugated to quercetin in onion have showed lower antioxidant capacity than that of the quercetin aglycone (Manach et al., 1998), the conversion of quercetin glucoside into quercetin by fermentation is a promising strategy to enhance the bioavailability and bioactivity of onion (Yang et al., 2012). In contrast with fermentation, tamping was responsible for great losses in polyphenols. These losses are mainly due to leaching from cut or bruised surfaces and enzymatic reactions, as after harvest or upon cellular fragmentation enzymes are free to react with polyphenols leading to significant losses.

In general, these fermentation results indicate the nutritional relevance of fermentation, which can be a promising stabilisation technique for leek. Furthermore, the introduction of fermented leek as a new food product or ingredient onto the market will require its acceptance by the consumer, a key factor for its potential marketing success (Sivakumar et al., 2010). In the study of Wouters (2013), the acceptability of fermented leek was evaluated through consumer tasting sessions. The sensory analysis of this study revealed that acceptable end-products were obtained through the fermentation of white and green leek parts together, masking the odour and flavour intensity of the fermented green parts.

In addition, a second possible stabilisation method was investigated, namely **drying**. Drying brings a substantial reduction in weight and volume, which minimises packaging, storage and transportation costs (Sobukola et al., 2007). Moreover, products with low moisture content can be stored at ambient temperature for longer periods of time due to a considerable decrease in the water activity of the material, reduced microbiological

activity and minimised physical and chemical changes (Ozgur et al., 2011). However, food products are sensitive to drying temperature, which can induce degradation (*e.g.* oxidation, loss of colour, shrinkage or loss in texture) of nutritional/functional properties (Attanasio et al., 2004). Many papers describe the poor quality of conventional air-dried products, compared to freeze-dried samples. Our results, however, are in contradiction with these studies, and suggest that this statement should not be generalised. Derived from the results of the antioxidant capacity assays, we can conclude that leek samples, subjected to 3 drying methods, retained the same antioxidant capacity, with the exception of the higher ORAC value of air-dried green leaves and the higher DPPH value of the air-dried white shaft. Similarly, air-drying resulted in the highest total phenolic content compared with freeze-drying, while freeze-dried and refractance window dried samples exhibited equal amount of polyphenols. The analysis of individual polyphenols revealed again that, air-dried samples contained higher quantities of polyphenols than freeze-dried leek, demonstrating the thermostability of polyphenols. Freeze-dried leek on his turn exhibited higher levels of polyphenols compared to refractance window dried samples. Although air-drying was the best drying technique in retaining the antioxidant capacity and polyphenols, air-drying resulted in high losses of the ACSOs compared to freeze-drying. In fact, refractance window drying was the best drying technique to retain methiin.

In addition, the present study reported the identification of 13 individual polyphenols in the white shaft and green leaves of leek with the U-HPLC-Orbitrap-MS/MS method (JHI). Six polyphenols, including kaempferol/quercetin 3-O-sophoroside, kaempferol 3-O-gentiobioside, kaempferol 3,7-O-diglucoside, kaempferol 4'-methylether and ferulic acid 4-O-glucoside, had not yet been identified in leek and other *Allium* species. In addition to the 13 polyphenols identified in leek, 5 extra compounds could be identified in the related *Allium* species, including quercetin 7-O-glucoside, quercetin 4'-O-glucoside, isorhamnetin 4'-O-glucoside, quercetin 3,7-O-diglucoside and isorhamnetin 3,4'-O-diglucoside. The U-HPLC-Orbitrap-MS (VITO) additionally identified the polyphenols luteolin, quercetin 3-galactoside, quercetin 3-rutinoside, quercetin 3-arabinoside, quercetin 3-rhamnoside, naringenin, dihydroquercetin, propyl gallate, ferulic acid, hydroferulic acid, caffeic acid, epicatechin and catechin in leek. In summary, 26 polyphenols were identified in leek using both U-HPLC-Orbitrap methods.

10.2 Innovative leek products

There is an obvious increase in demand for the valorisation of the green leek leaves, by companies such as Groentenhof (Bornem, Rudy Croket), Ons dagelijks groen (Meldert, Luc De Neef), Fracha (Meulebeke, Franky Neirynck) and Provalor (Vijfhuizen, Piet Nell). Therefore, dried leek powder can be part of the valorisation process and can create opportunities for applications in different food products. A preliminary investigation of different food products, fortified with different concentrations of dried (AD or FD) leek powder derived from green leaves was accomplished, *e.g.* leek bread, leek cheese. These food products were investigated in collaboration with Hogeschool Gent, Katholieke Hogeschool Sint-Lieven (Gent), Katholieke Hogeschool Zuid West-Vlaanderen (Roeselare), Bakkerij Schepens (Schelderode) and Beverse Kaasmakerij (Bever). Preliminary tests (texture, taste, etc.) were accomplished, but further studies and product development is needed to exploit the full potential of these products. Leek bread, cheese, pasta and croquettes are promising products.

Our explorative research resulted in the collaboration with 'Ons dagelijks groen', an innovative leek company. In October 2012, they started to sell leek soup, leek bread and leek cheese in roadside dispensers to create a direct relation with the consumer and contribute to a sustainable environment. Green leek leaves, dried using the excessive thermal energy available within the company 'Ons Dagelijks groen', are used in the production of leek bread and leek cheese which is marketed in this way.

The exploitation of by-products from vegetable processing for application in food is a promising field which requires interdisciplinary research. Many efforts have been made to valorise by-products of vegetables (Schieber et al., 2001). Similarly, because of the demand for processed onions, an increase was established in waste production (more than 500 000 tonnes annually). Therefore, onion producers and processors, regulatory authorities and consumer groups were all interested in developing alternative means for the valorisation of the onion waste to promote its profitable usage and it subsequent conversion into food grade products (Gonzalez-Saiz et al., 2008). The major by-products resulting from industrial peeling of onion bulbs are the brown skin, the outer 2 fleshy leaves and the top and bottom bulbs. Owing to their strong characteristic aroma and their susceptibility to phytopathogens, onion wastes are not suitable as fodder. However, they are a source of flavour components, fiber compounds and are particularly rich in quercetin glycosides (Schieber et al., 2001; Benitez et al., 2012). In fact, several studies have reported the valorisation of onion by-products as potential source of different valuable food ingredients, including several biological approaches dealing with ethanol, vinegar and lactic acid production from onions by fermentation (Horiuchi et al., 2000;

Roberts and Kidd, 2005). Production of snacks from onion pomace has also been reported (Kee et al., 2000).

This could be achieved for leek production as well. Figure 10.1 presents a possible strategy to valorise the green leek leaves. After a drying and milling step, the obtained powder can be used in many food commodities.

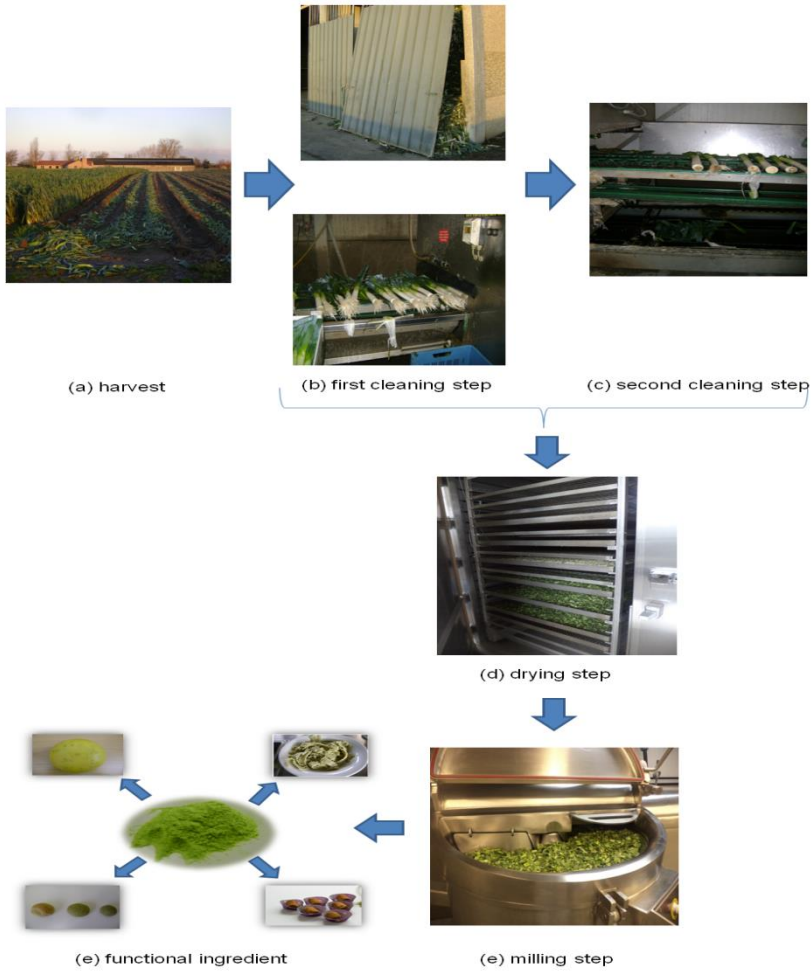


Figure 10.1 Strategy for the valorisation of the green leek leaves

However, in addition to the technical feasibility, the economic and commercial aspects deserve further attention.

To solve the challenges of implementing innovative products on the market, the GeNeSyS (Use of By-Products as System Innovation) project was developed. The GeNeSyS project is the first Coordinated Action within ILVOs research strategy, entitled ILVO2020. The aim of GeNeSyS is to perform trans disciplinary research in an innovative way: bundling the expertise present in various scientific disciplines. This study will include different analyses, including an economic assessment. Freeze-drying for example, demands additional energy consumption, and therefore it will result in a higher operational cost. An additional transportation cost should be accounted as well. Moreover, logistic aspects have to be taken into account. In general, the commercial implementation is a complex approach depending on several parameters that should be considered in future research projects.

Besides a better closure of the cycles of resource use, valorisation of green leek leaves could also contribute in solving some ecological problems. Such biowastes constitute an environmental problem on the field as they contain large quantities of nitrogen and phosphorous and they also have a high water content. This makes them susceptible to rapid modification by micro-organisms, with leachate formation and gas emission, *e.g*. N_2O, a major greenhouse gas and air-pollutant.

10.3 Relation with human health

Interest in the role of bioactive compounds in human health has promoted research in the field of horticulture and food science to evaluate fruit and vegetables antioxidants and to determine how their content and activity can be maintained or even improved through crop breeding, cultural practices and post-harvest storage and processing.

The present study elucidated the antioxidant capacity of 30 leek cultivars and different processed samples. Importantly, the antioxidant capacity cannot be used to predict what effect or benefit an antioxidant substance will have within the human body in attenuating free radical production or coping with oxidative stress. As an *in vitro* test, the antioxidant capacity assays cannot predict such *in vivo* effects. In addition to the antioxidant capacity, specific bioactive compounds were analysed which are attributed to the health benefits of *Allium* species.

The present study reported the identification of 26 individual polyphenols in the white shaft and green leaves of leek. Different health benefits are attributed to these polyphenolic compounds. For example, quercetin, kaempferol and isorhamnetin, 3 main flavonoid aglycones, have been shown to have inflammatory effect on activated macrophages (Hamalainen et al., 2007). In addition, quercetin and kaempferol show

chemopreventive properties in brain tumours and synergistically suppress cell proliferation in human gut cancer lines (Ackland et al., 2005; Labbe et al., 2009). The consumption of 300 g of onion per day was shown to clearly increase the amount of quercetin in plasma (Moon et al., 2000). High intakes of kaempferol resulted in a lower risk of coronary heart disease (Lin et al., 2007).

Ferulic acid, a phenolic acid, which increased during fermentation experiments, acts as a well-known antioxidant, effective in scavenging peroxyl radicals and other active oxygen species like superoxide and hydroxyl radical (Kikuzaki, 2003). It has an inhibitory effect on 4-nitroquinoline 1-oxide induced rat tongue carcinogenesis (Tanaka et al., 1993).

It is known that *Alliums* possess induced antiplatelet activity which is mainly attributed to the organosulfur compounds and flavonoids. Data show that 150 mg and 300 mg quercetin 4'-O-glucoside ingested orally in humans resulted in platelet inhibition 30 min and 120 min after ingestion. Subjects given a diet containing onion slices 3 times a day (260-360 g day^{-1}) for 1 week resulted in an equivalent of 67.6-93.6 mg day^{-1} of quercetin ingestion. It's concentration in the plasma increased from 0.04 ± 0.04 µM before the trial to 0.63 ± 0.72 µM after the trial. These studies suggest that onion quercetin conjugates are accumulated in the plasma and may provoke antiplatelet effects if ingested at a high enough dose (Moon et al. 2000; Hubbard et al. 2004). However, cooking can destroy this antiplatelet activity (Hansen et al. 2012).

Flavonol (subclass of the flavonoids) intakes have been reported to vary widely across countries, with some of the lowest intakes being reported for northern European populations, whereas populations from the United States and other European countries have among the highest reported intakes. In Western populations, estimated daily intake is in the range of 20-50 mg flavonols day^{-1} (Hertog et al., 1994; Knekt et al., 1996; Hertog et al., 1997), which corresponds with approximately 100 g of dried green leek leaves or 666 g fresh green leaves (85% water content), depending on the cultivar. However, a daily consumption of 180 mg of flavonoids expressed as the quercetin equivalent demonstrates a positive health effects (Harwood et al., 2007).

The recommended daily intake for an adult of vitamin C amounts 70 mg, which corresponds with approximately 12.5 g of dried green leaves or 83.3 g fresh green leaves (85% water content), depending on the cultivar.

Fructans stimulate the growth of specific microorganisms in the colon (*e.g.* bifidobacteria, lactobacilli) with generally positive health effects. A minimal dose of 2.5 g of fructans is a condition upon which a bifidogenuous effect takes place (Guigoz et al., 2002). This amount corresponds with 36.54 g dried green leaves or 243.6 g fresh green leaves, depending on the cultivar.

10.4 Originality

Reports on the effect of different parameters (genetics, harvest time and processing) on the antioxidant capacity, polyphenol, vitamin C, ACSO and fructan content of the white shaft and green leaves of leek are scarce. Moreover, this is the first study that revealed the antioxidant properties of a range of commercial and less common leek cultivars, a collection which was mainly realised by the ILVO research group Crop Husbandry and Environment. In addition, this study revealed the presence of polyphenolic compounds, which have never been identified in *Allium* species. To our knowledge this is the first report on the evolution of antioxidants based on leek season, post-harvest storage and processing. Moreover, the initial phase in the valorisation of the leek leaves, *i.e.* stabilisation by fermentation and drying, was executed. This study is original and can result in an added value to the leek breeding community, leek growers and processors and finally to the consumers.

10.5 Recommendations and future perspectives

Recent trends encourage breeders to use health benefits and antioxidant characteristics as a quality parameter of fruits and vegetables in plant breeding programs. Therefore, novel tools are developed to improve future coupling of genetic and metabolomic data. However, despite the fact that leek is an important vegetable crop, it is poorly known in the genetic and molecular aspects as compared to *A. cepa.* Conventional leek breeding could benefit from the use of molecular marker technology, the so-called marker-assisted breeding. At present, there are only a limited number of studies reporting about the development and use of molecular markers in leek breeding (HRI, 2004). For example, Filjushin et al. (2011) reported the development of 24 AFLP markers in leek in order to analyse the leek genome, *i.e*. the choice of restriction endonucleases and primer combinations for revealing polymorphism and genotyping of the accessions. A detailed genetic map of leek is not available within the public domain (Filjushin et al., 2011), but is most likely developed by commercial leek breeding companies. In the future, the knowledge of positions of markers closely linked to the presence of health-promoting compounds on the leek genetic maps, will help in marker assisted breeding towards genotypes with higher content of the phytochemicals of interest. Examples of this approach appear more and more and were *e.g*. demonstrated for apple (Khan, 2012).

The availability of data on antioxidant levels in leek can be considered as an important criterion for selection of genotypes from a gene bank for use in crop improvement or other research-related or commercial activities. This study revealed the variability in antioxidants between a range of 30 leek cultivars. This variability is necessary in

breeding experiments, because it allows to distinguish possible cross parents (cultivars), rich in a specific bioactive compounds or with complementary biochemical profiles.

Similarly, the Nunhems seed company has embarked on the challenging endeavour to identify and manage the host of plant compounds that influence flavour and nutrition (Nunhems, 2012). In addition, the seed company Bejo is also focussing on healthier cultivars, for example the 'scheutjebroccoli' (Figure 10.2). The 'scheutjesbroccoli' contains 10 times more glucoiberin, a glucosinolate, compared to standard broccoli (Bejo, 2012).

Health-promoting compounds can also be linked to the resistance of pests and diseases. Based on the results from the study of Hovius and Goldman (2005), breeders can screen onions for resistance to white rot (*Sclerotium cepivorum* Berk.) by comparing onion root or bulb isoalliin levels. White rot incidence in the field should be higher in those plants whose roots and bulbs have the highest levels of isoalliin.

In general, the present study can recommend leek growers to use specific cultivars, types and practices in order to maximise content of specific health-promoting compounds and antioxidant concentrations more specifically and as such improving existing leek cultivars.

