

*“Success consists of going from failure to failure without loss of enthusiasm”*

Winston Churchill



# **CHARACTERIZATION AND PROCESSING OF HORTICULTURAL BYPRODUCTS: A CASE STUDY OF TOMATO AND BELGIAN ENDIVE ROOTS**

IR. LIES KIPS

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



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Karakterisatie en verwerking van tuinbouwreststromen: toegepast op tomaat en witloofwortel

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## Thank you!

Performing this PhD can be compared to riding an intensive cycling tour. It takes a great amount of courage and perseverance and one cannot do it alone. For a kick-start, a great amount of enthusiasm and optimism was necessary. 175 kilometers and ain't no mountain high enough, Bart! To be able to continue for quite some time, a strict schedule and a critical but fair voice to help keeping course was crucial. It must be said, Katleen was a natural. When the kilometers were passing by, Els sacrificed to keep focus as a true guardian of accuracy with a great, absurd sense of humor. Domien and Nathalie generated the necessary slipstream to relieve the legs from time to time without slowing down. After 2 massive cols, one started to wonder about the exact reason of this insane course. Then, Marc came in the picture with a pep-talk and enormous amount of confidence and trust in the good result. 90 kilometers to go. To divert one's mind from sore legs, I could recommend Marjolein. With her exceptional level of activity and her (almost ever-lasting) happiness, she is just one indispensable element to complete the course. In order to stay focused, hold a regular pace and see how others are coping with the hard work. Here, the laboratory staff led the way, thank you all! Far over half way and still standing. This must be the result of regular food injections and confidence boosts. Keep up the good work, Grietje & Roger! "Are you under stress?", "Do you think you can make it?", "How do you estimate your chance of winning?". If not yet the case, one would become stressed. The only way to cope with this was to remain calm and have faith. All of this, coated with a good portion of "joy de vivre" was kindly provided by Guido and Lutje. The last 50 kilometers and doing great. Let's think about something else. Ward, Marie, Julie, Jan, Jelke, Myriam, Lise, Laura, Hanne, Melissa, Jessie, Nele, Julie, Lien, Valerie and Jo are all aboard to divert one's mind. Now, it's all about holding the line. One last esthetic discussion with Jana and a final critical assessment of the final kilometers with Julie L. Along comes my loyal, stubborn helper to complete those last and toughest kilometers. Jonas is blessed with an extremely regular climbing pace, the capacity to take an extraordinary amount of mood swings, a motivational tongue and an indispensable strategic perspective. There he is, the finish, on top of the mountain. Hurray, we made it, all together!! Het is gebeurd! Thank you all!!





## Summary

Both from an economic as well as from an environmental and social perspective, efficient reutilization of horticultural byproducts is necessary. This is in accordance with the vision of the circular economy that strongly encourages the food supply chain, amongst others, to upgrade their low-quality byproducts. Whereas this concept holds strong in theory, its translation in practice is hampered today. The aim in this dissertation was to facilitate and optimize this valorization.

In Chapter 1, horticultural byproducts were classified and their amounts estimated. This was coupled to literature data to identify their potential. Major conclusions are: (i) horticultural byproducts occur from many different crops under many different forms at different stages in the supply chain, (ii) based on their composition, they have great potential to be used in various industries but (iii) there are some traits hindering their practical valorization such as high moisture content, geographical and seasonal occurrence, difficult collectability and lack of purity. The choice for a specific type or source of byproduct can already partly alleviate some of these traits thus increasing the chances for successful valorization. For example, byproducts generated at the produce auctions and during food processing are characterized by a lower geographical spread, less collectability issues and little lack of purity. Therefore, in the first part of this dissertation (Chapter 2, 3 and 4), tomato surplus products occurring at the produce auctions were chosen as model crop to investigate valorization. Some other traits are however inherently present in almost all horticultural byproducts and still pose practical challenges for valorization, requiring a processing strategy appropriately tuned on these traits. Hence, a processing technology was proposed and evaluated for its suitability for valorizing horticultural byproducts.

Horticultural byproducts are characterized by a high moisture content, making them susceptible to a rapid deterioration. Therefore, a pressing technology was proposed to process the moist byproducts in liquid (juice) and solid (press residue) fractions and hence allow for their subsequent valorization. This is in accordance with the biorefinery principle, aiming for the entire biomass use and avoiding the production of residues. Furthermore, three additional constraints were imposed on this pressing technology in order to increase the chances of successful valorization towards food, which was primarily targeted, in line with the cascade principle (OVAM, 2012b; 2015). Firstly, the nature of horticultural byproducts, i.e. their relatively small and geographically dispersed volumes and the seasonality of their production can obstruct the feasibility of the valorization process. Therefore, a technology, flexible towards type and amount of input byproducts is necessary (Budzianowski & Postawa, 2016; Fava et al., 2015; Lin et al., 2014; Matharu et al., 2016). Secondly, consumers increasingly demand attractive products (i.e. products with attractive color, appearance and taste)

which thirdly, maximally retain the naturalness of the fruits and vegetables. Hence, a technology with a low process impact that is able to meet these demands is expected to generate products that are competitive and lead to a successful valorization of horticultural byproducts. Based on these characteristics, the spiral-filter press was proposed and evaluated in the first part of this dissertation for its ability to process horticultural byproducts (Chapters 2, 3 and 4).

### **I. Process optimization (Chapter 2)**

Using surplus tomatoes as a feedstock, insight in the working principle of the spiral-filter press was gained by changing the process parameters and evaluating their effect on the process performance and juice characteristics. A high juice yield was used as a primary criterion to optimize the solid-liquid separation (further also denoted by filtration or pressing). The results indicated that in case of soft berry-like matrices such as tomato, this juice yield could be maximized by using a high spiral and high vacuum frequency, whereas for more liquid products (e.g. after thermal pretreatment), it was found crucial to use a spiral with less steep channels. In addition to the juice yield, also the turbidity and the precipitate weight ratio were studied, allowing tuning of the process parameters in function of the output products. It was found that the production of cloudy juices with a large concentration of solid particles were promoted by the use of large screen sizes and high vacuum pump frequencies. Besides changing the process parameters, the introduction of a thermal pretreatment was found to further augment the juice yield and thus the separation of solid and liquid fractions. This resulted in a press residue almost only consisting of seeds and skins, which could be separated from each other, thereby increasing the valorization potential of both fractions. These observations were coupled to physical insights to improve understanding of the working principle of the technology and allow to tune the process parameters in function of different input streams.

### **II. Process impact on physical characteristics of the output products (Chapter 3)**

Focusing on the liquid tomato juice resulting from the optimized process in Chapter 2, the physical juice stability and related quality attributes were investigated in Chapter 3. Stable juices are highly demanded by consumers but are often difficult to obtain in practice. It was investigated whether a stable juice could be produced using the spiral-filter press and how different process parameters influence the water-insoluble solid characteristics known to influence this stability. The results showed that fast processing with a large filter size and maximum vacuum allowed the production of a tomato juice, stable for over 170 days. However, using exactly the same processing conditions on another variety rendered the juice unstable, illustrating that changing the process parameters alone is not sufficient to control juice stability. In order to fully understand the influence of process variables and

variety on the physical stability, this issue should be subjected to further investigation for example via the measurement of enzymatic activity or investigation of the particle interactions.

### **III. Process impact on chemical characteristics of the output products (Chapter 4)**

In Chapter 2, the antioxidative capacity was found to be preserved throughout processing which suggested a low process impact of the spiral-filter press. However, a more detailed investigation was necessary to determine the fate of the individual phenolic compounds, carotenoids and ascorbic acid content. These results are described in Chapter 4. The results showed that the ascorbic acid content was retained during spiral-filter processing. The weighed retention efficiency of the phenolic compounds and carotenoids was  $88 \% \pm 8 \%$  and  $122 \% \pm 15 \%$  respectively, confirming a low process impact of the spiral-filter press and illustrating its potential to maintain the native constitution of the tomatoes to a high extent. The distribution of compounds in the different fractions was also investigated, showing an enrichment of both phenolic compounds (on average 5 times) and carotenoids (on average 2.5 times) in the press residue compared to the mashed tomato. During thermal downstream processing of the tomato juice, the carotenoid content significantly decreased. Future research efforts should thus investigate (i) coupling the spiral-filter press to alternative, innovative mild processing technologies to conserve the high-quality of the spiral-filter-processed products as well as (ii) the valorization opportunities of the press residue, as the higher concentration of phytochemicals in this fraction was clearly shown.

Besides practical characteristics such as the high moisture content and the seasonal occurrence, hindering the valorization of horticultural byproducts, another aspect was found to impede their large scale valorization, i.e. the lack of knowledge on the composition of some horticultural byproducts. This was investigated in the second part of this dissertation (Chapter 5). This issue was addressed for forced Belgian endive roots (*Cichorium intybus* L. var. *foliosum*). Approximately 36,000 tonnes of these roots occur yearly in Belgium during the production of Belgian endive chicons. This byproduct is most often locally used as feed, however investigating their composition can lead to higher added-value applications in products such as food, pharmaceuticals or biocides.

### **IV. Profiling of forced Belgian endive roots (Chapter 5)**

Insight in the composition of forced Belgian endive roots was obtained by investigating their content of sesquiterpene lactones (bitter compounds) and phenolic compounds as well as their antioxidative capacity and elemental composition. The composition of 5 different Belgian endive cultivars and industrial chicory (*Cichorium intybus* L. var. *sativum*) was compared and forced roots were compared to non-forced roots, stored roots and chicons. The results indicate that the forced roots are enriched in sesquiterpene lactones compared to the non-forced roots and the chicons. The major phenolic

compound (chlorogenic acid) was present in levels about twice as high in the roots compared to the chiccons and the antioxidative capacity significantly increased upon forcing. Finally, the forced roots were found to contain predominantly K, P, Cl and Ca and appear to contain sufficient amounts of Fe and Cu to meet the requirements for the food label 'source of'. These findings illustrate the potential of forced Belgian endive roots for further valorization. However, a large variability of type and concentration of the measured phytochemicals was found in function of different cultivars. This should be taken into account when using this knowledge as a basis for further investigation of potential valorizations. Future research should focus on investigating the functionality and also the toxicity of the derived extracts or products as well as on the pilot-scale valorization of these roots.

In conclusion, efficient reutilization of horticultural byproducts is necessary, but the translation in practice is being hampered today. Two aspects that hinder this translation were identified i.e. (i) the need to cope with moist byproducts, occurring scattered, both in time and space and (ii) the lack of knowledge on the composition of some byproducts. These issues are addressed by two different approaches on two different model crops. As a result, the spiral-filter press was found suitable to valorize horticultural byproducts as it was able to process different types of moist byproducts with a low process impact on their phytochemical content. In future research, the downstream processing should be optimized in function of maintaining this low process impact throughout the whole process line. The forced Belgian endive roots were investigated to tackle the second issue concerning the lack of knowledge on the composition of byproducts. These roots were shown to be enriched in sesquiterpene lactones and phenolic compounds and based on literature, these compounds appear to be promising for application in the food, pharmaceutical and biocide sector. This detailed characterization of the composition can thus be used as starting point for further product development. The results are discussed in a bioeconomy context and critically discussed from a broader technical and socio-economic perspective in the reflective discussion (Chapter 6).

This dissertation thus clearly shows the possibilities of horticultural byproducts and accordingly the opportunities for their valorization. On the other hand, it indicates the complexity of valorizing horticultural byproducts and the necessity for transdisciplinary approaches. Today, we are only at the beginning and much work is still to be done. However, the interest keeps growing and the route is being paved.

## Samenvatting

Efficiënte valorisatie van tuinbouwreststromen is cruciaal vanuit economisch, ecologisch en sociaal perspectief. Dit komt overeen met de visie van de circulaire economie die onder meer de valorisatie van reststromen uit de voedingsketen stimuleert. Ondanks het feit dat dit concept in theorie mooi klinkt, blijkt de praktische vertaling moeilijk op de dag van vandaag. De doelstelling in dit doctoraat is om deze valorisatie te faciliteren en te optimaliseren.

In Hoofdstuk 1 werden tuinbouwreststromen geclassificeerd en werden hun hoeveelheden geschat. Dit werd gekoppeld aan literatuurdata om hun potentiële meerwaarde te identificeren. De voornaamste conclusies waren: (i) tuinbouwreststromen ontstaan bij verschillende gewassen onder diverse vormen doorheen verschillende stadia in de supply chain, (ii) hun samenstelling wijst op hun potentieel om gevaloriseerd te worden in diverse industrieën, maar (iii) ze worden gekarakteriseerd door een aantal eigenschappen die de praktische valorisatie verhinderen zoals hoge vochtinhoud, verspreide en seizoensale beschikbaarheid, moeilijke inzameling en gebrek aan zuiverheid. De keuze voor een specifieke reststroom of voor een specifiek type reststromen kan sommige van deze eigenschappen reeds deels wegnemen en dus de kansen voor succesvolle valorisatie verhogen. Bijvoorbeeld, reststromen die voorkomen op de veilingen en in voedselverwerkende bedrijven worden gekenmerkt door een kleinere geografische spreiding en minder problemen met inzameling en gebrek aan zuiverheid. Daarom werden in dit doctoraat de surplus tomaten op de veilingen gekozen als modelgewas om valorisatie te onderzoeken. Sommige eigenschappen zijn echter aanwezig in alle reststromen en vormen dus praktische uitdagingen voor valorisatie. Dit vereist een strategie die goed afgestemd is op deze eigenschappen. In dit doctoraat werd bijgevolg een veelbelovende technologie voorgesteld welke geëvalueerd werd op zijn capaciteit om tuinbouwreststromen te valoriseren.

Tuinbouwreststromen worden gekenmerkt door een hoog vochtgehalte, wat hen gevoelig maakt voor rottingsverschijnselen. Daarom werd een perstechnologie voorgesteld die vochtige reststromen perst in vloeibare (sap) en vaste (persresidu) fracties en hun verdere valorisatie mogelijk maakt. Dit past in het bioraffinage principe waarin gestreefd wordt om de volledige biomassa te gebruiken en de productie van reststromen te minimaliseren. Verder werden drie extra voorwaarden opgelegd aan deze perstechnologie om de kansen op succesvolle valorisatie richting voeding te verhogen, in lijn met het cascade principe (OVAM, 2012b; 2015). Ten eerste kunnen de relatief kleine hoeveelheden die geografisch verspreid zijn en seizoensaal voorkomen, de haalbaarheid van het valorisatieproces van tuinbouwreststromen bemoeilijken. Daarom is een technologie nodig die diverse types en hoeveelheden aan biomassa kan verwerken (Budzianowski & Postawa, 2016; Fava et al., 2015; Lin et

al., 2014; Matharu et al., 2016). Ten tweede vragen consumenten steeds meer naar aantrekkelijke producten (i.e. producten met een aantrekkelijk kleur, uitzicht en smaak) welke ten derde de natuurlijkheid van de groenten en fruit maximaal bewaren. Daarom is een technologie nodig met een beperkte procesimpact die competitieve producten kan genereren en tot een succesvolle valorisatie van tuinbouwreststromen kan leiden. Op basis van deze eigenschappen werd de spiraalfilterpers voorgesteld, welke geëvalueerd werd op zijn capaciteit om tuinbouwreststromen te valoriseren.

### **I. Procesoptimalisatie (Hoofdstuk 2)**

Gebruik makend van surplus tomaten werd inzicht in het werkingsprincipe van de spiraalfilterpers verkregen door het aanpassen van procesparameters en het evalueren van hun effect op de performantie van het proces en de eigenschappen van het sap. Een groot saprendement werd gebruikt als primair criterium om de fasescheiding in vloeibare en vaste fracties te optimaliseren. De resultaten geven aan dat in het geval van zachte bes-achtige structuren zoals tomaat, dit saprendement gemaximaliseerd kon worden door het gebruik van een hoge spiraal- en vacuümfrequentie, terwijl het voor meer vloeibare producten (vb. na thermische behandeling) belangrijker is om een spiraal te gebruiken met minder steile kanalen. Naast het saprendement werden ook de turbiditeit en de partikel massa ratio gemeten, wat toeliet om de procesparameters aan te passen in functie van de eindproducten. De productie van troebele sappen met een grote concentratie aan vaste deeltjes bleek bevorderd te worden door het gebruik van grote zeefporiën en hoge vacuüm frequenties. Naast het veranderen van de procesparameters bleek het gebruik van een thermische voorbehandeling het saprendement te verhogen, wat overeenkomt met een betere scheiding van vaste en vloeibare fracties. Dit resulteerde in een persresidu dat bijna uitsluitend bestond uit tomatenpitten en –schillen die gemakkelijk van elkaar konden worden gescheiden, wat het valorisatiepotentieel van beide fracties verhoogt. Deze waarnemingen werden gekoppeld aan fysische inzichten in het werkingsprincipe van de technologie zodat procesparameters konden aangepast worden in functie van verschillende input stromen.

### **II. Procesimpact op de fysische eigenschappen van de resulterende producten (Hoofdstuk 3)**

Met de focus op de vloeibare tomatenfractie die resulteerde uit het proces dat geoptimaliseerd werd in Hoofdstuk 2, werd in Hoofdstuk 3 de fysische stabiliteit en gerelateerde eigenschappen van de sappen onderzocht. Stabiele sappen zijn gegeerd bij consumenten maar zijn vaak moeilijk om te verkrijgen in de praktijk. Hier werd onderzocht of stabiele sappen verkregen konden worden door gebruik te maken van de spiraalfilterpers en hoe de verschillende procesparameters de eigenschappen van de water-onoplosbare deeltjes beïnvloeden, welke op hun beurt de stabiliteit beïnvloeden. De resultaten gaven aan dat een snelle procesvoering met een grote filterporiegrootte en een maximum

vacuüm de productie van een tomatensap mogelijk maakten, dat stabiel is voor meer dan 170 dagen. Echter, wanneer exact dezelfde procescondities gebruikt werden op een andere variëteit, werd het sap onstabiel. Dit illustreert dat enkel het veranderen van de procesparameters niet volstond om de sapstabiliteit te controleren. Om de invloed van zowel procesvariabelen en variëteit op de stabiliteit volledig te verstaan, moet verder onderzoek uitgevoerd worden waarin bijvoorbeeld enzymatische activiteit of partikel interacties onderzocht worden.

### **III. Procesimpact op de chemische eigenschappen van de resulterende producten (Hoofdstuk 4)**

In Hoofdstuk 2 werd aangetoond dat de antioxidantcapaciteit behouden bleef doorheen de procesvoering, wat suggereerde dat de spiraalfilterpers een beperkte procesimpact heeft. Echter, een meer gedetailleerde aanpak was nodig om de procesimpact op die individuele fenolische componenten, carotenoïden en ascorbinezuur concentratie na te gaan. Deze resultaten werden beschreven in Hoofdstuk 4 en tonen aan dat de ascorbinezuurconcentratie behouden bleef doorheen persing met de spiraalfilterpers. De gewogen retentie efficiëntie van de fenolische componenten en carotenoïden was  $88 \% \pm 8 \%$  en  $122 \% \pm 15 \%$ , respectievelijk, wat de beperkte procesimpact van de spiraalfilterpers bevestigt en zijn potentieel illustreert om de natuurlijke samenstelling van de tomaten te behouden. De verdeling van de componenten in de verschillende fracties werd ook onderzocht, waarin een aanrijking in het persresidu van zowel fenolische componenten (gemiddeld 5 keer) als carotenoïden (gemiddeld 2.5 keer) werd aangetoond. Doorheen de daaropvolgende thermische behandeling van het tomatensap daalde de concentratie aan carotenoïden significant. Verder onderzoek zou dus moeten focussen op (i) het koppelen van de spiraalfilterpers met alternatieve, innovatieve en milde technologieën alsook (ii) de valorisatie van het persresidu, gezien de aanrijking van de onderzochte componenten duidelijk aangetoond is.

Naast praktische eigenschappen die de valorisatie van tuinbouwreststromen verhinderen zoals hun hoge vochtinhoud en seizoenale voorkomen, werd een ander aspect geïdentificeerd dat hun grootschalige valorisatie bemoeilijkt, meer bepaald het gebrek aan kennis rond de samenstelling van bepaalde tuinbouwreststromen. Dit werd onderzocht in het tweede deel van dit doctoraat voor geforceerde witloofwortels (*Cichorium intybus* L. var. *foliosum*). Ongeveer 36,000 ton geforceerde witloofwortels ontstaan jaarlijks in België tijdens de productie van witloofkroppen (chicons). Deze reststroom wordt momenteel voornamelijk lokaal gebruikt als voeder. Echter, een beter inzicht in hun samenstelling kan leiden tot eindproducten met een hogere toegevoegde waarde zoals voeding, farmaceutica en biociden.

#### IV. Profilering van geforceerde witloofwortels (Hoofdstuk 5)

Inzicht in de samenstelling van geforceerde witloofwortels werd verkregen door het onderzoeken van de aanwezigheid van sesquiterpeenlactonen (bittere componenten) en fenolische componenten, alsook hun antioxidantcapaciteit en elementaire samenstelling. Zowel de samenstelling van 5 verschillende witloofcultivars en cichorei (*Cichorium intybus* L. var. *Sativum*) werd vergeleken als deze van de geforceerde wortels met niet geforceerde wortels, bewaarde wortels en chicons. De resultaten gaven aan dat geforceerde wortels verrijkt waren met sesquiterpeen lactonen in vergelijking met niet geforceerde wortels en chicons. De voornaamste fenolische component (chlorogeenzuur) was aanwezig in de wortels in een concentratie ongeveer dubbel zo hoog vergeleken met de chicons en de antioxidantcapaciteit steeg significant na forcering. Ten slotte bleek dat de geforceerde wortels voornamelijk K, P, Cl en Ca bevatten en dat hun gehaltes aan Fe en Cu aan de voorwaarden voor het voedingslabel 'bron van' voldoen. Deze bevindingen illustreren het potentieel van de geforceerde witloofwortels voor verdere valorisatie. Echter, er bleek een grote variabiliteit te zijn in type en concentratie van de gemeten componenten in functie van de verschillende cultivars. Hiermee moet rekening gehouden worden wanneer deze resultaten als basis voor verder onderzoek rond potentiële valorisatie gebruikt worden. Verder onderzoek moet focussen op het onderzoeken van de functionaliteit en de toxiciteit van de verkregen extracten of producten alsook op het testen van de valorisatie op pilotschaal.

Concluderend kan gesteld worden dat efficiënt gebruik van tuinbouwreststromen nodig is, maar dat de vertaling in de praktijk niet altijd even vlot verloopt. Twee aspecten die aan de oorzaak kunnen liggen, werden geïdentificeerd, namelijk (i) vochtige reststromen die seizoenaal en in beperkte hoeveelheden verspreid voorkomen en (ii) het gebrek aan kennis rond de samenstelling van bepaalde reststromen. Deze aspecten werden onderzocht aan de hand van twee verschillende aanpakken op twee modelgewassen. Uit de resultaten bleek dat de spiraalfilterpers geschikt was om tuinbouwreststromen te valoriseren aangezien deze verschillende types reststromen kon verwerken met een beperkte procesimpact. In verder onderzoek moeten de downstream technologieën onderzocht worden in functie van het behouden van de beperkte procesimpact doorheen het hele proces. Om het tweede aspect betreffende het gebrek aan kennis van de samenstelling van reststromen aan te pakken werden geforceerde witloofwortels onderzocht. Deze wortels bleken verrijkt te zijn in sesquiterpeenlactonen en fenolische componenten welke, gebaseerd op de literatuur, beloftevol zijn voor toepassing in de voeding, farmaceutische en biocidale sector. Deze gedetailleerde karakterisatie kan dus gebruikt worden als een startpunt voor verdere productontwikkeling. De resultaten worden beschreven in de context van de bioeconomie en kritisch besproken vanuit een breder technisch en socio-economisch perspectief in Hoofdstuk 6.



Het doctoraat geeft dus duidelijk de mogelijkheden aan voor het valoriseren van tuinbouwreststromen. Anderzijds wijst het op de complexiteit en de nood voor een transdisciplinaire en veelzijdige aanpak. We staan momenteel slechts aan het begin van dit verhaal, maar de interesse groeit en er worden stappen voorwaarts gezet.



## Abbreviations

% E <sub>JNT</sub>	juice extraction efficiency
% E <sub>PR</sub>	press residue extraction efficiency
% R	retention efficiency
ΔE	total color difference
a*	green (-a) to red (+a) in CIELAB color space
AA	ascorbic acid
AOC	antioxidative capacity
b*	blue (-b) to yellow (+b) in CIELAB color space
BX	total soluble solids
C	number of channels of the spiral
C1	chicons forced from NF1
C2	chicons forced from NF2
CH	chalcones
CHCH	dihydrochalcones
DHdoLAC	dihydrodeoxylactucin
DHdoLACglyc	dihydrodeoxylactucin glycoside
DHdoLACox	dihydrodeoxylactucin oxalate
DHLAC	dihydrolactucin
DHLACglyc	dihydrolactucin glycoside
DHLACox	dihydrolactucin oxalate
DHLCP	dihydrolactuopicrin
DHLCPox	dihydrolactuopicrin oxalate
doLAC	deoxylactucin
doLACglyc	deoxylactucin glycoside
doLACox	deoxylactucin oxalate
DOSS	degree of serum separation
DPI	dichlorophenolindophenol
F	feed pump frequency
F1	forced roots from NF1
F2	forced roots from NF2

## Abbreviations

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FLS	flavonols
FNS	flavanonols
HCA	hydroxycinnamic acids
HHP	high hydrostatic pressure
HPPA	hydroxyphenyl propanoic acids
JFO	juice filtered once
JFO30	juice filtered once, pasteurized for 30 sec
JFO60	juice filtered once, pasteurized for 60 sec
JFT	juice filtered twice
JFT30	juice filtered twice, pasteurized for 30 sec
JFT60	juice filtered twice, pasteurized for 60 sec
JnT	juice not thermally treated
JT	thermally treated juice
JT14	thermally treated juice, stored for 14 days
JT30	thermally treated juice, stored for 1 month
JT90	thermally treated juice, stored for three months
JY	juice yield
k	reaction rate value
$K_{part}$	partitioning coefficient in octan-1-ol or olive oil and water
$L^*$	lightness in CIELAB colorspace
LAC	lactucin
LACglyc	lactucin glycoside
LACox	lactucin oxalate
LCP	lactucopicrin
LCPOx	lactucopicrin oxalate
M	pore size of the filter element
$MC_{JFO}$	moisture content of the juice fraction
$MC_{MT}$	moisture content of the mashed tomatoes
$MC_{PR}$	moisture content of the press residue
MeOH	methanol
MT	mashed tomatoes
NF1	non-forced Belgian endive roots 6 months after harvest

## Abbreviations

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NF2	non-forced Belgian endive roots 10 months after harvest
PHAs	polyhydroxyalkanoates
PG	polygalacturonase
PME	pectin methylesterase
PPO	polyphenol oxidase
PR	press residue
PSD	particle size distribution
PWR	precipitate weight ratio
S	spiral frequency
SS	soluble solids
TH	throughput
TS	tomato solids & total solids
TSI	Turbiscan Stability Index
TSI <sub>eq</sub>	TSI equilibrium value
TSI <sub>init</sub>	TSI initial value
TT	thermally treated tomato
TU	turbidity
UHPLC-MS/MS	ultra-high performance liquid chromatography coupled to tandem mass spectrometry
UHT	ultra-high temperature
V	vacuum pump frequency
WIS	water insoluble solids



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## **I. Research context and objectives**

### **i. Context**

#### **a. From the 2030 Agenda to the circular economy and the bioeconomy**

In September 2015, the United Nations released the 2030 Agenda which represents an integrated approach to sustainable development, summarized in 17 sustainable development goals (SDGs) covering five different areas: people, planet, profit, peace and partnership (Gregersen et al., 2016). This strategy is introduced by the European Commission in response to the current challenges such as unemployment, price and supply risks, poverty, gender inequality, health, natural resource depletion and climate change (United Nations, 2015). It is believed that the circular economy can contribute to reaching these SDGs, and in particular goal 12 aiming at ensuring sustainable consumption and production patterns. This strives for a transition towards an economy where the value of products, materials and resources is maximally maintained and the generation of waste is minimized (European Commission, 2015c). The EU has assigned various priority areas in the circular economy that need to be addressed in a targeted way including plastic recycling, recovery of critical raw materials and recycling of construction and demolition material. Two other priority areas, particularly important for this dissertation are (i) food waste and (ii) biomass and biobased products (European Commission, 2015c). To address these topics, the bioeconomy can play a crucial role. Thus, whereas the circular economy principle is applicable in different areas, the bioeconomy focuses on biomass in particular (European Commission, 2015c).

#### **b. From the bioeconomy to biorefineries and the cascade principle**

This bioeconomy is conceived as a key element in the Europe 2020 strategy for smart and green growth. It implies “the production of renewable biological resources and the conversion of these resources and waste streams into value-added products, such as food, feed, biobased products and bioenergy” (European Commission, 2012). Sectors that can be considered to be active in this bioeconomy are (i) primary producers of biomass, e.g. agriculture, forestry, fisheries and aquaculture, (ii) sectors traditionally using biomass as feedstock, e.g. food, feed and paper production and (iii) sectors that conventionally rely on fossil resources but can shift to biomass inputs, e.g. chemical, biotechnological and energy industries (European Commission, 2012). The latter group is sometimes classified as biobased economy, and hence seen as a subdivision of the bioeconomy (IWG BE, 2013; Koop et al., 2014).