Moreover, the related species analysed in Chapter 6 can be used for interspecific hybridisation in order to widen the genetic variation of leek. More specifically, they can be introduced in leek breeding programmes in order to allow the introgression of desirable traits such as disease resistance, but also taste, flavour and health-promoting compounds (HRI, 2004; Chuda and Adamus, 2009). For example, Q 7-G, Q 4-G and I 4-G were polyphenols which were present in *A. cepa*, *A. fistulosum* and *A. ascalonicum*, but were not detected in *A*. *ampeloprasum* var. *porrum*. In addition, *A. odorum* had significantly higher levels of ascorbate and methiin compared with leek. Moreover, alliin,

an ACSO which was not detected in leek, was present in significant amounts in *A. odorum*.

Additionally, the present study can recommend consumers with regard to the maximum maintenance of antioxidants upon domestic processing. Moreover, the study can stimulate the valorisation of the green leaves of leek or waste streams of other plant material in general. In addition, the increasing demand from leek growers for the development of new possibilities for the limited used green part is a positive trend. 'Ons dagelijks groen' (Meldert, Belgium), is an innovative leek company, who started in October 2012 with a new concept relating to short chain sale. The combination of research on bioactive compounds and the increasing demand for innovation is a good basis for the realisation of leek innovation and diversification.

In the present study, the bioactive compound value of leek was described, but other crops in Flanders can be interesting as well. They can be interesting in terms of their bioactive compound profile, but moreover, they can be of interest relating to their valorisation potential. Leaves of cauliflower (16500 ton dw of waste) and Brussels sprouts stems (6500 tonnes dw of waste) are possible projects (CINBIOS, 2011).

In addition, the fructan analysis should be further optimized as some problems arise running the method.

Furthermore, this research fits in the scope of stimulation of the consumption of vegetables. According to the World Health Organization (WHO) an intake of minimum 400 g of fruit and vegetables per day is recommended (excluding potatoes and other starchy tubers) for the prevention of chronic diseases such as heart disease, cancer, diabetes and obesity, as well as for the prevention and alleviation of several micronutrient deficiencies, especially in less developed countries. In addition to antioxidants, dietary fibres play an important role in the prevention of these diseases. In line with the request by the WHO, a number of programs have been started in various countries all around the globe to encourage the sufficient consumption of fruit and vegetables each day. They are carried out under different names, but most widely known is probably the "5 A Day" campaign (eating at least 5 portions of fruits and vegetables per day). However, the general message is in many countries transformed into other campaign names, such as "2x2" in the Netherlands, "3x3" in Hungary or "6 om dagen" in Denmark and 'all day long' in Belgium (Enjoy Fresh, 2012), all promoting the consumption of fruits and vegetables.

In addition to promotion, diversification in product assortment will be another way to increase vegetable consumption around the world. That's why the seed company Rijk Zwaan has selected a number of varieties that suit the wishes of the modern consumer perfectly: ready-to-eat, bite-size vegetables with a great taste. Examples are ready-to-

eat, honey-sweet plum tomatoes and small, handy-sized and crunchy cocktail cucumbers (Rijk Zwaan, 2012).

10.6 Conclusion

This study focused on 2 aspects. First, the health promoting compounds were evaluated in leek, an important vegetable in Flanders, as a function of genetic diversity, harvest time and processing. Second, this project investigated potential valorisation routes for the large amount of leek by-products, generated during harvesting and processing.

Leek tissue, type of cultivar and harvest time had a clearly impact on the antioxidant properties. Leek could be stored for 13 days under refrigerated conditions without a negative impact on the antioxidant properties. Steaming of the green leaves resulted in an increase of antioxidant capacity, while boiling had a negative effect on total phenolic content in the white shaft and green leaves. Heating (boiling and steaming) leek samples had a negative influence on the content of methiin and isoalliin. In general, steaming seems to be responsible for a better retention of the bioactive compounds present in leek.

Moreover, we have shown evidence for the potential of stabilisation of the green leaves of leek in order to valorise the leek by-products. Two stabilisation methods, including fermentation and drying, were evaluated and revealed nutritional advantages. Using leek powder as ingredient in different food products can be a first step in the valorisation of the by-products in leek production. However, economic risks need to be significantly reduced, before companies and/or leek growers will be willing to invest in the stabilisation of leek and the commercialisation of leek products.

The combination of the two aspects in this study can help to strengthen the position of leek in Belgium and can stimulate the consumption of leek and vegetables in general.

SUMMARY

Leek (*Allium ampeloprasum* var. *porrum*) is one of the most important vegetables cultivated outdoors in Belgium. Besides their economic importance, they are a source of several bioactive or health-promoting compounds including 4 important chemical groups that have perceived benefits to human health, *i.e.* the *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs), polyphenols, vitamins and fructans. Moreover, epidemiologic studies elucidated the reduction of the risk of prostate, colorectal, stomach and breast cancer when leek is consumed. On the one hand, the present study identified and quantified bioactive compounds in fresh leek for a range of leek cultivars and as a function of harvest time. On the other hand, the behaviour of these compounds was investigated during different processing/stabilisation techniques.

Although leek is a popular vegetable in many European countries, the limited scientific knowledge concerning its health-promoting compounds is in sharp contrast with the well-documented health aspects of its related *Allium* species, *i.e*. onion, shallot, garlic. The objective of this PhD thesis was to develop knowledge on the presence of health-promoting compounds in leek in order to complement the many reports focusing on these compounds in related *Allium* species. On the basis of novel scientific knowledge on health-promoting compounds in leek obtained in the present study, the dissertation aims to stimulate innovation in leek breeding, production, marketing and the consumption pattern of leek.

Chapter 1 included a general introduction and the objectives of the present study. *Chapter 2* gave a brief summary concerning the current position of leek production in Belgium and abroad. Leek was described from two different angles: leek as a crop and leek as a food product. Moreover, the presence and properties of bioactive compounds was discussed in Chapter 2. *Chapter 3* described the experimental design, including the selected plant material, the sampling procedure and further sample preparation for each experiment. The analytical and statistical methods were discussed in this chapter as well.

In *Chapter 4*, statistically significant differences among 30 commercial and less common leek cultivars were discussed in terms of antioxidant capacity, polyphenols, ascorbate, ACSOs and fructan content. The antioxidant properties of the leek extracts were influenced by leek tissue (white shaft/green leaves) and type of cultivar (summer/autumn/winter type) to a large extent, whilst the manner of breeding (F1 hybrids/open pollinated) had no significant influence on the antioxidant properties. The green leaves of most cultivars contained a higher antioxidant capacity, ascorbate and

polyphenol content, while the white shaft was rich in ACSOs and fructans. The dissimilarities between the three leek types could be explained by their genetic background, but because of their different harvest times, stress factors such as temperature, solar radiation, pathogens etc. to which the plants were subjected may also partly explain the different accumulation patterns of health-promoting compounds between the summer, autumn and winter leek. Therefore, *Chapter 5* elucidated the effect of harvest time on the antioxidant properties in 9 F1 leek cultivars, harvested at 4 times during the leek growing season. Harvest time had a clear effect on antioxidant levels, in addition to the effect of cultivar. A clear distinction between harvest in September and harvest in November was observed, together with the difference between September/November and the other 2 months (January/March). Harvest in January or March resulted, for most of the cultivars and both parts, in a higher antioxidant capacity and polyphenol levels. Harvest in September had a positive influence on the ACSO content.

Chapter 6 elucidated the difference between leek and some of its related *Allium* species with regard to the antioxidant properties. Based on the results, the antioxidant properties of the white shaft of leek were closely related to the antioxidant potential of the bulb of *A. kurrat* (Egyptian leek) and *A. cepa* (onion), while the green leaves of leek resembled the antioxidant profile of *A. schoenoprasum* (chives) and *A. fistulosum* (bunching onion). *A. odorum* (Chinese leek) and *A. cepa* (cv. Red Creole, red onion) were the species with different antioxidant properties compared to leek. These species were higher in methiin and flavonoid content, respectively.

The evaluation of the impact of post-harvest storage and domestic food processing on the health benefits of vegetables is also of great practical importance and was demonstrated in *Chapter 7* and *Chapter 8,* respectively. The antioxidant capacity was highly influenced by cooking but not by storage. In general, the antioxidant capacity and the total phenolic content in the white shaft and green leaves of the entire and packaged leek was stable during 13 days of storage at 4 °C. A slight increase in isoalliin level could be observed after a cool storage period. The difference between the antioxidant properties of the white shaft of the entire leek and the packaged leek was minimal, except for the lower ACSO values in packaged leek.

An obvious increase could be observed in the antioxidant capacity of the steamed green leaves. Boiling had a negative effect on total phenolic content in the white shaft and green leaves. In contrast with boiling, steaming did not have an influence on the polyphenolic content. It is remarkable that blanching resulted in a slight increase in the ACSO content. Applying a longer-duration thermal treatment to leek samples negatively influenced the content of methiin and isoalliin. In general, steaming seemed to be responsible for a better retention of the bioactive compounds present in leek.

The white shaft is used in many culinary applications, whereas the green leaves are considered inferior and are, therefore, usually only used in soups or even left behind on the fields or during processing. The application of 2 alternative value-adding valorisation methods, including lactic acid fermentation and drying, were therefore investigated in *Chapter 9* with regard to the retention of the antioxidant properties. The results of *Chapter 9* demonstrate the higher antioxidant capacity and polyphenol content upon fermentation especially in the green leaves. These results indicated the nutritional relevance of fermentation, which can be a promising stabilisation technique for leek. Fermentation does not require extensive materials and is an inexpensive way to stabilise and preserve plant material such as leek.
Leek samples subjected to 3 drying methods retained their antioxidant capacity, with the exception of the higher ORAC value of air-dried green leaves and the higher DPPH value of the air-dried white shaft. Similarly, air-drying resulted in the highest total phenolic content compared with freeze-drying, while freeze-dried and refractance window dried samples exhibited equal amounts of polyphenols. The analysis of individual polyphenols revealed again that, air-dried samples contained higher quantities of polyphenols than freeze-dried leek, while freeze-dried leek exhibited higher levels of polyphenols compared to refractance window dried samples. Although air-drying was the best drying technique in retaining the antioxidant capacity and polyphenols, air-drying resulted in high losses of the ACSOs compared to freeze-drying. In fact, refractance window drying was the best drying technique to retain methiin.

The present study revealed novel scientific knowledge on the content of health-promoting compounds in leek. This information can help to stimulate innovation in leek breeding, production, marketing and the consumption pattern of leek. In addition, the present study can give the onset to valorise the green leaves of leek, plant material which is rich in antioxidants.

SAMENVATTING

Prei (*Allium ampeloprasum* var. *porrum*) is met zijn areaal van 4800 ha één van de voornaamste vollegrondsgroenten in België. Van een aantal gewassen uit dezelfde plantenfamilie – zoals ajuin en look – is de waaier aan bioactieve of gezondheidsbevorderende stoffen bekend. Fundamentele kennis omtrent inhoudstoffen bij prei ontbreekt daarentegen. *Allium* species zijn een bron van 4 belangrijke groepen van bioactieve componenten, nl. *S*-alk(en)yl-L-cysteine sulfoxides (ACSO's), polyfenolen, vitamines en fructanen. Dit onderzoeksproject identificeert en kwantificeert gezondheidsbevorderende componenten enerzijds in verse, rauwe prei en dit voor een brede waaier van verschillende preicultivars, zowel voor de witte schacht als de groene bladeren. In een volgende stap werd bepaald hoe deze componenten zich gedragen tijdens de verschillende verwerkingsprocessen (vb. bewaring, koken, drogen, fermentatie, etc.).

Hoofdstuk 1 lichtte naast een algemene inleiding, de objectieven van deze studie toe. *Hoofdstuk 2* vatte de positie van de groente prei samen in België en omstreken. Dit werd besproken in 2 delen, enerzijds 'prei als een gewas', anderzijds 'prei als een voedingsproduct'. In dit hoofdstuk werden ook de voornaamste bioactieve componenten in *Allium* species uitvoerig behandeld.

Hoofdstuk 3 beschreef de experimentele proefopzet, zoals het geselecteerde plantmateriaal, de staalnameprocedure en verdere staalvoorbereiding voor elk experiment. De statistische en analytische methodes werden ook in dit hoofdstuk uitvoerig besproken.

In *Hoofdstuk 4* werden statistische verschillen gevonden tussen een brede range van preicultivars naar antioxidantcapaciteit, totale fenolen, ascorbaat, ACSO's en fructanen. De groene preibladeren bevatten de hoogste antioxidantcapaciteit, de hoogste ascorbaat en polyfenoleninhoud, terwijl de witte schacht rijk was aan ACSO's en fructanen.

Naast verschil in preideel, werden ook verschillen vastgesteld tussen de drie preitypes. Deze verschillen tussen zomer, herfst en winter types kunnen verklaard worden door genetische achtergrond, maar ook mede door de verschillende oogsttijdstippen kunnen stressfactoren zoals temperatuur, zonnestraling, pathogenen, … een reden zijn voor de verschillen tussen de 3 types. Daarom werd in *Hoofdstuk 5* het effect van oogsttijdstip op de inhoud aan antioxidanten meer in detail onderzocht. Hiervoor werden negen preicultivars geoogst op 4 tijdstippen in het preigroeiseizoen. Oogsttijdstip had een significant effect op de antioxidanten, naast het effect van cultivar. Een duidelijk verschil kon vastgesteld worden tussen oogst in september en oogst in november, én tussen oogst in september/november en oogst in januari/maart. Oogst in januari of maart resulteerde voor de meeste cultivars en beide preidelen in een hogere

antioxidantcapaciteit en polyfenollevels. Oogst in September daarentegen had een positieve invloed op de ACSO inhoud.

Hoofdstuk 6 beschreef het verschil tussen prei en enkele van zijn verwante soorten. De antioxidanteigenschappen van de witte preischacht waren meest gerelateerd aan de bulb van *A. kurrat* (Egyptische prei) en *A. cepa (*ui*)*, terwijl de groene preibladeren dicht aansloten bij het antioxidantprofiel van *A. schoenoprasum* (bieslook) en *A. fistulosum* (stengelui). *A. odorum* (Chinese prei) en *A. cepa* (cv. Red Creole, rode ui) waren de species die het minst aanleunden bij de antioxidantwaarde van prei. Deze species scoorden hoger in methiin- en flavonoidinhoud, respectievelijk.

De evaluatie van de impact van naoogstbewaring en keukenbereidingen op de gezondheidsbevorderende componenten in prei is ook van groot belang en werd beschreven in *Hoofdstuk 7* en *Hoofdstuk 8,* respectievelijk. Keukenbereidingen hadden een significante invloed op de antioxidantcapaciteit, terwijl bewaring de antioxidanten nauwelijks beïnvloedde. De antioxidantcapaciteit en totale polyfenoleninhoud van de witte schacht en groene preibladeren was stabiel gedurende een bewaarperiode van 13 dagen (4 °C). Een lichte stijging van isoalliin kon echter vastgesteld worden na een koele bewaarperiode. Het verschil tussen antioxidanten van de witte schacht van volledige prei en verpakte prei was minimaal, behalve de lagere ACSO gehaltes in verpakte prei.

Een significante stijging kon vastgesteld worden in de antioxidantcapaciteit van gestoomde groene bladeren. Koken had echter een negatief effect op de totale polyfenoleninhoud van de witte schacht en groene bladeren. In tegenstelling tot koken, had stomen geen invloed op de totale polyfenoleninhoud. Het is opvallend dat blancheren resulteerde in een lichte stijging in de ACSO-inhoud. Wanneer een langere hittebehandelingsduur werd toegepast, werd een negatieve invloed op de ACSOs vastgesteld. In het algemeen bleek stomen een betere techniek te zijn dan koken naar behoud van bioactieve componenten in prei.

De witte schacht wordt gebruikt in vele culinaire gerechten, terwijl de groene bladeren vaak alleen in soepen worden gebruikt, of zelfs verwijderd worden op het veld bij oogst/verwerking. De toepassing van twee valorisatiemethodes, nl. melkzuurfermentatie en drogen, werd daarom onderzocht in *Hoofdstuk 9* naar behoud van bioactieve componenten. De resultaten van *Hoofdstuk 9* demonstreerden dat de toepassing van fermentatie resulteerde in een hogere antioxidantcapaciteit en totale polyfenoleninhoud, voornamelijk in de groene bladeren. Deze resultaten duidden de nutritionele relevantie van fermentatie aan, welke een veelbelovende stabilisatietechniek kan zijn voor de groene bladeren van prei. Fermentatie vereist weinig materiaal en is een goedkope manier om plantenmateriaal te stabiliseren en te bewaren.

Naast fermentatie, werden 3 droogtechnieken onderzocht als mogelijke stabilisatiemethode. Gedroogde prei behield zijn antioxidantcapaciteit, met uitzondering van de hogere ORAC waarde van luchtgedroogde bladeren en de hogere DPPH waarde van de luchtgedroogde witte schacht. Luchtdrogen resulteerde in de hoogste totale polyfenoleninhoud vergeleken met vriesdrogen, terwijl gevriesdroogde en refractance windowgedroogde stalen gelijke hoeveelheden bezaten. De analyse van de individuele polyfenolen toonde opnieuw aan dat luchtgedroogde stalen hogere polyfenolengehaltes bevatte dan gevriesdroogde stalen, terwijl gevriesdroogde stalen hogere gehaltes bevatten dan refractance windowgedroogde stalen. Ook al was luchtdrogen de beste droogtechniek naar behoud van de antioxidantcapaciteit en polyfenolen, luchtdrogen resulteerde in de grootste verliezen aan ACSO's vergeleken met vriesdrogen. Refractance windowdrogen was de beste droogtechniek naar behoud van methiin.
Deze studie leverde nieuwe wetenschappelijke kennis op naar de inhoud aan gezondheidsbevorderende componenten in prei. Deze informatie kan helpen om innovatie in preiveredeling, -productie, -marketing en -consumptie te stimuleren.

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REFERENCES

ABONYI, B. I., FENG, H., TANG, J., EDWARDS, C. G., CHEW, B. P., MATTINSON, D. S., & FELLMAN, J. K. (2002). Quality retention in strawberry and carrot purees dried with Refractance Window (TM) system. Journal of Food Science 67(3), 1051-1056.

ABRAMS, S. A., GRIFFIN, I. J., HAWTHORNE, K. M., LIANG, L., GUNN, S. K., DARLINGTON, G., & ELLIS, K. J. (2005). A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. American Journal of Clinical Nutrition 82(2), 471-476.

ACKLAND, M. L., VAN DE WAARSENBURG, S., & JONES, R. (2005). Synergistic antiproliferative action of the flavonols quercetin and kaempferol in cultured human cancer cell lines. In Vivo 19(1), 69-76.

ADAMS, M. & MITCHELL, R. (2002). Fermentation and pathogen control: a risk assessment approach. International Journal of Food Microbiology 79(1-2), 75-83.

ADITI, A. & GRAHAM, D. Y. (2012). Vitamin C, Gastritis, and Gastric Disease: A Historical Review and Update. Digestive Diseases and Sciences 57(10), 2504-2515.

ALI, M., BORDIA, T., & MUSTAFA, T. (1999). Effect of raw versus boiled aqueous extract of garlic and onion on platelet aggregation. Prostaglandins Leukotrienes and Essential Fatty Acids 60(1), 43-47.

AOR. (2012). Advanced Orthomolecular Research - Quercetin.

AOYAMA, S. & YAMAMOTO, Y. (2007). Antioxidant activity and flavonoid content of Welsh onion (Allium fistulosum) and the effect of thermal treatment. Food Science and Technology Research 13(1), 67-72.

ARDO. (2012). Productgamma's. Ardo

ARIFFIN, F., CHEW, S. H., BHUPINDER, K., KARIM, A. A., & HUDA, N. (2011). Antioxidant capacity and phenolic composition of fermented Centella asiatica herbal teas. Journal of the Science of Food and Agriculture 91(15), 2731-2739.

ARRIGONI, O. & DE TULLIO, M. C. (2000). The role of ascorbic acid in cell metabolism: between gene-directed functions and unpredictable chemical reactions. Journal of Plant Physiology 157(5), 481-488.

ARYA, S. P., MAHAJAN, M., & JAIN, P. (2000). Non-spectrophotometric methods for the determination of Vitamin C. Analytica Chimica Acta 417(1), 1-14.

ASAMI, D. K., HONG, Y. J., BARRETT, D. M., & MITCHELL, A. E. (2003). Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. Journal of Agricultural and Food Chemistry 51(5), 1237-1241.

ATTANASIO, G., CINQUANTA, L., ALBANESE, D., & DI MATTEO, M. (2004). Effects of drying temperatures on physico-chemical properties of dried and rehydrated chestnuts (Castanea sativa). Food Chemistry 88(4), 583-590.

AUGUSTI, K. T. (1990) Therapeutic and medicinal values of onions and garlic. Brewster, J. L. and Rabinowitch, H. D. 93-108. CRC Press. Onions and Allied Crops. vol III: Biochemistry, Food Science and Minor Crops.

AYALA-ZAVALA, J. F., WANG, S. Y., WANG, C. Y., & GONZALEZ-AGUILAR, G. A. (2004). Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology 37(7), 687-695.

BACON, J. R., MOATES, G. K., NG, A., RHODES, M. J. C., SMITH, A. C., & WALDRON, K. W. (1999). Quantitative analysis of flavour precursors and pyruvate levels in different tissues and cultivars of onion (Allium cepa). Food Chemistry 64(2), 257-261.

BAERT, J. R. A. (1997). The effect of sowing and harvest date and cultivar on inulin yield and composition of chicory (Cichorium intybus L) roots. Industrial Crops and Products 6(3-4), 195-199.

BARBERIS, A., FADDA, A., SCHIRRA, M., BAZZU, G., & SERRA, P. A. (2012). Detection of postharvest changes of ascorbic acid in fresh-cut melon, kiwi, and pineapple, by using a low cost telemetric system. Food Chemistry 135(3), 1555-1562.

BARTH, C., DE TULLIO, M., & CONKLIN, P. L. (2006). The role of ascorbic acid in the control of flowering time and the onset of senescence. Journal of Experimental Botany 57(8), 1657-1665.

BEATO, V. M., ORGAZ, F., MANSILLA, F., & MONTANO, A. (2011). Changes in Phenolic Compounds in Garlic (Allium sativum L.) Owing to the Cultivar and Location of Growth. Plant Foods for Human Nutrition 66(3), 218-223.

BEATO, V. M., SANCHEZ, A. H., DE CASTRO, A., & MONTANO, A. (2012). Effect of Processing and Storage Time on the Contents of Organosulfur Compounds in Pickled Blanched Garlic. Journal of Agricultural and Food Chemistry 60(13), 3485-3491.

BECKMAN, C. H. (2000). Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? Physiological and Molecular Plant Pathology 57(3), 101-110.

BEECHER, G. R. (2003). Overview of dietary flavonoids: Nomenclature, occurrence and intake. Journal of Nutrition 133(10), 3248S-3254S.

BENITEZ, V., MOLLA, E., MARTIN-CABREJAS, M. A., AGUILERA, Y., LOPEZ-ANDREU, F. J., & ESTEBAN, R. M. (2012). Onion (Allium cepa L.) by-products as source of dietary fiber: physicochemical properties and effect on serum lipid levels in high-fat fed rats. European Food Research and Technology 234(4), 617-625.

BENKEBLIA, N., ONODERA, S., & SHIOMI, N. (2004). Effect of gamma irradiation and temperature on fructans (fructo-oligosaccharides) of stored onion bulbs Allium cepa L. Food Chemistry 87(3), 377-382.

BERGQUIST, S. A., GERTSSON, U. E., NORDMARK, L. Y. G., & OLSSON, M. E. (2007). Effects of shade nettings, sowing time and storage on baby spinach flavonoids. Journal of the Science of Food and Agriculture 87(13), 2464-2471.

BIANCHINI, F. & VAINIO, H. (2001). Allium vegetables and organosulfur compounds: Do they help prevent cancer? Environmental Health Perspectives 109(9), 893-902.

BISAKOWSKI, B., ATWAL, A. S., GARDNER, N., & CHAMPAGNE, C. P. (2007). Effect of lactic acid fermentation of onions (Allium cepa) on the composition of flavonol glucosides. International Journal of Food Science and Technology 42(7), 783-789.

BLOCK, E. (2010). Garlic and Other Alliums - The Lore and the Science. RSC Publishing.

BLOEM, E., HANEKLAUS, S., & SCHNUG, E. (2004). Influence of nitrogen and sulfur fertilization on the alliin content of onions and garlic. Journal of Plant Nutrition 27(10), 1827-1839.

BONACCORSI, P., CARISTI, C., GARGIULLI, C., & LEUZZI, U. (2005). Flavonol glucoside profile of southern Italian red onion (Allium cepa L.). Journal of Agricultural and Food Chemistry 53(7), 2733-2740.

BONACCORSI, P., CARISTI, C., GARGIULLI, C., & LEUZZI, U. (2008). Flavonol glucosides in Allium species: A comparative study by means of HPLC-DAD-ESI-MS-MS. Food Chemistry 107(4), 1668-1673.

BOSWELL, J. J. (1983). English botany. London: George Bell and Sons.

BRAVO, L. (1998). Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews 56(11), 317-333.

BREENE, W. M. (1994). Healthfulness and nutritional quality of fresh versus processed fruits and vegetables: a review. Journal of Foodservice Systems 8, 1-45.

BREWSTER, J. L. & SUTHERLAND, R. A. (1993). The rapid determination in controlled environments of parameters for predicting seedling growth rates in natural conditions. Annals of Applied Biology 122, 123-133.

BROBERG, A., JACOBSSON, K., STROM, K., & SCHNURER, J. (2007). Metabolite profiles of lactic acid bacteria in grass silage. Applied and Environmental Microbiology 73(17), 5547-5552.

BUCHNER, N., KRUMBEIN, A., ROHN, S., & KROH, L. W. (2006). Effect of thermal processing on the flavonols rutin and quercetin. Rapid Communications in Mass Spectrometry 20(21), 3229-3235.

BURT, J. (2011). Growing leeks in Western Australia. Farmnote 52.

CAO, G. H., ALESSIO, H. M., & CUTLER, R. G. (1993). Oxygen-Radical Absorbency Capacity Assay for Antioxidants. Free Radical Biology and Medicine 14(3), 303-311.

CAPARINO, O. A., TANG, J., NINDO, C. I., SABLANI, S. S., POWERS, J. R., & FELLMAN, J. K. (2012). Effect of drying methods on the physical properties and microstructures of mango (Philippine 'Carabao' var.) powder. Journal of Food Engineering 111(1), 135-148.

CARSON, J. (1987). Chemistry and biological properties of onions and garlic. Food Reviews International 3, 71-103.

CHATTERTON, N. J. & HARRISON, P. A. (2003). Fructans in crested wheatgrass leaves. Journal of Plant Physiology 160(8), 843-849.

CHAU, C. F. & CHEUNG, P. C. K. (1997). Effect of various processing methods on antinutrients and in vitro digestibility of protein and starch of two Chinese indigenous legume seeds. Journal of Agricultural and Food Chemistry 45(12), 4773-4776.

CHU, Y. H., CHANG, C. L., & HSU, H. F. (2000). Flavonoid content of several vegetables and their antioxidant activity. Journal of the Science of Food and Agriculture 80(5), 561-566.

CHUDA, A. & ADAMUS, A. (2009). Aspects of interspecific hybridization within edible Alliaceae. Acta Physiologiae Plantarum 31(2), 223-227.

CINBIOS. (2011). Een zicht op de Vlaamse biogebaseerde economie, vandaag en morgen.

CISNEROS-ZEVALLOS, L. (2003). The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding-value of fresh fruits and vegetables. Journal of Food Science 68(5), 1560-1565.

CLARK, G. T., ZUTHER, E., OUTRED, H. A., MCMANUS, M. T., & HEYER, A. G. (2004). Tissue-specific changes in remobilisation of fructan in the xerophytic tussock species Festuca novae-zelandiae in response to a water deficit. Functional Plant Biology 31(4), 377-389.

COMPERNOL, N. & DE RYCK, B. (2011). Onderzoek naar de consumptie en het gebruik van prei in Vlaanderen en ontwikkeling van alternatieve receptuur. Katholieke Hogeschool Gent.

COOLONG, T. W. & RANDLE, W. M. (2003). Temperature influences flavor intensity and quality in 'Granex 33' onion. Journal of the American Society for Horticultural Science 128(2), 176-181.

COOLONG, T. W. & RANDLE, W. M. (2006). The influence of root zone temperature on growth and flavour precursors in Allium cepa L. Journal of Horticultural Science & Biotechnology 81(2), 199-204.

CORTELL, J. M. & KENNEDY, J. A. (2006). Effect of shading on accumulation of flavonoid compounds in (Vitis vinifera L.) pinot noir fruit and extraction in a model system. Journal of Agricultural and Food Chemistry 54(22), 8510-8520.

CORZO-MARTINEZ, M., CORZO, N., & VILLAMIEL, M. (2007). Biological properties of onions and garlic. Trends in Food Science & Technology 18(12), 609-625.

CROKET, R. (2011). Amounts of leek by-products during processing.

D'ARTA. (2012). Products.

DAVALOS, A., BARTOLOME, B., & GOMEZ-CORDOVES, C. (2004). Inhibition of methyl linoleate autoxidation by phenolics and other related compounds under mild oxidative conditions. Journal of the Science of Food and Agriculture 84(7), 631-638.

DAVEY, M. W., VAN MONTAGU, M., INZE, D., SANMARTIN, M., KANELLIS, A., SMIRNOFF, N., BENZIE, I. J. J., STRAIN, J. J., FAVELL, D., & FLETCHER, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. Journal of the Science of Food and Agriculture 80(7), 825-860.

DE CASTRO, A., MONTANO, A., SANCHEZ, A. H., & REJANO, L. (1998). Lactic acid fermentation and storage of blanched garlic. International Journal of Food Microbiology 39(3), 205-211.

DE CLERCQ, H., BAERT, J., & VAN BOCKSTAELE, E. (1999). Breeding potential of Belgian landraces of leek (Allium ampeloprasum L. var. porrum). Euphytica 106(2), 101-109.

DE CLERCQ, H., PEUSENS, D., ROLDAN-RUIZ, I., & VAN BOCKSTAELE, E. (2003). Causal relationships between inbreeding, seed characteristics and plant performance in leek (Allium porrum L.). Euphytica 134(1), 103-115.

DE CLERCQ, H. & VAN BOCKSTAELE, E. (2002). Leek: advances in agronomy and breeding. In Allium Crop Science: recent advances : CABI publishing.

DE CLERCQ, H. (2008). Cursus: vollegrondsgroenten, Hogeschool Gent.

DE CLERCQ, H. (2012). Breeding of leek.

DE PAEPE, D., SERVAES, K., NOTEN, B., DIELS, L., DE LOOSE, M., VAN DROOGENBROECK, B., & VOORSPOELS, S. (2013). An improved mass spectrometric method for identification and quantification of phenolic compounds in apple fruits. Food Chemistry 136, 368-375.

DE TULLIO, M. C., LISO, R., & ARRIGONI, O. (2004). Ascorbic acid oxidase: an enzyme in search of a role. Biologia Plantarum 48(2), 161-166.

DE WILDE-DUYFJES, B. E. E. (1976). A revision of the genus Allium L. (Liliaceae) in Africa. Belmontia 7, 75-78.

DEIGHTON, N., BRENNAN, R., FINN, C., & DAVIES, H. V. (2000). Antioxidant properties of domesticated and wild Rubus species. Journal of the Science of Food and Agriculture 80(9), 1307-1313.

DELCHIER, N., REICH, M., & RENARD, C. M. G. C. (2012). Impact of cooking methods on folates, ascorbic acid and lutein in green beans (Phaseolus vulgaris) and spinach (Spinacea oleracea). Lwt-Food Science and Technology 49(2), 197-201.

DEMIR, N., BAHCECI, K. S., & ACAR, J. (2006). The effects of different initial Lactobacillus plantarum concentrations on some properties of fermented carrot juice. Journal of Food Processing and Preservation 30(3), 352-363.

DEMIREZER, L. O., KARAHAN, N., UCAKTURK, E., KURUUZUM-UZ, A., GUVENALP, Z., & KAZAZ, C. (2011). HPLC Fingerprinting of Sennosides in Laxative Drugs with Isolation of Standard Substances from Some Senna Leaves. Records of Natural Products 5(4), 261-270.

DE NEEF, L. (2012). Market of leek in Japan.

DESAI, P. & SHETH, T. (1997). Controlled fermentation of vegetables using mixed inoculum of lactic cultures. Journal of Food Science and Technology-Mysore 34(2), 155-158.

DESOBRY, S. A., NETTO, F. M., & LABUZA, T. P. (1997). Comparison of spray-drying, drum-drying and freeze-drying for beta-carotene encapsulation and preservation. Journal of Food Science 62(6), 1158-1162.

DIAZ-MAROTO, M. C., PEREZ-COELLO, M. S., & CABEZUDO, M. D. (2002). Effect of different drying methods on the volatile components of parsley (Petroselinum crispum L.). European Food Research and Technology 215(3), 227-230.

DINI, I., TENORE, G. C., & DINI, A. (2008). S-Alkenyl Cysteine Sulfoxide and Its Antioxidant Properties from Allium cepa var. tropeana (Red Onion) Seeds. Journal of Natural Products 71(12), 2036-2037.

DLAMINI, N. R., TAYLOR, J. R. N., & ROONEY, L. W. (2007). The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. Food Chemistry 105(4), 1412-1419.

DORAN, J. A., O'DONNELL, J. S., LAIRSON, L. L., MCDONALD, M. R., SCHWAN, A. L., & GRODZINSKI, B. (2007). S-alk(en)yl-l-cysteine sulfoxides and relative pungency measurements of photosynthetic and non photosynthetic tissues of Allium porrum. Journal of Agricultural and Food Chemistry 55(20), 8243-8250.

DORDEVIC, T. M., SILER-MARINKOVIC, S. S., & DIMITRIJEVIC-BRANKOVIC, S. I. (2010). Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. Food Chemistry 119(3), 957-963.