No consensus on an exact definition of the term bioeconomy has been reached yet, hence various specifications with different nuances (such as requirement of biotechnology, focus on renewable feedstock or concentration on sustainability) are adopted by different instances (Brugge, Hansen & Klitkou, 2016; European Commission, 2012; IWG BE, 2013). Aspects predominantly agreed upon are (i) the use of biomass as feedstock (Brugge, Hansen & Klitkou, 2016; Pfau et al., 2014), (ii) the operation in a sustainable and eco-efficient way, thereby minimizing waste and maintaining the circular productions chains (European Commission, 2012; IWG BE, 2013) and (iii) the use of biorefineries to realize the biomass conversion, which are expected to follow a cascade principle in order to maximally valorize the available biomass (de Besi & McCormick, 2015; European Commission, 2012; McCormick & Kautto, 2013).

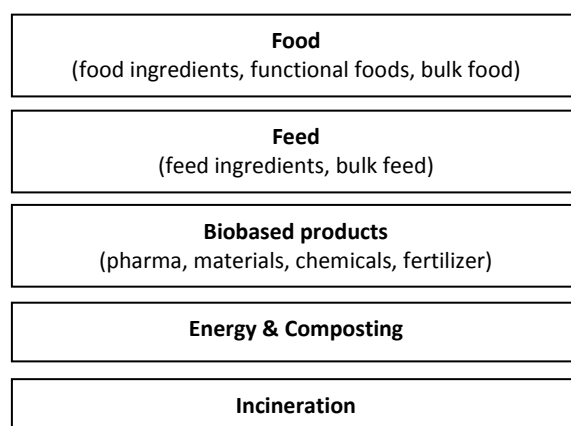
### c. The cascade principle and biorefineries

The cascade principle is proposed in order to optimally use biomass and byproducts (Giroto et al, 2015). It concerns a priority order with high-value applications on top followed by lower value applications, entailing systematic exploitation of biomass for higher-added-value products, before using it as an energy source (Keegan & Kretschmer, 2013). The definition of high and low value can be based on economic, social or ecologic criteria or a combination hereof (IWG BE, 2013; Mourad, 2016).

Depending on the adopted criteria and the priorities, different cascades exist for using biomass and biomass byproducts. They all agree on the use of materials over energy (Carus et al., 2015; de Besi & McCormick, 2015). Some focus on the economic added value of the biomass and prioritize health and lifestyle products (such as pharmaceuticals and fine chemicals) over food (WBBE, 2011; WTC-BBE, 2011). However, from an integrated perspective, taking into account the economic, social and ecologic criteria, food is often placed on top, together with feed as this indirectly leads to food. This comes forth out of the rationale that food security is of primordial importance and the development of the bioeconomy should not come at the expense of food security. Furthermore, the byproducts originate from the food producing sector and thus should flow back there in first instance. Therefore, valorization of biomass towards food is most often perceived as the highest integral added-value application and the predominant part of the cascades agree on food as a top priority (de Besi & McCormick, 2015; Giroto et al., 2015; IWG BE, 2013; Maciulevičius, 2016; OVAM, 2012; 2015b; SCAR, 2014). Applied on byproducts from the agricultural sector (IWG BE, 2013; OVAM, 2012; OVAM, 2015b) and more specifically food waste (Giroto et al., 2015; Keulemans et al., 2015), this rationale can be translated in a hierarchy as follows: food, feed, biobased products (including chemical compounds,

materials, pharmaceuticals), energy production and composting followed by incineration, which is the least desirable option (Figure I.1).

The main principle of the cascading thus lies in the optimal biomass valorization by stepwise utilizing the energy and material content of the biomass in such a way that all parts of the plant are optimally exploited, generating viable opportunities in different sectors. It is believed that this can be realized using strongly integrated biorefinery concepts, able to process biomass in different fractions, leading to a spectrum of marketable products (food, feed, materials and/or chemicals) and energy (fuels, power and/or heat) (IEA, 2012; McKormick & Kautto, 2013; Odegard, Croezen & Bergsma, 2012).



**Figure I.1** Hierarchy for valorizing agricultural byproducts and food waste in the bioeconomy (Figure based on Giroto et al., 2015; IWG BE, 2013; Keulemans et al., 2015; OVAM, 2012; 2015b).

#### d. Bioeconomy implementation remains challenging

It is generally acknowledged that the bioeconomy is currently still in its infancy (Golembiewski et al., 2014). Existing publications originate predominantly from governmental institutions and primarily concern strategic agendas, rather than identification of challenges and measures to allow implementation of the bioeconomy (Golembiewski et al., 2014). Hence, although the idea of developing and unfolding the bioeconomy is increasingly being adopted by nations, translating strategic objectives into industrial reality appears challenging and complex (Kircher, 2012).

In this regard, both research and innovation in the bioeconomy sector are considered crucial (FP7-framework, Horizon 2020 program) (European Commission, 2012; Golembiewski et al., 2015; IWG BE, 2013; OVAM, 2015b). Such research can be performed from several perspectives. A socio-economic perspective can consist of investigating economic aspects (e.g. price of substitutes, market potential, market acceptance, investment cost, added value), logistic aspects (e.g. transport, seasonal occurrence, storage) or legislative aspects (e.g. waste legislation, product safety, claims). Besides

studying socio-economic aspects, bioeconomy research can also be performed from a technical perspective as often a limited knowledge, technical unavailability of required processes or immaturity of novel technologies (e.g. lab scale, limited efficiency) obstruct the development of biorefineries (Golembiewski et al., 2014).

The research conducted in this doctoral dissertation, draws further on this lack of technical knowledge and the need for technologies that can biorefine biomass in order to stimulate the development of the bioeconomy. More specifically, this doctoral dissertation addresses the issue of horticultural byproducts that are not used to their full potential, although this is being strongly stimulated by the Flemish government (OVAM, 2015).

## **ii. Aim, objectives and outline**

The aim of this study is to facilitate the valorization of horticultural byproducts. Chapter 1 gives the reader an overview of the state of the art of horticultural byproducts, in which their occurrence in Flanders and Europe is described, complemented with their current use and their valorization potential. Cooperation with different stakeholders complemented with literature research allowed us to identify different aspects currently obstructing the large scale valorization of these byproducts. Two of such hindering aspects are tackled in this study i.e. (i) the need to cope with moist byproducts occurring scattered, both in time and space and (ii) the lack of knowledge on the composition of some byproducts. These aspects are addressed by two different approaches for two different model crops (Figure 1.2).

### **a. Research line 1**

The high moisture content (> 80 %) is a common denominator of almost all horticultural byproducts, leading to problems during storage. A pressing technology, able to perform a separation of liquid and solid fractions, can cope with this high moisture content, thereby optimally utilizing all fractions and generating a minimal amount of waste. Furthermore, three additional constraints were imposed on this pressing technology in order to increase the chances of successful valorization towards food, which was primarily targeted, in line with the cascade principle (OVAM, 2012b; 2015). Firstly, the relatively small and geographically dispersed volumes of byproducts and the seasonality of their production are characteristics that are inherently present in almost all horticultural byproducts and can obstruct the feasibility of the valorization process. This can be tackled by choosing a technology flexible in type of input product and thus able to process different byproducts throughout the year (Budzianowski &



Postawa, 2016; Fava et al., 2015; Lin et al., 2014; Matharu et al., 2016). Secondly, consumers increasingly demand attractive products (i.e. products with attractive color, appearance and taste) which thirdly, maximally retain the naturalness of the fruits and vegetables. Therefore, a technology that is able to meet these demands is expected to generate products that are competitive with the current ones available and may thus be able to lead to a successful valorization of horticultural byproducts. In this regard, the spiral-filter press is introduced as a promising technology that can cope with these aspects and perfectly fits within a biorefining strategy. The suitability of the spiral-filter press to biorefine horticultural byproducts is technically investigated based on these constraints in Chapters 2, 3 and 4 on surplus tomatoes (*Solanum lycopersicum* L.) present at the Flemish produce auctions. Byproducts occurring at the auctions have major advantages in terms of product quality, purity and concentrated occurrence. These aspects can be considered crucial for developing high-added-value applications such as food products (Budzianowski & Postawa, 2016; Ghatak, 2011; Hennig et al., 2016; KTN, 2016; OVAM, 2014; 2015b; Poltronieri & D'Urso, 2016; Sweet et al., 2016; Van Buggenhout et al., 2016). The choice of tomato surplus products originates from the fact that (i) these are one of the largest byproducts occurring at the produce auctions and (ii) they are very perishable thus in need of rapid processing. The processing of this tomato crop can be used as a model for other soft horticultural products. Furthermore, the experiments are performed on pilot scale to increase the industrial relevance and overcome the issues often encountered at extrapolating lab-scale results to industrial scale.

Consequently, the first objective of this study is to investigate how this technology can be utilized for refining soft horticultural byproducts containing a high moisture content into valuable products. This objective can be translated into the following research questions:

**1/ How can the spiral-filter press be used to biorefine a soft biomass matrix?**

Hereto, a stepwise optimization of a pilot-scale spiral-filter press refinery process is performed in Chapter 2, starting from a simple system towards a more complex formation, thereby maximizing the juice yield. Generally applicable insights in the effect of different process parameters are offered. These insights are complemented with an evaluation of the antioxidative capacity of the resulting products.

**2/ How can the physical stability of the resulting juice be influenced by the process parameters of the spiral-filter press?**

The juices resulting from the optimized process in Chapter 2 were investigated in Chapter 3 for their juice stability and related quality attributes by evaluating how different process parameters influence the water-insoluble solid characteristics known to influence this juice stability.

### **3/ Does the spiral-filter press retain the bioactive compounds present in the feedstock biomass throughout processing?**

The process impact of the spiral-filter press on the fate of three different phytochemicals present in tomato (phenolic compounds, carotenoids and ascorbic acid) is investigated in Chapter 4.

#### **b. Research line 2**

The second research focus relates to the necessary knowledge about the composition of the byproducts as a basis for designing promising valorization pathways. Although an increasing body of evidence is being built in the scientific literature about the composition of a wide range of byproducts, some compound groups and byproducts have been left under the radar, for which knowledge on the composition is necessary to explore their valorization options. This is exemplified by the forced Belgian endive roots (*Cichorium intybus* L. var. *foliosum*). To produce the commonly known ‘witloof’ chicons, Belgian endive roots are forced in the absence of light. The 36,000 tonnes of forced roots, remaining after forcing of the chicons are not used for human consumption and are currently predominantly used as feed for local cattle (Department of Agriculture and Fisheries, 2014). These byproducts have a large valorization potential based on their large amounts and logistic advantages compared to other horticultural byproducts (e.g. continuous occurrence throughout the year, geographical concentration, available in pure and collectable form, limited susceptibility to microbial degradation during storage). Furthermore, bitter compounds (i.e. sesquiterpene lactones) and phenolic compounds were found to be prevalent in the *Cichorium intybus* L. species (de Kraker, 2002; Jurgoński, et al., 2011; Milala et al., 2009; Sessa et al., 2000; Sinkovič et al., 2014; 2015). These compounds have been attributed with multiple bioactivities which could lead to an added value of the derived products (Azay-Milhau et al., 2013; Bischoff et al., 2004; Chadwick et al., 2013; Chaturvedi, 2011; Ghantous et al., 2010; Milala et al., 2009; Padilla-Gonzalez et al., 2016; Picman, 1986; Prakash & Gaikwad, 2012). However, in literature no specific information regarding the content of the forced Belgian endive roots is present. The predominant part of the literature available focused on (i) the species *Cichorium intybus* L. in general, to which the Belgian endives belong or (ii) the industrial root *Cichorium intybus* L. var. *sativum*. Although some reports on the composition of *Cichorium intybus* L. var. *foliosum* are present, they predominantly focus on the edible parts. Information on the roots and specifically the forced roots of Belgian endives has been found to be very scarce. Even though the information regarding the composition of the general *Cichorium intybus* L. species can be used to give an indication of the composition of the forced Belgian endive roots, translating this information to the case of forced Belgian endive roots and using it as a basis for designing a specific valorization pathway or target a

specific market or derived end product, can be misleading. Therefore, the focus in this dissertation lies on gaining a better insight in the composition of forced Belgian endive roots to facilitate their valorization. This second objective can be further converted into the following research questions:

**4/ What is the sesquiterpene lactone profile in forced Belgian endive roots?**

**5/ What is the phenolic content and the related antioxidative capacity of the forced Belgian endive roots?**

**6/ How does the composition change in function of cultivar, variety, matrix, forcing and storage?**

The composition of the currently underutilized forced Belgian endive roots is investigated in Chapter 5. The focus lies on the sesquiterpene lactones. Additionally, the phenolic content, the antioxidative capacity and the elemental composition are investigated to gain a broader insight into their composition. The analysis of the forced roots is complemented with other samples enabling an investigation of the effect of cultivar, variety, matrix, forcing and storage.

The insights obtained in these research chapters are put in a broader perspective in the general discussion in Chapter 6.

The following pages briefly elaborate on the adopted research approach.

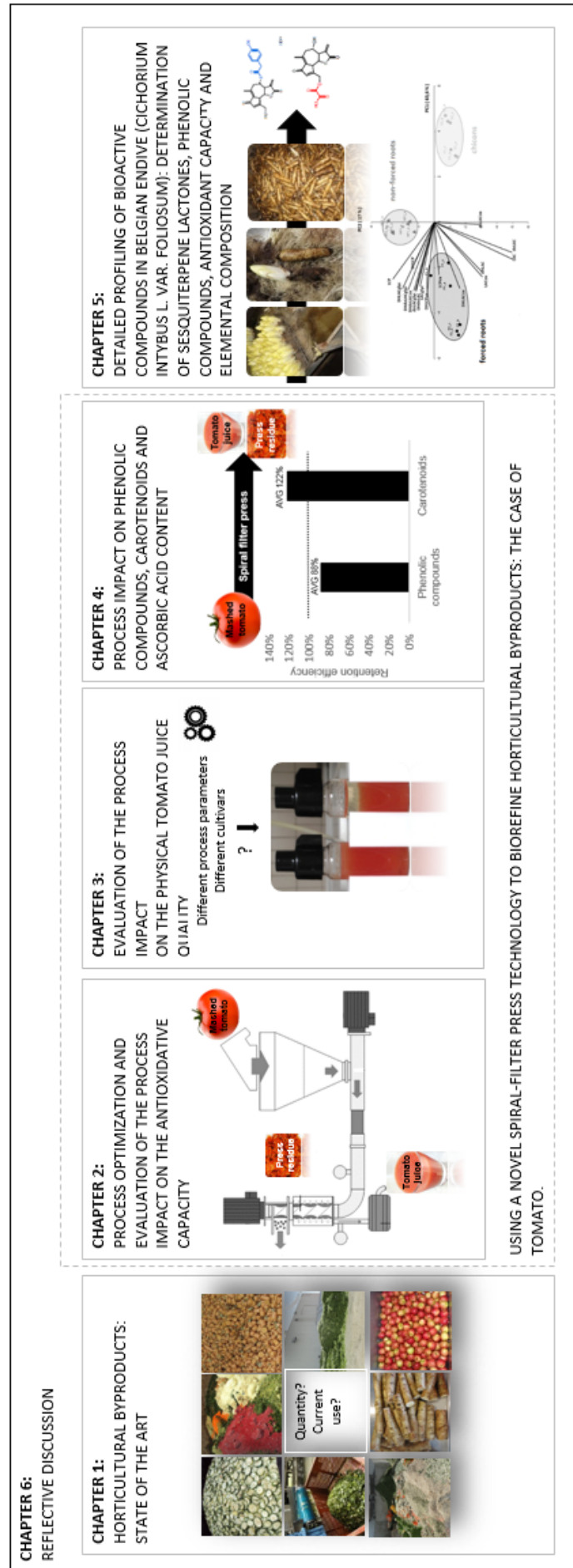


Figure 1.2 Schematic outline of this doctoral dissertation.

## II. Research approach

This doctoral dissertation was performed in the context of a research project called GeNeSys, in which three doctoral researchers investigated the technical valorization of horticultural byproducts, fishery-byproducts and valorization through composting, respectively. A fourth researcher investigated the management of innovation processes in the bioeconomy and developed a set of guiding principles (Van Lancker et al., 2016). The three technical cases attempted to follow the strategy thus proposed in order to develop innovative solutions, maximally responding to the opportunities and threats of the developing bioeconomy.

The followed research methodology was based on three important characteristics: (i) transdisciplinary, (ii) iterative and (iii) open to collaboration (Van Lancker et al., 2016). In order to generate the necessary knowledge from a variety of sectors and sciences, a transdisciplinary approach was advocated to successfully develop new ideas and assess their viability, set up integrated biorefineries, alter the required supply chains and identify obstructing legislation (European Commission, 2012; Golembiewski et al., 2015; Kircher, 2012). An iterative approach was proposed in order to create learning cycles, distinguish viable from less viable ideas and adjust the strategy for unforeseen developments and mistakes (Caraça et al., 2009; Golembiewski et al., 2015; Hadorn et al., 2006; Kroon et al., 2008). Finally, innovation processes open to collaboration within a network of diverse stakeholders such as governmental institutions, academics, competitors, suppliers and retailers were promoted as they are expected to contribute to an improved adaptation to the dynamic market, to a decreased time to market and to an increased identification of valuable opportunities and possibilities to expand to new markets (Chesbrough, 2012; Du et al., 2014).

This methodology defines three main phases within the innovation process: (i) the idea development phase, (ii) the invention phase and (iii) the commercialization phase (Figure I.3) (Van Lancker et al., 2016). This approach was predominantly adopted in the first idea development phase. Through cooperation with over 50 stakeholders from groups of different origin (research institutes, primary sector, food industry, government, sector associations) insights were gained in the different aspects, important for valorizing biomass. Based on this knowledge, ideas were generated and selected which lead us to identify promising research avenues and formulate research questions.

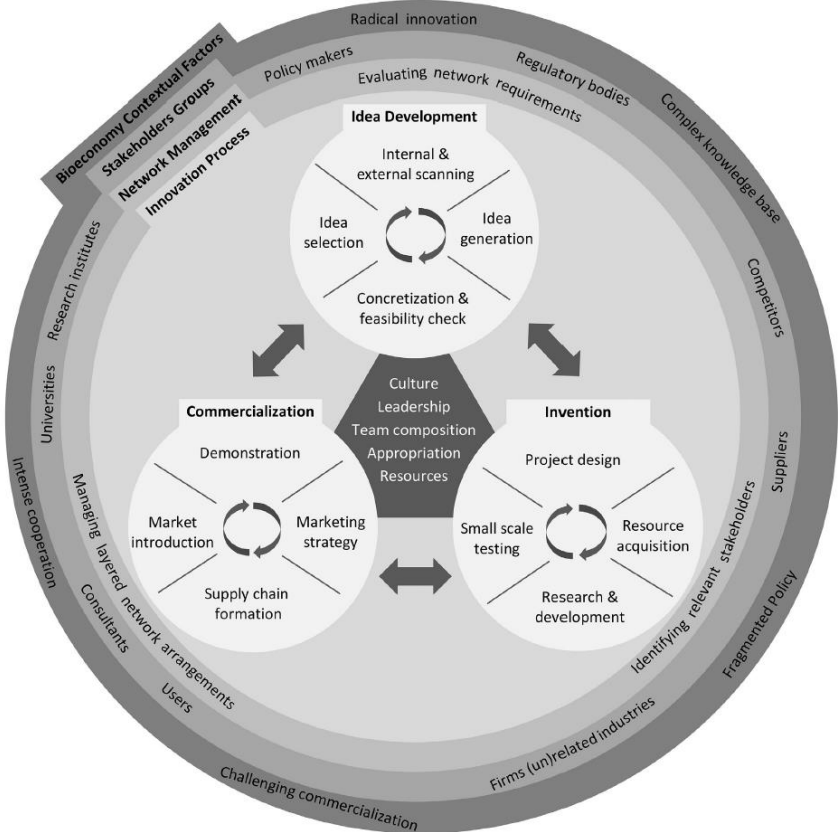


Figure I.3 Schematic representation of the innovation process in the bioeconomy context (Van Lancker et al., 2016).

## **Chapter 1**

Horticultural byproducts: state of the art





## **Chapter 1: Horticultural byproducts: state of the art**

Both from an economic as well as from an environmental and social perspective, efficient utilization of horticultural byproducts is needed. This is in accordance with the circular economy policy that strongly encourages the food supply chain to valorize their byproducts (European Commission, 2015c). Insight in the yearly available amounts of byproducts is often perceived as one of the prerequisites for successful valorizations (Hennig et al., 2016; Mirabella et al., 2014; Östergren et al., 2014; OVAM, 2014; 2015b). However, before these data can be collected and interpreted, a transparent terminology framework must be adopted (part 1.1). Many terms and definitions concerning byproducts, waste and losses circulate. While it is not within the scope of this study to give an exhaustive overview of these terms or judge their value, it is important to (i) make a comprehensive overview of the terminology, clearly defining the terms that will be used in this dissertation (part 1.1.1) and (ii) explicitly compare them to the terms adopted in other recently published reports (part 1.1.2). Based on the stage in which the byproducts occur throughout the food value chain, they are classified in three groups. In the subsequent part (1.2), an estimation is made of the amounts of byproducts occurring in each of these three groups, both in Flanders and in Europe. The third part (1.3) gives an overview of the current and potential applications of the horticultural byproducts, primarily focusing on food and feed as these applications are on top of the valorization cascade. It is not aimed to provide an exhaustive list of all current applications and research, rather it aims to illustrate the potential of these byproducts and give an insight in the focus and scale of the current research.

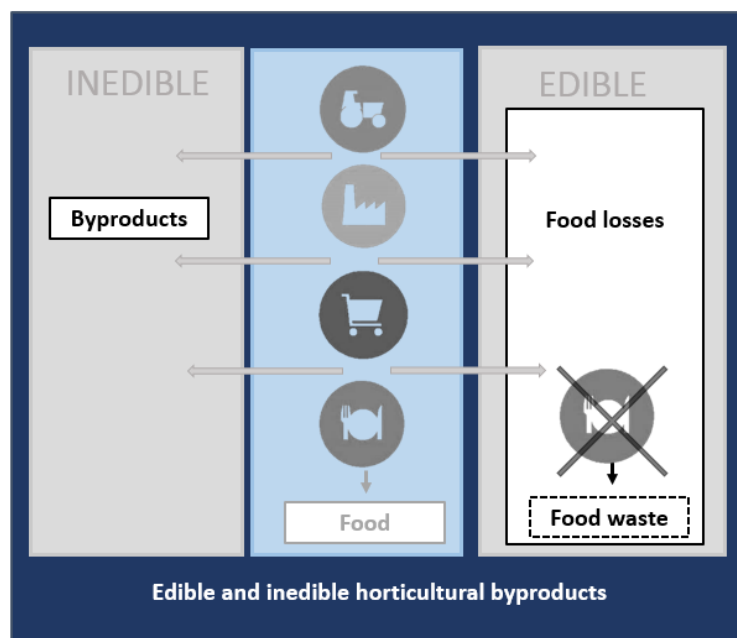
### **1.1 Defining and classifying horticultural byproducts**

#### **1.1.1 Terminology**

A product is defined as a material that is deliberately created in a production process (European Commission, 2007). Materials that are not the main objective of a production process are product residues. These can either be byproducts or wastes, differing in the fact that the former are considered non-waste whereas the latter should be treated as waste (European Commission, 2007).

Applied to the agricultural production and food chain, food is the product. Byproducts in this context can be considered as unavoidable and inedible products such as skins, seeds, peels and hulls (Lipinski et al., 2013). This is however not only confined to byproducts derived from horticultural products (also animal-derived products, dairy, fats, etc.). Besides inedible byproducts, also (traditionally considered) edible biomass is lost throughout the production chain (edible byproducts). The term food loss is often

used to refer to this edible material that is intended for human consumption but ultimately not consumed by people. Instead it is lost, discarded, degraded or diverted intentionally from human food into other applications such as animal feed. These food losses typically occur at the production, storage, processing and distribution stages of the food supply chain. When considering the later stages of the food chain (retail and consumption), food losses are often referred to as food waste (Gustavsson et al., 2011; Lipinski et al., 2013; Parfitt et al., 2010). As the focus in this dissertation lies on horticultural byproducts, the terms edible and inedible horticultural byproducts will be used. This is in line with the current trend to change the term waste into byproduct, to stress their valorization potential, yet indicating that they are not the primary goal of the production process (Galanakis, 2012). Figure 1.1 schematically illustrates the different terms.



**Figure 1.1** Schematic overview of the different terms related to products and product residues in the food supply chain.

### 1.1.2 How is the adopted terminology related to other reports?

Several recent projects and documents have been launched in the last decade to estimate the amount of food related byproducts (e.g. EU-FP7-projects FUSIONS<sup>1</sup>, NOSHAN<sup>2</sup> and REFRESH<sup>3</sup>, Interreg project ARBOR<sup>4</sup>, EUBIS Cost action<sup>5</sup>, IWT project CINBIOS<sup>6</sup>, and a study to be published in 2017 by the

<sup>1</sup> <http://www.eu-fusions.org/>

<sup>2</sup> <http://www.noshan.eu/index.php/en/>

<sup>3</sup> <http://eu-refresh.org/>

<sup>4</sup> <http://pomwvl.be/arbor>

<sup>5</sup> <http://costeubis.org/mapping>

<sup>6</sup> <http://cinbios.be/>

Department of Agriculture and Fisheries). These differ in the type of product studied (e.g. agricultural byproducts, horticultural byproducts, food losses), region (Flanders, Europe, world) and to some extent in the terminology adopted. This reduces the transparency of the data. Therefore, it is important to explicitly address these differences.

In the FUSIONS project, a definition framework and quantification manual have been developed to define and quantify food waste. This framework will be used to highlight some differences in the approach adopted in this dissertation with that used in several other reports.

In the FUSIONS project, food waste is defined as “any food and inedible parts of food, removed from the food supply chain to be recovered or disposed of (including composting, crops ploughed in/not harvested, anaerobic digestion, bioenergy production, co-generation, incineration, disposal to sewer, landfill or discarded to sea)” (Östergren et al., 2014). Our definitions and quantification differ from the proposed FUSIONS framework in two aspects, (i) the system boundaries of the food supply chain and (ii) the use of the final destination in the definition.

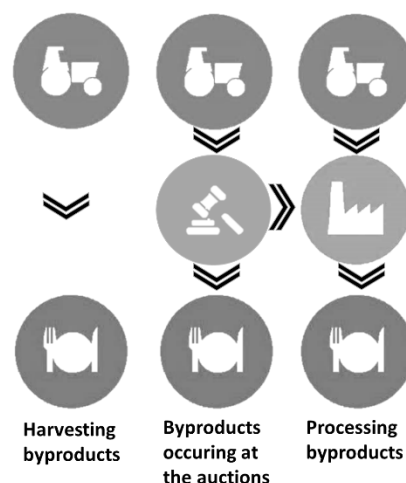
First, the start of the food supply chain considered in this dissertation differs from the one in the FUSIONS project. In this dissertation, the inedible parts that are typically removed during harvesting or that are not harvested at all, are considered as inedible byproducts (i.e. foliage and stems) as they have potential to be valorized. Consequently they are included in the database. The FUSIONS definition does not include these parts of the plants as they are not intended for consumption and do not enter the food chain. On the other hand, the inedible parts that are harvested and enter the food chain (e.g. olive pits), are considered as food waste according to FUSIONS (Tostivint et al., 2016). From the perspective of food waste, this FUSIONS rationale can be understood. Hence, this approach is often adopted in studies targeting food waste such as the study of the Department of Agriculture and Fisheries, to be published in 2017 (Roels, 2017) and the projects EUBIS, NOSHAN and REFRESH. However, in this dissertation, we are mapping the horticultural byproducts in order to valorize them. Therefore, all fractions related to horticultural production are included. This is in accordance with the approach used in the CINBIOS and ARBOR projects.

The second difference originates from the fact that the FUSION definition implies that the final destination defines whether or not a product is considered as food waste. Only products used for composting, plough-in, anaerobic digestion, bioenergy, cogeneration, incineration, sewer, landfill and discards are defined as food waste. Food and inedible parts diverted to animal feed or biobased materials are consequently not referred to as food waste but are termed ‘valorization and conversion’ (Östergren et al., 2014). In this dissertation, a stricter definition is adopted where all potentially valuable biomass produced along the fruit and vegetable supply chain that is not consumed nor

industrially utilized is considered as a byproduct. Hence the final destinations *per se* do not define the biomass to be a byproduct or not. This is in accordance with the approach used in the projects CINBIOS and ARBOR as well as in the study of Roels (2017). To what extent the FUSIONS definition is implemented in the other projects is less clear. However since the definitional framework has only been launched recently (2014), most of the data circulating today are expected not to be tuned yet with the FUSIONS framework.

### 1.1.3 Three different classes of byproducts

Based on the stage during which the byproducts occur throughout the food value chain, three different types of byproducts can be distinguished (Figure 1.2). The primary production is the first step in the supply chain. In a second step, the produced biomass can be commercialized, either directly from the producer to the market or via the produce auctions. A third option consists of processing the biomass before commercialization. Based on this simplified supply chain, three different categories of byproducts can be distinguished, characterized by different characteristics: (i) harvesting byproducts (e.g. not harvested biomass, discarded biomass failing to meet quality standards), (ii) byproducts resulting from the auctions (e.g. surplus products, products failing to meet quality standards) and (iii) food processing byproducts (e.g. process interruptions, products generated during processing such as press residues and skins, accidental spillage, products failing to meet quality standards, surplus products) (Giroto et al., 2015).



**Figure 1.2** Schematic food supply chain leading to three categories of byproducts: (i) harvesting byproducts, (ii) byproducts occurring at the produce auctions and (iii) processing byproducts.

In this chapter, the focus will thus be on these three classes of byproducts as these have been shown to be the predominant sources of byproducts in Flanders (IWG BE, 2012). Losses occurring at the retail, during transport and at consumption stage will not be specifically addressed. Reducing food losses at

the consumer stage is however perceived to be very important. It is influenced by a series of factors, mainly socio-demographic characteristics of the household, consumption behavior and food patterns (Giroto et al., 2015). Hereto, a change in attitude for example via education of consumers is often proposed, which is stressed and addressed in other studies (e.g. OVAM, 2012; 2015a; Van Geffen et al., 2016).