DUCKSTEIN, S. M., LORENZ, P., & STINTZING, F. C. (2012). Conversion of Phenolic Constituents in Aqueous Hamamelis virginiana Leaf Extracts During Fermentation. Phytochemical Analysis 23(6), 588-597.

DUENAS, M., FERNANDEZ, D., HERNANDEZ, T., ESTRELLA, I., & MUNOZ, R. (2005). Bioactive phenolic compounds of cowpeas (Vigna sinensis L). Modifications by fermentation with natural microflora and with Lactobacillus plantarum ATCC 14917. Journal of the Science of Food and Agriculture 85(2), 297-304.

DUH, P. D. (1998). Antioxidant activity of burdock (Arctium lappa Linne): Its scavenging effect on free-radical and active oxygen. Journal of the American Oil Chemists Society 75(4), 455-461.

DUVAL, B., SHETTY, K., & THOMAS, W. H. (1999). Phenolic compounds and antioxidant properties in the snow alga Chlamydomonas nivalis after exposure to UV light. Journal of Applied Phycology 11(6), 559-566.

ENGELEN, C. (2003). Zaadteelt van ui, kool en peen. Een praktische gids, rapport

ENJOY FRESH. (2012). Enjoy fresh fruits and vegetables.

ERDMAN, J. W., BALENTINE, D., ARAB, L., BEECHER, G., DWYER, J. T., FOLTS, J., HARNLY, J., HOLLMAN, P., KEEN, C. L., MAZZA, G., MESSINA, M., SCALBERT, A., VITA, J., WILLIAMSON, G., & BURROWES, J. (2007). Flavonoids and heart health: Proceedings of the ILSI North America Flavonoids Workshop, May 31-June 1, 2005, Washington, DC. Journal of Nutrition 137(3), 718S-737S.

EUROSTAT (2012). Agricultural statistics - Fruits and vegetables (annual data). Publications Office of the European Community .

EVERETTE, J. D., BRYANT, Q. M., GREEN, A. M., ABBEY, Y. A., WANGILA, G. W., & WALKER, R. B. (2010). Thorough Study of Reactivity of Various Compound Classes toward the Folin-Ciocalteu Reagent. Journal of Agricultural and Food Chemistry 58(14), 8139-8144.

EWALD, C., FJELKNER-MODIG, S., JOHANSSON, K., SJOHOLM, I., & AKESSON, B. (1999). Effect of processing on major flavonoids in processed onions, green beans, and peas. Food Chemistry 64(2), 231-235.

FAO. (2012). FAO statistics.

FATTORUSSO, E., LANZOTTI, V., TAGLIALATELA-SCAFATI, O., & CICALA, C. (2001). The flavonoids of leek, Allium porrum. Phytochemistry 57(4), 565-569.

FERRERES, F., LLORACH, R., & GIL-IZQUIERDO, A. (2004). Characterization of the interglycosidic linkage in di-, tri-, tetra- and pentaglycosylated flavonoids and differentiation of positional isomers by liquid chromatography/electrospray ionization tandem mass spectrometry. Journal of Mass Spectrometry 39(3), 312-321.

FILJUSHIN, M. A., KHOLDA, O. A., KOCHIEVA, E. Z., & RYZHOVA, N. N. (2011). AFLP marking of the genotypes of leek (Allium porrum) varieties. Russian Journal of Genetics 47(4), 492-496.

FOLIN, C. and CIOCALTEU, V. (1927). Tyrosine and tryptophan determination in protein. Journal of Biology and Chemistry 73, 627-650.

FREEMAN, G. G. & MOSSADEG, N. (1973). Studies on Relationship Between Water Regime and Flavor Strength in Watercress (Rorippa-Nasturtium-Aquaticum (L) Hayek), Cabbage (Brassica-Oleracea-Capitata) and Onion (Allium-Cepa). Journal of Horticultural Science & Biotechnology 48(4), 365-378.

FRIEDMAN, M. (1997). Chemistry, biochemistry, and dietary role of potato polyphenols. A review. Journal of Agricultural and Food Chemistry 45(5), 1523-1540.

FRITSCH, R. M. & KEUSGEN, M. (2006). Occurrence and taxonomic significance of cysteine sulphoxides in the genus Allium L. (Alliaceae). Phytochemistry 67(11), 1127-1135.

FUCHS, A. (1993). Inulin and inulin containing crops: studies in Plant Science. Amsterdam: Elsevier.

GALDON, B. R., RODRIGUEZ, C. T., RODRIGUEZ, E. M. R., & ROMERO, C. D. (2009). Fructans and major compounds in onion cultivars (Allium cepa). Journal of Food Composition and Analysis 22(1), 25-32.

GALDON, B. R., RODRIGUEZ, E. M. R., & ROMERO, C. D. (2008). Flavonoids in Onion Cultivars (Allium cepa L.). Journal of Food Science 73(8), C599-C605.

GANSKE, F. & DELL, E. J. (2006). ORAC assay on the FLUOstar OPTIMA to Determine Antioxidant Capacity - BMG LABTECH. Application note 148 .

GAZZANI, G., DAGLIA, M., PAPETTI, A., & GREGOTTI, C. (2000). In vitro and ex vivo anti- and prooxidant components of Cichorium intybus. Journal of Pharmaceutical and Biomedical Analysis 23(1), 127-133.

GENNARO, L., LEONARDI, C., ESPOSITO, F., SALUCCI, M., MAIANI, G., QUAGLIA, G., & FOGLIANO, V. (2002). Flavonoid and carbohydrate contents in Tropea red onions: Effects of homelike peeling and storage. Journal of Agricultural and Food Chemistry 50(7), 1904-1910.

GERM, M., STIBILJ, V., KREFT, S., GABERSCIK, A., & KREFT, I. (2010). Flavonoid, tannin and hypericin concentrations in the leaves of St. John's wort (Hypericum perforatum L.) are affected by UV-B radiation levels. Food Chemistry 122(3), 471-474.

GOKCE, A. F., KAYA, C., SERCE, S., & OZGEN, M. (2010). Effect of scale color on the antioxidant capacity of onions. Scientia Horticulturae 123(4), 431-435.

GONZALEZ-SAIZ, J. M., ESTEBAN-DIEZ, I., RODRIGUEZ-TECEDOR, S., & PIZARRO, C. (2008). Valorization of Onion Waste and By-Products: MCR-ALS Applied to Reveal the Compositional Profiles of Alcoholic Fermentations of Onion Juice Monitored by Near-Infrared Spectroscopy. Biotechnology and Bioengineering 101(4), 776-787.

GORINSTEIN, S., PARK, Y. S., HEO, B. G., NAMIESNIK, J., LEONTOWICZ, H., LEONTOWICZ, M., HAM, K. S., CHO, J. Y., & KANG, S. G. (2009). A comparative study of phenolic compounds and antioxidant and antiproliferative activities in frequently consumed raw vegetables. European Food Research and Technology 228(6), 903-911.

GOWEN, A. A., ABU-GHANNAM, N., FRIAS, J. M., BARAT, J. M., ANDRES, A. M., & OLIVEIRA, J. C. (2006). Comparative study of quality changes occurring on dehydration and rehydration of cooked chickpeas (Cicer arietinum L.) subjected to combined microwave-convective and convective hot air dehydration. Journal of Food Science 71(6), E282-E289.

GRIFFITHS, G., TRUEMAN, L., CROWTHER, T., THOMAS, B., & SMITH, B. (2002). Onions - A global benefit to health. Phytotherapy Research 16(7), 603-615.

GROTEWOLD, E. (2006). The Science of Flavonoids. Springer, 173p

GRUBBEN, G. J. H. & DENTON, O. A. (2004). Plant resources of Tropical Africa 2, vegetables. Wageningen: Backhuys Leiden.

GRZELAK-BLASZCZYK, K., KOLODZIEJCZYK, K., BADELEK, E., & ADAMICKI, F. (2011). Changes in the contents of mono-, di- and oligosaccharides in leek plants stored in cold room. European Food Research and Technology 232(6), 1027-1033.

GU, L. W., HOUSE, S. E., WU, X. L., OU, B. X., & PRIOR, R. L. (2006). Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products. Journal of Agricultural and Food Chemistry 54(11), 4057-4061.

GUIGOZ, Y., ROCHAT, F., PERRUISSEAU-CARRIER, G., ROCHAT, I., & SCHIFFRIN, E. J. (2002). Effects of oligosaccharide on the faecal flora and non-specific immune system in elderly people. Nutrition Research 22(1-2), 13-25.

HALVORSEN, B. L., HOLTE, K., MYHRSTAD, M. C. W., BARIKMO, I., HVATTUM, E., REMBERG, S. F., WOLD, A. B., HAFFNER, K., BAUGEROD, H., ANDERSEN, L. F., MOSKAUG, J. O., JACOBS, D. R., & BLOMHOFF, R. (2002). A systematic screening of total antioxidants in dietary plants. Journal of Nutrition 132(3), 461-471.

HAMALAINEN, M., NIEMINEN, R., VUORELA, P., HEINONEN, M., & MOILANEN, E. (2007). Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappa B activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappa B activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. Mediators of Inflammation .

HAMINIUK, C. W. I., MACIEL, G. M., PLATA-OVIEDO, M. S. V., & PERALTA, R. M. (2012). Phenolic compounds in fruits - an overview. International Journal of Food Science and Technology 47(10), 2023-2044.

HAMMAMI, C. & RENE, F. (1997). Determination of freeze-drying process variables for strawberries. Journal of Food Engineering 32(2), 133-154.

HANCOCK, R. D., GALPIN, J. R., & VIOLA, R. (2000). Biosynthesis of L-ascorbic acid (vitamin C) by Saccharomyces cerevisiae. Fems Microbiology Letters 186(2), 245-250.

HANCOCK, R. D. & VIOLA, R. (2005). Biosynthesis and catabolism of L-ascorbic acid in plants. Critical Reviews in Plant Sciences 24(3), 167-188.

HANHINEVA, K., ROGACHEV, I., KOKKO, H., MINTZ-ORON, S., VENGER, I., KARENLAMPI, S., & AHARONI, A. (2008). Non-targeted analysis of spatial metabolite composition in strawberry (Fragaria x ananassa) flowers. Phytochemistry 69(13), 2463-2481.

HANSEN, E. A., FOLTS, J. D., & GOLDMAN, I. L. (2012). Steam-cooking rapidly destroys and reverses onion-induced antiplatelet activity. Nutrition Journal 11.

HARBAUM, B., HUBBERMANN, E. M., ZHU, Z. J., & SCHWARZ, K. (2008). Impact of fermentation on phenolic compounds in leaves of pak choi (Brassica campestris L. ssp chinensis var. communis) and Chinese leaf mustard (Brassica juncea coss). Journal of Agricultural and Food Chemistry 56(1), 148-157.

HARBORNE, J. B. & WILLIAMS, C. A. (2000). Advances in flavonoid research since 1992. Phytochemistry 55(6), 481-504.

HART, D. J. & SCOTT, K. J. (1995). Development and Evaluation of An Hplc Method for the Analysis of Carotenoids in Foods, and the Measurement of the Carotenoid Content of Vegetables and Fruits Commonly Consumed in the Uk. Food Chemistry 54(1), 101-111.

HARWOOD, M., DANIELEWSKA-NIKIEL, B., BORZELLECA, J. F., FLAMM, G. W., WILLIAMS, G. M., & LINES, T. C. (2007). A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcino genic properties. Food and Chemical Toxicology 45(11), 2179-2205.

HAVEY, M. J., GALMARINI, C. R., GOKCE, A. F., & HENSON, C. (2004). QTL affecting soluble carbohydrate concentrations in stored onion bulbs and their association with flavor and health-enhancing attributes. Genome 47(3), 463-468.

HAVEY, M. J. & LEITE, D. L. (1999). Toward the identification of cytoplasmic male sterility in leek: Evaluation of organellar DNA diversity among cultivated accessions of Allium ampeloprasum. Journal of the American Society for Horticultural Science 124(2), 163-165.

HENDRY, G. A. F. & WALLACE, R. K. (1993). The origin, distribution, and evolutionary significance of fructans. In Science and Technology of fructans (Eds M. Suzuki & N. J. Chatterton), pp. 119-139. Boca Raton: CRC Press.

HERCHI, W., SAWALHA, S., ARRBEZ-ROMBN, D., BOUKHCHINA, S., SEGURA-CARRETERO, A., KALLEL, H., & FERNBNDEZ-GUTIERREZ, A. (2011). Determination of phenolic and other polar compounds in flaxseed oil using liquid chromatography coupled with time-of-flight mass spectrometry. Food Chemistry 126(1), 332-338.

HERRMANN, K. (1988). On the Occurrence of Flavonol and Flavone Glycosides in Vegetables. Zeitschrift fur Lebensmittel-Untersuchung Und-Forschung 186(1), 1-5.

HERTOG, M. G. L., FESKENS, E. J. M., HOLLMAN, P. C. H., KATAN, M. B., & KROMHOUT, D. (1994). Dietary Flavonoids and Cancer Risk in the Zutphen Elderly Study. Nutrition and Cancer-An International Journal 22(2), 175-184.

HERTOG, M. G. L., HOLLMAN, P. C. H., & KATAN, M. B. (1992a). Content of Potentially Anticarcinogenic Flavonoids of 28 Vegetables and 9 Fruits Commonly Consumed in the Netherlands. Journal of Agricultural and Food Chemistry 40(12), 2379-2383.

HERTOG, M. G. L., HOLLMAN, P. C. H., & VENEMA, D. P. (1992b). Optimization of A Quantitative Hplc Determination of Potentially Anticarcinogenic Flavonoids in Vegetables and Fruits. Journal of Agricultural and Food Chemistry 40(9), 1591-1598.

HERTOG, M. G. L., SWEETMAN, P. M., FEHILY, A. M., ELWOOD, P. C., & KROMHOUT, D. (1997). Antioxidant flavonols and ischemic heart disease in a Welsh population of men: The Caerphilly Study. American Journal of Clinical Nutrition 65(5), 1489-1494.

HIDALGO, M., SANCHEZ-MORENO, C., & DE PASCUAL-TERESA, S. (2010). Flavonoid-flavonoid interaction and its effect on their antioxidant activity. Food Chemistry 121(3), 691-696.

HIGUCHI, O., TATESHITA, K., & NISHIMURA, H. (2003). Antioxidative activity of sulfur-containing compounds in allium species for human low-density lipoprotein (LDL) oxidation in vitro. Journal of Agricultural and Food Chemistry 51(24), 7208-7214.

HINCHA, D. K., LIVINGSTON, D. P., PREMAKUMAR, R., ZUTHER, E., OBEL, N., CACELA, C., & HEYER, A. G. (2007). Fructans from oat and rye: Composition and effects on membrane stability during drying. Biochimica et Biophysica Acta-Biomembranes 1768(6), 1611-1619.

HIROTA, S., SHIMODA, T., & TAKAHAMA, U. (1998). Tissue and spatial distribution of flavonol and peroxidase in onion bulbs and stability of flavonol glucosides during boiling of the scales. Journal of Agricultural and Food Chemistry 46(9), 3497-3502.

HISANO, H., KANAZAWA, A., YOSHIDA, M., HUMPHREYS, M. O., IIZUKA, M., KITAMURA, K., & YAMADA, T. (2008). Coordinated expression of functionally diverse fructosyltransferase genes is associated with fructan accumulation in response to low temperature in perennial ryegrass. New Phytologist 178(4), 766-780.

HOGAN, S., ZHANG, L., LI, J. R., ZOECKLEIN, B., & ZHOU, K. Q. (2009). Antioxidant properties and bioactive components of Norton (Vitis aestivalis) and Cabernet Franc (Vitis vinifera) wine grapes. Lwt-Food Science and Technology 42(7), 1269-1274.

HONG, S. I. & KIM, D. M. (2001). Influence of oxygen concentration and temperature on respiratory characteristics of fresh-cut green onion. International Journal of Food Science and Technology 36(3), 283-289.

HORIUCHI, J. I., YAMAUCHI, N., OSUGI, M., KANNO, T., KOBAYASHI, M., & KURIYAMA, H. (2000). Onion alcohol production by repeated batch process using a flocculating yeast. Bioresource Technology 75(2), 153-156.

HORNICKOVA, J., KUBEC, R., CEJPEK, K., VELISEK, J., OVESNA, J., & STAVELIKOVA, H. (2010). Profiles of S-Alk(en)ylcysteine Sulfoxides in Various Garlic Genotypes. Czech Journal of Food Sciences 28(4), 298-308.

HOSSAIN, M. B., BARRY-RYAN, C., MARTIN-DIANA, A. B., & BRUNTON, N. P. (2010). Effect of drying method on the antioxidant capacity of six Lamiaceae herbs. Food Chemistry 123(1), 85-91.

HOVIUS, M. H. Y. & GOLDMAN, I. L. (2005). Flavor precursor [S-alk(en)yl-L-cysteine sulfoxidel concentration and composition in onion plant organs and predictability of field white rot reaction of onions. Journal of the American Society for Horticultural Science 130(2), 196-202.

HRI. (2004). DNA markers in leek improvement.

HSING, A. W., CHOKKALINGAM, A. P., GAO, Y. T., MADIGAN, M., DENG, J., GRIDLEY, G., & FRAUMENI, J. F. (2002). Allium vegetables and risk of prostate cancer: A population-based study. Journal of the National Cancer Institute 94(21), 1648-1651.

HUANG, D. J., OU, B. X., & PRIOR, R. L. (2005). The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry 53(6), 1841-1856.

HUBACEK, J. (1970). Paper Chromatography of Flavonoids from Hops (Humulus-Lupulus L) .3. Identification of Apigenin, Apigenin 7-Glucoside, and Quercetin 7-Glucoside. Collection of Czechoslovak Chemical Communications 35(10), 3119

HUGHES, J., COLLIN, H. A., TREGOVA, A., TOMSETT, A. B., COSSTICK, R., & JONES, M. G. (2006). Effect of low storage temperature on some of the flavour precursors in garlic (Allium sativum). Plant Foods for Human Nutrition 61(2), 81-85.

HUYNH, B. L., PALMER, L., MATHER, D. E., WALLWORK, H., GRAHAM, R. D., WELCH, R. M., & STANGOULIS, J. C. R. (2008). Genotypic variation in wheat grain fructan content revealed by a simplified HPLC method. Journal of Cereal Science 48(2), 369-378.

ICHIKAWA, M., IDE, N., & ONO, K. (2006). Changes in organosulfur compounds in garlic cloves during storage. Journal of Agricultural and Food Chemistry 54(13), 4849-4854.

IKAWA, M., SCHAPER, T. D., DOLLARD, C. A., & SASNER, J. J. (2003). Utilization of Folin-Ciocalteu phenol reagent for the detection of certain nitrogen compounds. Journal of Agricultural and Food Chemistry 51(7), 1811-1815.

IOANNOU, I., HAFSA, I., HAMDI, S., CHARBONNEL, C., & GHOUL, M. (2012). Review of the effects of food processing and formulation on flavonol and anthocyanin behaviour. Journal of Food Engineering 111(2), 208-217.

IOKU, K., AOYAMA, Y., TOKUNO, A., TERAO, J., NAKATANI, N., & TAKEI, Y. (2001). Various cooking methods and the flavonoid content in onion. Journal of Nutritional Science and Vitaminology 47(1), 78-83.

IRKIN, R. & KORUKLUOGLU, M. (2009). Control of Some Filamentous Fungi and Yeasts by Dehydrated Allium Extracts. Journal fur Verbraucherschutz und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety 4(1), 3-6.

JAIME, L., MARTIN-CABREJAS, M. A., MOLLA, E., LOPEZ-ANDREU, F. J., & ESTEBAN, R. M. (2001). Effect of storage on fructan and fructooligosaccharide of onion (Allium cepa L.). Journal of Agricultural and Food Chemistry 49(2), 982-988.

JASTRZEBSKI, Z., LEONTOWICZ, H., LEONTOWICZ, M., NAMIESNIK, J., ZACHWLEJA, Z., BARTON, H., PAWELZIK, E., ARANCIBLA-AVILA, P., TOLEDO, F., & GORINSTEIN, S. (2007). The bioactivity of processed garlic (Allium sativum L.) as shown in vitro and in vivo studies on rats. Food and Chemical Toxicology 45(9), 1626-1633.

JONES, H. A. & MANN, L. K. (1963). Onions and Their Allies. New York: Interscience Publishers.

JONES, M. G., HUGHES, J., TREGOVA, A., MILNE, J., TOMSETT, A. B., & COLLIN, H. A. (2004). Biosynthesis of the flavour precursors of onion and garlic. Journal of Experimental Botany 55(404), 1903-1918.

JORDAN, B. R., JAMES, P. E., & MACKERNESS, S. A. H. (1998). Factors affecting UV-B-induced changes in Arabidopsis thaliana L. gene expression: the role of development, protective pigments and the chloroplast signal. Plant and Cell Physiology 39(7), 769-778.

JULKUNEN-TIITTO, R. & SORSA, S. (2001). Testing the effects of drying methods on willow flavonoids, tannins, and salicylates. Journal of Chemical Ecology 27(4), 779-789.

JUSTESEN, U., KNUTHSEN, P., & LETH, T. (1998). Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. Journal of Chromatography A 799(1-2), 101-110.

KAHKONEN, M. P., HOPIA, A. I., VUORELA, H. J., RAUHA, J. P., PIHLAJA, K., KUJALA, T. S., & HEINONEN, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry 47(10), 3954-3962.

KALT, W., FORNEY, C. F., MARTIN, A., & PRIOR, R. L. (1999). Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. Journal of Agricultural and Food Chemistry 47(11), 4638-4644.

KASPAR, K. L., PARK, J. S., MATHISON, B. D., BROWN, C. R., MASSIMINO, S., & CHEW, B. P. (2012). Processing of pigmented-flesh potatoes (Solanum tuberosum L.) on the retention of bioactive compounds. International Journal of Food Science and Technology 47(2), 376-382.

KASPAR, S., MATROS, A., & MOCK, H. P. (2010). Proteome and Flavonoid Analysis Reveals Distinct Responses of Epidermal Tissue and Whole Leaves upon UV-B Radiation of Barley (Hordeum vulgare L.) Seedlings. Journal of Proteome Research 9(5), 2402-2411.

KATSUBE, T., TSURUNAGA, Y., SUGIYAMA, M., FURUNO, T., & YAMASAKI, Y. (2009). Effect of air-drying temperature on antioxidant capacity and stability of polyphenolic compounds in mulberry (Morus alba L.) leaves. Food Chemistry 113(4), 964-969.

KEE, H. J., RYY, G. H., & PARK, Y. K. (2000). Preparation and quality properties of extruded snack using onion pomace and onion. Korean Journal of Food Science and Technology 32, 578-583.

KEINANEN, M. & JULKUNENTIITTO, R. (1996). Effect of sample preparation method on birch (Betala pendula Roth) leaf phenolics. Journal of Agricultural and Food Chemistry 44(9), 2724-2727.

KEUSGEN, M., SCHULZ, H., GLODEK, J., KREST, I., KRUGER, H., HERCHERT, N., & KELLER, J. (2002). Characterization of some Allium hybrids by aroma precursors, aroma profiles, and aillinase activity. Journal of Agricultural and Food Chemistry 50(10), 2884-2890.

KEVERS, C., FALKOWSKI, M., TABART, J., DEFRAIGNE, J. O., DOMMES, J., & PINCEMAIL, J. (2007). Evolution of antioxidant capacity during storage of selected fruits and vegetables. Journal of Agricultural and Food Chemistry 55(21), 8596-8603.

KHALLOUFI, S. & RATTI, C. (2003). Quality deterioration of freeze-dried foods as explained by their glass transition temperature and internal structure. Journal of Food Science 68(3), 892-903.

KIKUZAKI, H. (2003). Antioxidants from some tropical spices. Oriental Foods and Herbs 859, 176-189.

KIM, S. M., KUBOTA, K., & KOBAYASHI, A. (1997). Antioxidative activity of sulfur-containing flavor compounds in garlic. Bioscience Biotechnology and Biochemistry 61(9), 1482-1485.

KMI. (2012). Current climate Belgium: past months.

KMIECIK, W. & LISIEWSKA, Z. (1999). Effect of pretreatment and conditions and period of storage on some quality indices of frozen chive (Allium schoenoprasum L.). Food Chemistry 67(1), 61-66.

KNEKT, P., JARVINEN, R., REUNANEN, A., & MAATELA, J. (1996). Flavonoid intake and coronary mortality in Finland: A cohort study. British Medical Journal 312(7029), 478-481.

KOLOTA, E., ADAMCZEWSKA-SOWINSKA, K., & UKLANSKA-PUSZ, C. (2012). Yield and Nutritional Value of Japanese Bunching Onion (Allium Fistulosum L.) Depending on the Growing Season and Plant Maturation Stage. Journal of Elementology 17(4), 587-596.

KOPSELL, D. E., RANDLE, W. M., & EITEMAN, M. A. (1999). Changes in the S-alk(en)yl cysteine sulfoxides and their biosynthetic intermediates during onion storage. Journal of the American Society for Horticultural Science 124(2), 177-183.

KORUS, A. (2011). Effect of preliminary processing, method of drying and storage temperature on the level of antioxidants in kale (Brassica oleracea L. var. acephala) leaves. Lwt-Food Science and Technology 44(8), 1711-1716.

KREST, I., GLODEK, J., & KEUSGEN, M. (2000). Cysteine sulfoxides and alliinase activity of some Allium species. Journal of Agricultural and Food Chemistry 48(8), 3753-3760.

KRIS-ETHERTON, P. M., HECKER, K. D., BONANOME, A., COVAL, S. M., BINKOSKI, A. E., HILPERT, K. F., GRIEL, A. E., & ETHERTON, T. D. (2002). Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine 113, 71-88.

KRISHNAMACHARI, V., LEVINE, L. H., & PARE, P. W. (2002). Flavonoid oxidation by the radical generator AIBN: A unified mechanism for quercetin radical scavenging. Journal of Agricultural and Food Chemistry 50(15), 4357-4363.

KSOURI, R., MEGDICHE, W., FALLEH, H., TRABELSI, N., BOULAABA, M., SMAOUI, A., & ABDELLY, C. (2008). Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. Comptes Rendus Biologies 331(11), 865-873.

KUBEC, R., CODY, R. B., DANE, A. J., MUSAH, R. A., SCHRAML, J., VATTEKKATTE, A., & BLOCK, E. (2010). Applications of Direct Analysis in Real Time-Mass Spectrometry (DART-MS) in Allium Chemistry. (Z)-Butanethial S-Oxide and 1-Butenyl Thiosulfinates and Their S-(E)-1-Butenylcysteine S-Oxide Precursor from Allium siculum. Journal of Agricultural and Food Chemistry 58(2), 1121-1128.

KUBEC, R. & DADAKOVA, E. (2008). Quantitative determination of S-alk(en)ylcysteine-S-oxides by micellar electrokinetic capillary chromatography. Journal of Chromatography A 1212(1-2), 154-157.

KUBEC, R., DRHOVA, V., & VELISEK, J. (1998). Thermal degradation of S-methylcysteine and its sulfoxide - Important flavor precursors of Brassica and Allium vegetables. Journal of Agricultural and Food Chemistry 46(10), 4334-4340.

KUBEC, R., KIM, S., MCKEON, D. M., & MUSAH, R. A. (2002). Isolation of S-n-butylcysteine sulfoxide and six n-butyl-containing thiosulfinates from Allium siculum. Journal of Natural Products 65(7), 960-964.

KUBEC, R., KREJCOVA, P., SIMEK, P., VACLAVIK, L., HAJSLOVA, J., & SCHRAML, J. (2011). Precursors and Formation of Pyrithione and Other Pyridyl-Containing Sulfur Compounds in Drumstick Onion, Allium stipitatum. Journal of Agricultural and Food Chemistry 59(10), 5763-5770.

KUBEC, R., SVOBODOVA, M., & VELISEK, J. (2000). Distribution of S-alk(en)ylcysteine sulfoxides in some Allium species. Identification of a new flavor precursor: S-ethylcysteine sulfoxide (ethiin). Journal of Agricultural and Food Chemistry 48(2), 428-433.

KUCEROVA, P., KUBEC, R., SIMEK, P., VACLAVIK, L., & SCHRAML, J. (2011). Allium Discoloration: The Precursor and Formation of the Red Pigment in Giant Onion (Allium giganteum Regel) and Some Other Subgenus Melanocrommyum Species. Journal of Agricultural and Food Chemistry 59(5), 1821-1828.

KUMARI, K., MATHEW, B. C., & AUGUSTI, K. T. (1995). Antidiabetic and Hypolipidemic Effects of S-Methyl Cysteine Sulfoxide Isolated from Allium-Cepa Linn. Indian Journal of Biochemistry & Biophysics 32(1), 49-54.

KURECHI, T., KIKUGAWA, K., & KATO, T. (1980). Studies on the Antioxidants .13. Hydrogen Donating Capability of Antioxidants to 2,2-Diphenyl-1-Picrylhydrazyl. Chemical & Pharmaceutical Bulletin 28(7), 2089-2093.

KUSZNIEREWICZ, B., SMIECHOWSKA, A., BARTOSZEK, A., & NAMIESNIK, J. (2008). The effect of heating and fermenting on antioxidant properties of white cabbage. Food Chemistry 108(3), 853-861.

LABBE, D., PROVENCAL, M., LAMY, S., BOIVIN, D., GINGRAS, D., & BELIVEAU, R. (2009). The Flavonols Quercetin, Kaempferol, and Myricetin Inhibit Hepatocyte Growth Factor-Induced Medulloblastoma Cell Migration. Journal of Nutrition 139(4), 646-652.

LAMPE, J. W. (1999). Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. American Journal of Clinical Nutrition 70(3), 475S-490S.

LANCASTER, J. E. & BOLAND, M. J. (1990). Flavor biochemistry. In Alliums and allied crops (Eds H. D. Rabinowitch & J. L. Brewster), pp. 33-72: Boca Raton, FL: CRC Press.

LANCASTER, J. E. & KELLY, K. E. (1983). Quantitative-Analysis of the S-Alk(En)Yl-L-Cysteine Sulfoxides in Onion (Allium-Cepa L). Journal of the Science of Food and Agriculture 34(11), 1229-1235.

LANCASTER, J. E., REAY, P. F., MANN, J. D., BENNETT, W. D., & SEDCOLE, J. R. (1988). Quality in New-Zealand-Grown Onion Bulbs - A Survey of Chemical and Physical Characteristics. New Zealand Journal of Experimental Agriculture 16(3), 279-285.

LANCASTER, J. E. & SHAW, M. L. (1989). Gamma-Glutamyl-Transferase Peptides in the Biosynthesis of S-Alk(En)Yl-L-Cysteine Sulfoxides (Flavor Precursors) in Allium. Phytochemistry 28(2), 455-460.

LANCASTER, J. E., SHAW, M. L., & RANDLE, W. M. (1998). Differential hydrolysis of alk(en)yl cysteine sulphoxides by alliinase in onion macerates: Flavour implications. Journal of the Science of Food and Agriculture 78(3), 367-372.

LANZOTTI, V. (2006). The analysis of onion and garlic. Journal of Chromatography A 1112(1-2), 3-22.

LAVA. Het veilingsysteem in België. 2012.

LAWSON, L. D. Phytomedicines of Europe Chemistry and Biological Activity. Lawson, L. D. and Bauer, R. 176-209. 1998. Washington, DC, American Chemical Society. Garlic: a review of its medicinal effects and indicated active compounds. Lawson, L. D.

LEE, E. J., YOO, K. S., JIFON, J., & PATIL, B. S. (2009). Application of extra sulfur to high-sulfur soils does not increase pungency and related compounds in shortday onions. Scientia Horticulturae 123(2), 178-183.

LEE, J. H., LEE, S. J., PARK, S., JEONG, S. W., KIM, C. Y., JIN, J. S., JEONG, E. D., KWAK, Y. S., KIM, S. T., BAE, D. W., KIM, G. S., & SHIN, S. C. (2012). Determination of flavonoid level variation in onion (Allium cepa L.) infected by Fusarium oxysporum using liquid chromatography-tandem mass spectrometry. Food Chemistry 133(4), 1653-1657.

LEE, J. Y. & MITCHELL, A. E. (2011a). Quercetin and Isorhamnetin Glycosides in Onion (Allium cepa L.): Varietal Comparison, Physical Distribution, Coproduct Evaluation, and Long-Term Storage Stability. Journal of Agricultural and Food Chemistry 59(3), 857-863.

LEE, J. Y. & MITCHELL, A. E. (2011b). Quercetin and Isorhamnetin Glycosides in Onion (Allium cepa L.): Varietal Comparison, Physical Distribution, Coproduct Evaluation, and Long-Term Storage Stability. Journal of Agricultural and Food Chemistry 59(3), 857-863.

LEE, S. K. & KADER, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biology and Technology 20(3), 207-220.

LEE, S. U., LEE, J. H., CHOI, S. H., LEE, J. S., OHNISI-KAMEYAMA, M., KOZUKUE, N., LEVIN, C. E., & FRIEDMAN, M. (2008). Flavonoid content in fresh, home-processed, and light-exposed onions and in dehydrated commercial onion products. Journal of Agricultural and Food Chemistry 56(18), 8541-8548.

LEJA, M., MARECZEK, A., & BEN, J. (2003). Antioxidant properties of two apple cultivars during long-term storage. Food Chemistry 80(3), 303-307.

LEJA, M., MARECZEK, A., STARZYNSKA, A., & ROZEK, S. (2001). Antioxidant ability of broccoli flower buds during short-term storage. Food Chemistry 72(2), 219-222.

LEMPEREUR, I., ROUAU, X., & ABECASSIS, J. (1997). Genetic and agronomic variation in arabinoxylan and ferulic acid contents of durum wheat (Triticum durum L.) grain and its milling fractions. Journal of Cereal Science 25(2), 103-110.

LEON, H. C. L., GALDON, B. R., RODRIGUEZ, E. M. R., & ROMERO, C. D. (2009). Antioxidant capacity of different onion cultivars. Cyta-Journal of Food 7(1), 53-58.

LEUSTEK, T. & SAITO, K. (1999). Sulfate transport and assimilation in plants. Plant Physiology 120(3), 637-643.