## **1.2 Amount of horticultural byproducts in Flanders and the EU**

### **1.2.1 Amount of horticultural byproducts in Flanders**

The horticultural sector is of major importance in Flanders. Although occupying only 7.5 % of the arable acreage, the horticultural sector is characterized by high production rates leading to high revenues, responsible for 20 % of the total value of Flemish agricultural production (Platteau et al., 2016). Whereas the relative economic importance of agriculture in the Flemish economy is limited (0.9 %), the relative share of the vegetable sector is greater than the mean in the EU (Platteau et al., 2014). In 2014, approximately 74 % of the produced vegetables were outdoor vegetables (63 % of the horticultural acreage) whereas only 26 % were greenhouse vegetables (Platteau et al., 2016). Beans, leek, cauliflower, carrots, peas, Brussels sprouts, Belgian endive roots and spinach are the vegetables predominantly cultivated outdoors (based on the covered area), whereas tomatoes and lettuce are the main greenhouse vegetables (Platteau et al., 2012; Roels & Van Gijsegem, 2011). The fruit sector is smaller (37 % of the horticultural acreage) and predominantly concerns outdoor cultivation of apples and pears. The floriculture is also a part of the horticultural sector, however these data will not be included in this dissertation. Hence, whenever referred to the horticultural sector or to horticultural byproducts, this solely applies to fruit and vegetables.

Besides the primary production, the food industry is of major importance in Belgium, with a large and increasing share of export of frozen vegetables, potato-based products and pome fruit. Approximately 65 % of the horticultural production is used for food processing, which is primarily located in regional clusters in Flanders (80 %) (Platteau, et al., 2012; 2014; 2016).

It is thus obvious that the horticultural sector and related food industry are important assets of Flanders. Therefore, in light of the current evolution towards more sustainability, valorization of the according byproducts is of major importance for the competitiveness of Flanders.

### 1.2.1.1 Harvesting byproducts in Flanders

The harvesting byproducts can be considered as losses during harvest. Limited processing towards marketable products at the farm is also included. Examples are separating edible and inedible biomass parts, washing, sorting, drying, storing or packing. The harvesting byproducts can consist of both edible and inedible biomass (Roels & Van Gijsegheem, 2011). Depending on factors such as the type of crop, the degree of spoilage, cosmetic defects and purity (remaining on the field or collected during harvesting), the edible byproducts are to a greater or lesser extent suitable for further valorization towards food (Sweet et al., 2016). Irrespective of their condition, these will be denoted as edible byproducts hereafter, to make the distinction with the inedible biomass such as foliage and stems.

In Table 1.1, the areas, primary production volumes and related byproducts are given for the predominant (based on production volume) outdoor vegetables, greenhouse vegetables and fruit in Flanders. The data collection was performed in collaboration with the Department of Agriculture and Fisheries and in interaction with experts (listed in the heading of Table 1.1). The selected crops represent approximately 80 % and 89 % of the production volume of the outdoor vegetables and greenhouse vegetables, respectively. The data are shown in fresh biomass weight since the high moisture content is characteristic for horticultural (by-)products. The moisture content depends on the biomass and byproduct type, but is in most of the cases between 80 % - 90 % (Mirabella et al., 2014). The fractions of edible and inedible byproducts are calculated relative to the primary production, based on two different calculation methods. The first method is used to calculate the percentage of edible byproduct. This can be considered as lost primary production. Therefore, the reported primary production figure does not represent the entire production. This can be illustrated by using a fictive example. Suppose a marketable production of 1,000 tonnes is characterized by a loss of 10 % byproduct. This means that the 1,000 tonnes is only 90 % of the primary production and the real production is 1,111 tonnes. Consequently, the byproduct fraction (10 %) is 111 tonnes in absolute amounts, which is slightly larger compared to 100 tonnes (10 % of 1,000 tonnes) (Roels & Van Gijsegheem, 2011). Hence, the byproduct fraction can be calculated based on the sum of (i) primary production and (ii) the amount of byproduct. In contrast, the inedible byproduct cannot be considered as lost primary production. Therefore, its fraction is determined by using a second calculation method, relative to the primary production. For example, a byproduct mass of 100 tonnes on a primary production of 1,000 tonnes represents a 10 % fraction (Roels & Van Gijsegheem, 2011).

#### a. Fractions of edible and inedible byproducts

Edible byproducts can occur due to different causes, which can be classified in three categories, (i) prerequisites set by fresh market, (ii) size requirements set by industrial processors and (iii) preliminary preparation for industrial processors.

**Table 1.1** Vegetables, greenhouse vegetables and fruit with their related byproducts occurring during harvesting and subsequent preparation for fresh market or industry in Flanders. In cooperation with Roels (2017). Sources based on personal communication with [1] National estimations, translated for Flanders (2015) [2] ARBOR (2015), [3] Debussche (2016), [4] Holmstock (2010), [5] ILVO (2016), [6] National Chicory Research Center (2016), [7] Department of Agricultural and Fisheries (2014), [8] VeGeBe (2016), [9] Ongena (2016), [10] SUNNIVA (2016), [11] Leenknecht (2016), [12] Wittemans (2016), [13] Mertens (2016), [14] Roels & Van Gijsegem (2011), [15] Van Hemelrijck (2016).

Primary product					Byproduct					
Type	Destination	Area (ha)	Primary production (10 <sup>3</sup> tonnes)		Main type of byproduct	Yield (tonnes/ha)		Total amount (10 <sup>3</sup> tonnes)	Edible fraction	Inedible fraction
Beans	Industry	3,566 [1]	48.1	[1]	Foliage	10-30	[2]	71		148 %
					Beans (remaining on field)	0.5	[3]	2	3.6 %	
Belgian endive & roots	Fresh	-	35.4	[1]	Outer leaves and low quality chicons	-	[6]	10	23 %	
		1,212 [7]	36.4	[1]	Forced roots	30	[6]	36		100 %
Brussels sprouts	Industry	2,371 [1]	54.5	[1]	Foliage	50-70	[2]	142		261 %
					Oversized and low quality sprouts	3	[3]	6	10 %	
Cabbage (white, green and red)	Industry & fresh	1,044 [1]	62.4	[1]	Foliage	36	[2]	38		61 %
					Outer cabbage leaves	14	[10]	14	19 %	
Carrots	Industry	2,727 [1]	177.2	[1]	Foliage	20-30	[2]	68		38 %
	Fresh	662 [1]	39.7	[1]	Carrot tops	5 - 7	[3]	16	8.5 %	
Cauliflower	Industry	3,856 [1]	84.5	[1]	Foliage	20-30	[2]	17		42 %
	Fresh	386 [1]	8.5	[1]	Undersized carrots	0.9	[3]	0.6	1.5 %	
Cauliflower	Industry	3,856 [1]	84.5	[1]	Foliage and stems	30 - 50	[4]	154		182 %
	Fresh	386 [1]	8.5	[1]	Cauliflower hearts	3.5 - 5	[5]	16	16 %	
Leek	Industry	711 [1]	35.6	[1]	Foliage and stems	30 - 50	[4]	15		182 %
	Fresh	3,811 [1]	152.4	[1]	Undersized and low quality product	1	[3]	0.4	5.0 %	
Onions	Industry	1,554 [1]	77.7	[1]	Upper green parts, outer leaves, roots	20 - 30	[2]	18	33 %	
	Fresh	3,811 [1]	152.4	[1]	Upper green parts, outer leaves	20 - 30	[2]	95	38 %	
Spinach	Industry	3,188 [1]	79.7	[1]	Foliage	1.5	[9]	2		3.0 %
					Undersized and low quality onions	1.5	[9]	2	2.9 %	
					Peels and outer parts	20	[3]	31	29 %	
Bell pepper	Fresh	89 [1]	25.0	[1]	Unharvested spinach leaves	6	[8]	19	20 %	
Bell pepper	Fresh	89 [1]	25.0	[1]	Foliage	25	[12]	2		8.9 %
					Low quality bell peper	6	[12]	0.5	2.0 %	
Lettuce	Fresh	939 [1]	42.1	[1]	Low quality lettuce	11	[11]	11	20 %	
Mushroom	Fresh	-	30.4	[1]	Stems	-	[13]	3	8.0 %	
					Low quality mushrooms	-	[13]	0.8	2.5 %	
Tomato	Fresh	505 [1]	247.5	[1]	Foliage	30	[12]	15		6.1 %
					Low quality tomatoes	10	[12]	5	2.0 %	
Apple	Fresh	7,106 [1]	264.4	[1]	Low quality apples	2.0	[14]	14	5.0 %	
Pear	Fresh	9,287 [1]	322.0	[1]	Low quality pears	1.8	[14]	17	5.0 %	
Strawberry	Fresh	1,442 [1]	34.9	[1]	Low quality strawberries	1.3	[15]	2	5.0 %	

Firstly, the fresh market demands products of a certain size and appearance. Hence, the outer leaves of Belgian endive chicons for example are often manually removed to make the chicons fit in certain size classes (23 % byproduct). The same goes for leaves of cabbages (19 % byproduct). Also the outer leaves and part of the green top of the leek are cut in order to fit the quality prescriptions of the fresh market (38 % byproduct). Greenhouse vegetables generate byproducts in this category in the order of 2 %. This is in accordance with figures from the Netherlands reporting approximately 2.5 % edible byproducts for greenhouse vegetables (VMT, 2010). In most cases, these losses are caused by (partial) spoilage and cosmetic defects such as skin spots, odd shapes and colors. As lettuce is a leafy vegetable, it is more susceptible to spoilage and damage (20 %). Finally, on average 5 % of the fruits are not commercialized due to spoilage, parasites, climate and physiological damage (Roels & Van Gijssels, 2011).

Secondly, also the industry demands a certain size and appearance. Undersized, oversized or low quality products are sorted during harvesting or at the agricultural company and become byproducts. This is for example the case for beans (4 % byproduct), Brussels sprouts (10 % byproduct), cauliflower (5 % byproduct) and onions (2.9 % byproduct).

A preliminary preparation before the industrial processing can give rise to a third category of edible byproducts. Examples are the cauliflower hearts (16 % byproduct) which are mechanically removed at the field and onions, peeled before supplied to the freezing industry, which leads to 29 % of byproduct. Also the carrot tops are removed when supplied to industrial processors (8.5 % byproduct).

Besides edible byproducts, large masses of inevitable and inedible biomass (crop residues) also occur during harvest, for example in case of cabbages as these are cut out of the plant, leaving the remainder on the field. Sprouts are harvested from the stems, after which the latter are milled and left on the field. These harvesting practices thus result in biomass such as foliage, leaves and stems. The masses of these crop residues are sometimes even larger than the actual primary (edible) production, with for example 148 % for bean foliage, 261 % for Brussels sprouts and 182 % for cauliflower foliage.

### **b. Absolute amounts**

Analogous to the aforementioned fractions of byproducts, the absolute amounts of inedible byproducts are largest for cauliflower foliage (154 10<sup>3</sup> tonnes) and Brussels sprouts foliage (142 10<sup>3</sup> tonnes), followed by bean foliage (71 10<sup>3</sup> tonnes) and carrot foliage (68 10<sup>3</sup> tonnes).

The absolute amount of edible byproducts is the highest for outdoor vegetables, predominantly green parts of leek (95 10<sup>3</sup> tonnes) and onion peels (31 10<sup>3</sup> tonnes), followed by unharvested spinach (19 10<sup>3</sup> tonnes), low quality pears (17 10<sup>3</sup> tonnes), carrot tops and cauliflower hearts (both 16 10<sup>3</sup> tonnes) and low quality apples and outer cabbage leaves (both 14 10<sup>3</sup> tonnes).



Besides total amount, also the byproduct yield is important, especially regarding valorization as high yields imply concentrated occurrence, which may offer logistic advantages (Budzianowski & Postawa, 2016; Ghatak, 2011; Hennig et al., 2016; OVAM, 2014; Poltronieri & D'Urso, 2016; Sweet et al., 2016). Edible byproducts such as leek (20 – 30 tonnes/ha), onion peels (20 tonnes/ha), cabbage outer leaves (14 tonnes/ha), lettuce (11 tonnes/ha) and tomato (10 tonnes/ha) score high. Also the yields of the inedible byproducts of Brussels sprouts (50 – 70 tonnes/ha), cauliflower (30 – 50 tonnes/ha), cabbage (36 tonnes/ha), tomato (30 tonnes/ha) and carrot (20 – 30 tonnes/ha) are high.

#### 1.2.1.2 Byproducts occurring at the produce auctions in Flanders

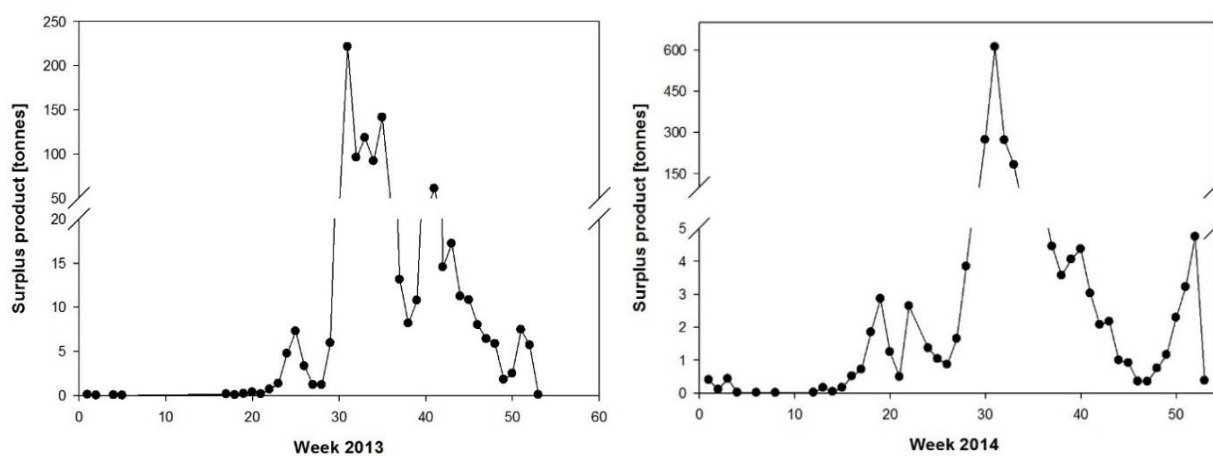
In Flanders, selling vegetables and fruit predominantly occurs at the produce auctions. In order to avoid market destabilization, the auctions agree upon a minimum price per product. Products that are not sold at this price are removed from the circuit and considered surplus products. This is regulated at the European level by Regulation (EC) No 1234/007 and Commission Implementing Regulation (EU) No 543/2011. These surplus products thus consist of perfectly consumable vegetables and fruit (often lowest quality segment) as they have gone through the same quality control as the commercially sold products and are thus of high quality. They can be classified under the term food loss (edible horticultural byproduct) as determined in section 1.1.1. The food loss category is however larger and comprises also edible byproducts occurring for example at the farm, through transportation, in the shop or at the consumers plate. Besides these surplus products, also goods damaged during transport or storage occur at the auctions.

Collective data from six produce auctions in 2015 show a non-sold vegetable and fruit share of 1.5 % (e.g. surplus product, damage during transport) equaling 16 10<sup>3</sup> tonnes (VBT, 2015). The relative contribution of the different crops is deduced from crop-specific data of three of the largest auctions in Flanders (Table 1.2). The largest amounts of byproducts in 2015 occur from apples (4,299 tonnes), tomatoes (3,110 tonnes), lettuce (2,666 tonnes) and pears (1,117 tonnes), followed by Belgian endives (807 tonnes) and bell peppers (768 tonnes).

It can be seen that the occurrence of byproducts strongly fluctuates between different years. This can be explained by factors such as temperature and rain, simultaneous ripening of different cultivars and external factors such as the EHEC crisis in 2011 and the Russia boycott established since 2014. Variation during the year is also present. In Figure 1.3, the surplus production for tomato from one auction is shown throughout two consecutive years.

**Table 1.2** Amounts of vegetable and fruit byproducts occurring at the three major produce auctions in Flanders.

	Byproduct (tonnes)		
	2013	2014	2015
Asparagus	42	0	38
Beans	0	2	8
Belgian endive	1,233	954	807
Bell pepper	456	793	768
Broccoli	19	4	6
Brussels sprouts	6	17	53
Cabbage	103	75	141
Carrot	0	0	1
Cauliflower	900	616	418
Celeriac	34	41	33
Celery	111	61	108
Cucumber	189	240	86
Eggplant	9	6	4
Endive	34	39	65
Fennel	16	10	28
Leek	155	695	114
Lettuce	985	738	2,666
Radish	3	26	1
Tomato	4,884	6,381	3,110
Zucchini	666	358	517
Apple	0	13,239	4,299
Pear	0	1,108	1,117
Strawberry	25	59	173

**Figure 1.3** Surplus production of tomato at a produce auction in Flanders for two consecutive years.

### 1.2.1.3 Food processing byproducts in Belgium

The processing industry for fruit and vegetables is strongly developed in Belgium. In general, approximately 65 % of the outdoor vegetable production is used for industrial processing. Especially the Belgian production of frozen vegetables has a leading position worldwide (Platteau et al., 2012; Roels & Van Gijsegem, 2011).

During processing of fruit and vegetables, an array of different byproducts are generated, depending on the end product and the technology used (e.g. biomass unfit for processing, peels, seeds, but also oils, wastewater, etc.). Due to its importance in Flanders, more specific figures were collected for the frozen vegetable processing sector, consisting of peeling and production losses (e.g. suboptimal processing conditions, low quality vegetables unfit for processing due to color, shape, etc.) (Table 1.3). These processing byproducts are often clean, homogeneous and still qualitative. The collected figures apply to Belgium, but as processing of fruit and vegetables is predominantly located in Flanders, these figures can be considered highly relevant for Flanders. Since the processing industry also utilizes imported crops (predominantly coming from the Netherlands (18.5 %), France (11.7 %), Spain (8.5 %) and Germany (6.8 %)), estimations of the import production volumes were also included in Table 1.3 (Platteau et al., 2016). The data were obtained from the 'Union of the Belgian vegetables processing sector and the trade in vegetables for processing' (VeGeBe).

**Table 1.3** Primary products used and byproducts generated in the processing industry of frozen vegetables in Belgium. Sources: personal communication VeGeBe (2013) and Elst & Gheyskens (2013).

Type	Primary product		Byproduct	
	Primary Belgian production (10 <sup>3</sup> tonnes/year)	Total amount of primary product processed in Belgium (10 <sup>3</sup> tonnes/year)	Fraction (%)	Amount (10 <sup>3</sup> tonnes)
Beans	78	107	20	21
Carrot	233	306	5	15
Cauliflower	73	76	5	4
Leek, Brussels sprouts, zucchini	94	34	5	2
Peas	67	130	18	23
Spinach	86	96	5	5

From Table 1.3, it can be seen that great differences in relative and absolute amounts of processing byproducts exist. Freezing of beans and peas is associated with the greatest relative production loss (18 % - 20 %). In absolute amounts, the peas and beans are joined by the carrot byproducts (15 – 23 10<sup>3</sup> tonnes/year).

Besides frozen vegetables, there is also one large canned vegetables producer in Flanders. They report byproducts for carrots of 5.1 10<sup>3</sup> tonnes of sorted raw and blanched carrots per year (19 %). For beans,

about  $3.3 \cdot 10^3$  sorted raw and blanched beans occur per year (19 %). Black salsify, an important crop for the canning industry generates  $3.0 \cdot 10^3$  tonnes (13 %). In absolute amounts, the amounts of byproducts in the canning industry are thus in the lower range compared to the frozen vegetables, but the fractions of byproducts are similar for beans and larger for carrots (Personal communication Lambrechts, 2016). The order of magnitude and the trends in these data are in agreement with the processing industry from France (personal communication in light of Sunniva project, November 2015).

## 1.2.2 Byproducts occurring throughout the supply chain in the EU

On a European level, the fruit and vegetable byproducts occurring throughout the whole production chain are shown in Table 1.4. These results were adapted from unpublished results of the FP7-project Noshan. They give an overview of the byproducts associated with important crops that are sold either raw to the fresh market or under a processed form. Beside the European scale and the inclusion of a larger amount of crop types, the added value of these figures lies in the fact that they give an indication of the different shares of the supply chain.

In absolute amounts, the predominant byproducts occurring throughout the supply chain in the EU are tomatoes (raw,  $18 \cdot 10^6$  tonnes), grapes (fruit and stalks,  $8 \cdot 10^6$  tonnes), onions (raw,  $7.2 \cdot 10^6$  tonnes), apples (raw,  $5.9 \cdot 10^6$  tonnes), cabbage (raw,  $5.7 \cdot 10^6$  tonnes), oranges (raw,  $3.8 \cdot 10^6$  tonnes) and olives (raw,  $3.07 \cdot 10^6$  tonnes).

The raw marketed products are generally characterized by a 20 % loss at cultivation stage (i.e. harvesting losses). A limited processing towards a marketable raw product is often necessary and is associated with an extra loss ranging from 2 % (cauliflower, watermelon) to 50 % (peas). For these raw products, the data derived from the sum of the cultivation and processing stages, can be compared to the Flemish data on the 'edible harvesting byproducts', as reported in Part 1.2.1.1. A general comparison of these European and Flemish data shows that the European data are characterized by larger losses for both vegetables and fruit. On average about 30 %, 25 % and 20 % of byproducts are reported by Europe during harvesting and preliminary processing of outdoor vegetables, greenhouse vegetables and fruits, respectively. In comparison, the Flemish data of the same categories show 16 %, 7 % and 5 %, respectively. This comparison can be partly biased due to the fact that more crop types are included in the European database. However, a crop-wise comparison between both spreadsheets shows an analogous trend. Factors related to different measurement methodologies (e.g. differing assumptions), inaccurate estimations and different practices in other countries (e.g. different cultivation techniques) can lie at the basis of these differences.

**Table 1.4** Horticultural byproducts in the EU. Stage 1, cultivation; stage 2, transport & storage; stage 3, processing; stage 4, transport, storage & distribution to population; stage 5, consumption. Adapted from FP7-project Noshan (unpublished).

Primary product		Byproduct						
Type	Primary product	Main type of byproduct	Supply chain stages					10 <sup>6</sup> tonnes
			1	2	3	4	5	
Asparagus	Raw	Raw asparagus	20%	5%	12%	10%	19%	<b>0.16</b>
	Processed (preserved)	Asparagus, peels,...	-	-	51%	10%	19%	<b>0.05</b>
Cabbage	Raw	Cabbage (raw)	20 - 40%	5%	40%	10%	19%	<b>5.74</b>
Carrots and turnips	Raw	Carrot (raw)	20%	9%	-	7%	17%	<b>2.98</b>
	Processed (juice, frozen)	Pomace & frozen carrot	-	-	20% - 65%	7%	17%	<b>0.29</b>
Cauliflowers/ broccoli	Raw	Cauliflower	20%	5%	2%	10%	19%	<b>1.13</b>
	Processed (frozen)	Frozen cauliflower	-	-	48%	10%	19%	<b>0.43</b>
Leek	Raw	Leek	20%	9%	-	-	-	<b>2.38</b>
	Processed (frozen)	Leek (processed)	-	-	15%	7%	17%	<b>2.41</b>
Olive	Raw	Olives	3%	5%	24%	0%	19%	<b>3.07</b>
	Processed (olive oil)	Olive pomace	3%	5%	77%	0%	50%	<b>1.56</b>
Onions	Raw	Entire onion	20%	5%	-	10%	19%	<b>7.22</b>
	Processed (sliced, frozen)	Onion peels	-	-	35%	10%	19%	<b>0.13</b>
Peas	Raw	Pea cobs	10%	1%	54%	1%	4%	<b>0.34</b>
	Processed (prepared and preserved)	Peas, hulls	-	-	10%	1%	4%	<b>0.05</b>
Pumpkins & squash	Raw	Pumpkins	20%	5%	-	10%	19%	<b>0.82</b>
Peppers and bell peppers	Raw	Peppers and bell peppers	20%	5%	-	10%	19%	<b>1.04</b>
	Processed (prepared and preserved)	Processed bell peppers	-	-	10%	10%	19%	<b>0.89</b>
Cucumbers	Raw	Cucumbers	20%	5%	2%	10%	19%	<b>2.38</b>
Lettuce	Raw	Lettuce	20%	5%	-	10%	19%	<b>1.76</b>
Mushrooms and truffles	Raw	Whole with roots	21%	5%	-	10%	19%	<b>0.75</b>
	Processed (canned)	Prepared mushrooms	-	-	40%	10%	19%	<b>0.24</b>
Tomato	Raw	Entire tomatoes	49%	5%	-	5%	19%	<b>18.29</b>
	Processed (canned, juice, peeled, puree)	Tomato retails, peel, paste, puree	-	-	4% (juice) - 22,5% (canned)	5%	19%	<b>3.10</b>
Apples	Raw	Entire apples	20%	5%	-	10%	19%	<b>5.91</b>
	Processed (dried, puree, compote juice)	Apple peels, seeds, pulp	-	-	16% (pulp) - 35% (peels)	10%	19%	<b>1.44</b>
Apricots and cherries	Raw	Apricots and cherries	20%	5%	-	10%	19%	<b>0.70</b>
	Processed (dried, juice, jam, frozen)	Processed products, pit, peels	-	-	8% (juice) - 14% (frozen)	10%	19%	<b>0.29</b>
Grapes	Raw	Grape fruit and stalks	20%	5%	10%	10%	10%	<b>8.03</b>
	Processed (dried, juice, wine)	Stems, seeds, skins, pomace, mashed pulp	-	-	4% (dried) - 38% (pomace)	10%	19%	<b>6.05</b>
Lemons and limes	Raw	Lemon (fresh)	20%	5%	-	10%	19%	<b>0.73</b>
	Processed (juice & jam)	Peel and jam	-	-	25% (jam) - 50% (juice)	10%	19%	<b>0.09</b>
Oranges	Raw	Orange fruit	20%	5%	-	10%	19%	<b>3.82</b>
	Processed (juice, jam)	Peel, juice, jam	-	-	25% (jam) - 50% (juice)	10%	19%	<b>5.64</b>
Peaches and nectarines	Raw	Peach	20%	5%	-	10%	19%	<b>2.06</b>
	Processed (canned, juice)	Peach canned, dried peels	-	-	25%	10%	19%	<b>0.60</b>
Pears	Raw	Pears	20%	5%	-	10%	19%	<b>1.47</b>
	Processed (dried, canned, juice)	Dried pears, canned pears, pear pomace	-	-	34%	10%	19%	<b>0.79</b>
Plums and sloes	Raw	Plum (fresh)	20%	5%	-	10%	19%	<b>0.79</b>
	Processed (purées, dried)	Plum cake, pits	-	-	10%	10%	19%	<b>0.09</b>
Tangerines, mandarins	Raw	Tangerines	20%	5%	-	10%	19%	<b>1.68</b>
	Processed (canned, juice)	Canned mandarines, peel	-	-	39% (canned) - 50% (peel)	10%	19%	<b>0.52</b>
Watermelon	Watermelon (from harvesting)	Watermelon (from harvesting)	20%	5%	2%	10%	19%	<b>2.36</b>

In contrast to the raw marketed products, the processing stage (e.g. juiced, sliced, frozen) in the processed products contributes to a larger extent to the formation of byproducts. In case of outdoor vegetables, these amounts range from 10 % (prepared peas) to 77 % (olive oil). During the processing of greenhouse vegetables, about 4 % (tomato juice) to 40 % (prepared mushrooms) are converted to byproducts whereas in case of fruit this ranges from 8 % (cherry juice and jam) to 50 % (lemon juice). In most cases, processing of fruit and vegetables thus leads to the greatest amount of byproducts, although the specific amounts are very process specific. Consequently, a comparison with the Flemish data in section 1.2.1.3 cannot be made as it only represents the byproducts occurring in the freezing industry.

Finally, about 15 % of the raw and processed fruit and vegetable products are converted to byproducts during transport and 20 % during consumption. Unfortunately, no crop-specific data were found for the relative share of this distribution and consumption stage. However, when studying the edible and inedible byproducts occurring in Europe in the food industry in general, the overall trend showing a large contribution of consumption in the generation of byproducts is confirmed. Expressed on the total amount of byproducts (instead of relative to the primary production), households are reported to generate about 53 % of byproducts and distribution about 17 % (Monier et al., 2010; Stenmark et al., 2016).

### **1.3 Potential for using horticultural byproducts in the bioeconomy**

It becomes clear that there are a myriad of fruit and vegetable byproducts available. From an economic, environmental and social point of view, their valorization is necessary. Current applications predominantly include feed and ploughing in the field, complemented by food and anaerobic digestion (Braekevelt & Schelfhout, 2012; Fava et al., 2015; Kips & Van Droogenbroeck, 2014; VBT, 2015). However, other applications with a higher added value are promising. To illustrate the potential of these horticultural byproducts, an overview of the current applications and research of horticultural byproducts is given below, categorized per sector. The goal is not to give an exhaustive list of all nutrients present in byproducts and their possible valorizations, but rather to demonstrate the potential of the horticultural byproducts and illustrate some of the current practices. The main focus lies on food (and feed) valorization in accordance with the cascade principle for valorization, adopted in Flanders and the EU (European Commission, 2015c; IWG BE, 2013; Flemish government, 2015). Special attention will be given to the different levels at which the valorization actions are operating, varying from lab scale to pilot scale and industrial implementation.

### 1.3.1 Food

A balanced diet providing the required amounts of micro- and macronutrients is essential in view of a healthy life style. An increased awareness and interest in these balanced diets can be observed nowadays with focus on a diet rich in fruit and vegetables and ingredients from natural sources (O'Shea et al., 2012; WHO, 2002; WHO, 2015a). Besides the primary products resulting from horticultural production and processing industry, byproducts can also be considered as promising sources of compounds to produce these high-quality, nutritious food products (Galanakis, 2012; Mirabella et al., 2014; O'Shea et al., 2012; Schieber et al., 2001; Sharma et al., 2016).

#### 1.3.1.1 Current applications of horticultural byproducts in food

The current applications of byproducts in the food industry can be classified in three categories, (i) whole byproducts directly used for food purposes, (ii) whole byproducts used for food purposes after limited processing and (iii) refined ingredients derived from byproducts.

The direct use of whole byproducts occurs for example via social charity initiatives, offering surplus byproducts or products unfit for commercialization or processing to people in need. This donation is the most straightforward way of byproduct valorization and is believed to be the most appropriate response to surplus products (OVAM, 2012; EC, 2008; Mourad, 2016). The Flemish produce auctions donate on average 8 % of their surplus products to charity, varying from 0.3 % to 10 % depending on the policy of the auction (personal communication auctions, 2016; VBT, 2015). Recently, this donation was facilitated by the Belgian federal government by adapting the fiscal policy. Furthermore, an online platform was founded to assist in the interaction process of supply (agricultural farms, supermarkets, processing industries) and demand (charities and social organizations) of these byproducts (Schenkingsbeurs<sup>7</sup>). An analogous initiative has been launched at the European level for biomass in general (Biocontact<sup>8</sup>). Byproducts that are processed to a limited extent into meals can also be classified in this first category of directly using whole byproducts, as often the service is targeted instead of specific end products. Such activities are performed for example by catering companies or environmental organizations (e.g. Soepcarrousel in Belgium, Culinary Misfits in Germany, Feed the 5000 from Feedback in the UK). Another example are the supermarkets providing 'ugly' fruit and vegetables (e.g. Albert Hein, Delhaize). Analogous concepts are adopted in other European countries (e.g. Intermarché in France and the 'Outletsupermarkt' in the Netherlands).

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<sup>7</sup> <https://www.schenkingsbeurs.be/>

<sup>8</sup> <https://www.biocontact.eu>

A second category can be distinguished in which preservation technologies are used to process whole byproducts into commercial food products. Culinary processes used to produce soups, juices and smoothies are a first example. In contrast to the above, the end products are targeted here, rather than the service. Provalor and the Greenery produce vegetable juices from byproducts derived from the vegetable processing industry. 'Overlekker' and 'Barstensvol' are two Dutch brands of soups produced in the Verspillingsfabriek using surplus horticultural products. Kromkommer is an analogous Dutch initiative selling soup, which is based on surplus vegetables. Besides culinary processes, also industrial drying technologies are often used for preserving and valorizing horticultural byproducts and transforming them into flavorings and colorants. An example is FoPo (Food Powder<sup>9</sup>), an initiative that dries fruit and vegetable byproducts into powders to add into food products. Another example is Scelta, a company that cooks, presses and dries or concentrates mushroom stems into flavorings for soups and sauces (Soethoudt & Timmermans, 2013).

Refining byproducts into ingredients for the food industry is a last category of current byproduct applications. A well-established example is the use of citrus peels and apple pomace for the production of pectin, which is suited for a range of food products such as jams, dairy products, beverages, pastries and confectioneries (CIR, 2015; Lario et al., 2004; May, 1990). Citrus peels and grape seeds are also used to produce oils and sweeteners, which are 'Generally Recognized as Safe' (GRAS) for intended use in foods for human consumption (CIR, 2014; Deng et al., 2011; Galanakis, 2012; Wadhwa & Bakshi, 2013). Also seed oils from a range of fruits are allowed in food formulation. Ecotreasures for example presses a variety of fruit seeds to oil for the food (and cosmetic) industry. Deriving food ingredients with specific health-beneficial properties from byproducts is less widespread. There are some existing commercial products with a registered health claim, based on bioactive compounds that are also present in fruit and vegetable byproducts. Examples are (i) hydroxytyrosol from olives (Hytolive<sup>TM</sup>), contributing to the protection of blood lipids from oxidative stress (Ciriminna et al., 2016), (ii) cocoa flavanols (Acticoa<sup>TM</sup>) which help maintain the elasticity of blood vessels, thereby contributing to normal blood flow (Tallon, 2015) and (iii) tomato phenolic compounds (in combination with nucleoside derivatives in Fruitflow<sup>R</sup>) which lead to natural cardio-protective effects (O'Kennedy et al., 2016).

### 1.3.1.2 Current research on the application of horticultural byproducts in food

Due to the increased awareness of the problems related to the occurrence of these horticultural byproducts, a lot of research is being performed on their potential use as food ingredients. Generally, this research can be classified in four different groups according to the subject and level of detail: (i) elucidating the composition of byproducts, (ii) optimizing stabilization and extraction of byproducts to

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<sup>9</sup> <http://www.hellofopo.com/>



food ingredients, (iii) evaluating the technical functions of horticultural-byproduct-derived ingredients and (iv) evaluating the health-promoting attributes of byproducts and derived food products.

**a. Research focused on elucidating the composition of the byproducts**

Many studies focus on characterizing the byproducts. A myriad of byproducts have been analyzed in this way, especially recently due to the increased interest in the value of recycling and integral exploitation of agro-food products (Galanakis, 2012; Mirabella et al., 2014; O'Shea et al., 2012; Russ & Meyer-Pittroff, 2004; Schieber et al., 2001; Sharma et al., 2016). This information is used to demonstrate the high nutritional value of byproducts and is often used as a means to screen and explore the opportunities of the byproducts.

It has been found that in general phytochemicals are predominantly present in higher levels in the peels and seeds of the fruit compared to the actual flesh (Kalt, 2005). Hence, press residues from industrial juice processing are often investigated. Examples are apple pomace, grape pomace, citrus pomace, mango peel, carrot pomace, cauliflower florets and stems, onion skins, tomato pomace and olive pomace (Mirabella et al., 2014; Panouillé et al., 2007; Schieber et al., 2001). Compounds most often investigated are dietary fibers and phytochemicals such as phenolic compounds, carotenoids and glucosinolates (Galanakis, 2012; Larrauri, 1999; Mirabella et al., 2014; O'Shea et al., 2012; Schieber et al., 2001).

The approach adopted in the aforementioned studies determines the level of detail of the composition that is obtained. On the one hand, screening the content is often performed using general, rapid and easy-to-use assays, for example spectrophotometric assays detecting specific groups of chemically similar reactive compounds, thereby not specifying between the different individual compounds (Tarbart et al., 2009). An example is the spectrophotometric assay for estimating the total phenolic content (Folin-Ciocalteu) by measuring the absorbance at a certain wavelength (Tarbart et al., 2009; Ignat et al., 2011). However, due to its lack of selectivity, this is reported to overestimate the phenolic content (Escarpa & González, 2001). On the other hand, more specific analytical methods such as for example liquid chromatography coupled to mass spectrometry, enables one to separate, identify and quantify the individual compounds (Ignat et al., 2011; Tabart et al., 2009). For multiple byproducts, a wide range of compositional information is thus present with different levels of detail.

**b. Research focused on the stabilization of horticultural byproducts and extraction of specific compounds as food ingredients or end products**

A lot of research focuses on methods for stabilizing byproducts and extraction and recovery of specific compounds (Galanakis, 2012). Stabilization technologies are generally used to reduce the moisture content of the product, to stop enzymatic activity and/or to improve permeability of the tissues

(Galanakis, 2012). These predominantly consist of size reduction, concentrating, pressing, drying, centrifuging, fermentation and microfiltration (Galanakis, 2012; Laufenberg et al., 2003; O'Shea et al., 2012). These technologies are well-established as they have been used in the food industry for many decades (Galanakis, 2013). Also novel technologies are being investigated such as radiation treatments (UV light, high-intensity light pulses,  $\gamma$ -irradiation), electrical treatments (pulsed electric fields, radiofrequency electric fields, microwave heating, ohmic heating), ultrasound treatment, high hydrostatic pressure, inert gas treatments (supercritical carbon dioxide, ozonation) and combinations thereof (Barrett & Lloyd, 2012; Jiménez-Sánchez et al., 2017a; 2017b; Pereira & Vicente, 2010; Turk et al., 2012). Besides pretreatment, a large body of literature focuses on the optimization of the extraction and recovery of bioactive compounds from the byproducts. Attention is often given to environmentally friendly methods such as pressurized solvent extraction, enzyme-assisted extraction, supercritical fluid extraction, membrane separation, microwave-assisted extraction, ultrasound-assisted extraction, high hydrostatic pressure pretreatment, pulsed electric fields and combinations of these extraction technologies such as supercritical fluid extraction with ultrasound, enzymatic of high pressure solvents (Ferrentino et al., 2016; Galanakis, 2012; Gil-Chávez et al., 2013). Both optimization of technical parameters (such as type of solvent, time, temperature, pressure, power, extraction steps depending on the extraction method) and the effect of the utilized technology on the concentration and nature of the targeted compounds are often studied. Despite increasing scientific research generating promising results, the industrial implementation of these technologies is still limited.

These novel technologies are however often only tested at lab scale. Furthermore, a lack of standardization in operation conditions make comparisons between different studies difficult. More research on their performance on pilot and industrial scale is necessary to allow for broad industrial applications, thereby investigating the performance and stability of the resulting extracts (Ferrentino et al., 2016; Jiménez-Sánchez et al., 2017a; Gañan et al., 2015).

**c. Research focused on evaluating the technical functions of horticultural-byproduct-derived ingredients**  
Gathering information about the composition, stabilization and/or extraction is often followed by tests incorporating the byproduct-derived ingredients in food products and evaluating their technical functions in the food product.

These studies predominantly investigate the addition of stabilized fractions (e.g. dried powders derived from apple pomace, grape pomace, carrot peels, cauliflower stems and stalks, onion skins) or to a lesser extent fresh fractions (e.g. pastes) to products such as juices, meat products and bakery products. They are predominantly investigated for the technical effect of their fiber content to the food products. Fiber addition to food products can enable a partial replacement of the flour, fat or

sugar content and improve the viscosity, texture, sensory characteristics and shelf-life of food products (Elleuch et al., 2011; Quiles et al., 2016; Sharma et al., 2016). The aspects predominantly studied in this regard are sensory traits (color, taste, texture, viscosity) and technological characteristics (water holding capacity, oil holding capacity, gelling properties, antioxidative properties) of the formulated products (Elleuch et al., 2011; Laufenberg et al., 2003; Mirabella et al., 2014; O'Shea et al., 2012; Schieber et al., 2001; Sharma et al., 2016). Besides fiber, also the function of other byproduct-derived ingredients such as antioxidants (preventing browning and lipid oxidation), antimicrobials, colorants, flavorings and thickener agents have been investigated (Ayala-Zavala et al., 2011; Balasundram et al., 2006; Moure et al., 2001; Schieber et al., 2001; Shahidi & Ambigaipalan, 2015).

d. Research focused on evaluating the health-promoting attributes of byproducts and derived food products

Related to the search for more naturalness in food is the trend for health-promoting food attributes in products such as functional foods and nutraceuticals. Multiple studies on horticultural byproduct valorization in food respond to this trend (Kammerer et al., 2014; Mirabella et al., 2014; O'Shea et al., 2012; Schieber et al., 2001). However, actual measurements of the specific health-promoting attributes of these byproduct-derived foods in the human body are scarce in these studies. They can be split into two major groups, i.e. (i) the studies that solely rely on literature evidence to explore the potential health-benefits of the byproducts and (ii) the studies that use *in vitro* tests to actually test the health-beneficial effects.

The first group of studies are confined to investigating the composition of the byproduct without actually testing the functionality. Therefore, they rely solely on literature to link the composition to compound-specific health-beneficial effects. Accordingly, the potential of byproducts as novel sources of these compounds is stressed as well as the opportunities of using the byproducts as food ingredients to obtain functional foods. Research for dietary fiber content for example, often relates to their beneficial effects in reducing the risk of cancer and coronary heart disease (Larrauri, 1999; O'Shea et al., 2012; Panouillé et al., 2007). Investigation of the presence of phenolic compounds in byproducts is another example that is often complemented with literature information regarding their strong antioxidative potential, their potential reduction of inflammation and cardiovascular diseases, cancer and coronary heart diseases (Balasundram et al., 2006; Mirabella et al., 2014; O'Shea et al., 2012; Panouillé et al., 2007; Schieber et al., 2001; Wadhwa & Bakshi, 2013).

Actually testing the potential health-beneficial effects of byproduct-derived ingredients is performed to a lesser extent. There are some studies that screen the phytochemical and pharmacological profiles of phytochemicals and gain insight in their mechanisms of action using *in vitro* tests (e.g. Babbar et al.,

2015; Kabuki et al., 2000; Obied et al., 2009; Ramos et al., 2013; Sanz-Puig et al., 2006; Yanagida et al., 2000), whereas examples of human *in vivo* tests using horticultural-byproduct-derived foods are very scarce (D'Archivio et al., 2010; Dillard & German, 2000; Moran et al., 2013; Moure et al., 2001; Schieber et al., 2000). Rather the *in vivo* effect of specific plant-derived compounds (not necessarily derived from byproducts) have been shown in clinical studies of which the knowledge is often used in studies on byproducts, serving as a basis for suggesting the potential of a byproduct containing the specific compound, as also mentioned above.

### 1.3.2 Feed

In light of the global challenge to ensure the food production, valorization towards feed applications is another important aspect that can indirectly contribute to this objective.

#### 1.3.2.1 Current applications of horticultural byproducts in feed

Traditionally, horticultural byproducts have been used directly, as a whole, in feed without extensive processing (Fava et al., 2015; Braekevelt & Schelfhout, 2012; OVAM, 2014). This often arises from the economic, environmental and legal restrictions associated with disposal of the byproducts (Russ & Mayer-Pitroff, 2004). Instead of technical processing features, practical aspects such as variability in nutritional levels, seasonal availability, logistics, storage and legislation are more important to address to realize these valorization pathways (Kusch et al., 2014).

For example, edible harvesting byproducts, whether or not after a preservation process, are often valorized towards feed (e.g. carrots, peas, bell pepper, beans, chicory roots, Belgian endive roots and surplus rebut apples) (Kips & Van Droogenbroeck, 2014; VBT, 2015). Also (conventionally considered) inedible harvesting byproducts are sometimes directed to feed. For example forced Belgian endive roots generate about € 10 - € 15 per tonne when valorized as feed (Kips & Van Droogenbroeck, 2014). Many auctions also divert their byproducts to feed purposes. This varies from 0 % - 100 % depending on the policy of the auction (personal communication auctions, 2016). Finally, processing byproducts are mainly reused as feed within Flanders and abroad (e.g. apple pomace, orange peel, tomato pomace, edible parts of carrots, peas, beans, red cabbage, salsify and turnip rooted celery) (Sweet et al., 2016; personal communication industrial processor Flanders, 2016; unpublished results FP7-project Noshan). These types of valorization predominantly occur in local set-ups, aiming to close the resource cycle at the farm or at a regional level.

### 1.3.2.2 Current research on the application of horticultural byproducts in feed

In order to meet the nutrient requirements of livestock and sustain their productivity and profitability, feed resources are increasingly being explored. Therefore, a broad array of fruit and vegetable byproducts have been investigated. The focus often lies on high voluminous byproducts such as apple, grape, tomato, olive and citrus pomace.

As was the case in food valorization, the composition is often the starting point of research in this domain. This compositional information is widespread and does not specifically differ when targeting valorization towards feed instead of food (Gowe, 2015; Kasapidou et al., 2015; O'Shea et al., 2012; Schieber et al., 2001; Wadhwa & Bakshi, 2013).

Horticultural byproducts are often investigated for their use as a main feed ingredient providing energy, fibers and proteins, for which they are used as a whole, either fresh or after limited processing such as drying or ensiling (Angulo et al., 2012; Bampidis & Robinson, 2006; Kasapidou et al., 2015; Mirzaei-Aghsaghali & Maheri-Sis, 2008; Wadhwa & Bakshi, 2013). No extraction nor complex conversion processes are thus required.

In contrast to using them as a whole for main feed ingredients, horticultural byproducts can also be used as a source of phytochemicals. These natural-derived functional feed ingredients comply with consumer requests for the production of clean label animal-derived products (Kasapidou et al., 2015). The research for these novel feedstocks is predominantly focused on their effect on animal performance (e.g. growth performance, organ size, protein, fat digestibility, resistance to infections, digestibility, palatability, performance) and the derived food products (e.g. meat oxidative stability, meat discoloration, meat shelf-life, lipid profile in milk, color of egg yolk) (e.g. Brenes et al., 2008; Centre of Expertise for Plant compounds, 2016; Gladine et al., 2007; Vasta & Luciano, 2011).

## 1.3.3 Biobased products

### 1.3.3.1 Pharmaceuticals and cosmetics

Horticultural products and byproducts contain a wide variety of compounds that can be interesting for the pharmaceutical and/or cosmetic industry. Various products (extracts, powders, juices and oils) from different byproducts (resulting from fruit, bark, flower, leaf, peel or pulp of apple, citrus, tomato, grape, olive, cucumber, pumpkin, strawberry, apricot and peach) have been reported as safe and are being used in cosmetics (CIR, 2011; 2015; 2016; Fiume et al., 2014). Examples of byproduct-derived phytochemicals used in pharmaceuticals are less present.

The research into pharmaceutical and cosmetic uses of horticultural byproducts is growing. A myriad of scientific studies (Deng et al., 2012; Korthout & van der Meulen, 2012; Peschel et al., 2006; Schieber et al., 2001; Wadhwa & Bakshi, 2013) and various databases (Dr. Dukes phytochemical and ethnobotanical database<sup>10</sup>, Extractenbibliotheek<sup>11</sup> and Napralert<sup>12</sup>) list the present compounds in plants (often including byproducts of fruit and vegetables) and their potential functionality in various markets such as cosmetics and pharmaceuticals. As the health-promoting attributes of vegetable and fruit byproducts are also of importance for the food industry (e.g. functional food, nutraceuticals), a parallel structure (as in part 1.3.1.2. d) with often overlapping results is present in the research for the cosmetic and pharmaceutical activities with varying levels of specificity (e.g. showing the presence of a certain compound and linking it to literature knowledge on specific activities vs. human tests).

### 1.3.3.2 Materials

Also within the material sector, there are possibilities for horticultural byproducts serving as sustainable and qualitative substitutes for fossil-based materials. Ligno-cellulosic byproducts can for example be used for natural materials such as paper and cardboard. An example is the production of cardboard based on fibrous pulp from tomato leaves or tomato stems (OVAM, 2014; Center of Expertise for Plant Compounds, 2016a). They can also be used for the production of biobased polymers. Today, polymers and plastics are almost entirely based on fossil sources. Only 1 % of the existing polymers is biobased, however interest is growing and various renewable resources can be used for this purpose (Dietrich et al., 2016; Keegan & Kretschmer, 2013; Voevodina & Kržan, 2011). These biobased plastics can be divided into three classes: (i) modification of natural polymers (e.g. cellulose acetate, thermoplastic starch), (ii) direct production in plants (e.g. polyhydroxyalkanoates (PHAs) accumulated in bacteria that can be fed with byproducts) and (iii) two-step biomass conversion (e.g. biopolyethylene, biopolypropylene, polylactic acid) (Fava et al., 2015; Follonier et al., 2015; Girotto et al., 2015; Naranjo et al. 2014; Storz & Vorlop, 2013; Voevodina & Kržan, 2011).

Currently, the majority of the biobased materials are based on agricultural crops rich in carbohydrates, otherwise used as food or feed (so called 1<sup>st</sup> generation bioresources). However, in order not to compromise life-sustaining production, non-food and non-feed crops are increasingly being explored (often called the 2<sup>nd</sup> generation bioresources) such as ligno-cellulosic resources, agricultural waste and food waste (Girotto et al., 2015; Storz & Vorlop, 2013; Voevodina & Kržan, 2011). Some examples of (predominantly agricultural) byproducts used for production of materials are maize byproducts

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<sup>10</sup> <https://phytochem.nal.usda.gov/phytochem/search>

<sup>11</sup> <http://plantenstoffen.nl/extractenbibliotheek/>

<sup>12</sup> <https://www.napralert.org/>

(Futero, NatureWorks, Novamont) and potato byproducts (Rodenburg biopolymers, Avebe, Biotec) (Bos & van Rees, 2004; Bolck et al., 2012).

### 1.3.3.3 Biopesticides and biostimulantia

According to the European definition, pesticides are used to prevent, destroy or control harmful organisms or diseases or to protect plants during production, storage and transport. Examples are herbicides, fungicides, insecticides and biocides. This term is often used interchangeably with the term plant protection products, which is used specifically for plants. The indiscriminate use of synthetic pesticides has given rise to many problems including genetic resistance, toxic residues, hazards from handling and environmental pollution (Adeyemi, 2010; Burketova et al., 2015). This has increased the interest in biopesticides, i.e. pesticides of natural origin, derived from animals, plants, bacteria or minerals (Chojnaka et al., 2015; European Commission, 2016a; O'Brien et al., 2009; Schuurbijs et al., 2013). While biopesticides protect against biotic stress (e.g. attack by pests), biostimulants are used to protect the plant against abiotic stress and to stimulate natural processes enhancing the plants nutrient uptake, nutrient efficiency and crop quality (Chojnaka et al., 2015; European Biostimulants Industry Council, 2016). Although the added value of these biobased products is generally recognized from a human and environmental hazard perspective and even though they have been investigated for more than 50 years, only 0.1 % of the developed formulations have been put on the market, due to a variety of factors such as high development costs and complex elucidation of working mechanisms (Chojnaka et al., 2015; O'Brien et al., 2009).

Scientific literature shows many examples of the efficacy of biobased agricultural products (Adeyemi, 2010; Chojnaka et al., 2015; Seiber et al., 2014). Copping & Duke (2007) give an overview of plant-derived products, going from fungicides and bactericides to herbicides and insecticides. Analogously, plant-derived byproducts could form an alternative feedstock for biopesticides. Tomato and paprika stems and leaves are being investigated for example for their potential action against various molds and mildew (Schuurbijs et al., 2013).

## 1.4 In conclusion

The aim of this introduction was to give more insight in three issues related to valorizing horticultural byproducts, i.e. (i) the adopted terminology and classification of byproducts, (ii) the amounts of horticultural byproducts and (iii) the current applications and potential valorizations of horticultural byproducts.

Firstly, a transparent terminology framework is necessary for generating qualitative data on horticultural byproducts and enabling comparison with other countries or measurement of evolutions throughout time. The terms edible and inedible horticultural byproducts are used in this dissertation. These include non-consumed horticultural products occurring throughout the supply chain. Based on the stage during which they occur throughout the supply chain, they have been grouped in three categories: (i) harvesting byproducts, (ii) byproducts resulting from the produce auctions and (iii) food processing byproducts.

Secondly, horticultural byproducts occur throughout the supply chain in different amounts and under different forms. The total amounts of edible byproducts occurring in Flanders were largest for the harvesting byproducts (274 10<sup>3</sup> tonnes), followed by processing byproducts (70 10<sup>3</sup> tonnes) and losses at the auctions (16 10<sup>3</sup> tonnes). The inedible byproducts occur predominantly during harvesting and preliminary processing (562 10<sup>3</sup> tonnes), with the largest amounts for the foliage and leaves of the Brussels sprouts and cauliflower. The largest edible byproducts are the harvesting byproducts of leek, onion peels and the processing byproducts of peas and beans.

Valorization of these horticultural byproducts in Flanders today consists predominantly of feed applications and ploughing in the field. According to the cascade principle, other applications are possible that can create a higher added value. The current and potential valorizations were illustrated in the third part of this introduction. Based on the immense amount of scientific studies and small scale projects, it can be seen that awareness has been raised regarding the potential added value of converting fruit and vegetable byproducts to food, feed and functional material products. However, despite this recognition, the actual successful implementations are scarce and scientific studies do not equally lead to practical implementations. For food and feed purposes, a different stage of adoption can be seen for valorizing the byproducts as a whole versus refining them in ingredients with specific functionalities. Whereas the former is already commercialized under different forms and on different scales, the latter remains predominantly in the research stage, “despite the omnipresence of hypothetic scenarios, high quality studies and patented methodologies” as stated by Galanakis (2012). This can also be seen for biobased products, where the possibilities, cited in scientific literature are often not converted to actual industrial applications.

On the one hand, this has been related to the technical execution of scientific research, consisting predominantly of specific lab-scale studies with only few cases containing technical feasibility on pilot or industrial scale (Mirabella et al., 2014; Peschel et al., 2006). Practical aspects such as extraction efficiency, recovery efficiency, performance, variable composition of the feedstock (induced by origin, storage and processing conditions), stability of the derived products, functionality, bioavailability,



bioactivity and toxicology are often not (yet) investigated. Furthermore, more generally, a unilateral techno-scientific approach is often adopted when investigating the development of the bioeconomy (Golembiewski et al. 2015; Pfau et al., 2014). Hence, research is often driven by and focused on the byproduct itself, based on the available amounts or other attractive characteristics, without incorporating socio-economic issues. Although the impact of these economic (e.g. price of substitutes, market potential, market acceptance investment cost, added value), logistic (e.g. transport, seasonal occurrence, storage) and legislative aspects (e.g. waste legislation, product safety, claims) as prerequisites for successful implementations are being increasingly recognized (Ayala-Zavala et al., 2011; Galanakis, 2012; Peschel et al., 2006), their investigation at the start of the research phase is limited. Hence, research is often still focused on technical aspects. Integral, transdisciplinary studies are thus key in order to secure industrial exploitation of horticultural byproducts. These aspects are further elaborated upon in the reflective discussion (Chapter 6).



## **Chapter 2**

Using a novel spiral-filter press to biorefine horticultural byproducts: the case of tomato. Part I: process optimization and evaluation of the process impact on the antioxidative capacity



## **Chapter 2: Using a novel spiral-filter press technology to biorefine horticultural byproducts: the case of tomato. Part I: process optimization and evaluation of the process impact on the antioxidative capacity**

### **Redrafted from**

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### **2.1 Abstract**

With tomato as a model crop, the use of a novel, low-oxygen spiral-filter press technology for juice production was demonstrated on pilot scale. The results showed that a robust process could be developed with a juice yield of 82.5 % which can be increased to 97.0 % with an additional mild thermal pretreatment (40 °C for 3 minutes). A comprehensive insight was gained in the underlying mechanisms through which process parameters can affect juice yield and juice quality parameters such as turbidity and precipitate weight ratio. Additionally, the antioxidative capacity (AOC) was investigated, showing a preservation of antioxidants during pressing (102 % ± 12 %) which may be attributed to the low-oxygen processing. Finally, also an insight was gained in the antioxidative distribution of the resulting fractions, demonstrating the potential of the press residue and confirming the relevance of designing a biorefinery system where all fractions are valorized.

## 2.2 Introduction

Globally, one third of the edible food is lost ( $1.3 \cdot 10^6$  tonnes.year<sup>-1</sup>) (Gustavsson et al., 2011). The fruit and vegetable processing sector, with losses of 40 % – 60 % in their production process (e.g. overproduction, edible and inedible processing byproducts and waste fractions), is a sector where one of the largest quantities of healthy and potentially high-value biomass remains unused (Bos-Brouwers et al., 2012; Gustavsson et al., 2011). Conversely, biomass plays a key role in the emerging bioeconomy where it is used as input for the production of a wide range of products. It is conceived that in this more sustainable economy, products are produced via biorefineries, following a cascade principle in order to maximally valorize the available biomass (Flemish government, 2013; McCormick & Kautto, 2013). Combining both factors, i.e. using food losses in the bioeconomy through a biorefinery process, would thus convert a problem into an opportunity for the emerging bioeconomy.

However, there are a number of factors currently impeding the valorization of fruit and vegetable byproducts. A literature screening (e.g. HLPE, 2014; Schieber et al., 2001) and interviews with stakeholders show that their valorization is currently mainly impeded by their high moisture content (often > 90 %) and corresponding fast decay, their relatively small and geographically dispersed volumes and the seasonality of their production. The combination of these factors makes their collection, conservation and processing a major challenge (OVAM, 2014).

In this chapter, the capability of a novel low-oxygen spiral-filter press to biorefine fruit and vegetable biomass is evaluated. Due to its flexibility and modular design, this spiral-filter press seems to be able to tackle the above mentioned impeding factors, making it a promising technology. Firstly, using a pressing technology in general for valorizing fruit and vegetable byproducts optimally addresses the first challenge mentioned, namely the high moisture content. Instead of stabilizing the biomass by using expensive or quality-reducing drying techniques such as hot air drying (Jangam, 2011), juice pressing extracts a large part of the liquid content that subsequently can be valorized as fruit or vegetable juice or related products. Secondly, the spiral-filter press can deal with the seasonality and variable volumes of horticultural byproducts. Preliminary experiments have shown that the press is able to process a range of volumes ( $300 - 28,000$  kg.h<sup>-1</sup>) as well as handle a multitude of different textures (e.g. apples, berries, corn, carrots, nuts,... ), due to its modular nature and its flexible process parameters (Siewert, 2013). Hence, multiple biomass streams can be processed in function of the harvesting season, which is not the case with some conventionally used presses such as the widely used belt-press which is only suitable for hard biomass matrices such as apples and pears or the Bucher horizontal piston press, working in batch mode (Beveridge & Rao, 1997; Barrett et al., 2005). Moreover, due to its flexibility, it can be used as a key technology in biorefineries, because it allows a further

unraveling and dissection of the resulting solid and liquid streams into multiple fractions, thereby creating a higher added value compared to the direct use of the whole byproduct as such (Baiano, 2014; Bos-Brouwers et al., 2012). An important attribute of the spiral-filter press is its ability to generate premium, cloudy juices, in contrast to the Bucher horizontal piston press and the decanter centrifuge generating rather clarified juices with low soluble solids content (Barrett et al., 2005; De Paepe et al., 2015a; 2015b) (further investigated in Chapter 3). This creates diversity in the different types of juices in the market. Each type of press has its own value and typical end product which provides the consumer with a choice in a variety of juices.