LEWIS, S., BURMEISTER, S., & BRAZIER, J. (2005). Effect of the prebiotic oligofructose on relapse of Clostridium difficile-associated diarrhea: A randomized, controlled study. Clinical Gastroenterology and Hepatology 3(5), 442-448.

LIN, J., REXRODE, K. M., HU, F., ALBERT, C. M., CHAE, C. U., RIMM, E. B., STAMPFER, M. J., & MANSON, J. E. (2007). Dietary intakes of flavonols and flavones and coronary heart disease in US women. American Journal of Epidemiology 165(11), 1305-1313.

LIN, L. Z., LEI, F. F., SUN, D. W., DONG, Y., YANG, B., & ZHAO, M. M. (2012). Thermal inactivation kinetics of Rabdosia serra (Maxim.) Hara leaf peroxidase and polyphenol oxidase and comparative evaluation of drying methods on leaf phenolic profile and bioactivities. Food Chemistry 134(4), 2021-2029.

LOEWUS, F. A. (1999). Biosynthesis and metabolism of ascorbic acid in plants and of analogs of ascorbic acid in fungi. Phytochemistry 52(2), 193-210.

LOEWUS, F. A. & LOEWUS, M. W. (1987). Biosynthesis and Metabolism of Ascorbic-Acid in Plants. Crc Critical Reviews in Plant Sciences 5(1), 101-119.

LOMBARDO, S., PANDINO, G., IERNA, A., & MAUROMICALE, G. (2012). Variation of polyphenols in a germplasm collection of globe artichoke. Food Research International 46(2), 544-551.

LU, X. N., WANG, J., AL-QADIRI, H. M., ROSS, C. F., POWERS, J. R., TANG, J. M., & RASCO, B. A. (2011). Determination of total phenolic content and antioxidant capacity of onion (Allium cepa) and shallot (Allium oschaninii) using infrared spectroscopy. Food Chemistry 129(2), 637-644.

LUGASI, A. & HOVARI, J. (2000). Flavonoid aglycons in foods of plant origin I. Vegetables. Acta Alimentaria 29(4), 345-352.

LUNDEGARDH, B., BOTEK, P., SCHULZOV, V., HAJSLOV, J., STROMBERG, A., & ANDERSSON, H. C. (2008). Impact of different green manures on the content of S-alk(en)yl-L-cystelne Sulfoxides and l-ascorbic acid in leek (Allium porrum). Journal of Agricultural and Food Chemistry 56(6), 2102-2111.

MA, X. W., WU, H. X., LIU, L. Q., YAO, Q. S., WANG, S. B., ZHAN, R. L., XING, S. S., & ZHOU, Y. G. (2011). Polyphenolic compounds and antioxidant properties in mango fruits. Scientia Horticulturae 129(1), 102-107.

MAEDA, T., KAKUTA, H., SONODA, T., MOTOKI, S., UENO, R., SUZUKI, T., & OOSAWA, K. (2005). Antioxidation capacities of extracts from green, purple, and white asparagus spears related to polyphenol concentration. Hortscience 40(5), 1221-1224.

MAES, S., ELSEN, A., TITS, M., BOON, W., DECKERS, S., BRIES, J., VOGELS, N., & VANDENDRIESSCHE, H. (2012). Wegwijs in bodemvruchtbaarheid van de Belgische akkerbouw-en weilandpercelen (2008-2011). Bodemkundige Dienst van België.

MAGRA, T. I., BLOUKAS, J. G., & FISTA, G. A. (2006). Effect of frozen and dried leek on processing and quality characteristics of Greek traditional sausages. Meat Science 72(2), 280-287.

MAKRIS, D. P. & ROSSITER, J. T. (2000). Heat-induced, metal-catalyzed oxidative degradation of quercetin and rutin (quercetin 3-O-rhamnosylglucoside) in aqueous model systems. Journal of Agricultural and Food Chemistry 48(9), 3830-3838.

MAKRIS, D. P. & ROSSITER, J. T. (2001). Domestic processing of onion bulbs (Allium cepa) and asparagus spears (Asparagus officinalis): Effect on flavonol content and antioxidant status. Journal of Agricultural and Food Chemistry 49(7), 3216-3222.

MANACH, C., WILLIAMSON, G., MORAND, C., SCALBERT, A., & REMESY, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. American Journal of Clinical Nutrition 81(1), 230S-242S.

MANZOCCO, L., ANESE, M., & NICOLI, M. C. (1998). Antioxidant properties of tea extracts as affected by processing. Food Science and Technology-Lebensmittel-Wissenschaft & Technologie 31(7-8), 694-698.

MARAIS, E., JACOBS, G., & HOLCROFT, D. A. (2001). Colour response of 'Cripps' Pink' apples to postharvest irradiation is influenced by maturity and temperature. Scientia Horticulturae 90(1-2), 31-41.

MAROTTI, M. & PICCAGLIA, R. (2002). Characterization of flavonoids in different cultivars of onion (Allium cepa L.). Journal of Food Science 67(3), 1229-1232.

MARTIN, L. J. & MATAR, C. (2005). Increase of antioxidant capacity of the lowbush blueberry (Vaccinium angustifolium) during fermentation by a novel bacterium from the fruit microflora. Journal of the Science of Food and Agriculture 85(9), 1477-1484.

MARTINEZ-VILLALUENGA, C., PENAS, E., FRIAS, J., CISKA, E., HONKE, J., PISKULA, M. K., KOZLOWSKA, H., & VIDAL-VALVERDE, C. (2009). Influence of Fermentation Conditions on Glucosinolates, Ascorbigen, and Ascorbic Acid Content in White Cabbage (Brassica oleracea var. capitata cv. Taler) Cultivated in Different Seasons. Journal of Food Science 74(1), C62-C67.

MARTINEZ-VILLALUENGA, C., PENAS, E., SIDRO, B., ULLATE, M., FRIAS, J., & VIDAL-VALVERDE, C. (2012). White cabbage fermentation improves ascorbigen content, antioxidant and nitric oxide production inhibitory activity in LPS-induced macrophages. Lwt-Food Science and Technology 46(1), 77-83.

MASAMURA, N., YAGUCHI, S., ONO, Y., NAKAJIMA, T., MASUZAKI, S., IMAI, S., YAMAUCHI, N., & SHIGYO, M. (2011). Characterization of Amino Acid and S-alk(en)yl-L-cysteine Sulfoxide Production in Japanese Bunching Onion Carrying an Extra Chromosome of Shallot. Journal of the Japanese Society for Horticultural Science 80(3), 322-333.

MASEFIELD, G. B., WALLIS, M., HARRISON, S. G., & NICHOLSON, B. E. (1969). The oxford book of food plants. Oxford University Press.

MASRIZAL, M. A., GIRAUD, D. W., & DRISKELL, J. A. (1997). Retention of vitamin C, iron, and beta-carotene in vegetables prepared using different cooking methods. Journal of Food Quality 20(5), 403-418.

MATHEW, S. & ABRAHAM, T. E. (2008). Characterisation of ferulic acid incorporated starch-chitosan blend films. Food Hydrocolloids 22(5), 826-835.

MEJIA-MEZA, E. I., YANEZ, J. A., REMSBERG, C. M., TAKEMOTO, J. K., DAVIES, N. M., RASCO, B., & CLARY, C. (2010). Effect of Dehydration on Raspberries: Polyphenol and Anthocyanin Retention, Antioxidant Capacity, and Antiadipogenic Activity. Journal of Food Science 75(1), H5-H12.

MICHIELS, J. A., KEVERS, C., PINCEMAIL, J., DEFRAIGNE, J. O., & DOMMES, J. (2012). Extraction conditions can greatly influence antioxidant capacity assays in plant food matrices. Food Chemistry 130(4), 986-993.

MITRA, J., SHRIVASTAVA, S. L., & RAO, P. S. (2012). Onion dehydration: a review. Journal of Food Science and Technology-Mysore 49(3), 267-277.

MLADENOVIC, J. D., MASKOVIC, P. Z., PAVLOVIC, R. M., RADOVANOVIC, B. C., ACAMOVIC-DOKOVIC, G., & CVIJOVIC, M. S. (2011). Antioxidant activity of ultrasonic extracts of leek Allium porrum L. Hemijska Industrija 65(4), 473-477.

MOGREN, L. M., OLSSON, M. E., & GERTSSON, U. E. (2007a). Effects of cultivar, lifting time and nitrogen fertiliser level on quercetin content in onion(Allium cepa L.) at lifting. Journal of the Science of Food and Agriculture 87(3), 470-476.

MOGREN, L. M., OLSSON, M. E., & GERTSSON, U. E. (2007b). Quercetin content in stored onions (Allium cepa L.): effects ot storage conditions, cultivar, lifting time and nitrogen fertiliser level. Journal of the Science of Food and Agriculture 87(8), 1595-1602.

MONTI, A., AMADUCCI, M. T., PRITONI, G., & VENTURI, G. (2005). Growth, fructan yield, and quality of chicory (Cichorium intybus L.) as related to photosynthetic capacity, harvest time, and water regime. Journal of Experimental Botany 56(415), 1389-1395.

MOON, J. H., NAKATA, R., OSHIMA, S., INAKUMA, T., & TERAO, J. (2000). Accumulation of quercetin conjugates in blood plasma after the short-term ingestion of onion by women. American Journal of Physiology-Regulatory Integrative and Comparative Physiology 279(2), R461-R467.

MOON, J. S., KIM, H. D., HA, I. J., LEE, S. Y., LEE, J. T., & LEE, S. D. (2010). Chemical Component of Red Onion (Allium cepa L.) according to Cultivars and Growing Areas. Korean Journal of Horticultural Science & Technology 28(6), 921-927.

MOOR, U., POLDMA, P., TONUTARE, T., KARP, K., STARAST, M., & VOOL, E. (2009). Effect of phosphite fertilization on growth, yield and fruit composition of strawberries. Scientia Horticulturae 119(3), 264-269.

MORI, K., SUGAYA, S., & GEMMA, H. (2005). Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. Scientia Horticulturae 105(3), 319-330.

MOSTAFA, M. G., MIMA, T., OHNISHI, S. T., & MORI, K. (2000). S-allylcysteine ameliorates doxorubicin toxicity in the heart and liver in mice. Planta Medica 66(2), 148-151.

MOTA, C. L., LUCIANO, C., DIAS, A., BARROCA, M. J., & GUINE, R. P. F. (2010). Convective drying of onion: Kinetics and nutritional evaluation. Food and Bioproducts Processing 88(C2-3), 115-123.

MUIR, J. G., SHEPHERD, S. J., ROSELLA, O., ROSE, R., BARRETT, J. S., & GIBSON, P. R. (2007). Fructan and free fructose content of common Australian vegetables and fruit. Journal of Agricultural and Food Chemistry 55(16), 6619-6627.

MULLER-MOULE, P., GOLAN, T., & NIYOGI, K. K. (2004). Ascorbate-deficient mutants of arabidopsis grow in high light despite chronic photooxidative stress. Plant Physiology 134(3), 1163-1172.

MUMINOVA, B. A., BATIROV, E. K., YULDASHEV, M. P., & INAMOVA, Z. G. (2006). Kaempferol glycosedes from Allium cepa and Raphanus sativus. Chemistry of Natural Compounds 42(1), 110-111.

MURAKAMI, M., YAMAGUCHI, T., TAKAMURA, H., & MATOBA, T. (2004). Effects of thermal treatment on radical-scavenging activity of single and mixed polyphenolic compounds. Journal of Food Science 69(1), C7-C10.

NACZK, M. & SHAHIDI, F. (2004). Extraction and analysis of phenolics in food. Journal of Chromatography A 1054(1-2), 95-111.

NAYAK, B., BERRIOS, J. D. J., POWERS, J. R., TANG, J. M., & JI, Y. L. (2011). Colored Potatoes (Solanum Tuberosum L.) Dried for Antioxidant-Rich Value-Added Foods. Journal of Food Processing and Preservation 35(5), 571-580.

NAZZARO, F., FRATIANNI, F., SADA, A., & ORLANDO, P. (2008). Synbiotic potential of carrot juice supplemented with Lactobacillus spp. and inulin or fructooligosaccharides. Journal of the Science of Food and Agriculture 88(13), 2271-2276.

NEEFS, V., & MEULEMEESTER, P. (2010). Preisector hoopvol gestemd. Boer&Tuinder.

NENCINI, C., MENCHIARI, A., FRANCHI, G. G., & MICHELI, L. (2011). In vitro Antioxidant Activity of Aged Extracts of some Italian Allium Species. Plant Foods for Human Nutrition 66(1), 11-16.

NICOLI, M. C., ANESE, M., PARPINEL, M. T., FRANCESCHI, S., & LERICI, C. R. (1997). Loss and/or formation of antioxidants during food processing and storage. Cancer Letters 114(1-2), 71-74.

NINDO, C. I., SUN, T., WANG, S. W., TANG, J., & POWERS, J. R. (2003). Evaluation of drying technologies for retention of physical quality and antioxidants in asparagus (Asparagus officinalis, L.). Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology 36(5), 507-516.

NINDO, C. I. & TANG, J. (2007). Refractance window dehydration technology: A novel contact drying method. Drying Technology 25(1-3), 37-48.

NUNHEMS. (2012). Research and Innovation.

NYMAN, M. (2002). Fermentation and bulking capacity of indigestible carbohydrates: the case of inulin and oligofructose. British Journal of Nutrition 87, S163-S168.

ODRIOZOLA-SERRANO, I., HERNANDEZ-JOVER, T., & MARTIN-BELLOSO, O. (2007). Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits. Food Chemistry 105(3), 1151-1158.

OLTHOF, M. R., HOLLMAN, P. C. H., VREE, T. B., & KATAN, M. B. (2000). Bioavailabilities of quercetin-3-glucoside and quercetin-4 '-glucoside do not differ in humans. Journal of Nutrition 130(5), 1200-1203.

OU, B. X., HUANG, D. J., HAMPSCH-WOODILL, M., FLANAGAN, J. A., & DEEMER, E. K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric

reducing antioxidant power (FRAP) assays: A comparative study. Journal of Agricultural and Food Chemistry 50(11), 3122-3128.

OZGUR, M., AKPINAR-BAYIZIT, A., OZCAN, T., & YILMAZ-ERSAN, L. (2011). Effect of Dehydration on Several Physico-Chemical Properties and the Antioxidant Activity of Leeks (Allium porrum L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca 39(1), 144-151.

PAPETTI, A., DAGLIA, M., ACETI, C., QUAGLIA, M., GREGOTTI, C., & GAZZANI, G. (2006). Isolation of an in vitro and ex vivo antiradical melanoidin from roasted barley. Journal of Agricultural and Food Chemistry 54(4), 1209-1216.

PAPOULIAS, E., SIOMOS, A. S., KOUKOUNARAS, A., GERASOPOULOS, D., & KAZAKIS, E. (2009). Effects of Genetic, Pre- and Post-Harvest Factors on Phenolic Content and Antioxidant Capacity of White Asparagus Spears. International Journal of Molecular Sciences 10(12), 5370-5380.

PARVIAINEN, M. T. & NYYSSONEN, K. (1992). Ascorbic acid. In Modern Chromatographic Analysis of Vitamins (Eds A. P. D. Leenheer, W. E. Lambert, & H. Nelis). New York: Marcel Dekker.

PASTORI, G. M., KIDDLE, G., ANTONIW, J., BERNARD, S., VELJOVIC-JOVANOVIC, S., VERRIER, P. J., NOCTOR, G., & FOYER, C. H. (2003). Leaf vitamin C contents modulate plant defense transcripts and regulate genes that control development through hormone signaling. Plant Cell 15(4), 939-951.

PATIL, B. S., PIKE, L. M., & HAMILTON, B. K. (1995). Changes in Quercetin Concentration in Onion (Allium-Cepa L) Owing to Location, Growth Stage and Soil Type. New Phytologist 130(3), 349-355.

PAYET, B., SING, A. S. C., & SMADJA, J. (2005). Assessment of antioxidant activity of cane brown sugars by ABTS and DPPH radical scavenging assays: Determination of their polyphenolic and volatile constituents. Journal of Agricultural and Food Chemistry 53(26), 10074-10079.

PEREZ-GREGORIO, M. R., REGUEIRO, J., GONZALEZ-BARREIRO, C., RIAL-OTERO, R., & SIMAL-GANDARA, J. (2011). Changes in antioxidant flavonoids during freeze-drying of red onions and subsequent storage. Food Control 22(7), 1108-1113.

PEREZ-GREGORIO, R. M., GARCIA-FALCON, M. S., SIMAL-GANDARA, J., RODRIGUES, A. S., & ALMEIDA, D. P. F. (2010). Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest. Journal of Food Composition and Analysis 23(6), 592-598.

PEREZ-JIMENEZ, J., ARRANZ, S., TABERNERO, M., DIAZ-RUBIO, M. E., SERRANO, J., GONI, I., & SAURA-CALIXTO, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of. Food Research International 41(3), 274-285.

PILONSMITS, E. A. H., EBSKAMP, M. J. M., PAUL, M. J., JEUKEN, M. J. W., WEISBEEK, P. J., & SMEEKENS, S. C. M. (1995). Improved Performance of Transgenic Fructan-Accumulating Tobacco Under Drought Stress. Plant Physiology 107(1), 125-130.