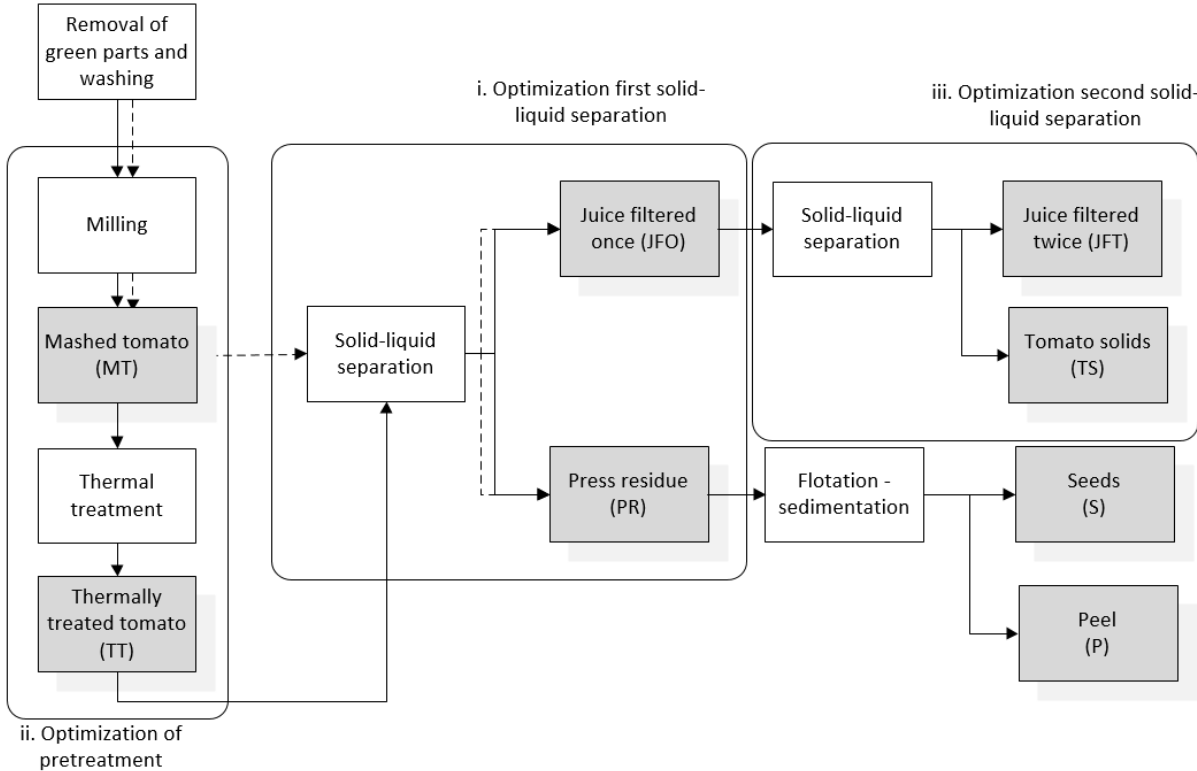
Finally, it has been reported that the spiral-filter press can conserve the phenolic composition of the input biomass throughout processing, which can be attributed to the juice extraction under low-oxygen atmosphere, preventing oxidation from taking place (De Paepe et al., 2015a; 2015b) (further investigated in Chapter 4). In general, conventional presses such as the belt-press and the Bucher horizontal piston press work open to the atmosphere, allowing oxidation and subsequent product degradation. For apples, a comparison of the performance of the spiral-filter press with the belt-press has been performed, showing (i) a higher juice yield, (ii) a higher juice turbidity and (iii) a higher retention of phenolic compounds during downstream processing steps and storage for the former (De Paepe et al., 2015b). Also the juice extraction process used for tomato juice processing using pulper and finisher to separate the peels and seeds from the juice, is characterized by a high rate of oxygen absorption caused by a high rotation speed open to the atmosphere (Noomhorm & Tansakul, 1992). However, oxidation is not always disastrous as this can generate aroma components which are typical for certain juices. Many of the aliphatic esters, alcohols, acids and carbonyls, which are important aroma compounds found in fruits are formed by (i) oxidative degradation of fatty acids or (ii) via the lipoxygenase pathway (El Hadi et al., 2013). In apple juice for example, the temperatures which are used to activate pectinases seemed to activate other enzymes like lipoxygenase, which oxidized the lipids in apple juice and produced flavor compounds such as hexanal and *trans*-2-hexenal (Su & Wiley, 1998). In tomato, this lipoxygenase pathway is also reported to catalyze the production of typical tomato flavors (Barrett et al., 2010; Rodrigo et al., 2007). However, also rancid off-flavors can be produced. Thus, as mentioned above, each press has its own characteristics and typical juices, suitable for different niche markets.

The spiral-filter press thus seems to have the potential to serve in small and medium size enterprises (SME) context to produce premium juices derived from multiple biomass feedstocks and byproducts. Even though being flexible towards the nature of the starting material, biomass processing with the spiral-filter press does require an optimization per matrix and comprehensive insights in the working principle of the press are needed in order to exploit its broad working range. To date, only limited

scientific studies have evaluated the performance of the spiral-filter press, focusing on the processing of apple, pear and strawberry (De Paepe et al., 2015a; 2015b; Possner et al., 2015). As a result, detailed process parameters and knowledge about the press' working principle, that are broadly applicable to other matrices, are particularly uncommon. This chapter offers a comprehensive insight in these aspects by using underutilized tomatoes (e.g. surplus product, low quality tomatoes) as a model crop to investigate the use of the spiral-filter press to biorefine and valorize horticultural byproducts. In 2015, over 3,000 tonnes of healthy tomatoes remained unsold at the produce auctions in Belgium, leaving high-value valorization options unused (personal communication, Belgian produce auctions). In addition, the conventional tomato processing industry (i.e. washing, sorting, crushing, preheating (hot/cold break), pulping/finishing using screens and evaporation) in Belgium is absent, impeding a potential valorization of these tomatoes (Hayes et al., 1998; Heutink, 1986). Furthermore, in contrast to apples and pears, it is a soft matrix which implies that other technical processing challenges have to be addressed, which are in their turn applicable for similar berry-like matrices. The focus in this chapter thus lies in understanding the underlying mechanisms of the spiral-filter press through which process parameters affect juice yield and juice quality when processing soft matrices, by comparing different pilot-scale experiments with different parameter sets. The antioxidative capacity of the resulting samples was related to the feedstock, in order to discern the impact of processing on the raw fruit/vegetable and achieve a relevant indication of the process impact.

The approach adopted here consisted of a pilot-scale optimization of the parameters necessary for a basic solid-liquid separation using the spiral-filter press (Figure 2.1, dotted line). The resulting end products were analyzed for their antioxidative capacity in order to evaluate the process impact of the low-oxygen spiral-filter press on the oxidation of the biomass. In a second step, the optimized system was developed towards further refining the tomato biomass, using the insights gained in the first step (Figure 2.1, solid line). This was achieved by (ii) applying a thermal pretreatment (analogous to conventional tomato processing using cold/hot break) to produce a press residue, separable into whole seeds and peels and (iii) by performing a second solid-liquid separation of the juice obtained in the first phase, in order to isolate a firm tomato puree (tomato solids) from the tomato juice.





**Figure 2.1** Visualization of the optimized biorefinery process. The processes are represented by white boxes and the resulting products by grey boxes. The optimizations for the different subprocesses are indicated by grouped boxes. The dotted lines represent the start of the optimization using a simplified process.

## 2.3 Materials and methods

The optimization of the biorefinery process was performed in several steps (Figure 2.1). These steps will be subsequently covered hereafter.

### 2.3.1 Optimization of the first solid-liquid separation

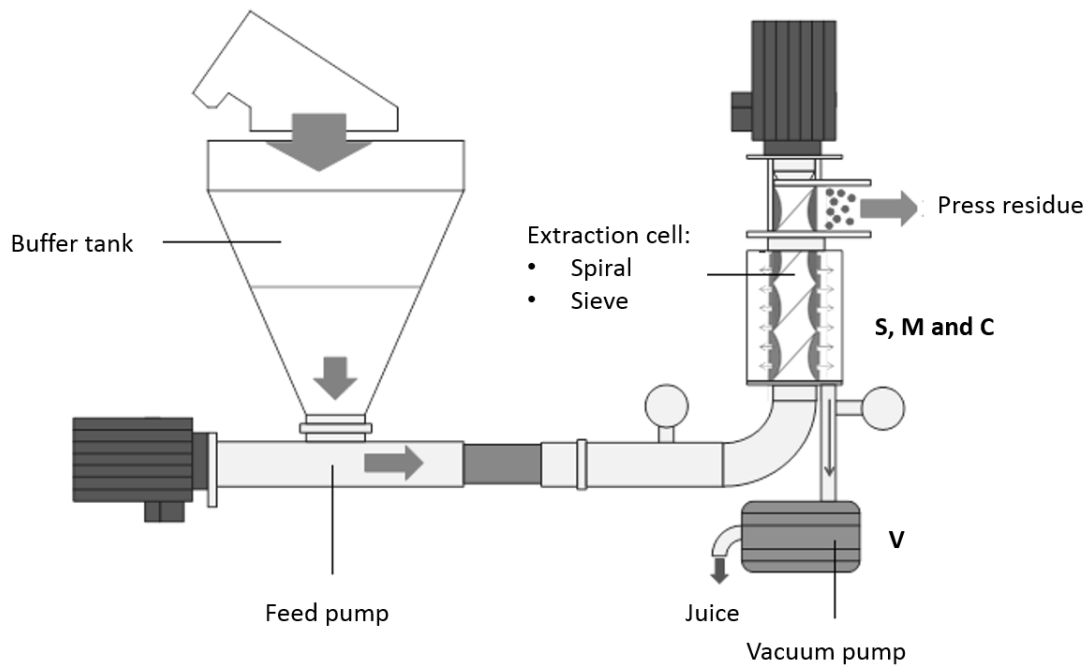
The first step in the optimization process was to investigate if the juice yield obtained by the spiral-filter press could be maximized without using an additional heat pretreatment. Therefore, the process depicted in Figure 2.1 was simplified and comprised only the milling step and the first solid-liquid separation (shown by the dotted line).

#### 2.3.1.1 Description of the machinery and the optimization process

Intact tomato fruits, resulting from overproduction and consisting of a mixture of cultivars (predominantly Kanavaro flesh tomato), were provided by a Belgian produce auction (REO, Roeselare, Belgium) (Figure 2.2). The majority (~95 %) of the tomatoes was at commercial maturity. A homogenized batch of 500 kg was collected in a water bath, filled with cold tap water until all tomatoes were submerged. Subsequently, they were transported by a conveyor into a mill, rotating at a constant angular speed ( $20 \text{ kg}\cdot\text{h}^{-1}$ ) (KWEM 1000, Kreuzmayr, Wallem, Germany). The mashed tomatoes were ejected into the buffer tank of the spiral-filter press, which was the central part in the biorefinery process (Figure 2.3). This system consists of a buffer tank, a screw pump (feed pump), an extraction cell, a spiral that rotates in a cylindrical sieve, a vacuum pump and two exits for liquid and solid fractions, respectively (De Paepe et al., 2015a; 2015b).



**Figure 2.2** Tomatoes used as feedstock, resulting from overproduction and consisting of a mixture of cultivars.



**Figure 2.3** Schematic representation of the spiral-filter press with spiral frequency (S), pore size of the filter element (M), number of channels of the spiral (C) and vacuum pump frequency (V).

The optimization process started with evaluating the system parameters for the first solid-liquid separation: feed pump frequency (F [Hz], 0-50 Hz), spiral frequency (S [Hz], 0-50 Hz), vacuum pump frequency (V [Hz], 0-50 Hz), pore size of the filter element (M [ $\mu\text{m}$ ], 60, 100, 150 and 300  $\mu\text{m}$ ) and number of channels of the spiral (C [-], 3, 4 or 7). The shaft inclination angle was kept at 45°. Furthermore, due to practical considerations, a constant feed pump frequency F of 20 Hz was used in all experiments, leading to a system with four variable system parameters. The effect of varying these system parameters was evaluated on the juice yield (JY) and the moisture content of the press residue ( $\text{MC}_{\text{PR}}$ ). However, in order to get a better insight in the process, various other dependent variables were also recorded such as the moisture contents of the other fractions (moisture content of the mashed tomato,  $\text{MC}_{\text{MT}}$ ; moisture content of the juice filtered once,  $\text{MC}_{\text{JFO}}$ ), the total throughput (TH), the turbidity of the juice (TU) and the precipitate weight ratio (PWR). A total of sixteen combinations were tested according to a screening design configuration (Table 2.1). It has to be noted that the mentioned frequencies represent a rescaled value of the real rotation frequency. The correlations are as follows (De Paepe et al., 2015a):

- Volumetric feed flow rate of the feed pump:  $F_v [L \cdot h^{-1}] = 25 \times F [\text{Hz}]$
- Angular velocity of the spiral  $S_r [\text{rad} \cdot \text{s}^{-1}] = 12 \cdot 10^{-2} \times S [\text{Hz}]$
- Absolute underpressure in the extraction cell  $V_u [\text{bar}] = 3.0 \cdot 10^{-4} \times V^2 - 3.86 \cdot 10^{-2} \times V + 3.25 \cdot 10^{-1}$

**Table 2.1** Screening design used in the optimization of the first solid-liquid separation with the real (C, M, S, V) and the coded (c, m, s, v) independent variables.

Experiment	Number of	Pore size filter	Spiral frequency	Vacuum pump	Measured juice yield [%] JY
	channels [-]	[ $\mu\text{m}$ ]	[Hz]	frequency [Hz]	
	C (c)	M (m)	S (s)	V (v)	
1	7 (+1)	100 (-1)	10 (-1)	0 (-1)	24.1
2	7 (+1)	100 (-1)	50 (+1)	0 (-1)	42.8
3	7 (+1)	100 (-1)	10 (-1)	50 (+1)	20.3
4	7 (+1)	100 (-1)	50 (+1)	50 (+1)	49.8
5	7 (+1)	300 (+1)	10 (-1)	0 (-1)	30.5
6	7 (+1)	300 (+1)	50 (+1)	0 (-1)	43.2
7	7 (+1)	300 (+1)	10 (-1)	50 (+1)	30.3
8	7 (+1)	300 (+1)	50 (+1)	50 (+1)	76.8
9	4 (-1)	100 (-1)	10 (-1)	0 (-1)	23.9
10	4 (-1)	100 (-1)	50 (+1)	0 (-1)	31.3
11	4 (-1)	100 (-1)	10 (-1)	50 (+1)	24.7
12	4 (-1)	100 (-1)	50 (+1)	50 (+1)	54.1
13	4 (-1)	300 (+1)	10 (-1)	0 (-1)	25.2
14	4 (-1)	300 (+1)	50 (+1)	0 (-1)	27.8
15	4 (-1)	300 (+1)	10 (-1)	50 (+1)	59.1
16	4 (-1)	300 (+1)	50 (+1)	50 (+1)	82.5

### 2.3.1.2 Description of the sampling and recording of the dependent variables

After each well-defined process step, samples were taken to investigate the process impact on the quality of the end products. These are represented by the grey boxes in Figure 2.1 and consist of mashed tomatoes (MT), juice filtered once (JFO) and press residue (PR) in the first solid-liquid separation process. The analyses of JY, MC, TU and PWR were performed on all freshly taken samples, whereas only the samples resulting from the conditions generating the highest JY were frozen at -20 °C, freeze-dried (Epsilon 2-10 D LSC, Martin Christ, Osterode am Harz, Germany) and subsequently analyzed for their antioxidative capacity (AOC).

The JY of the first solid-liquid separation process was determined by recording mass balances of the JFO and PR during the steady-state phase of the process. The JY was determined as  $JY = \frac{M_{JFO}}{M_{JFO} + M_{PR}} \times 100 \%$  with  $M_{JFO}$  the net mass of the juice and  $M_{PR}$  the net mass of the press residue (calculation in Appendix 1). The total TH was calculated analogously:  $TH = \frac{M_{JFO} + M_{PR}}{t}$  with t the time during which both fractions were collected.

The MC measurements were performed in duplicate using a halogen moisture analyzer (HB43-S, Mettler Toledo, Schwerzenbach, Switzerland). The TU was measured nephelometrically using a light scattering photometer (Micro1000 Laboratory Turbidity meter, HF scientific, Florida, USA). These TU measurements were performed three times on a homogeneous sample. PWR was measured gravimetrically (PB3002-S, Mettler-Toledo, Greifensee, Switzerland) by calculating the ratio of the mass of 30 g of juice ( $M_0$ ) and the net mass of sediment resulting after centrifugation (4200 g, 15 min) of the juice and subsequent decantation the supernatans ( $M_c$ ):  $PWR = \frac{M_c}{M_0} * 100 \%$ .

The AOC was determined by a modified oxygen radical absorbance capacity (ORAC) assay (Prior et al., 2005), as described by Bernaert et al. (2013). Analysis was performed in triplicate (n=3) (Clariostar, BMG labtech, Ortenberg, Germany). Results were expressed in  $\mu\text{moles}$  of Trolox equivalents per gram of dry weight ( $\mu\text{mol TE.g}^{-1}$  DW) and converted per gram of fresh weight ( $\mu\text{mol TE.g}^{-1}$  FW) (calculation in Appendix 2) using both the moisture contents of wet and dry products (Table 2.2). The impact of the spiral-filter press on the AOC was evaluated by calculating the retention efficiency (% R). This represents the ratio of the AOC present after the process and before the process and is calculated by dividing the yield-corrected-AOC in JFO and PR by the AOC in MT. Also the juice and press residue extraction efficiencies were calculated (%  $E_{\text{JFO}}$  and %  $E_{\text{PR}}$ ) representing the percentage of the AOC that ends up in the juice fraction or the press residue, respectively (calculations in Appendix 2).

**Table 2.2** Moisture contents [%] of the three obtained fractions, i.e. mashed tomato (MT), juice filtered once (JFO) and press residue (PR) and with  $MC_{\text{wet}}$  and  $MC_{\text{dry}}$  the moisture contents of the fresh and the freeze-dried products, respectively.

	MT [%]	JFO [%]	PR [%]
$MC_{\text{wet}}$	$95.0 \pm 0.2$	$96.1 \pm 0.02$	$90.1 \pm 0.2$
$MC_{\text{dry}}$	$11.8 \pm 0.03$	$14.7 \pm 0.03$	$5.9 \pm 0.02$

Based on the generated knowledge and the resulting products, it was concluded that even under optimal conditions, no complete solid-liquid separation between juice on the one hand and peel and seeds on the other hand was achieved. This led to the investigation of an additional thermal pretreatment step.

### 2.3.2 Optimization of the thermal pretreatment

In order to obtain a better solid-liquid separation, a thermal pretreatment step was included in the biorefinery process (Figure 2.1). From this point onwards, the optimization process was executed stepwise using 75 kg of tomatoes per treatment. After each solid-liquid separation, an evaluation of

the JY and the  $MC_{PR}$  was performed and a corresponding decision for the next set of conditions was taken.

A thermal pretreatment was performed by a mix/homogenize/emulgate system (UMSK 60 E, Stephan Food Service Equipment GmbH, Hamelin, Germany) wherein the mashed tomatoes were heated batchwise ( $35 \text{ kg}\cdot\text{batch}^{-1}$ ) and additionally milled under vacuum. This was performed in two steps. Initially, three temperatures were tested, based on cold and hot break used in conventional tomato processing (Goodman et al., 2002; Hayes et al., 1998; Heutink, 1986):  $40 \text{ }^\circ\text{C}$ ,  $60 \text{ }^\circ\text{C}$  or  $90 \text{ }^\circ\text{C}$  which were all applied for 3 or 6 minutes. Subsequently, the duration was varied (3, 6 or 9 minutes) for two temperature treatments ( $40 \text{ }^\circ\text{C}$  and  $50 \text{ }^\circ\text{C}$ ). The tomato mashes treated at  $50 \text{ }^\circ\text{C}$ ,  $60 \text{ }^\circ\text{C}$  or  $90 \text{ }^\circ\text{C}$ , were cooled down to  $40 \text{ }^\circ\text{C}$  in order to generate a product with a constant temperature for subsequent application to the spiral-filter press. Subsequently, a solid-liquid separation of this thermally treated tomato mash (TT) was performed by means of the spiral-filter press using the same conditions as used in the optimized conditions in the first solid-liquid filtration (M, F, S, V) only varying the spirals (7-C ( $45^\circ$ ), 4-C ( $45^\circ$ ) and 4-C ( $38^\circ$ )) in function of an optimal yield and a continuous operation.

### 2.3.3 Optimization of the second solid-liquid separation

In order to test the flexibility of the spiral-filter press, a second solid-liquid separation was performed, using JFO as a feedstock, in order to obtain a tomato solids fraction (TS) and a less viscous juice filtered twice (JFT). Using JFO as an input stream means processing a very liquid product, conversely it was processed with the 3-C spiral (inclination angle  $32^\circ$ ). The optimization included the testing of multiple filter sizes ( $100 \text{ }\mu\text{m}$ ,  $80 \text{ }\mu\text{m}$  and  $60 \text{ }\mu\text{m}$ ) and multiple vacuum frequencies (0 Hz, 10 Hz and 50 Hz). F and S were kept constant at 15 Hz and 50 Hz, respectively.

### 2.3.4 Statistical analysis

A screening design was performed with four independent factors (S, V, M and C) to investigate their effect on the dependent variables JY,  $MC_{PR}$ , TH, TU and PWR. A contrast analysis was conducted in which the main effects and the first order interaction effects were estimated by means of the following first order linear regression model:

$$y = b_0 + b_{1.S} + b_{2.V} + b_{3.M} + b_{4.C} + b_{5.SV} + b_{6.SM} + b_{7.SC} + b_{8.VM} + b_{9.VC} + b_{10.MC}$$

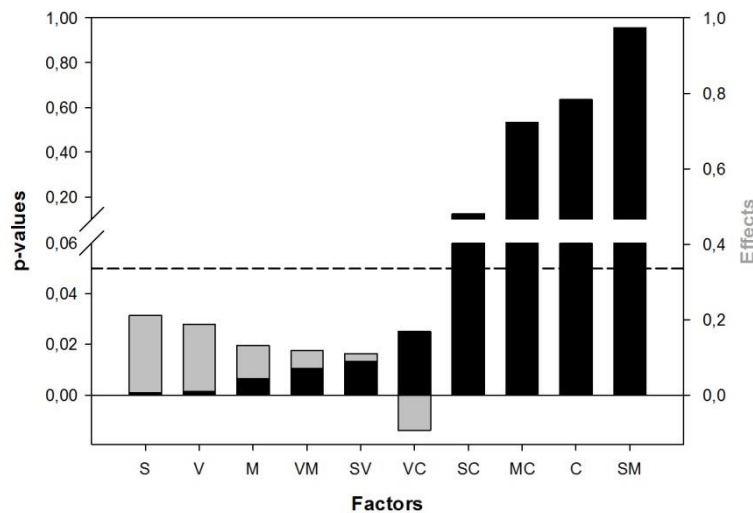
In this equation, the coefficients  $b_{1 \rightarrow 4}$  represent the main effects of the corresponding factors, while the coefficients  $b_{5 \rightarrow 10}$  describe the interaction effects of the factors.  $b_0$  is the intercept and represents the grand mean of the dependent variable  $y$ . The coefficients represent half of the effects that are

induced in the dependent variable upon changing the independent variable from a low to a high level. One-way analysis of variance (ANOVA) was conducted to identify effects with a level of significance of  $p < 0.05$ . Thereby, the best subset of independent variables was determined for each response function based on the Akaike information criterion (for finite sample sizes) which takes into account both model fit and complexity, obviating any overfitting problems. All statistical analyses of the optimization processes were performed with R 3.0.1 (R Foundation, Auckland, New Zealand). The AOC of the end products was statistically evaluated using ANOVA analysis followed by a Scheffé post-hoc test in SPSS Statistics 22 ( $p < 0.05$ ). Sigmaplot 13 was used to visualize the data.

## 2.4 Results and discussion

### 2.4.1 Understanding the process impact of the independent variables during the first solid-liquid separation

Due to its economic importance at the processing plant, JY was used as a primary criterion to optimize the system. The higher the JY, the better the dewatering and the dryer the press residue. The JY-values, corresponding to the different experiments varied from 20.3 % (exp. 3, Table 2.1) to 82.5 % (exp. 16, Table 2.1), whereas reported tomato JY-values from a paddle or screw type extractor with hot break pretreatment range from 70 % to 95 % (Bates et al., 2001; Hayes et al., 1998; Min & Zhang, 2003). The large range in JY obtained throughout the experiment indicates a major effect of the system parameters. Figure 2.4 shows the main effects and the interaction effects influencing the JY.



**Figure 2.4** Visualization of the significance and the magnitude of the effects of different factors, i.e. spiral frequency (S), vacuum (V), filter pore size (M) and number of channels of the spiral (C) and their interactions on the juice yield. *P*-values are represented by black bars and shown on the primary y-axis. The dotted line represents a *p*-value of 0.05 (95 % significance level). The magnitudes of the effects are represented by grey bars and shown on the secondary y-axis.

From this figure, it can be concluded that the factors S, V and M all exert a significant positive effect on JY (grey bars). The largest effects (smallest black bars) are ascribed to the spiral rotation frequency S. This positive effect can be explained by the tomato peel fraction that accumulates on the inner side of the sieve and blocks the pores. The rotating spiral can induce a scraping effect on the sieve thus removing the peel from the pores and increasing the juice extraction. It has to be noted that this effect is matrix dependent. In case of apples and pears for example, S exerts a negative influence on the JY as S is also negatively correlated with the biomass residence time in the extraction cell (De Paepe et al., 2015a). The positive effect of V on JY can be explained by an extra extraction force as V is correlated

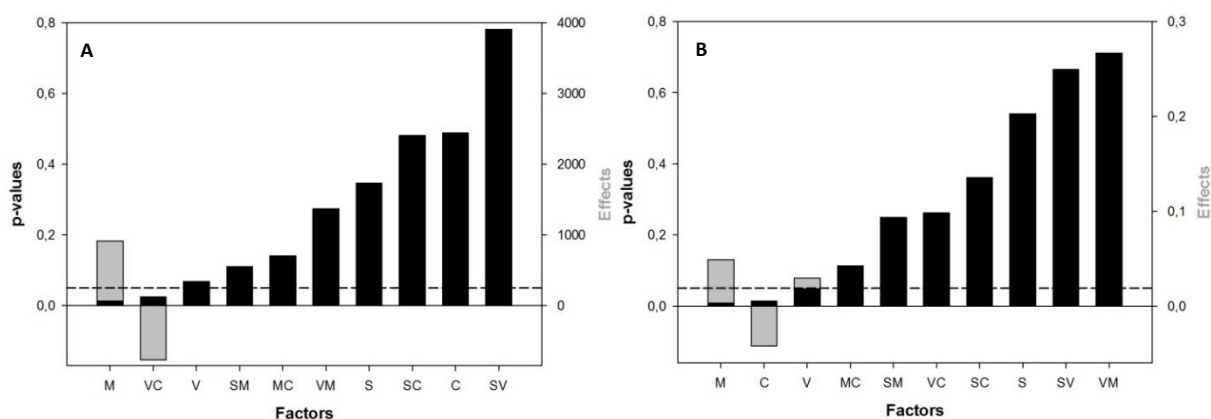


with the underpressure in the extraction cell. Also M exerts a significantly positive effect on the JY, as increasing the pore size of the filter, allows juice to pass through the filter more easily within the residence time. The choice of M will also influence the turbidity in the juice as more and larger particles are allowed to pass (Figure 2.5), which will be discussed hereafter in more detail. Besides these three main effects, three significant interaction effects also were identified. The positive V-M effect implies that the effect of the vacuum is higher when the filter pore size is enlarged. An explanation could be that the vacuum exerts a larger driving force on the juice through larger pores, as there is more “open space” to pull the liquid through. At smaller M, the juice is already blocked by the smaller pores and an increase of the vacuum can only offer a limited added value. The positive interaction effect of V and S is of a similar nature. The higher the frequency of the spiral, the more effect of V on the JY. Indeed, the higher S, the more the filter is scraped, the better the filter pores remain unblocked by particulate matter, hence the more juice can be extracted by increasing V. The V-C interaction effect is negative, implying that an increase of V will lead to a less pronounced increase in JY when C is high. This can be explained by the larger compression forces that are exerted on the material in a 7-C spiral. Increasing V will only lead to a small extra driving force on the JY in this system, which is already characterized by a high compression.

The JY can also be related to other dependent variables. The higher the JY, the better the solid-liquid separation and the lower the moisture content of the press residue. This inverse relation of  $MC_{PR}$  with JY is also visible in the significantly negative effects of S, V and M on  $MC_{PR}$  (results not shown). In each pressing system, the JY is related to the TH (Beveridge & Rao, 1997). In this experiment however, no significant factors nor interactions were found to influence TH. This could be caused by the soft tomato matrix for which only the feed pump frequency is determining the TH. As F is kept constant in this experiment, also constant TH values were found ( $475 - 498 \text{ kg}\cdot\text{h}^{-1}$ ). Increasing this F in regard to industrial scale systems, and maintaining the other process parameters might slightly decrease the JY but generally only in the order of 3 % – 4 %. This TH increase is limited however, as at a certain feed pump frequency, the system will “break-through”. In that case, a the parallel placement of identical extraction cells is needed. This implies a multiplication of TH by the number of extraction cells whereas the juice yield and juice quality remain constant, as also stated by De Paepe et al. (2015a). It has to be noted that the limiting technology to achieve higher TH is often the milling technology instead of the juice pressing technology.

Values for TU varied from  $3,310 \pm 40 \text{ NTU}$  to  $6,965 \pm 76 \text{ NTU}$  in this experiment. These values are found to be only significantly influenced by the main effect of M and the interaction effect of V-C (Figure 2.5A). M exerts a positive effect on the TU indicating that larger pore sizes yield a more turbid juice, confirming the results obtained in pear juice production (De Paepe et al., 2015a). The interaction effect

V-C has a negative origin, which means that using a vacuum has a larger effect on the TU when a 4-C spiral is used compared to a 7-C. This effect has also been seen on the JY and can be explained by the 7-C spiral system that already exerts a high compression force on the mashed tomatoes, leading only to a small extra driving force of V for extracting juice and small particles out of the mash. The high TU values can be attributed to the extraction of soft tomato tissue which collapses easily under pressure, creating cloud particles that contribute to the turbidity. In other presses, these cloud particles can clog juice escape channels and reduce the JY. Earlier, press aids (such as rice hulls, ground wool pulp or shredded paper) have been used to avoid this clogging and thereby increase juice yield by (i) scraping the screen during pressing which prevents clogging, (ii) adding mass to the fruit mash to better transmit the pressing forces and (iii) providing juice escape channels to increase the juice yield (Beveridge & Rao, 1997; Roberts et al., 2004). Nowadays, the use of press aids is mostly replaced by enzymatic pretreatment, for example by using pectinases which break down the pectins, preventing the blockage of filters (Echavarría et al., 2011; Pagán, 2014; Urlaub, 2002). In the spiral-filter press however, cloudy particles cause no problems, as the rotating spiral scrapes the solid material away from the filter pores, clearing the juice extraction channels.



**Figure 2.5** Visualization of the significance and the magnitude of the effects of different factors, i.e. spiral frequency (S), vacuum (V), filter pore size (M) and number of channels of the spiral (C) and their interactions on A) the turbidity (TU) and B) the precipitate weight ratio (PWR). P-values are represented by black bars (primary y-axis). The dotted line represents a p-value of 0,05 (95 % significance level). The magnitude of the effects are represented by grey bars and shown on the secondary y-axis.

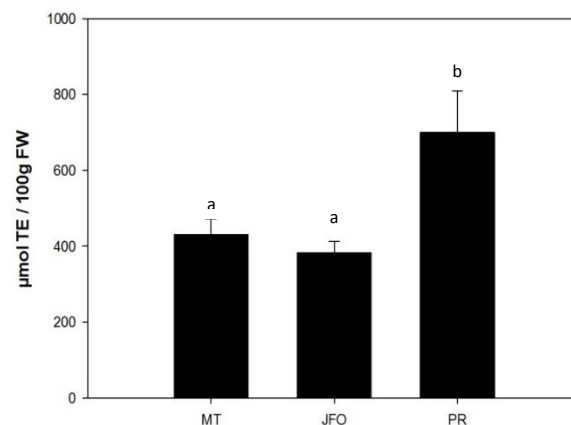
Finally, a last parameter evaluated throughout the experiments is the PWR, referring to the water insoluble solids comprised of intact cells, broken cell walls and middle lamella compounds. This parameter has been found to correlate to the physical stability of the juice, where a higher PWR leads to less sedimentation and serum separation. Hence, the PWR is an important quality parameter for tomato-derived products (Kaur et al., 2007). Experimental values varied between 20 % and 33 %, which are higher or at least within the same range compared to the values observed by Anthon & Barrett

(2010). Figure 2.5B shows that the PWR is significantly influenced by M, C and V. As can be expected, the larger the filter pore size, the more particles are found in the juice. The number of channels exerts a negative influence on PWR. In other words, the more channels present to compress the tomato mash, the more difficult particles are released to migrate to the juice fraction. Lastly, a significantly positive effect is found for the effect of V on PWR. Thus the higher the vacuum, the larger the driving force for juice extraction and the more particles are drawn into the liquid fraction.

By means of the experimental screening experiment, it thus became clear that the studied parameters all show a JY optimum for a 4-C (45°) spiral operating with a large S, V and M, corresponding to experiment 16 (Table 2.1). These conditions were therefore chosen for the determination of the AOC of the end products.

#### 2.4.2 Antioxidative capacity of the end products resulting from the first solid-liquid separation without thermal pretreatment

The low-oxygen spiral-filter press has already shown to impede oxidative degradation in the production of apple and pear juice, which is in part allocated to its extraction under low oxygen levels (De Paepe et al., 2015a; 2015b). In order to evaluate the process impact of the spiral-filter press during tomato juice production, samples resulting from the optimized first solid-liquid separation (exp. 16, Table 2.1) were analyzed for their antioxidative capacity. The AOCs of the end products (JFO and PR) were therefore compared to the AOC of the input product (MT), in order to calculate the spiral-filter press process impact on the AOC. The ORAC-values of the three fractions resulting from the optimized solid-liquid separation are depicted on a fresh weight basis in Figure 2.6. Here, it is shown that there was no significant decrease in AOC in juice compared to fresh fruit. Furthermore, the PR was characterized by a significantly ( $p < 0.001$ ) higher AOC compared to the other two fractions.



**Figure 2.6** ORAC-values ( $\mu\text{mol TE}/100\text{g FW}$ ) of the different fractions obtained after solid-liquid separation ( $n=3$ ), i.e. mashed tomato (MT), juice filtered once (JFO) and press residue (PR).

This is confirmed in Toor and Savage (2005) who separated tomatoes in different fractions (skin, pulp and seeds) and subsequently determined their individual AOC. The ORAC-values of MT were in the same order of magnitude compared to reported ORAC-values for raw tomatoes. The USDA database reports ORAC-values for raw tomato ranging between 216 and 457  $\mu\text{mol TE}\cdot 100\text{ g}^{-1}\text{ FW}$  (Haytowitz & Bhagwat, 2010). Also the results of Ou et al. (2002) and Zhou & Yu (2006) are within the same range. The large fluctuations in the reported ORAC-values can be ascribed to a dependency on variety, ripening stage, location and harvesting season (Ou et al., 2002). This stresses the importance of evaluating AOCs within the process relative to the input product, when evaluating process impact. The relative change of the AOC throughout processing was calculated by means of the retention efficiency (% R). An % R-value of  $102\% \pm 12\%$  showed that the ORAC-values of JFO and PR, expressed on the basis of their actual weight fraction, resulted in the ORAC-value of MT. This implies a conservation of the AOC which could be related to the use of the low-oxygen spiral-filter press, preventing oxidative degradation. Furthermore, an insight was also gained in the distribution of the AOC within the three products. Despite its small volume (17.5 %), the press residue was found to contribute  $28\% \pm 5\%$  to the total ORAC-value of the tomato. This shows the potential value of this so-called waste fraction and confirms the relevance of designing a biorefinery system where these fractions can also be valorized.

These results however have to be interpreted carefully as the AOC does not reflect individual antioxidative compound shifts (Martínez-Valverde et al., 2002). Therefore, in order to gain more insight in the impact of the spiral-filter press on the chemical composition of the end products, a multifaceted approach is necessary where additional investigations of the individual antioxidative compounds are performed. Therefore, detailed investigation of the obtained fractions using LC-MS analysis for determination of phenolics and carotenoids and titrimetric measurements for determination of vitamin C are investigated in Chapter 4.

### 2.4.3 Optimization of the pretreatment

The best case in the previous optimization resulted in a 82.5 % juice yield and a press residue with a moisture content of  $90.1\% \pm 0.17\%$ . However, it was visually determined that the solid-liquid separation was not completely carried out as the press residue still contained tomato flesh and juice. An inherent consequence from this incomplete solid-liquid separation, was a press residue that was not separable in a homogeneous seeds and peel fraction. This was due to the tomato flesh fraction that interfered with a flotation-sedimentation process which was used to separate seeds and peel. As a result, biobased product development starting from pure seeds and/or peel fractions was hindered. The seeds have the potential to produce vegetable oil as an ingredient in food or cosmetic products

whereas the peels can serve as a feedstock for carotenoid extraction (Alvarez & Rodríguez, 2000; Eller et al., 2010; Schieber et al., 2001). This led to the conclusion that a thermal pretreatment was necessary, as often applied in industry (Hayes et al., 1998).

In the first explorative thermal pretreatment experiment, three temperatures (40 °C, 60 °C and 90 °C) were tested for 3 and 6 minutes (results not shown). The two highest temperatures are often applied in industrial tomato processing using cold and hot break (Goodman et al., 2002). However, a lower temperature (40°C) was also included in the experiment as it is known that elevated temperatures can alter the flavor, color and nutritional quality of the juice (Min & Zhang, 2003; Sánchez-Moreno et al., 2006). The choice of the spiral of the following solid-liquid separation was reconsidered since the consistency of the input material changed compared to the non-thermally treated tomato mash. Explorative experiments showed that a 7-C spiral performed better on thermally pretreated material. Solid-liquid separation (7-C (45°)) of these thermally pretreated mashes all resulted in juice yields significantly larger than 82.5 %, indicating that the introduction of a thermal treatment indeed led to a significant increase in the extent of solid-liquid separation. No significant differences were however observed in the JY and MC<sub>PR</sub> between the different combinations. Furthermore, an ad hoc sensory evaluation showed that the juices smelled and tasted more “cooked” at higher temperatures. Therefore, an additional experiment was conducted at two lower temperatures (40 °C and 50 °C) for 3, 6 and 9 minutes, in order to investigate if a longer treatment could lead to a higher extent of solid-liquid separation. The resulting JY and MC<sub>PR</sub> are shown in Table 2.3. Here, also the conditions leading to the highest yield in the non-thermally treated experiments were added as reference.

**Table 2.3** Time and temperature of the applied thermal pretreatments and the corresponding juice yields (JY) and moisture contents of the press residue (MC<sub>PR</sub>) using a 7-C (45°) spiral.

Time (minutes)	Temperature (°C)	JY (%)	MC <sub>PR</sub> (%)
0	0	82.5	90.1
3	40	98.2	70.5
6	40	98.7	62.5
9	40	98.7	60.4
3	50	98.5	61.0
6	50	98.5	69.5
9	50	98.7	62.8

From this experiment, it can be concluded that applying a thermal treatment led to a significant increase in JY and a significant decrease in MC ( $p < 0.001$ ). However between the thermal treatments, no significant effect of temperature nor time was found. This implies that applying a heat treatment is sufficient to detach the peel from the flesh, independent of the duration or the temperature of the

heat treatment. Consequently, the minimum temperature-duration combination (40 °C – 3 min) was selected in order to affect the quality of the tomato product as little as possible, yet obtaining a thorough solid-liquid separation. However, due to the larger compression forces in the 7-C spiral, a compression build-up was often encountered, due to an increasingly dry press residue, which resulted in system blocking. Therefore, the 4-C spiral with inclination angle of 38° was selected for further operation. Although resulting in a slightly lower juice yield (97.0 %), it was able to work on a continuous basis. The choice of 38° instead of 45°, as optimized in the first-solid-liquid separation, can be explained by the fact that the more liquid products are less susceptible to compression, which consequently implies that an increased steepness of the channels (45° versus 38°) does not generate any extra driving force. What is more, less steep channels lead to an increased residence time of the tomato mash in the extraction cell, thereby improving the juice extraction (De Paepe et al., 2015a).

Using the proposed thermal pretreatment enabled the production of a press residue that consisted solely out of peel and seeds, which allowed their further separation and valorization (Figure 2.7)



**Figure 2.7** Press residue obtained from the optimized spiral-filter process with thermal pretreatment.

#### 2.4.4 Optimization of the second solid-liquid separation

In order to investigate the flexibility of the spiral-filter press, further refinement of the tomato was investigated by stripping the tomato solids (TS) fraction from the viscous JFO leading to a less viscous JFT and a TS fraction with a firm puree texture. The input stream JFO was more liquid compared to both input streams MT and TT from the previous experiments. In general, thermally treated mashes are difficult to process using conventional pressing technologies as they tend to slide through the press cloth or block the pores leading to very low juice yields, often less than 50 % (Beveridge & Rao, 1997). The spiral-filter press is however able to process these liquid streams and based on the conclusions drawn above on the low compressibility of the liquid biomass, a 3-C spiral with small inclination angle (32°) was used for optimal yield. Subsequently, vacuum and filter pore size were optimized. In the first experiment a filter pore size of 60 µm was used and three different vacuums were tested (0 Hz, 10 Hz

and 50 Hz). Here, it was concluded that a higher vacuum led to less TS fraction with a more solid structure. In the extreme condition of 50 Hz vacuum, even the whole input stream was pulled through the filter, exiting the system at the juice side. The vacuum thus exerted a too large extraction force. When applying no vacuum, the whole input stream tended to exit the system at the press residue side. A smaller vacuum (10 Hz) therefore appeared to be optimal. Finally, two filter sizes of 80  $\mu\text{m}$  and 60  $\mu\text{m}$  were compared. In the first case, almost no TS were extracted from JFO (TS yield  $4.2\% \pm 0.9\%$ ). Due to the larger pore size, more small particles that otherwise would end up in the TS fraction, passed through the filter and ended up in the JFT fraction. Using the 60  $\mu\text{m}$  filter led to more TS mass (TS yield  $8.9\% \pm 0.9\%$ ). On the one hand, this could be allocated to the fraction of small particles (theoretically between 60  $\mu\text{m}$  and 80  $\mu\text{m}$ ) that were not allowed to pass the 60  $\mu\text{m}$  filter and thus ended up in the TS fraction. On the other hand, it could be caused by a larger fraction of tomato juice, that could theoretically pass the filter pores, but of which the flow was obstructed by the small pore size. The choice of this filter should be made in function of the aimed application: smaller amounts of less liquid TS could be obtained using a 80  $\mu\text{m}$  filter, whereas a 60  $\mu\text{m}$  filter could generate a larger TS-mass with a slightly smaller dry weight content. Besides being flexible towards the biomass input, the spiral-filter press is thus also able to produce a variety of textures in its end products (juice, smoothie, puree).

## 2.5 Conclusion

The spiral-filter press is proposed in the context of food losses as a promising technology, able to adequately refine a variety of biomass matrices, facilitating the valorization of all the obtained fractions. Using tomato as a model crop, a robust refinery process was developed, consisting of a light thermal pretreatment (40 °C, 3 minutes) followed by a spiral-filter pressing which proves to be flexible towards input biomass as well as adjustable in function of the desired generated end product (juice, smoothie, puree). Generally applicable insights in the working of the spiral-filter press were obtained by elucidating the effects of different process parameters on the juice yield and juice quality parameters (turbidity, precipitate weight ratio). These results are crucial for further product formulation and processing of biomass with a similar soft texture, and can be easily scaled to larger systems by increasing the feed pump frequency or parallel placement of identical extraction cells. Furthermore, the research suggests that the spiral-filter press is a qualitative technique, able to conserve the antioxidative potential of the raw tomato ( $102 \% \pm 12 \%$ ) during pressing.



## **Chapter 3**

Using a novel spiral-filter press to biorefine horticultural byproducts: the case of tomato. Part II: evaluation of the process impact on the physical tomato juice quality



## **Chapter 3: Using a novel spiral-filter press technology to biorefine horticultural byproducts: the case of tomato. Part II: evaluation of the process impact on the physical tomato juice quality**

### **Redrafted from**

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### **3.1 Abstract**

The spiral-filter press offers potential to minimize food losses by allowing the biorefinery of a multitude of food waste matrices into qualitative, healthy food products. This chapter focuses on the effect of different unit operations on the physical juice quality, which was illustrated for tomato biomass. Using optimized process conditions (derived from Chapter 2), a physically and microbiologically stable juice with a high juice yield ( $97.9 \% \pm 0.2 \%$ ) could be obtained. Thereby, the intense red color of the tomato was preserved throughout the process. Furthermore, by varying the filtration and pasteurization conditions juices of different turbidity and stability were produced, increasing insight in the processes underlying these phenomena. However, using exactly the same process conditions on another tomato cultivar generated an unstable juice, subject to sedimentation. This indicates that changing process parameters alone was not sufficient to control all the parameters that affect the juice stability and that more research is necessary to fully elucidate the phenomenon.

## 3.2 Introduction

Currently, 40 – 60 % of the biomass in the fruit and vegetable processing sector is lost at different stages of the supply chain throughout the world, leading to large losses of healthy and potentially high-value biomass (Gustavsson et al., 2011). Consequently, valorization of this food waste is listed high on the European agenda which translates into novel legislation and many related project calls expressing the need for technologies that allow stabilization and valorization of food waste (European Commission, 2013; 2015a; 2015b). Biorefining fruit and vegetable byproducts is a promising option that has the potential to result in different fractions with a high added value.

In Chapter 2, such a biorefinery process was developed and optimized for surplus tomato fruit, which served as a model crop. The process consisted of separating the solid content from the liquid content using a novel low-oxygen spiral-filter press. Application of this novel technology on a soft vegetable like tomato was described there for the first time. Process parameters were optimized in order to obtain a robust process, characterized by a large juice yield and a press residue separable in pure seeds and peel, suitable for further valorization (Figure 3.1).

Chapter 3 elaborates further on the optimized biorefinery process by studying the effect of adjustable process parameters on juice stability and related quality attributes. Due to the combination of compression forces and underpressure in the extraction cell, juices produced by the spiral-filter press are particularly turbid (De Paepe et al., 2015a; 2015b). This cloudiness is caused by a dispersion of insoluble macromolecules (e.g. pectins, proteins) in a serum, containing water-soluble components (Oszmianski et al., 2009). High intake of this fibrous fraction is associated with numerous health benefits, including reduced risk of coronary heart disease, diabetes, obesity and some forms of cancer (Elleuch et al., 2011). The production of cloudy, turbid juices by means of the spiral-filter press thus contributes to maintaining maximum health benefits from the unconsumed fruits or vegetables. However, producing a cloudy juice that is also physically stable remains difficult and is of particular importance in the food juice industry (Laratta et al., 1995). Often sedimentation of the fibrous fraction is provoked, leading to juices that are visually less attractive to the consumer (Sila et al., 2009). This juice stability is strongly influenced by the structural characteristics of the suspension (particle concentration, particle size and particle morphology), which are in their turn affected by the feedstock used and the processing operations (e.g. heating, mixing, sieving) (Moelants et al., 2014). Understanding the process impact on the structure of the tissue is thus crucial to control the stability of the juices produced by the spiral-filter press and to enable targeted product design. Therefore, the particle size distribution was investigated and followed through the different production steps as well as the related physical attributes, such as juice stability, turbidity and color.

Although process-induced changes in juice viscosity and rheology have been widely studied, there are only a few studies in the literature dealing with the stability of tomato juice as affected by processing, and none covering the novel spiral-filter press. Furthermore, the link with structural characteristics is often absent. This chapter provides knowledge for a better understanding of the structure and stability of the resulting juices and how these can be influenced by tomato cultivar and processing settings. Furthermore, insights gained in the case of tomato contribute to the necessary knowledge to process similar biomass feedstocks into stable juices by means of the spiral-filter press.

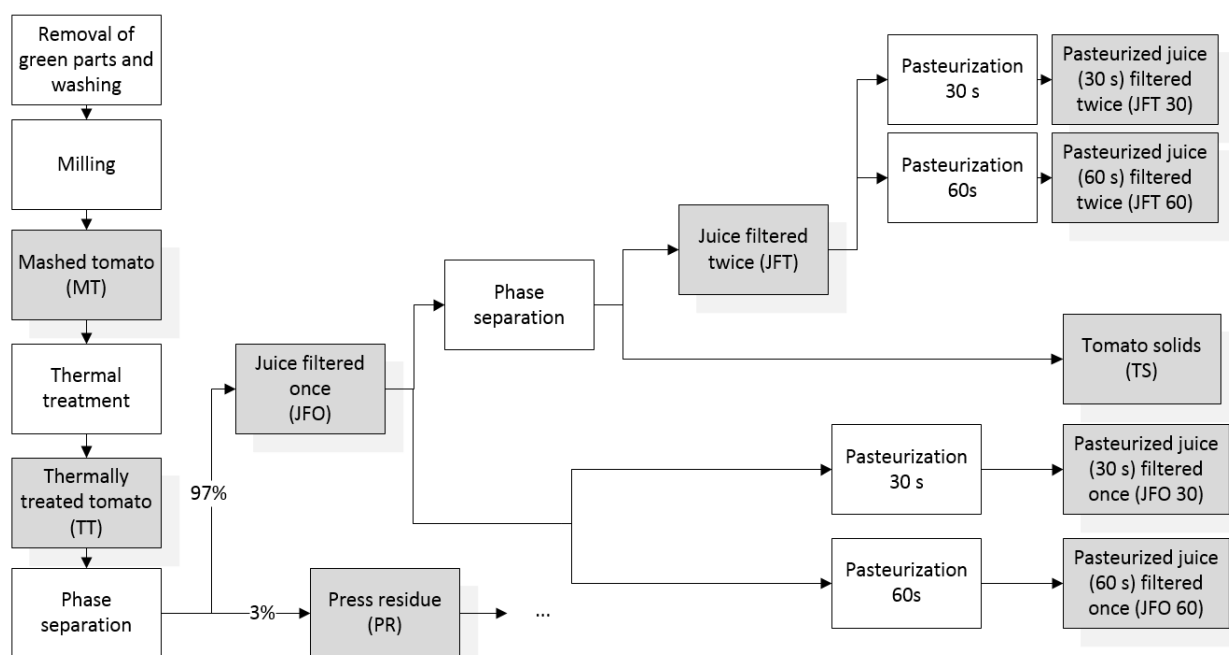
### 3.3 Materials and methods

#### 3.3.1 Description of the feedstock material

Tomatoes at commercial maturity were purchased from a Belgian produce auction (Bel'Orta, Sint-Katelijne-Waver, Belgium). The experiments were conducted with two different cultivars: Growdena and Merlice, a flesh type tomato and a truss type tomato, respectively. For each cultivar, 200 kg was used. Tomatoes were stored for four days at 4 °C before conducting the experiments.

#### 3.3.2 Pilot-scale machinery

The biorefinery process consists of a sequence of pilot-scale batch processes. The experimental set-up is presented in Figure 3.1 and will be described stepwise below.



**Figure 3.1** Experimental setup of the biorefinery process investigated in Chapter 3 with the unit processes represented by white boxes and the resulting products by grey boxes.

After manually removing the green stems, the tomato batches were washed in a water bath filled with cold tap water until all tomatoes were submerged. Subsequently, they were transported ( $20 \text{ kg}\cdot\text{h}^{-1}$ ) by a conveyor into a rasp mill (KWEM 1000, Kreuzmayr, Wallem, Germany). The mashed tomatoes (MT) were collected in plastic jars. These jars ( $35 \text{ kg}\cdot\text{batch}^{-1}$ ) were transferred batchwise to a mix/homogenize/emulgate system (UMSK 60 E, Stephan Food Service Equipment GmbH, Hamelin, Germany) where an additional heated milling was conducted under vacuum (3 min, 40 °C). The parameters used (batch size, temperature, holding time) were optimized in Chapter 2, in function of an optimal juice yield in the subsequent solid-liquid separation, with minimal energy input.

An innovative low-oxygen spiral-filter press (VacuIQ 1000, VacuIQ, Hamminkeln, Germany) was used to perform the solid-liquid separation of the thermally treated tomato fraction (TT) into a juice fraction (juice filtered once - JFO) and a press residue (PR). The process parameters are given in Table 3.1. Briefly, the TT was collected in a buffer tank, from where it was transferred to the extraction cell of the spiral-filter press by means of a feed pump. In the extraction cell, a plastic spiral rotates in a cylindrical filter element, carrying the mashed tomato biomass upwards. Due to the combination of compression forces exerted by both feed pump and spiral rotation and an underpressure acting on the mash through the filter element, the mash is dewatered resulting in two fractions leaving the system: JFO and PR. This solid-liquid separation will be further denoted as the first filtration. Part of the obtained juice fraction (JFO) was subsequently processed a second time by the spiral-filter press using adapted process parameters (Table 3.1) in order to separate the tomato solids (TS) from the remaining juice fraction (juice filtered twice - JFT). The parameters of both solid-liquid separations have been optimized in Chapter 2.

**Table 3.1** Spiral-filter press parameters used for the experiments in Chapter 3.

	First filtration	Second filtration
Filter size	300 $\mu\text{m}$	60 $\mu\text{m}$
Spiral (channels - inclination angle)	#4 – 38 °	#3 – 32°
Feed pump frequency	20 Hz	15 Hz
Spiral frequency	30 Hz	40 Hz
Vacuum pump frequency	50 Hz	10 Hz

After production, both JFO and JFT were transferred immediately to a multipurpose UHT pilot equipment (APV SPP, SPX Corporation, Gatwick, United Kingdom) where they were subjected to a pasteurization treatment at 90 °C, applied for 30 s (mild heat pasteurization) or for 60 s (high heat pasteurization) as proposed by Odriozola-Serrano et al. (2009). These pasteurized juices will be further denoted as JFO30, JFO60, JFT30 and JFT60. Afterwards, they were cooled to 4 °C and cold-filled into bag-in-box aluminum laminate aseptic bags (Rapak Bag-in-Box, 2 L).

### 3.3.3 Characterization of the semi-finished and finished products

#### 3.3.3.1 Sampling

After each well-defined process step, samples were taken to investigate the process impact on multiple juice quality parameters (grey boxes in Figure 3.1). The sampling during the solid-liquid separation process was performed during the steady-state phase. Samples were taken for the measurement of

pH, moisture content, soluble solids content, color, turbidity, particle size distribution and physical stability. Furthermore, pasteurized juice samples were stored in fivefold at three different temperatures (4 °C, 20 °C and 30 °C) for a microbial analysis at 0 days, 15 days, 1 month, 4 months and 6 months.

### 3.3.3.2 Juice yield

The juice yield of the solid-liquid separation process (JY) was determined by recording mass balances of the resulting fractions during the steady-state phase of the process:  $JY [\%] = \frac{M_j}{M_j + M_p} \times 100 \%$ , with  $M_j$  the net mass of the liquid fraction (JFO or JFT) and  $M_p$  the net mass of the solid fraction (PR or TF). Masses were recorded in fourfold both for the first filtration (JFO and PR) and for the second filtration (JFT and TF).

### 3.3.3.3 Tomato feedstock characteristics and tomato juice quality parameters

A texture analyzer equipped with a cylindrical 3.5 mm probe was used to measure the tomato firmness by means of recording the force necessary to penetrate the tomato for 3 cm (Personal communication, Flanders Centre of Postharvest Technology). This was performed on 20 whole tomatoes of each cultivar, randomly selected and measured at three points along the diameter.

Color was measured spectrophotometrically (CM-5 spectrophotometer, Konica Minolta optics inc, Tokyo, Japan) reporting values of a CIEL\*a\*b\* color system (illuminant D65, 10° standard observer, 45°/0° geometry, reflection modus, automatic white calibration using an internal white calibration plate). The color measurements of the intact feedstock tomatoes were conducted for each cultivar on 20 whole tomatoes at three points along the diameter. The color measurements of the liquid samples were conducted in triplicate and each sample was measured three times. From the standard  $L^*$ ,  $a^*$  and  $b^*$  parameters, a total color difference ( $\Delta E$ ) between different samples could be calculated:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

The pH was measured with a pH-meter (S220 SevenCompact™, Mettler Toledo, Schwerzenbach, Switzerland) in triplicate. The moisture content (MC) was determined by means of a halogen moisture analyzer (HB43-S, Mettler Toledo, Schwerzenbach, Switzerland) in duplicate. The amount of total solids (TS) can be calculated from the moisture content:  $TS = 100 - MC$  (Barrett et al., 1998). Soluble solids content (SS) was measured in triplicate by means of digital refractometry and expressed as °BX at 20 °C (RM 40, Mettler-Toledo, Greifensee, Switzerland). The amount of water insoluble solids (WIS) can be calculated by subtracting the soluble solids content from the total solids content  $WIS = TS - SS$  (Barrett et al., 1998).



Turbidity (TU) was measured nephelometrically using a light scattering photometer (Micro1000 Laboratory Turbidity meter, HF scientific, Florida, USA). The turbidity measurements were only performed on the pasteurized juices and were repeated three times on one homogeneous sample.

The particle size distribution (PSD) was analyzed by using a Malvern Mastersizer (Model 2000, Malvern Instruments Limited, Worcestershire, U.K.). The values for particle refractive index and particle absorption index were respectively 1.52 and 0.10 and an obscuration index around 8 % was pursued. The recorded parameters were the equivalent diameters  $d(0.1)$ ,  $d(0.5)$  and  $d(0.9)$  as well as the volume-based mean diameter  $d(4.3)$  and the area-based mean diameter  $d(3.2)$ . Particle size analysis was performed in duplicate on all juice and tomato solids samples.

Stability towards settling was measured using the Turbiscan LAB (Formulation, L'union, France). Triplicates were analyzed over a period of 170 days. A Turbiscan Stability Index (TSI) was calculated using the Turbiscan software. This TSI-parameter evaluates the stability of dispersions by measuring variations in light intensity of a sample from the bottom to the top and is calculated as  $TSI = \sum_j |scan_{ref}(h_j) - scan_i(h_j)|$  where  $scan_{ref}$  and  $scan_i$  are the initial backscattering value and the backscattering value at a given time respectively and  $h_j$  is a given height in the measuring cell.

### 3.3.4 Microbial juice parameters

Total colony count at 30 °C was determined by a surface plating technique in accordance with the ISO 4833 method, carried out in a BELAC-certified laboratory. This was performed for the juices after 0 days, 15 days, 1 month, 4 months and 6 months of storage at different temperatures. Additionally, after 4 and 6 months, also yeast and mold counts were performed at 25°C conform the ISO 7954 method.