PINEAU, B., LAYOUNE, O., DANON, A., & DE PAEPE, R. (2008). L-Galactono-1,4-lactone Dehydrogenase Is Required for the Accumulation of Plant Respiratory Complex I. Journal of Biological Chemistry 283(47), 32500-32505.

PINELI, L. D. D., MORETTI, C. L., DOS SANTOS, M. S., CAMPOS, A. B., BRASILEIRO, A. V., CORDOVA, A. C., & CHIARELLO, M. D. (2011). Antioxidants and other chemical and physical characteristics of two strawberry cultivars at different ripeness stages. Journal of Food Composition and Analysis 24(1), 11-16.

PLATTEAU, J., VAN GIJSEGHEM, D., & VAN BOGAERT, T. (2010). Landbouwrapport 2010. Departement Landbouw en Visserij . Brussel.

PRAJAPATI, J. B. & NAIR, B. M. (2003). The history of fermented foods. In Fermented Functional Foods (Ed E. R. Farnworth), pp. 1-25: CRC Press, Boca Raton.

PRAKASH, D., SINGH, B. N., & UPADHYAY, G. (2007). Antioxidant and free radical scavenging activities of phenols from onion (Allium cepa). Food Chemistry 102(4), 1389-1393.

PRICE, K. R., CASUSCELLI, F., COLQUHOUN, I. J., & RHODES, M. J. C. (1998). Composition and content of flavonol glycosides in broccoli florets (Brassica olearacea) and their fate during cooking. Journal of the Science of Food and Agriculture 77(4), 468-472.

PRIOR, R. L. & CAO, G. H. (1999). In vivo total antioxidant capacity: Comparison of different analytical methods. Free Radical Biology and Medicine 27(11-12), 1173-1181.

PRIOR, R. L., HOANG, H., GU, L. W., WU, X. L., BACCHIOCCA, M., HOWARD, L., HAMPSCH-WOODILL, M., HUANG, D. J., OU, B. X., & JACOB, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen

radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. Journal of Agricultural and Food Chemistry 51(11), 3273-3279.

PRIOR, R. L., WU, X. L., & SCHAICH, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53(10), 4290-4302.

PROTEGGENTE, A. R., PANNALA, A. S., PAGANGA, G., VAN BUREN, L., WAGNER, E., WISEMAN, S., VAN DE PUT, F., DACOMBE, C., & RICE-EVANS, C. A. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radical Research 36(2), 217-233.

PRZYBYLSKI, C., JUNGER, M. A., AUBERTIN, J., RADVANYI, F., AEBERSOLD, R., & PFLIEGER, D. (2010). Quantitative Analysis of Protein Complex Constituents and Their Phosphorylation States on a LTQ-Orbitrap Instrument. Journal of Proteome Research 9(10), 5118-5132.

RANDLE, W. M. (2000). Increasing nitrogen concentration in hydroponic solutions affects onion flavor and bulb quality. Journal of the American Society for Horticultural Science 125(2), 254-259.

RANDLE, W. M., LANCASTER, J. E., SHAW, M. L., SUTTON, K. H., HAY, R. L., & BUSSARD, M. L. (1995). Quantifying Onion Flavor Compounds Responding to Sulfur Fertility - Sulfur Increases Levels of Alk(En)Yl Cysteine Sulfoxides and Biosynthetic Intermediates. Journal of the American Society for Horticultural Science 120(6), 1075-1081.

RATTI, C. (2001). Hot air and freeze-drying of high-value foods: a review. Journal of Food Engineering 49(4), 311-319.

RAUTENBACH, F. & VENTER, I. (2010). Hydrophilic and lipophilic antioxidant capacity of commonly consumed South African fruits, vegetables, grains, legumes, fats/oils and beverages. Journal of Food Composition and Analysis 23(7), 753-761.

REDOVNIKOVIC, I. R., BOGOVIC, M., BELKO, D., DELONGA, K., FABEK, S., NOVAK, B., & TOTH, N. (2012). Influence of potassium fertilisation on the levels of phenolic compounds in sweet potato (Ipomoea batatas L.) leaves. Journal of Horticultural Science & Biotechnology 87(1), 47-51.

RICEEVANS, C. A., MILLER, N. J., & PAGANGA, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 20(7), 933-956.

RIJK ZWAAN. (2012). Sensational Snacks.

RITSEMA, T. & SMEEKENS, S. C. M. (2003). Engineering fructan metabolism in plants. Journal of Plant Physiology 160(7), 811-820.

ROBERTS, J. S. & KIDD, D. R. (2005). Lactic acid fermentation of onions. Lwt-Food Science and Technology 38(2), 185-190.

RODRIGUES, A. S., PEREZ-GREGORIO, M. R., GARCIA-FALCON, M. S., SIMAL-GANDARA, J., & ALMEIDA, D. P. F. (2010). Effect of post-harvest practices on flavonoid content of red and white onion cultivars. Food Control 21(6), 878-884.

RODRIGUES, A. S., PEREZ-GREGORIO, M. R., GARCIA-FALCON, M. S., SIMAL-GANDARA, J., & ALMEIDA, D. P. F. (2011). Effect of meteorological conditions on antioxidant flavonoids in Portuguese cultivars of white and red onions. Food Chemistry 124(1), 303-308.

RODRIGUEZ, H., CURIEL, J. A., LANDETE, J. M., DE LAS RIVAS, B., DE FELIPE, F. L., GOMEZ-CORDOVES, C., MANCHENO, J. M., & MUNOZ, R. (2009). Food phenolics and lactic acid bacteria. International Journal of Food Microbiology 132(2-3), 79-90.

ROMANRAMOS, R., FLORESSAENZ, J. L., & ALARCONAGUILAR, F. J. (1995). Anti-Hyperglycemic Effect of Some Edible Plants. Journal of Ethnopharmacology 48(1), 25-32.

ROSE, P., WHITEMAN, M., MOORE, P. K., & ZHU, Y. Z. (2005). Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus Allium: the chemistry of potential therapeutic agents. Natural Product Reports 22(3), 351-368.

ROY, M. K., TAKENAKA, M., & ISOBE, S. (2007). Anti-radical activity and reduces pro-oxidant activity in water-soluble fraction of selected Allium vegetables. Journal of the Science of Food and Agriculture 87(12), 2259-2265.

SAARI, N. B., FUJITA, S., MIYAZOE, R., & OKUGAWA, M. (1995). Distribution of ascorbate oxidase activities in the fruits of family cucurbitaceae and some of their properties. Journal of Food Biochemistry 19, 321-327.

SANCHEZ-MORENO, C., LARRAURI, J. A., & SAURA-CALIXTO, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture 76(2), 270-276.

SCHIEBER, A., STINTZING, F. C., & CARLE, R. (2001). By-products of plant food processing as a source of functional compounds - recent developments. Trends in Food Science & Technology 12(11), 401-+.

SELMA, M. V., ESPIN, J. C., & TOMAS-BARBERAN, F. A. (2009). Interaction between Phenolics and Gut Microbiota: Role in Human Health. Journal of Agricultural and Food Chemistry 57(15), 6485-6501.

SERRANO, J., GONI, I., & SAURA-CALIXTO, F. (2007). Food antioxidant capacity determined by chemical methods may underestimate the physiological antioxidant capacity. Food Research International 40(1), 15-21.

SERUGA, M., NOVAK, I., & JAKOBEK, L. (2011). Determination of polyphenols content and antioxidant activity of some red wines by differential pulse voltammetry, HPLC and spectrophotometric methods. Food Chemistry 124(3), 1208-1216.

SHAHIDI, F., JANITHA, P. K., & WANASUNDARA, P. D. (1992). Phenolic Antioxidants. Critical Reviews in Food Science and Nutrition 32(1), 67-103.

SICARD, D. & LEGRAS, J. L. (2011). Bread, beer and wine: Yeast domestication in the Saccharomyces sensu stricto complex. Comptes Rendus Biologies 334(3), 229-236.

SILVERTAND, B. (1996). Induction, maintenance and utilization of male sterility in leek (Allium ampeloprasum L).

SINCLAIR, P. J., BLAKENEY, A. B., & BARLOW, E. W. R. (1995). Relationships Between Bulb Dry-Matter Content, Soluble Solids Concentration and Nonstructural Carbohydrate-Composition in the Onion (Allium-Cepa). Journal of the Science of Food and Agriculture 69(2), 203-209.

SINGH, S., PANDEY, M. B., SINGH, J. P., & PANDEY, V. B. (2006). Flavonoids of Echinops echinatus. Journal of the Indian Chemical Society 83(3), 297-298.

SINGH, A. & SHUKLA, Y. (1998). Antitumor activity of diallyl sulfide in two-stage mouse skin model of carcinogenesis. Biomedical and Environmental Sciences 11(3), 258-263.

SINGLETON, V. L. & ROSSI, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture 16, 144-158.

SIVAKUMAR, P. S., PANDA, S. H., RAY, R. C., NASKAR, S. K., & BHARATHI, L. K. (2010). Consumer Acceptance of Lactic Acid-Fermented Sweet Potato Pickle. Journal of Sensory Studies 25(5), 706-719.

SLIMESTAD, R. & VAGEN, I. M. (2009). Distribution of non-structural carbohydrates, sugars, flavonols and pyruvate in scales of onions, Allium cepa L. Journal of Food Agriculture & Environment 7(3-4), 289-294.

SMIRNOFF, N. (2000). Ascorbate biosynthesis and function in photoprotection. Philosophical Transactions of the Royal Society B-Biological Sciences 355(1402), 1455-1464.

SMIRNOFF, N. & WHEELER, G. L. (2000). Ascorbic acid in plants: Biosynthesis and function. Critical Reviews in Plant Sciences 19(4), 267-290.

SOBUKOLA, O. P., DAIRO, O. U., SANNI, L. O., ODUNEWU, A. V., & FAFIOLU, B. O. (2007). Thin layer drying process of some leafy vegetables under open sun. Food Science and Technology International 13(1), 35-40.

SOININEN, T. H., JUKARAINEN, N., JULKUNEN-TIITTO, R., KARJALAINEN, R., & VEPSALAINEN, J. J. (2012). The combined use of constrained total-line-shape H-1 NMR and LC-MS/MS for quantitative analysis of bioactive components in yellow onion. Journal of Food Composition and Analysis 25(2), 208-214.

STAJNER, D., MILIC, N., CANADANOVIC-BRUNET, J., KAPOR, A., STAJNER, M., & POPOVIC, B. M. (2006). Exploring Allium species as a source of potential medicinal agents. Phytotherapy Research 20(7), 581-584.

STAJNER, D., POPOVIC, B. M., CALIC-DRAGOSAVAC, D., MALENCIC, D., & ZDRAVKOVIC-KORAC, S. (2011). Comparative Study on Allium schoenoprasum Cultivated Plant and Allium schoenoprasum Tissue Culture Organs Antioxidant Status. Phytotherapy Research 25(11), 1618-1622.

STEARN, W. T. (1978). European species of Allium and Allied genera of Alliaceae: a synonymic enumeration. Annual Musei Goulandis 4, 83-198.

STEWART, A. J., BOZONNET, S., MULLEN, W., JENKINS, G. I., LEAN, M. E. J., & CROZIER, A. (2000). Occurrence of flavonols in tomatoes and tomato-based products. Journal of Agricultural and Food Chemistry 48(7), 2663-2669.

STRATIL, P., KLEJDUS, B., & KUBAN, V. (2006). Determination of total content of phenolic compounds and their antioxidant activity in vegetables - Evaluation of spectrophotometric methods. Journal of Agricultural and Food Chemistry 54(3), 607-616.

SUNTORNSUK, L., GRITSANAPUN, W., NILKAMHANK, S., & PAOCHOM, A. (2002). Quantitation of vitamin C content in herbal juice using direct titration. Journal of Pharmaceutical and Biomedical Analysis 28(5), 849-855.

SVENSSON, L., SEKWATI-MONANG, B., LUTZ, D. L., SCHIEBER, A., & GANZLE, M. G. (2010). Phenolic Acids and Flavonoids in Nonfermented and Fermented Red Sorghum (Sorghum bicolor (L.) Moench). Journal of Agricultural and Food Chemistry 58(16), 9214-9220.

TAFULO, P. A. R., QUEIROS, R. B., DELERUE-MATOS, C. M., & SALES, M. G. F. (2010). Control and comparison of the antioxidant capacity of beers. Food Research International 43(6), 1702-1709.

THOMAS, L., LEUNG, S., CUMMING, M., SHAW, M., ALBERT, N., MCCALLUM, J., & MCMANUS, M. T. (2011). Genotypic variation in sulphur assimilation and metabolism of onion (Allium cepa L.). II: Characterisation of ATP sulphurylase activity. Phytochemistry 72(9), 888-896.

THOMPSON, R. & LOBRUTTO, R. (2007). Role of HPLC in Progress development. In HPLC for Pharmaceutical scientists (Ed John Wiley and sons), pp. 641-677. New Yersey.

TOLONEN, M., TAIPALE, M., VIANDER, B., PIHLAVA, J. M., KORHONEN, H., & RYHANEN, E. L. (2002). Plant-derived biomolecules in fermented cabbage. Journal of Agricultural and Food Chemistry 50(23), 6798-6803.

TOPUZ, A., DINCER, C., OZDEMIR, K. S., FENG, H., & KUSHAD, M. (2011). Influence of different drying methods on carotenoids and capsaicinoids of paprika (Cv., Jalapeno). Food Chemistry 129(3), 860-865.

TREUTTER, D. (2006). Significance of flavonoids in plant resistance: a review. Environmental Chemistry Letters 4(3), 147-157.

TSANGALIS, D., ASHTON, J. F., MCGILL, A. E. J., & SHAH, N. P. (2002). Enzymic transformation of isoflavone phytoestrogens in soymilk by beta-glucosidase-producing bifidobacteria. Journal of Food Science 67(8), 3104-3113.

TSOUVALTZIS, P., GERASOPOULOS, D., & SIOMOS, A. S. (2007). Effects of base removal and heat treatment on visual and nutritional quality of minimally processed leeks. Postharvest Biology and Technology 43(1), 158-164.

TSOUVALTZIS, P., SIOMOS, A. S., GERASOPOULOS, D., & BOSABALIDIS, A. M. (2010). Extension, anatomy and metabolic activity of leaves in minimally processed leek stalks. Postharvest Biology and Technology 57(3), 149-154.

TUDELA, J. A., CANTOS, E., ESPIN, J. C., TOMAS-BARBERAN, F. A., & GIL, M. I. (2002). Induction of antioxidant flavonol biosynthesis in fresh-cut potatoes. Effect of domestic cooking. Journal of Agricultural and Food Chemistry 50(21), 5925-5931.

TURKMEN, N., SARI, F., & VELIOGLU, Y. S. (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chemistry 93(4), 713-718.

U.S.DEPARTMENT OF AGRICULTURE (2010). Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods, Release 2. Nutrient Data laboratory Home .

URUSHIBARA, S., KITAYAMA, Y., WATANABE, T., OKUNO, T., WATARAI, A., & MATSUMOTO, T. (1992). New Flavonol Glycosides, Major Determinants Inducing the Green Fluorescence in the Guard-Cells of Allium-Cepa. Tetrahedron Letters 33(9), 1213-1216.

VALLEJO, F., TOMAS-BARBERAN, F. A., & FERRERES, F. (2004). Characterisation of flavonols in broccoli (Brassica oleracea L. var. italica) by liquid chromatography-UV diode-array detection-electrospray ionisation mass spectrometry. Journal of Chromatography A 1054(1-2), 181-193.

VALLVERDU-QUERALT, A., JAUREGUI, O., MEDINA-REMON, A., ANDRES-LACUEVA, C., & LAMUELA-RAVENTOS, R. M. (2010). Improved characterization of tomato polyphenols using liquid chromatography/electrospray ionization linear ion trap quadrupole Orbitrap mass spectrometry and liquid chromatography/electrospray ionization tandem mass spectrometry. Rapid Communications in Mass Spectrometry 24(20), 2986-2992.

VAN ARKEL, J., VERGAUWEN, R., SEVENIER, R., HAKKERT, J. C., VAN LAERE, A., BOUWMEESTER, H. J., KOOPS, A. J., & VAN DER MEER, I. M. (2012). Sink filling, inulin metabolizing enzymes and carbohydrate status in field grown chicory (Cichorium intybus L.). Journal of Plant Physiology 169(15), 1520-1529.

VAN BOEKEL, M., FOGLIANO, V., PELLEGRINI, N., STANTON, C., SCHOLZ, G., LALLJIE, S., SOMOZA, V., KNORR, D., JASTI, P. R., & EISENBRAND, G. (2010). A review on the beneficial aspects of food processing. Molecular Nutrition & Food Research 54(9), 1215-1247.

VAN DEN ELZEN, G. (2012). Producten.

VAN DER MEER, Q. P. & HANELT, P. (1990). Leek (Allium ampeloprasum). In Onions and Allied Crops, vol III. Biochemistry, Food Science, and Minor Crops (Eds J. L. Brewster & H. D. Rabinowitch), pp. 179-196. Boca Raton: CRC Press.