### 3.3.5 Statistical analysis

Statistical analysis was carried out using SPSS Statistics 22. Treatments were compared using one-way analysis of variance (ANOVA) where appropriate two and three way interactions were used, followed by a Scheffé post-hoc test (significance level  $p < 0.05$ ). The dependent variables were firmness, color ( $a^*$ ,  $b^*$ ,  $L^*$  and  $a^*/b^*$ ), JY, TU, particle size ( $d(0.1)$ ,  $d(0.5)$ ,  $d(0.9)$  and  $d(4.3)$ ), pH, MC and BX. For the  $\Delta E$ -values, a one-sampled t-test was performed. Finally a nonlinear regression procedure (R 3.0.1, T Foundation, Auckland, New Zealand) was used to describe the TSI-data:  $TSI = TSI_{eq} + (TSI_{init} - TSI_{eq}) \cdot e^{-k \cdot t}$  (Kubo et al., 2013) with estimated model parameters:  $TSI_{eq}$ , the equilibrium TSI-value,  $TSI_{init}$ , the initial TSI-value and  $k$ , the reaction rate. Sigmaplot 12.5 was used to visualize the data.

## 3.4 Results

### 3.4.1 Physical characterization of the feedstock

The two used tomato cultivars belonged to the same caliber class (diameter 70-82 mm). The firmness of Growdena (flesh) and Merlice (truss) tomatoes did not differ significantly ( $6.95 \pm 1.11$  N). Growdena tomatoes were significantly lighter ( $p < 0.001$ ) and less red ( $p < 0.001$ ) compared to the batch of Merlice tomatoes, while no significant differences in  $b^*$ -value were recorded (Table 3.2).

### 3.4.2 Juice yield and throughput

The JY in the first filtration was  $97.9 \pm 0.2$  % and no significant differences were observed between cultivars. The JY in the second filtration was significantly lower for both cultivars compared to the first filtration, i.e.  $94.7 \pm 0.3$  % for Growdena and  $93.1 \pm 0.7$  % for Merlice. The corresponding juice throughput was  $550 \text{ L}\cdot\text{h}^{-1}$  for the first filtration and  $375 \text{ L}\cdot\text{h}^{-1}$  for the second filtration.

### 3.4.3 Juice quality parameters

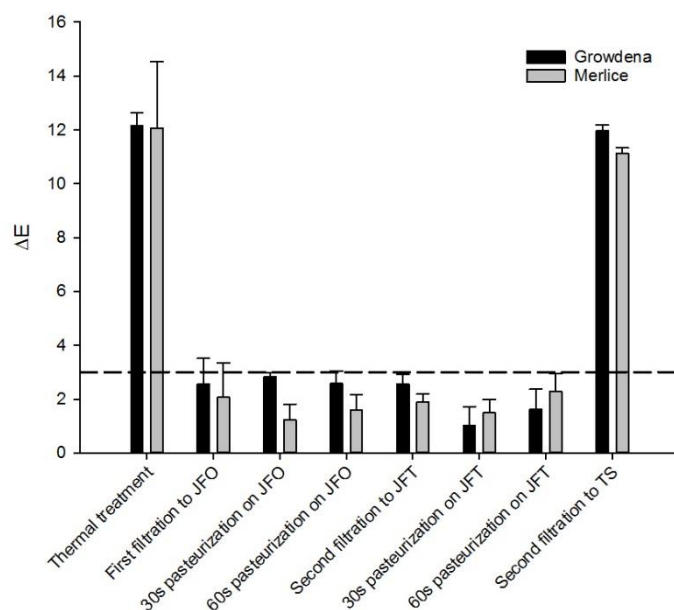
#### 3.4.3.1 Color

Table 3.2 shows the mean  $L^*$ ,  $a^*$  and  $b^*$  values of each sample in the production process for both cultivars. Both Growdena and Merlice tomatoes underwent a significant increase in  $L^*$  after milling and a significantly larger decrease in  $L^*$  after thermal treatment. No additional significant differences in  $L^*$  were observed further on. Milling led to a significant increase in  $a^*$  and a significant decrease in  $b^*$  of the samples, however throughout further processing (thermal treatment, filtration, pasteurization) both  $a^*$  and  $b^*$  remained constant. The overall redness, represented by the ratio  $a^*/b^*$  followed the same trend (data not shown).

In Figure 3.2,  $\Delta E$  relative to the previous process step is shown. The data clearly show that the thermal treatment had the largest impact on the tomato color. In addition, the color of the tomato solids fraction obtained after the second filtration differed significantly from the juice color. The  $\Delta E$ -value for both filtrations was approximately 3 for both cultivars, indicating a visually perceptible color difference upon filtration (Vervoort et al., 2012). The subsequent pasteurization processes were all characterized by a  $\Delta E$ -value smaller than 3.

**Table 3.2** Color parameters of the different samples, i.e. mashed tomato (MT), thermally treated tomato (TT), juice filtered once (JFO), juice filtered once and pasteurized for 30 sec (JFO30), juice filtered once and pasteurized for 60 sec (JFO60), juice filtered twice (JFT), juice filtered twice and pasteurized for 30 sec (JFT30), juice filtered twice and pasteurized for 60 sec (JFT60) and tomato solids (TS). Different letters in the same column for each color parameter indicate statistically significant differences ( $p < 0.001$ ).

Processing condition	Growdena			Merlice		
	L*	a*	b*	L*	a*	b*
Feedstock	43 ± 2 <sup>c</sup>	18 ± 4 <sup>a</sup>	30 ± 4 <sup>b</sup>	37 ± 2 <sup>c</sup>	24 ± 2 <sup>a</sup>	29 ± 2 <sup>b</sup>
MT	48 ± 0.3 <sup>d</sup>	24 ± 0.4 <sup>b</sup>	22 ± 1 <sup>a</sup>	44 ± 2 <sup>d</sup>	30 ± 1 <sup>c</sup>	25 ± 1 <sup>a</sup>
TT	36 ± 0.2 <sup>b</sup>	22 ± 0.3 <sup>a,b</sup>	20 ± 1 <sup>a</sup>	32 ± 1 <sup>b</sup>	28 ± 1 <sup>b,c</sup>	23 ± 2 <sup>a</sup>
JFO	34 ± 1 <sup>a,b</sup>	22 ± 1 <sup>a,b</sup>	19 ± 1 <sup>a</sup>	30 ± 0.2 <sup>a,b</sup>	28 ± 1 <sup>b,c</sup>	23 ± 1 <sup>a</sup>
JFO30	37 ± 1 <sup>b</sup>	21 ± 1 <sup>a,b</sup>	18 ± 1 <sup>a</sup>	31 ± 1 <sup>a,b</sup>	27 ± 1 <sup>b,c</sup>	23 ± 1 <sup>a</sup>
JFO60	33 ± 0.2 <sup>a</sup>	22 ± 0.2 <sup>a,b</sup>	21 ± 0.4 <sup>a</sup>	30 ± 0.2 <sup>a,b</sup>	27 ± 0.1 <sup>b,c</sup>	24 ± 0.3 <sup>a</sup>
JFT	32 ± 0.1 <sup>a</sup>	22 ± 0.1 <sup>a,b</sup>	18 ± 0.1 <sup>a</sup>	28 ± 0.1 <sup>a</sup>	27 ± 0.1 <sup>b,c</sup>	23 ± 0.2 <sup>a</sup>
JFT30	33 ± 1 <sup>a</sup>	21 ± 0.3 <sup>a,b</sup>	19 ± 1 <sup>a</sup>	29 ± 0.2 <sup>a,b</sup>	27 ± 0.3 <sup>b,c</sup>	24 ± 0.4 <sup>a</sup>
JFT60	32 ± 0.3 <sup>a</sup>	21 ± 0.3 <sup>a,b</sup>	20 ± 0.2 <sup>a</sup>	29 ± 0.3 <sup>a,b</sup>	27 ± 0.4 <sup>b,c</sup>	25 ± 0.4 <sup>a</sup>
TS	46 ± 0.1 <sup>c,d</sup>	20 ± 0.1 <sup>a,b</sup>	19 ± 0.1 <sup>a</sup>	41 ± 0.2 <sup>d</sup>	26 ± 0.3 <sup>a,b</sup>	22 ± 0.2 <sup>a</sup>



**Figure 3.2** Total color difference ( $\Delta E$ ) encountered through the production process, calculated relatively to the previous process step. The abbreviations JFO, JFT and TS denote juice filtered once, juice filtered twice and tomato solids respectively. The horizontal line ( $\Delta E=3$ ) represents a color difference, perceptible by most people (Vervoort et al., 2012) and the asterisks refer to values significantly smaller than 3. Vertical error bars represent the standard deviation.

### 3.4.3.2 pH and total soluble solids

The pH of the obtained tomato juices was  $4.4 \pm 0.03$  and was not significantly affected by cultivar nor processing conditions (results not shown).

The SS-values of all the analyzed samples ranged between 3.4 °BX and 4.1 °BX (Table 3.3). The largest differences in soluble solids occurred between the cultivars, where the SS-values of the Merlice tomatoes were all significantly lower compared to Growdena tomatoes, ranging from 3.4 to 3.5 °BX and from 3.8 to 4.1 °BX respectively. No consistent significant effects of filtration nor pasteurization duration were visible throughout the cultivars.

**Table 3.3** Moisture content (MC), total & soluble solids (TS and SS, respectively) and water insoluble solids (WIS) of the different samples, i.e. juice filtered once (JFO), juice filtered once and pasteurized for 30 sec (JFO30), juice filtered once and pasteurized for 60 sec (JFO60), juice filtered twice (JFT), juice filtered twice and pasteurized for 30 sec (JFT30) and juice filtered twice and pasteurized for 60 sec (JFT60).

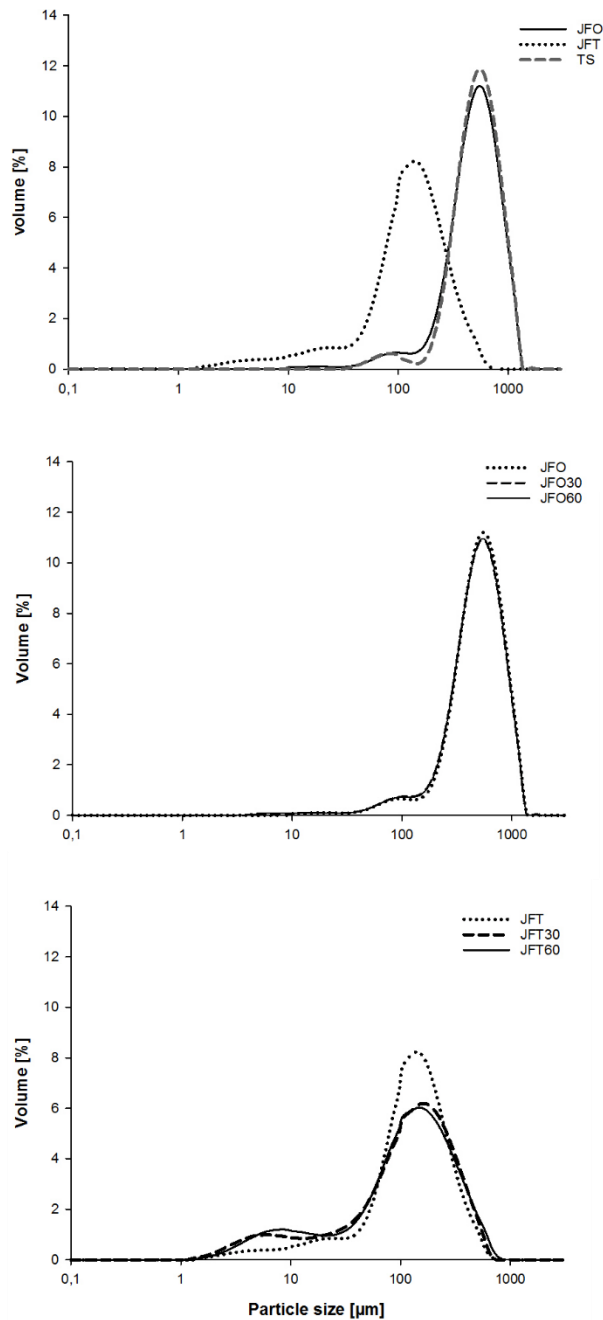
Processing condition	Growdena				Merlice			
	MC	TS	SS (BX)	WIS	MC	TS	SS (BX)	WIS
JFO	95 ± 0.1	4.5 ± 0.1	4.1 ± 0.04	0.40 ± 0.04	96 ± 0.01	3.9 ± 0.01	3.5 ± 0.01	0.40 ± 0.01
JFO30	96 ± 0.01	4.5 ± 0.01	4.0 ± 0.01	0.44 ± 0.01	96 ± 0.03	3.8 ± 0.03	3.5 ± 0.05	0.33 ± 0.05
JFO60	96 ± 0.1	4.4 ± 0.1	4.0 ± 0.01	0.42 ± 0.01	96 ± 0.1	3.8 ± 0.1	3.5 ± 0.01	0.29 ± 0.01
JFT	96 ± 0.01	4.0 ± 0.01	4.0 ± 0.01	0.03 ± 0.01	97 ± 0.01	3.5 ± 0.01	3.5 ± 0.01	0.07 ± 0.01
JFT30	96 ± 0.1	4.0 ± 0.1	3.9 ± 0.01	0.10 ± 0.01	97 ± 0.1	3.5 ± 0.1	3.4 ± 0.01	0.08 ± 0.01
JFT60	96 ± 0.01	3.9 ± 0.01	3.8 ± 0.01	0.10 ± 0.01	97 ± 0.1	3.5 ± 0.1	3.4 ± 0.01	0.08 ± 0.01

### 3.4.3.3 Turbidity

The TU-values of JFO ranged from 7,160 to 8,500 NTU depending on the cultivar and the pasteurization condition (data not shown). Increasing pasteurization time resulted in a significantly ( $p < 0.001$ ) lower TU in both cultivars (14 % reduction in Growdena versus 5 % reduction in Merlice). JFT was characterized by a significantly ( $p < 0.001$ ) lower TU compared with JFO, with values between 5,200 and 5,400 NTU. No consistent effects of pasteurization duration nor cultivar were observed.

### 3.4.3.4 Particle size distribution

JFO was filtered a second time resulting in JFT and TS. In the PSD of these three fractions (Figure 3.3A), it is clear that the majority of the particles larger than 200  $\mu\text{m}$  (intersection of both curves) moved to the TF fraction, where the JFT experienced an enrichment of smaller particles ( $< 200 \mu\text{m}$ ). This is also visible in the particle diameters depicted in Table 3.4, which were reduced upon filtration. Moreover, the reduction after filtration in  $d(3.2)$  (85 % – 90 %) was larger compared to the reduction in  $d(4.3)$  (71 % – 77 %), indicating that the filtration predominantly affected the smaller suspended particles. After pasteurization (not pasteurized vs. 30 s/60 s), the particle size of both tomato juices, irrespective of the filtration condition, significantly decreased, indicating that more small(er) particles were present and the amount of modus-sized particles was reduced (Figure 3.3B and C). For the particles in the outer



**Figure 3.3** Particle size distribution of Growdena tomato juice depicting (A) the effect of solid-liquid separation of juice filtered once (JFO) into juice filtered twice (JFT) and tomato solids (TS), (B) the effect of pasteurization duration (30s and 60s) on JFO and (C) the effect of pasteurization duration (30 s and 60 s) on JFT.

right hand side of the distribution ( $d(0.9)$ ), this trend is not visible for the JFO and even reversed for the JFT. Here, the pasteurized juices have a PSD shifted to larger particle sizes. Combined with the increase in small particles, this is translated in a broader PSD of pasteurized JFT.  $D(4.3)$  showed no distinct trend as the opposing trends visible in  $d(0.5)$  and  $d(0.9)$  are combined. The effect of a longer pasteurization duration (30 s vs. 60 s) is limited and differs between both filtration conditions. The abovementioned trends hold for the two tested cultivars, however their specific PSD's differ. In case

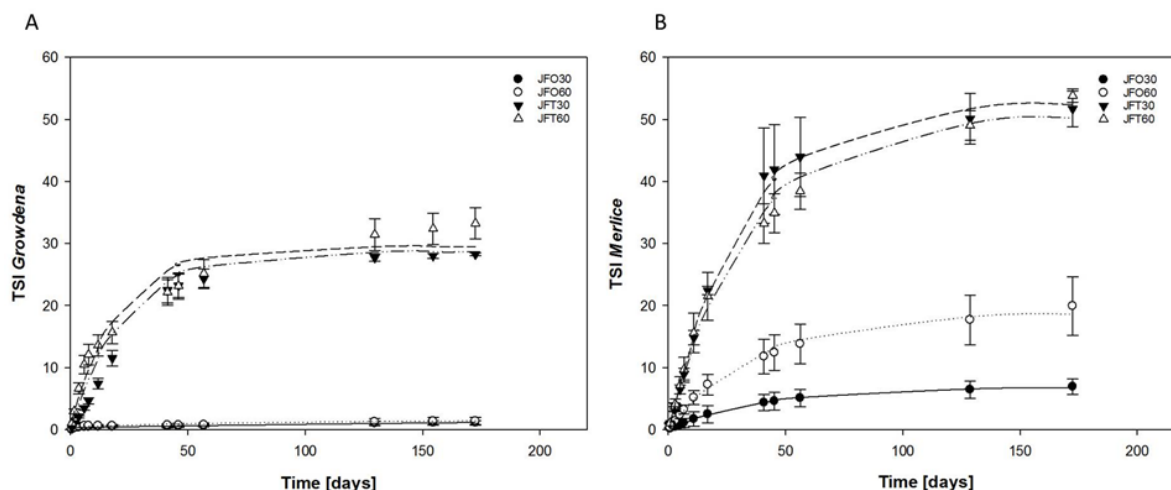
of JFO, the Merlice juices are characterized by less small particles (larger  $d(0.1)$  and  $d(3.2)$ ) and less large particles (smaller  $d(0.9)$  and  $d(4.3)$ ) indicating a more narrow distribution compared to the Growdena juice (Table 3.4). The PSD of JFT showed larger differences between cultivars compared to JFO, where all particle sizes in Merlice JFT were shifted towards smaller values.

**Table 3.4** Particle size distribution ( $\mu\text{m}$ ) in function of juice filtered once (JFO), juice filtered once and pasteurized for 30 sec (JFO30), juice filtered once and pasteurized for 60 sec (JFO60), juice filtered twice (JFT), juice filtered twice and pasteurized for 30 sec (JFT30), juice filtered twice and pasteurized for 60 sec (JFT60) and tomato solids (TF). The parameters  $d(0.1)$ ,  $d(0.5)$  and  $d(0.9)$  are the equivalent particle diameters for which respectively 10 %, 50 % and 90 % of the present particles are smaller.  $D(3.2)$  and  $d(4.3)$  are the surface weighed and the volume weighed particle diameter, respectively. Different letters in the same column of each particle size parameter indicate significant differences ( $p < 0.01$ ).

Processing condition	Growdena					Merlice				
	d (0.1)	d (0.5)	d (0.9)	d (3.2)	d (4.3)	d (0.1)	d (0.5)	d (0.9)	d (3.2)	d (4.3)
JFO	234 ± 3 <sup>e</sup>	507 ± 3 <sup>e</sup>	890 ± 10 <sup>d,e</sup>	323 ± 5 <sup>e</sup>	535 ± 4 <sup>c</sup>	240 ± 2 <sup>e</sup>	487 ± 3 <sup>e</sup>	854 ± 9 <sup>d</sup>	324 ± 5 <sup>e</sup>	517 ± 4 <sup>d</sup>
JFO30	210 ± 1 <sup>c</sup>	493 ± 2 <sup>c</sup>	874 ± 2 <sup>c</sup>	253 ± 1 <sup>c</sup>	519 ± 1 <sup>b</sup>	222 ± 3 <sup>d</sup>	478 ± 3 <sup>d</sup>	845 ± 6 <sup>c,d</sup>	305 ± 2 <sup>d</sup>	507 ± 3 <sup>c</sup>
JFO60	214 ± 2 <sup>d</sup>	497 ± 3 <sup>d</sup>	882 ± 7 <sup>c,d</sup>	263 ± 5 <sup>d</sup>	524 ± 3 <sup>b</sup>	218 ± 3 <sup>c</sup>	473 ± 3 <sup>c</sup>	836 ± 5 <sup>c</sup>	280 ± 7 <sup>c</sup>	501 ± 3 <sup>c</sup>
JFT	29.8 ± 0.4 <sup>b</sup>	131 ± 1 <sup>b</sup>	299 ± 5 <sup>a</sup>	47.7 ± 0.6 <sup>b</sup>	153 ± 2 <sup>a</sup>	16.1 ± 0.4 <sup>b</sup>	112 ± 1 <sup>b</sup>	232 ± 10 <sup>a</sup>	32.7 ± 1.0 <sup>b</sup>	121 ± 3 <sup>b</sup>
JFT30	10.5 ± 0.3 <sup>a</sup>	123 ± 1 <sup>a</sup>	332 ± 6 <sup>b</sup>	28.6 ± 0.4 <sup>a</sup>	152 ± 2 <sup>a</sup>	6.7 ± 0.1 <sup>a</sup>	89.2 ± 0.5 <sup>a</sup>	238 ± 4 <sup>a,b</sup>	19.1 ± 0.3 <sup>a</sup>	108 ± 2 <sup>a</sup>
JFT60	10.3 ± 0.1 <sup>a</sup>	120 ± 1 <sup>a</sup>	343 ± 9 <sup>b</sup>	30.5 ± 0.2 <sup>a</sup>	154 ± 3 <sup>a</sup>	7.2 ± 0.1 <sup>a</sup>	88.3 ± 0.8 <sup>a</sup>	250 ± 6 <sup>b</sup>	20.0 ± 0.3 <sup>a</sup>	113 ± 3 <sup>a</sup>
TF	275 ± 2 <sup>f</sup>	527 ± 2 <sup>f</sup>	903 ± 8 <sup>e</sup>	397 ± 3 <sup>f</sup>	557 ± 3 <sup>d</sup>	243 ± 1 <sup>e</sup>	494 ± 1 <sup>f</sup>	860 ± 6 <sup>d</sup>	342 ± 1 <sup>f</sup>	523 ± 2 <sup>d</sup>

#### 3.4.3.5 Stability towards settling

A graphical representation of the change in TSI with time at different processing conditions is illustrated in Figure 3.4. In Growdena JFO, there was no significant increase in TSI over 170 days for both pasteurization conditions, which is also visualized in low reaction rate values (Table 3.5). All other juices showed a similar TSI-kinetic profile with a distinctly steep period in the first 50 days, followed by a less steep or constant rate period leading to an equilibrium TSI-value. However, both the slope of the curve and the  $\text{TSI}_{\text{eq}}$ -value differed in function of cultivar and processing condition. In JFT's, the slope was higher and the TSI increased already after 24 hours, in contrast to the JFO's. Also the  $\text{TSI}_{\text{eq}}$ -values of JFT's were significantly larger compared to JFO's. Both higher  $k$  and  $\text{TSI}_{\text{eq}}$ -value thus indicate less stable JFT's. Between cultivars, Growdena juice was always characterized by significantly smaller  $\text{TSI}_{\text{eq}}$ -values compared to Merlice juice for the same conditions. Furthermore, pasteurization duration did not yield significantly different stabilities, except for Merlice JFO where a pasteurization duration of 60 s resulted in a less stable tomato juice compared to 30 s (higher  $\text{TSI}_{\text{eq}}$ ).



**Figure 3.4** The effect of filtration, i.e. juice filtered once (JFO) & juice filtered twice (JFT) and pasteurization conditions (30s & 60s) on sedimentation behavior (TSI) in Growdena tomato juice (A) and Merlice tomato juice (B). Vertical bars represent the standard deviation (n=3) in each value and the dashed curves are the nonlinear regression models described in Table 3.5.

**Table 3.5** Mathematical modeling of the sedimentation process in both Growdena and Merlice tomato juices during 170 days of storage (4 °C) in function of both cultivar and processing condition with TSI the Turbiscan Stability Index,  $TSI_{eq}$  the equilibrium TSI-value,  $TSI_{init}$  the initial TSI-value and  $k$  the reaction rate. The processing condition refers to samples, i.e. juice filtered once and pasteurized for 30 sec (JFO30), juice filtered once and pasteurized for 60 sec (JFO60), juice filtered twice and pasteurized for 30 sec (JFT30), juice filtered twice and pasteurized for 60 sec (JFT60).

Cultivar	Processing condition	Model: $TSI = TSI_{eq} + (TSI_{init} - TSI_{eq}) \cdot e^{-k \cdot t}$		
		$TSI_{eq}$	$TSI_{init}$	$k$ (h <sup>-1</sup> )
Growdena	JFO30	2.12 ± 1.91	0.30 ± 0.04***	0.003 ± 0.005
	JFO60	1.51 ± 0.28***	0.42 ± 0.06***	0.007 ± 0.01
	JFT30	28.7 ± 1.56***	-0.04 ± 0.52	0.04 ± 0.01***
	JFT60	30.8 ± 1.61***	3.17 ± 0.94**	0.06 ± 0.01***
Merlice	JFO30	6.81 ± 0.11***	-0.02 ± 0.02	0.03 ± 0.0009***
	JFO60	18.8 ± 0.62***	-0.02 ± 0.10	0.03 ± 0.002***
	JFT30	52.6 ± 1.40***	-0.72 ± 0.27*	0.03 ± 0.002***
	JFT60	50.5 ± 1.83***	-0.02 ± 0.34	0.03 ± 0.002***

Significance level: \*\*\*\*: 0.0001, \*\*\*: 0.001, \*\*: 0.01, \*: 0.05, -: not significant

### 3.4.4 Microbial juice parameters

Results of the total aerobic plate count were lower than 100 CFU.mL<sup>-1</sup> for all the samples. Yeast and mold count yielded values consistently lower than 1 CFU.mL<sup>-1</sup>.

### 3.5 Discussion

The juice yields obtained are equivalent or higher compared to conventional industrial non-enzymatic tomato juicing practices (Bates et al., 2001; Hayes et al., 1998; Min & Zhang, 2003). The correspondance with the results obtained in Chapter 2 as well as the accordance between the two tested cultivars, indicates a robust performance of the system, capable of handling a varying feedstock.

The European fruit juice association (AIJN) prescribes a minimum SS-content in tomato juice of 4.2 °BX (AIJN, 2016). The juices produced in Chapter 3 had consistently lower BX-values. As the SS-level in tomato juice consists approximately for 65 % of reducing sugars, low SS levels indicate a low sugar content in the tomatoes used (Yelle et al., 1988). There are two possible explanations for the low BX-values observed here. Year-averaged data of both cultivars indicate a low SS-content of 4.30 °BX and 4.05 °BX for Growdena and Merlice tomatoes, respectively (Pinxteren et al., 2014). Furthermore, the SS-level has also been reported to increase with color and maturity (Tigist et al., 2013). Following a comparison of the year-averaged color and texture data of the same cultivars in Belgium, it was found that the tomatoes used in the experiments in this chapter were light red with a yellow-green touch (classified as commonly marketed light red tomatoes) and slightly harder in texture compared to ripe tomatoes of both cultivars (Pinxteren et al., 2014). Hence, the not fully ripened tomatoes, used as feedstock here, match with the observed low SS-content.

Color analysis throughout further processing was examined by following the same batch of tomatoes throughout the process, assuring that any changes observed, resulted from process conditions and not from tomato characteristics such as maturity, cultivar, etc. Based on the total color difference, almost each unit operation provoked a color difference perceptible by most people. However, the individual redness ( $a^*$ ) and yellowness ( $b^*$ ) were maintained from milling onwards throughout the process, whereas the lightness ( $L^*$ ) remained constant after thermal processing. The significant decrease in lightness caused by the thermal treatment can be attributed to a dark color formation due to non-enzymatic or enzymatic browning reactions (De Paepe et al., 2015b; Min & Zhang, 2003). In acid media such as tomato juice, heat is often reported to induce the oxidation of ascorbic acid leading to brown color formation (Min & Zhang, 2003; Odriozola-Serrano et al., 2009). Enzymatic browning on the other hand can occur from the oxidative degradation of phenolic compounds by the enzymatic action of polyphenol oxidase by which the phenolic compounds are converted to  $o$ -quinones and further to brown colored pigments (Queiroz et al., 2008). Additionally, color changes during processing can also result from carotenoid degradation, mainly due to oxidation and isomerization of lycopene (Capanoglu et al., 2008; Chanforan et al., 2012; Shi & Le Maguer, 2000). Accordingly, the red color of tomato juice is commonly used as an indicator for lycopene concentration (Arias et al., 2000). The observed



conservation of the red color throughout the processing could thus imply a preservation of lycopene throughout the biorefinery process, illustrating the potential of the spiral-filter press in maintaining the initial quality of the biomass. In order to confirm this preservation, a more detailed chemical analysis is needed to further elucidate the fate of the phytochemicals (Chapter 4).

Besides color, also the particle size of the cloudy juices has been determined. The particles constituting JFO were characterized by diameters ranging between  $\sim 40 \mu\text{m}$  and  $\sim 1,100 \mu\text{m}$  in a bimodal distribution. The average diameter of tomato cells has been estimated to range from 250 to 1,000  $\mu\text{m}$  (Lopez-Sanchez et al., 2011a; Moelants et al., 2014; Redgwell et al., 2008). Based on the dimensions reported, JFO is thus expected to be constituted partly out of whole cells and partly out of cell fragments, disrupted by processing. This is in accordance with results from other studies where tomato material was already disrupted to the level of single cells (intact and broken) upon a simple blending without any heat input (Lopez-Sanchez et al., 2011b).