VAN LOO, J., CLUNE, Y., BENNETT, M., & COLLINS, J. K. (2005). The SYNCAN project: goals, set-up, first results and settings of the human intervention study. British Journal of Nutrition 93, S91-S98.

VAN PAMEL, E., VERBEKEN, A., VLAEMYNCK, G., DE BOEVER, J., & DAESELEIRE, E. (2011). Ultrahigh-Performance Liquid Chromatographic-Tandem Mass Spectrometric Multimycotoxin Method for Quantitating 26 Mycotoxins in Maize Silage. Journal of Agricultural and Food Chemistry 59(18), 9747-9755.

VANDEKINDEREN, I., VAN CAMP, J., DEVLIEGHERE, F., RAGAERT, P., VERAMME, K., BERNAERT, N., DENON, Q., & DE MEULENAER, B. (2009). Evaluation of the use of decontamination agents during fresh-cut leek processing and quantification of their effect on its total quality by means of a multidisciplinary approach. Innovative Food Science & Emerging Technologies 10(3), 363-373.

VANDOORNE, B., MATHIEU, A. S., VAN DEN ENDE, W., VERGAUWEN, R., PERILLEUX, C., JAVAUX, M., & LUTTS, S. (2012). Water stress drastically reduces root growth and inulin yield in Cichorium intybus (var. sativum) independently of photosynthesis. Journal of Experimental Botany 63(12), 4359-4373.

VIJN, I. & SMEEKENS, S. (1999). Fructan: More than a reserve carbohydrate? Plant Physiology 120(2), 351-359.

VINSON, J. A., HAO, Y., SU, X. H., & ZUBIK, L. (1998). Phenol antioxidant quantity and quality in foods: Vegetables. Journal of Agricultural and Food Chemistry 46(9), 3630-3634.

VINSON, J. A., PROCH, J., & BOSE, P. (2001). Determination of quantity and quality of polyphenol antioxidants in foods and beverages. Flavonoids and Other Polyphenols 335, 103-114.

VLAM. (2011). Het groente- en fruitverbruik gestegen ondanks hogere prijs.

VLM. (2012). Vlaamse Landmaatschappij: Regelgeving. VLM.

WALTON, N. J., NARBAD, A., FAULDS, C. B., & WILLIAMSON, G. (2000). Novel approaches to the biosynthesis of vanillin. Current Opinion in Biotechnology 11(5), 490-496.

WANG, S. Y. & ZHENG, W. (2001). Effect of plant growth temperature on antioxidant capacity in strawberry. Journal of Agricultural and Food Chemistry 49(10), 4977-4982.

WANGCHAROEN, W. & MORASUK, W. (2009). Effect of heat treatment on the antioxidant capacity of garlic. Maejo International Journal of Science and Technology 3(1), 60-70.

WATERMAN, P. G. & MOLE, S. (1994). Analysis of Phenolic plant metabolites. Oxford: Blackwell Scientific Publications.

WHEELER, G. L., JONES, M. A., & SMIRNOFF, N. (1998). The biosynthetic pathway of vitamin C in higher plants. Nature 393(6683), 365-369.

WICZKOWSKI, W., ROMASZKO, J., BUCINSKI, A., SZAWARA-NOWAK, D., HONKE, J., ZIELINSKI, H., & PISKULA, M. K. (2008). Quercetin from shallots (Allium cepa L. var. aggregatum) is more bioavailable than its glucosides. Journal of Nutrition 138(5), 885-888.

WILLIAMSON, G., PLUMB, G. W., UDA, Y., PRICE, K. R., & RHODES, M. J. C. (1996). Dietary quercetin glycosides: Antioxidant activity and induction of the anticarcinogenic phase II marker enzyme quinone reductase in Hepalclc7 cells. Carcinogenesis 17(11), 2385-2387.

WINKEL-SHIRLEY, B. (2001). Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiology 126(2), 485-493.

WOO, K. S., HWANG, I. G., KIM, T. M., KIM, D. J., HONG, A. T., & JEONG, H. S. (2007). Changes in the antioxidant activity of onion (Allium cepa) extracts with heat treatment. Food Science and Biotechnology 16(5), 828-831.

WOUTERS, D. (2013). Species diversity, community dynamics and metabolite target analysis of Romanian vegetable and Belgian leek fermentations. Vrije Universiteit Brussel.

WU, X. L., BEECHER, G. R., HOLDEN, J. M., HAYTOWITZ, D. B., GEBHARDT, S. E., & PRIOR, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. Journal of Agricultural and Food Chemistry 52(12), 4026-4037.

XIAO, D. H., PINTO, J. T., GUNDERSEN, G. G., & WEINSTEIN, I. B. (2005). Effects of a series of organosulfur compounds on mitotic arrest and induction of apoptosis in colon cancer cells. Molecular Cancer Therapeutics 4(9), 1388-1398.

XU, B. J. & CHANG, S. K. C. (2008). Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. Food Chemistry 110(1), 1-13.

YAMAZAKI, Y., IWASAKI, K., MIKAMI, M., & YAGIHASHI, A. (2011). Distribution of Eleven Flavor Precursors, S-Alk(en)yl-L-Cysteine Derivatives, in Seven Allium Vegetables. Food Science and Technology Research 17(1), 55-62.

YANG, B., HALTTUNEN, T., RAIMO, O., PRICE, K., & KALLIO, H. (2009). Flavonol glycosides in wild and cultivated berries of three major subspecies of Hippophae rhamnoides and changes during harvesting period. Food Chemistry 115(2), 657-664.

YANG, E. J., KIM, S. I., PARK, S. Y., BANG, H. Y., JEONG, J. H., SO, J. H., RHEE, I. K., & SONG, K. S. (2012). Fermentation enhances the in vitro antioxidative effect of onion (Allium cepa) via an increase in quercetin content. Food and Chemical Toxicology 50(6), 2042-2048.

YE, X. D., WU, X. L., ZHAO, H., FREHNER, M., NOSBERGER, J., POTRYKUS, I., & SPANGENBERG, G. (2001). Altered fructan accumulation in transgenic Lolium multiflorum plants expressing a Bacillus subtilis sacB gene. Plant Cell Reports 20(3), 205-212.

YIN, M. C., HWANG, S. W., & CHAN, K. C. (2002). Nonenzymatic antioxidant activity of four organosulfur compounds derived from garlic. Journal of Agricultural and Food Chemistry 50(21), 6143-6147.

YOO, K. S., LEE, E. J., & PATIL, B. S. (2012). Changes in Flavor Precursors, Pungency, and Sugar Content in Short-Day Onion Bulbs during 5-Month Storage at Various Temperatures or in Controlled Atmosphere. Journal of Food Science 77(2), C216-C221.

YOO, K. S. & PIKE, L. M. (1998). Determination of flavor precursor compound S-alk(en)yl-L-cysteine sulfoxides by an HPLC method and their distribution in Allium species. Scientia Horticulturae 75(1-2), 1-10.

YOUSIF, A. N., SCAMAN, C. H., DURANCE, T. D., & GIRARD, B. (1999). Flavor volatiles and physical properties of vacuum-microwave- and air-dried sweet basil (Ocimum basilicum L.). Journal of Agricultural and Food Chemistry 47(11), 4777-4781.

ZHAN, L. J., HU, J. Q., AI, Z. L., PANG, L. Y., LI, Y., & ZHU, M. Y. (2013). Light exposure during storage preserving soluble sugar and L-ascorbic acid content of minimally processed romaine lettuce (Lactuca sativa L.var. longifolia). Food Chemistry 136(1), 273-278.

ZHOU, Y., ZHUANG, W., HU, W., LIU, G. J., WU, T. X., & WU, X. T. (2011). Consumption of Large Amounts of Allium Vegetables Reduces Risk for Gastric Cancer in a Meta-analysis. Gastroenterology 141(1), 80-89.

ZILL-E-HUMA, VIAN, M. A., FABIANO-TIXIER, A. S., ELMAATAOUI, M., DANGLES, O., & CHEMAT, F. (2011). A remarkable influence of microwave extraction: Enhancement of antioxidant activity of extracted onion varieties. Food Chemistry 127(4), 1472-1480.

CURRICULUM VITAE

PERSONALIA

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EDUCATION

2013	**Particle Characterization,** Belgian Particle, Colloid & Interface Society, Ghent
2010	**Analysis of Variance**, IPVW, Ghent **Quality Research Skills,** doctoral schools, University Ghent
2009	**Metabolomics**, University Ghent
2003-2008	**Master Bio-Engineering, option Chemistry,** University Ghent**,** Faculty of Bioscience Engineering Dissertation: '*Het optimaliseren van nieuwe decontaminatietechnieken naar decontaminatie-effeciëntie, nutritionele kwaliteit en chemische veiligheid*' (Promotoren: Prof. Dr. ir. John Van Camp, Prof. Dr. ir. Bruno De Meulenaer, Prof. Dr. ir. Frank De Vlieghere Department of Food Safety and Food Quality)
1998-2003	**Secondary school, Science-Mathematics**, Don Boscocollege, Zwijnaarde

PROFESSIONAL CAREER

2008-2012	Institute for Agricultural and Fisheries Research (ILVO) Technology and Food Science Unit Product Quality and Innovation Function: PhD student (scholarship granted by IWT)

PUBLICATIONS

Peer review

Bernaert, N., De Paepe, D., Bouten, C., De Clercq, H., Stewart D., Van Bockstaele, E., De Loose, M. and Van Droogenbroeck, B. (2012). Antioxidant capacity, total phenolic and ascorbate content as a function of the genetic diversity of leek (*Allium ampeloprasum* var. *porrum*). Food Chemistry, 134, 669-677.

Bernaert, N., Goetghebeur, L., De Clercq, H., De Loose, M., Daeseleire, E., Van Pamel, E., Van Bockstaele, E. and Van Droogenbroeck, B. (2012). S-alk(en)yl-L-cysteine sulfoxides as a function of the genetic diversity and maturity of leek (*Allium ampeloprasum* var. *porrum*). Journal of Agricultural and Food Chemistry, 60, 10910-10919.

Bernaert, N., Wouters, D., De Vuyst, L., De Paepe, D., De Clercq, H., Van Bockstaele, E. De Loose, M. and Van Droogenbroeck, B. (2013). Antioxidant changes during spontaneous fermentation of the white shaft and green leaves of leek (*Allium ampeloprasum* var. *porrum*). Journal of The Science of Food and Agriculture, doi: 10.1002/jsfa.6020.

Bernaert, N., De Clercq, H., Van Bockstaele, E., De Loose, M. and Van Droogenbroeck, B. Antioxidant changes during post-harvest processing and storage of leek (*Allium ampeloprasum* var. *porrum*). Accepted with revisions in Postharvest Biology and Technology

Wouters, D., **Bernaert, N.,** Conjaerts, W., Van Droogenbroeck, B., De Loose, M., and De Vuyst, L. (2013). Species diversity, community dynamics, and metabolite kinetics of spontaneous leek fermentations. Food Microbiology, 33, 185-196.

Wouters, D., **Bernaert, N.,** Anno, N., Van Droogenbroeck, B., De Loose, M., Van Bockstaele, E., and De Vuyst, L. (Unpublished results). Application and validation of autochthonous lactic acid bacteria starter cultures for controlled leek fermentations and their influence on the antioxidant properties of leek (*Allium ampeloprasum* var. *porrum*), Submitted in Food Microbiology.

Vandekinderen, I., Van Camp, J., Devlieghere, F., Veramme, K., **Bernaert, N.,** Denon, Q., Ragaert, P. and De Meulenaer, B. (2009). Effect of decontamination on the microbial load, the sensory quality and the nutrient retention of ready-to-eat white cabbage. European Food Research and Technology, 229, 443-455.

Vandekinderen, I., Van Camp, J., Devlieghere, F., Ragaert, P., Veramme, K., **Bernaert, N.,** Denon, Q., and De Meulenaer, B. (2009). Evaluation of the use of decontamination agents during fresh-cut leek processing and quantification of their effect on its total quality by means of a multidisciplinary approach; Innovative Food Science & Emerging Technologies, 10, 363-373.

Vandekinderen, I., Van Camp, J., De Meulenaer, B., Veramme, K., **Bernaert, N.,** Denon, Q., Ragaert, P., and Devlieghere, F. (2009). Moderate and high doses of sodium hypochlorite, neutral electrolyzed oxidizing water, peroxyacetic acid and gaseous chlorine dioxide did not affect the nutritional and sensory qualities of fresh-cut iceberg lettuce (Lactuca sativa Var. capitata L.) after washing. Journal of Agricultural and Food Chemistry, 57, 4195-4203.

Publication in conference proceedings

Bernaert, N., Van Droogenbroeck, B., Bouten, C., De Paepe, D., Van Bockstaele, E., De Clercq, H., Stewart, D., De Loose, M. (2010). The antioxidant capacity of leek (Allium ampeloprasum var. porrum). Communications in Agricultural and Applied Biological sciences, 75

National magazines

Proeftuinnieuws (2010) "Prei: lekker en goed voor uw gezondheid" (PCG-ILVO)

Groene Kring Tijdschrift (2013): "Valorisatie van preinevenstromen"

INTERNATIONAL CONFERENCES

Determination of bioactive compounds in leek (*Allium ampeloprasum* var. *porrum*) (poster)

Trends in Food Analyses, Ghent, May 2009

Determination of bioactive compounds in leek (*Allium ampeloprasum* var. *porrum*) (lecture)
Future Trends in Phytochemistry in the Global Era of Agri-food and Health, Murcia, Spain, May 2009

Determination of bioactive compounds in leek (*Allium ampeloprasum* var. *porrum*) (lecture)
PhD symposium James Hutton Institute, Dundee
January 2010

Determination of bioactive compounds in leek (*Allium ampeloprasum* var. *porrum*) (poster)
Exchange conference of feed and food, Ghent, Belgium, September 2010

Identification of Flavonoid Glycosides and Phenolic Acids in Leek (*Allium ampeloprasum* var. *porrum*) and Related Species (poster)
Mass spectrometry in food and feed, Merelbeke, Belgium, 9 June 2011

Antioxidant Capacity of Different Leek Types and the Comparison with its Related Species (Lecture)
6th symposium on Edible Alliaceae, Fukuoka, Japan, 21-25 May 2012

INTERNATIONAL MOBILITY

U-HPLC-Orbitrap-MS analyses.
James Hutton Institute, April 2009, June 2010 and June 2011.

PROMOTORSHIP

Dissertations

Charlotte Bouten: Bepaling van bioactieve componenten in functie van de genetische diversiteit van *Allium porrum* L.
Master in de biowetenschappen: voedingsindustrie. Departement Biowetenschappen en Landschapsarchitectuur, Hogeschool Gent, 2009-2010.

Sophie Van Ranst: Bepaling van bioactieve componenten in functie van de genetische diversiteit van *Allium porrum* L.
Master in de biowetenschappen: voedingsindustrie. Departement Biowetenschappen en Landschapsarchitectuur, Hogeschool Gent, 2010-2011.

Nele Compernol en Brigitte De Ryck: Onderzoek naar de consumptie en het gebruik van prei in Vlaanderen en ontwikkeling van alternatieve receptuur.
Professionele Bachelor Voedings- en dieetkunde, Katholieke Hogeschool Sint-Lieven, 2010-2011.

Bert Michels: Antioxidanten in prei: analyse in functie van oogsttijdstip.
Professionele Bachelor Biomedische laboratoriumtechnologie, afstudeerrichting farmaceutische en biologische laboratoriumtechnologie, Katholieke Hogeschool Sint-Lieven, 2010-2011.

Lien Goetghebeur: Bioactieve componenten in functie van de genetische diversiteit, oogsttijdstip en verwerking in *Allium porrum* L.
Master in de biowetenschappen: voedingsindustrie. Departement Biowetenschappen en Landschapsarchitectuur, Hogeschool Gent, 2011-2012.

Nick Glorieux: bepaling van zwavelverbindingen in prei.
Katholieke Hogeschool Zuid-West Vlaanderen, 2011-2012

Liesbeth Colpaert: Bioactieve componenten in prei (*Allium porrum* L.): Analyses in functie van de verwerking.
Professionele Bachelor Biomedische laboratoriumtechnologie, afstudeerrichting farmaceutische en biologische laboratoriumtechnologie, Katholieke Hogeschool Sint-Lieven, 2011-2012.

Shari Monival en Sara Ratajczak: De valorisatie van reststromen in de agrovoedingsindustrie: een inventarisatie van projecten binnen onderzoeksinstellingen.
Professionele Bachelor Voedings- en dieetkunde, Katholieke Hogeschool Leuven, 2011-2012.

Project:

Vincent Batjoens, Annabelle Cassiman, Grégory Lekeux en Dieter Vermeir: Project voeding: Ontwikkelin en optimalisatie van preibrood en-pistolets
Bachelor Agro-en Biotechnologie, Departement Biowetenschappen en Landschapsarchitectuur, Hogeschool Gent, 2010-2011.

Stage:

Dries Segers: Totaal fenol-, flavonoid- en pyruvaatgehalte van *Allium porrum* L. (prei) in functie van genetische diversiteit
Master in bio-ingenieurswetenschappen, Universiteit Ghent, 2010.

AWARDS

ECOTROPHELIA competition (Fevia Vlaanderen): second prize ‘pretkroket, 2012