As expected, further filtration with a smaller filter mesh size, shifts the particle sizes in JFT towards smaller particles, ranging from  $\sim 1 \mu\text{m}$  to  $\sim 600 \mu\text{m}$ . A redistribution of the particles, originally present in JFO, took place with an accumulation of smaller particles in JFT and a depletion of these smaller particles in TF. Interestingly, the maximum particle size of the juices was always larger than the filter pores, which has also been observed by Den Ouden & Van Vliet (1997). This can be explained by the highly deformable parenchyma cells, which constitute the major fraction of cells present in tomato juice and which are able to pass through the pores of a sieve that are significantly smaller than the size of the cell itself (Den Ouden & Van Vliet, 1997; Moelants et al., 2014). Additionally, the exerted vacuum is also expected to aid in pulling larger particles through the filter pores. By varying the filter mesh sizes, changes in the constitution of the obtained end products can thus be induced, going from very liquid juices to highly viscous purees. Pasteurization of tomato juice (not pasteurized vs. 30 s/60 s) led to a significant increase in small particles, visible in both filtration conditions but more pronounced in JFT. This decreasing particle size can be allocated to a partial detachment of the cell walls due to solubilization of pectin in the middle lamellae during heating (Zhang et al., 2015b). Similar effects of thermal treatment were found by others such as Lopez-Sanchez et al. (2011b). Interestingly, the larger particles on the right hand side of the distribution were hardly affected in both filtration cases ( $d(0.9)$ ,  $d(4.3)$ ). Instead, the concentration of particles with a size around the modus of the PSD were mostly reduced upon pasteurization, yielding smaller particles. This can indicate a difference in susceptibility of modus-sized particles towards thermal degradation or it can represent merely a larger statistical chance of being broken down. The effect of prolonging the pasteurization duration (30 s vs. 60 s) exerted only minor effects on PSD's of the obtained juices. Finally, it should be noted that besides the controllable filtration and pasteurization conditions, also the tomato cultivar and the underlying

differences such as growing conditions or ripening stage clearly influence the PSD of the resulting juices as shown in Table 3.4.

From the same batch of tomatoes, both stable and unstable juices were produced. This can be partly explained by the enzymatic activity during processing. It is known that the enzyme PME (pectin methyl esterase) cleaves methylesters of the pectin, resulting in negatively charged pectin that can bind to naturally present divalent cations (e.g.  $\text{Ca}^{2+}$ ) (Fachin et al., 2002). This physico-chemical modification of the cloud particles in tomato juice can result in aggregate formation and consequent sedimentation leading to serum separation (Laratta et al., 1995; Sarr & Tsai, 2008; Schultz et al., 2014). Also PG (polygalacturonase) is present in large amounts in tomatoes causing depolymerization and solubilization of pectin, although its role in serum separation is not completely resolved (Laratta et al., 1995; Moelants et al., 2014). In order to inactivate this pectinolytic activity (and to soften the tissue), the conventional tomato processing industry uses thermal pretreatments (hot break around 90°C and cold break around 70°C). It has been reported that this pectinolytic activity is not completely inhibited at temperatures below 82°C (Hayes et al., 1998). Interestingly, in this chapter, a stable Growdena JFO could be produced that did not undergo a serum separation throughout 170 days, despite the mild thermal pretreatment (< 82°C). Using the low-oxygen spiral-filter press in combination with a fast thermal pasteurization thus enabled the production of a stable Growdena tomato juice without the need for a hot break pretreatment. Related to this, the enzymatic content has been reported to be function of the cultivar and maturity of the fruit, which could explain the lower stability of the Merlice JFO which was produced using exactly the same process (Moelants et al., 2014). Also the thermal stability of the PME has been shown to differ depending on the cultivar (Aghajanzadeh et al., 2016; Laratta et al., 1995). This accords with the findings from tomato puree producers reporting that cloud instability seemed to depend more on tomato cultivar than on production technology utilized (Laratta et al., 1995). Besides the Merlice JFO, also the JFT's underwent a serum separation within 24 hours. Consequently, this could be explained by the combination of (i) the mild thermal pretreatment and (ii) the longer time gap between juice pressing and thermal pasteurization in JFT due to an additional pressing step and the absence of in-line connected equipment. Therefore, JFT might have been subjected to more enzymatic activity, resulting in a faster serum separation.

Various other parameters have been linked to this degree of serum separation (DOSS) and could help to further elucidate the observed differences in stability, such as the content of water insoluble solids (WIS). This WIS-content is represented by the cell wall and middle lamella components such as cellulose, hemicellulose and pectates and can be calculated by subtracting the SS from the TS (Barrett et al., 1998; Kaur et al., 2007). A higher WIS content was found to be negatively correlated with the DOSS, thus leading to more stable juices and ketchups (Kaur et al., 2007; Stoforos & Reid, 1992). This

was confirmed in Chapter 3 and can account for the observed differences in stability between Growdena juices and Merlice juices as well as for the higher instability of Merlice JFO60. Heutink (1986) has stated that indeed tomato variety and processing method (hot break, cold break, finishing type, finishing screen size) influence the amount of WIS. Not only the total amount, but also the particle size distribution of these WIS are reported to play a role in the DOSS (Kubo et al., 2013). According to Stokes' hydrodynamic law, solutions with smaller particles are reported to form more stable solutions (Kubo et al., 2013; Thakur & Singh, 1994). However, the opposite seems to apply for the data obtained in this chapter, as the most unstable juices were always characterized by smaller particles. Accordingly, investigators have reported better consistency of tomato products when larger screen sizes of the finisher were used (Heutink, 1986; Kimball and Kertesz, 1952; Stoforos & Reid, 1992). A possible explanation may lie in the larger particle-particle interactions associated with small particles caused by their increased surface area, leading to larger aggregates which may result in faster sedimentation (Augusto et al., 2012; Kubo et al., 2013; Rojas et al., 2016). Importantly, the thus formed aggregates may not be detected in the particle size analysis as inter-particle forces are relatively weak and may be disrupted during particle size analysis due to the pumping and mixing forces of the instrument (Kubo et al., 2013). Finally, besides the amount and size, the particle nature or morphology is also an important factor in serum separation (Heutink, 1986; Kimball and Kertesz, 1952). It is for example known that elongated particles as created by homogenization, improve the consistency and reduce settling in the product (Stoforos & Reid, 1992; Tanglertpaibul & Rao, 1987). In that manner, the lower exerted vacuum and/or the smaller pores during the second filtration, could have changed the particle shape compared to JFO, leading to more unstable juices. Thus, as cited in literature and confirmed in this research, the physical juice stability seems to be influenced by the interplay of the enzymatic activity on the one hand and the characteristics of the WIS components (amount, distribution, shape, aggregate formation) on the other hand (Kaur et al., 2007). These juice characteristics are to some extent controllable by varying specific parameters in the applied processing such as filter size, applied vacuum and pasteurization duration which can be used to customize end products. Another important factor is the tomato variety which appears to interact in some cases with the effect of processing (Heutink, 1986; Stoforos & Reid, 1992). Specific measurements of the concentration and activity of the enzymes, active in the cloud stability of tomato juice (pectin methylesterase and polygalacturonase) should be included in the future. This will allow to further elucidate the influence of varying process variables and different varieties on both WIS characteristics and enzymatic activity. Consequently, such information can be used more effectively to control the juice stability.

### **3.6 Conclusion**

Producing qualitative, healthy products while meeting the challenges related to food losses proves difficult. The proposed juice production process aims at producing attractive products using the spiral-filter press. The results showed that a stable tomato juice could be produced with a high yield using a very light thermal pretreatment and a mild pasteurization. During juice pressing, the red color was maintained which indicates the preservation of the juice quality. Furthermore, by varying the filtration and pasteurization conditions juices of different turbidity and stability were produced. Comparing the characteristics of the diverse juices allowed us to gain more insight in the processes underlying these phenomena, contributing to the knowledge for better understanding the relation between the structure and the stability of the resulting juices. These insights are crucial for further process design and product formulation of biomass with a similar soft texture. However, changing the tomato feedstock cultivar showed that the same process conditions led to unstable juices. Therefore, more research is necessary to fully elucidate the juice stability phenomenon.

## **Chapter 4**

A novel spiral-filter press for tomato processing: process impact on phenolic compounds, carotenoids and ascorbic acid content



## **Chapter 4: A novel spiral-filter press for tomato juice processing: fate of phenolic compounds, carotenoids and ascorbic acid content during spiral-filter processing, downstream processing and storage**

### **Redrafted from**

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### **4.1 Abstract**

Industrial processing of fruit and vegetables can have detrimental effects on health-promoting phytochemicals. Here, a novel pilot-scale process using an innovative spiral-filter press followed by a thermal treatment was evaluated on tomato for the production of tomato juice. Three-month storage of the resulting juice was also evaluated. The process impact of the different unit processes, with emphasis on the novel spiral-filter pressing, was investigated for the three major compound classes present in tomato (ascorbic acid, phenolic compounds and carotenoids). The spiral-filter press processing did not seem to cause degradation of ascorbic acid, phenolic compounds or carotenoids, which can be ascribed to the fast processing in a low-oxygen atmosphere. Maintaining the native constitution of tomato to a great extent, the spiral-filter press thus offers potential for processing tomatoes and other vegetables into juices, smoothies and purees.

## 4.2 Introduction

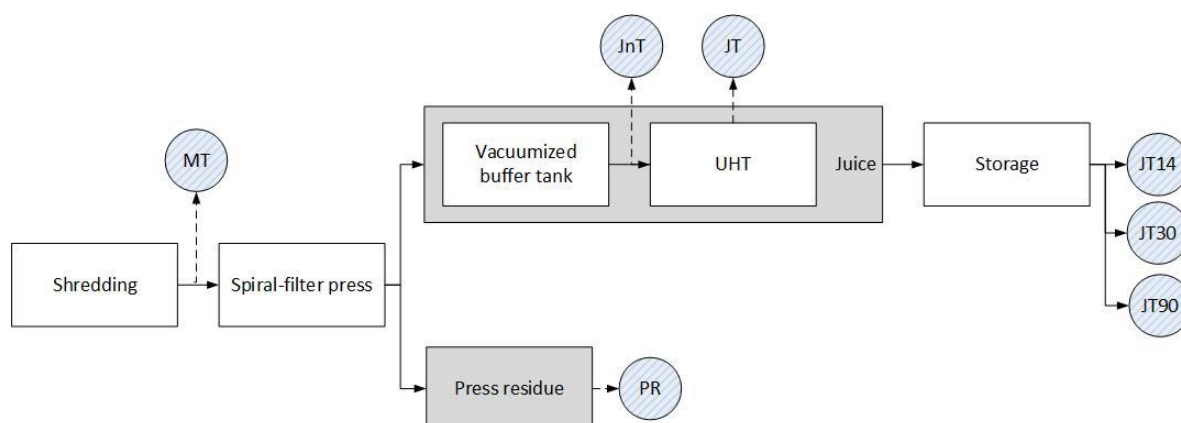
Fruits and vegetables are important sources of bioactive compounds that have multiple beneficial health effects. Year-round consumption is enabled by processing fresh and perishable products into juices, smoothies and pastes. Various types of processing have been shown to affect the sensorial qualities of the end products as well as the fate of phytochemicals (Chanforan et al., 2012; Georgé et al., 2011; Martínez-Hernández et al., 2016; Nayak et al., 2015; Vallverdú-Queralt et al., 2012; 2014). This has led to a growing interest in minimally processed fruit and vegetable products and the introduction of novel processing techniques (Ragaert et al., 2004). Non-thermal technologies (e.g. electric treatments, ultrasound treatments, high hydrostatic pressure treatments) have for example been developed and studied in order to inactivate enzymes and microorganisms and minimize adverse effects on food nutritional and quality parameters (Barrett & Lloyd, 2012; Jiménez-Sánchez et al., 2017a; 2017b; Oms-Oliu et al., 2012; Pereira & Vicente, 2010; Turk et al., 2012). Although less frequently studied, the processing steps before the thermal treatment can also cause degradations in the nutritional profile. Examples in the case of tomato processing are the use of a breaking process (thermal pretreatment), which is used to inactivate the enzymes or the straining process, which is characterized by a high rate of oxygen absorption caused by a high rotation speed (Noomhorm & Tansakul, 1992). Other conventional fruit and vegetable juice pressing techniques (such as belt press and decanter) have been proven to negatively affect the bioactive functionalities or require special modifications to minimize this degradation (De Paepe et al., 2015b; García-Torres et al., 2009; Turk et al., 2012). Depending on the processing conditions, diverse chemical changes including oxidation or isomerization can be provoked for different compounds such as ascorbic acid (AA), phenolic compounds and carotenoids and influence the physical appearance and nutritional characteristics of the end products (Abushita et al., 2000; Capanoglu et al., 2008; Chanforan et al., 2012; Odriozola-Serrano et al., 2009; Rickman et al., 2007a; 2007b).

The spiral-filter press is a novel technology to produce juices, smoothies and purees. This press avoids oxidative degradation by extracting the juice in a low-oxygen extraction cell under vacuum. The beneficial effects of this type of processing have already been described for apple and pear juice, in which the phenolic compounds are highly conserved (De Paepe et al., 2015a; 2015b). Significantly higher vitamin C and anthocyanin concentrations were demonstrated when processing strawberry on a spiral-filter press system as compared to the classical way using a finisher (Possner et al., 2015). To the best of our knowledge, there is currently no information available about how the spiral-filter press influences the constitution of other matrices and whether it conserves other compounds such as carotenoids. Therefore, in order to fully estimate the potential of this technology for the production



of innovative, tasty and nutritional food products, it is crucial to assess the processing impact on a different matrix (tomato) and study its effect on additional phytochemicals (carotenoids). Furthermore, as the type of juice extraction is known to influence the phytochemical retention during downstream processing and storage (De Paepe et al., 2015b), these unit processes should also be included in the analysis.

In this chapter, a novel juice processing line was investigated for its process impact on the resulting products. This consisted of a shredding pretreatment, filtration with the novel spiral-filter press, thermal treatment and 3-month storage of the resulting juices. Care was taken to minimize the presence of air throughout all process steps (Figure 4.1). Per unit process, a detailed study of the three main compound classes in tomato (AA, phenolic and carotenoid content) was carried out, to generate information about the impact of each process on the fate of these parameters. The aim of this chapter is not to compare the spiral-filter press with other conventional juice processing industries as the raw tomato feedstock was used exclusively as a reference point to evaluate the process impact. Comparison with other technologies should be subject of future research.



**Figure 4.1** Schematic overview of the tomato processing line where white boxes represent unit processes, grey boxes represent resulting products and circles represent samples that were taken throughout the process with MT, mashed tomato; PR, press residue; JnT, juice non thermally treated; JT, Juice thermally treated; JT14, juice thermally treated and stored for 14 days; JT30, juice thermally treated and stored for 30 days; JT90, juice thermally treated and stored for 90 days.

## 4.3 Materials and methods

A batch of 400 kg Merlice tomatoes (*Solanum lycopersicum* L. cv. Merlice, truss type tomato) at commercial maturity was purchased at a Belgian produce auction (Bel'Orta, Sint-Katelijne-Waver, Belgium). These tomatoes were stored at 4°C for 24 hours until the experiments were conducted.

### 4.3.1 Pilot-scale machinery

The process consists of a sequence of pilot-scale batch processes, as described above (Figure 4.1). After manually removing the green stems, the tomatoes were washed in cold tap water and transferred to a shredding device (Multicut, Bruckner Liquid Food Tech GmbH, Abstatt, Germany). Detailed process parameters are shown in Table 4.1.

**Table 4.1** Spiral-filter press parameters used for the experiments in Chapter 4.

Shredding and filtration parameters	Values
Filter size	500 µm
Spiral (channels - inclination angle)	#5 – variable
Milling pump frequency	11.1 rpm
Feed pump frequency	14.6 rpm
Spiral frequency	55.8 rpm

The resulting mashed tomato (MT) was pumped to the buffer tank of the spiral-filter press and into the extraction cell, where a plastic spiral rotates within a cylindrical filter element (VacuIQ 1000, VacuIQ, Hamminkeln, Germany). The milling was tuned to avoid accumulation of MT in the buffer tank. In the extraction cell, as a result of both the compression forces in the spiral and the underpressure created by the vacuum pump, the MT was dewatered through the filter element resulting in a juice fraction (JnT). The increasingly dry MT was carried upwards to exit the system as press residue (PR). JnT was collected in a vacuum buffer tank (100 L, VacuIQ, Hamminkeln, Germany). From there, it briefly passed a small open buffer tank for sampling and subsequently entered a multipurpose ultra-high temperature (UHT) pilot machine (APV SPP, SPX Corporation, Gatwick, UK) where it was subjected to a thermal treatment at 108°C for 30 s. The thermally treated juice (JT) was cooled to 4°C and cold-filled in bag-in-box aluminum laminate aseptic bags (Rapak Bag-in-box, 2L).

### 4.3.2 Characterization of the intermediate and end products

#### 4.3.2.1 Sampling

At four points along the tomato juice processing line, samples (MT, PR, JnT and JT, circles in Figure 4.1) were taken using 3-way-valves in order to avoid disturbing the system. All four samples (~1000 mL)

were taken twice without interrupting the processing. Approximately 500 mL of these samples were analyzed in triplicate immediately after collection for moisture content (MC) and AA. The remainder was immediately snap frozen using liquid nitrogen (Air Liquide, Liège, Belgium) and subsequently freeze-dried (Epsilon 2-10 D LSC, Martin Christ, Osterode am Harz, Germany) and milled. The resulting dry powders were stored at -80°C in amber glass bottles under a nitrogen atmosphere before analysis of carotenoids and phenolic compounds. Finally, the thermally treated juice samples were stored in aluminum bag-in-box bags at 20°C for 14 days (JT14), 1 month (JT30) and 3 months (JT90). At these time intervals, the stored samples were (i) subjected to microbial and AA analysis and (ii) freeze-dried for analysis of carotenoids and phenolic compounds.

#### 4.3.2.2 Juice yield

The juice yield (JY) of the filtration was calculated gravimetrically by recording the masses of both juice and press residue during the steady-state phase of the process:  $JY [\%] = \frac{M_j}{M_j + M_p} \times 100 \%$ , with  $M_j$  the net mass of the juice and  $M_p$  the net mass of the press residue. The masses were recorded in triplicate.

#### 4.3.2.3 Physico-chemical characterization of the intermediate and end products

Moisture content (MC) was determined by means of a halogen moisture analyzer (HB43-S, Mettler Toledo, Schwerzenbach, Switzerland). AA content was determined by voltametric titration with 2,6-dichlorophenolindophenol (DPI) on all liquid samples according to the Mettler-Toledo method M411-2006 (T70, Mettler-Toledo, Greifensee, Switzerland). All measurements were conducted in triplicate.

Phenolic compounds were extracted from the freeze-dried samples in triplicate using the extraction method of De Paepe et al. (2013). Briefly, 0.5 g freeze-dried sample was extracted with 5 mL of 100 % MeOH in a first step and 5 mL MeOH:water (20/80, v/v) in a second step. The ultrasound-assisted extraction (Transsonic Digital S, Elma, Germany) lasted 60 minutes (stirred after 30 minutes). Following centrifugation (3000 rpm, 15 min) (Sigma Laboratory Centrifuges 4K12, Germany), the supernatans were collected and stored at 4 °C. After the second extraction cycle, 1000 µL of both supernatans were combined, centrifuged (14000 rpm, 10 min), filtered (0.22 µm, Millipore, Overijse, Belgium) and stored in capped vials prior to injection. An internal standard (daidzein, 1 µg.g<sup>-1</sup>) was added before extraction. The analytical separation and detection were performed by means of reversed-phase, ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), based on the method described by De Paepe et al. (2013), using an Acquity™ UPLC (Waters) coupled to a Xevo™ TQ-S mass spectrometer (Waters) and a Waters UPLC BEH C<sub>18</sub> chromatographic column (150 mm x 2.1 mm, 1.7 µm, Waters) with a flow rate of 196 µL.min<sup>-1</sup>. The MS detector operated in ESI-

mode with a cone voltage of 40 V and a capillary potential of 2.8 kV. Source and desolvation temperatures were 130 °C and 450 °C, respectively. Desolvation gas flow was set at 800 L.h<sup>-1</sup>. Quantification was performed based on relative peak areas and using external standard curves with reference standards. Due to the presence of high amounts of chlorogenic acid, quantification of this compound was performed using 1/10 dilution (MeOH:water, 60/40, v/v) of the extracts and on an absolute basis (i.e. without using the signal of the internal standard as this was too diluted). Correct compound identification was assured based on the ion ratio and the relative retention time taking into account the criteria stipulated in Commission Decision 2002/657/EC. Data recording was performed by MassLynx™ (v.4.1) while the integration was performed with TargetLynx™ (v.4.1) (Waters).

The extraction, separation and identification of the carotenoids (*trans*-lycopene, 5-*cis*-lycopene, 9-*cis*-lycopene, 13-*cis*-lycopene and β-carotene) were performed using a HPLC-DAD procedure adapted from Cucu et al. (2012). Briefly, freeze-dried samples (0.1 g) were extracted in triplicate with 1 mL hexane/acetone/ethanol (2:1:1, v/v/v). After mixing on ice and centrifugation (1,000 g, 5 min), the upper orange organic phase was recovered. This procedure was repeated twice until the pellet was white. The pooled supernatant (3 mL) was thoroughly mixed with 3 mL of a saturated NaCl solution (200 g.L<sup>-1</sup>), the upper organic phase was recovered (1 mL) and evaporated under a nitrogen stream until dry. The dry extracts were resuspended in 250 μL of extraction buffer. Twenty microliters of the extract were separated on a C30 RP-column (3 μm, 250 x 4.6 mm) from YMC corporation (Kyoto, Japan) using a mobile phase (methanol/isopropyl alcohol/tetrahydrofuran) (30:30:35, v/v/v), stabilized with 250 mg.L<sup>-1</sup> butylated hydroxytoluene and 0.05 % trimethylamine and compounds were detected by measuring absorbance at 475 nm. The flow rate was 0.8 mL.min<sup>-1</sup>. For the JT90 sample, due to technical problems with the C<sub>30</sub> column, analysis was carried on a C<sub>18</sub> column with the method developed by Daood et al. (2014), using an elution gradient from water and acetone. Therefore, only β-carotene and *trans*-lycopene could be analyzed. Equal performance was confirmed by analysis of three quality control samples on both columns with deviations of maximum 10 %. Quantification was performed using authentic reference analytical standards for lycopene and β-carotene. The nature of the lycopene isomers was proposed based on the absorbance spectra (specific for lycopene) and retention times with regard to Cucu et al. (2012) and Ishida & Chapman (2006). As lycopene is a very unstable molecule, the concentration of the lycopene stock solution was checked in advance by measuring the absorbance at 502 nm and recalculating the exact concentration by using the Beer-Lambert law  $DO = \epsilon \cdot c \cdot l$ , with  $DO$ =optical density,  $\epsilon_{502}$ =absorption coefficient =  $1.72 \cdot 10^5$  L.mol<sup>-1</sup>.cm<sup>-1</sup>,  $c$ =concentration of the solution and  $l$ =length of the light pad=1 cm.

Following analytical standards were purchased from Sigma (Diegem, Belgium): 3,4,5-trimethoxycinnamic acid, 4-p-hydroxyphenyl acetic acid, apigetrin, avicularin, caffeic acid, chicoric

acid, chlorogenic acid, cyanidin chloride, epicatechin, daidzein, dihydrocafeic acid, dihydroferulic acid, ferulic acid, gallic acid, gentisic acid, hesperetin, hesperidin, kaempferol, luteolin, miquelianin, naringenin, o-coumaric acid, p-coumaric acid, phloretin, propyl gallate, quercetin, salicylic acid, sinapinic acid and vanillic acid. Aromadendrin, catechin, cynaroside, phloridzin, procyanidin B2, protocatechuic acid, taxifolin and naringenin chalcone were purchased from Phytolab GmbH & Co (Vestenbergsgreuth, Germany). Apigenin, astragalin,  $\beta$ -carotene, galangin, lycopene, naringin, quercitrin and rutin were provided by Extrasynthese (Genay, France). Ascorbic acid, isoquercitrin and isorhamnetin were provided by Carl Roth GmbH (Karsruhe, Germany). Dichloroindophenol was purchased from Merck (Kenilworth, USA).

#### 4.3.2.4 Calculation of retention and extraction efficiencies of the unit processes

The impact of the various unit processes on the phenolic and carotenoid compounds was evaluated by calculating the retention efficiency (% R) for each compound, representing the ratio of the concentration of the compound present after and before the process. This % R was calculated for both the filtration process and thermal process. The former can be divided in juice and press residue extraction efficiency (%  $E_{JnT}$  and %  $E_{PR}$ ) representing the percentage of the compound that ends up in the juice fraction or the press residue, respectively (calculations in Appendix 2). The % R and % E-values of the filtration process were only calculated for compounds with concentrations larger than their corresponding quantification limit in the MT fraction. Corresponding concentrations in JnT or PR that were lower than their detection limit were considered zero. The % R values of the thermal process were only calculated for compounds with concentrations larger than their quantification limit in the JnT fraction.

#### 4.3.2.5 Microbial juice characterization

Total colony count at 30°C was determined by a surface plating technique in accordance with the ISO 4833 method, carried out in a BELAC-certified laboratory. Further, yeast and mold counts were performed at 25°C in accordance with the ISO 7954 method. These parameters were assessed for thermally treated tomato juices which were stored at 20°C for 0 days, 14 days, 1 month and 3 months.

### 4.3.3 Statistical analysis

Statistical analysis was carried out using SPSS Statistics 22 (IBM, Brussels, Belgium). Treatments were compared using one-way analysis of variance (ANOVA) followed by a Scheffé post-hoc test. The dependent variables were MC, AA content and the concentrations of the measured phenolic compounds and carotenoids. The independent variable was the sample treatment, where the data

were split into three subsets. In the first subset, the effect of processing with the spiral-filter press was investigated (MT, JnT and PR). In the second dataset, the effect of thermal treatment was assessed (JnT and JT) and the third subset was used to examine the effect of storage by comparing samples JT, JT14, JT30 and JT90. A significance level of  $p < 0.05$  was used. Sigmaplot 12.5 (Systat Software GmbH, Erkrath, Germany) was used to visualize the data.

## 4.4. Results and discussion

### 4.4.1 Microbial juice characterization

Throughout the entire 3-month storage, both the total colony count and the yeast and mold counts were in all cases smaller than  $1.0 \text{ kve.ml}^{-1}$ , indicating adequate microbial inactivation in the final products.

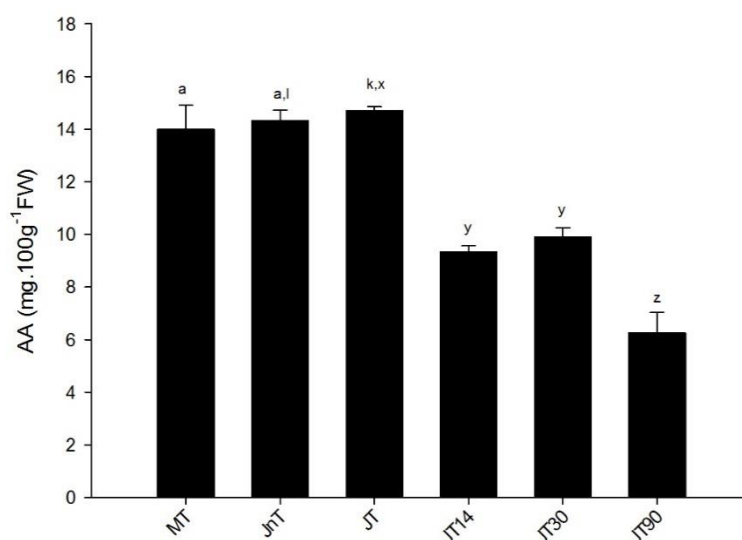
### 4.4.2 Ascorbic acid

Ascorbic acid (AA) is one of the most important vitamins in fruits and vegetables and has many beneficial biological functions such as antioxidative properties. However, it is a very labile molecule that is easily oxidized, both chemically and enzymatically, during processing into dehydroascorbic acid and further hydrolyzed to 2,3-diketogluconic acid. This conversion is to be minimized as the latter is devoid of vitamin C activity. The extent of degradation is influenced by many factors such as temperature, pH and the presence of oxygen and metal ions (Dewanto et al., 2002; Jayathunge et al., 2015; Munyaka et al., 2010; Phillips et al., 2016). The AA content therefore serves as an excellent indicator for the quality of processed tomato products.

The AA content of the mashed tomatoes was  $14 \pm 1 \text{ mg.100 g}^{-1}$  FW (fresh weight) which is in accordance with values published for red ripe tomatoes, which vary from 9 to 16 mg.  $100 \text{ g}^{-1}$  FW (moisture contents in Table 4.2 and calculation for wet based concentrations in Appendix 2) (Frusciante et al., 2007). The AA content did not significantly decrease upon filtration (Figure 4.2). However, the presence of oxidative enzymes (e.g. ascorbic acid oxidase and ascorbic acid peroxidase) have been known to catalyze the enzymatic oxidation of AA upon exposure to oxygen, particularly after matrix disruption (Munyaka et al., 2010). Hence, it can be assumed that the fast processing with the spiral-filter press in a low-oxygen atmosphere suppressed oxidation, leading to a retention of AA.

**Table 4.2** Moisture contents (%) of the four obtained fractions MT, mashed tomato, PR, press residue, JnT, juice not thermally treated and JT, juice thermally treated.

Sample	MC (%)
MT	$96 \pm 0.2^b$
PR	$90 \pm 0.2^c$
JnT	$96 \pm 0.1^a$
JT	$97 \pm 0.1^a$



**Figure 4.2** AA content of the tomato juice from mashed tomato (MT) throughout filtration (juice not thermally treated, JnT), thermal treatment (juice thermally treated, JT) and storage (juice stored for 14 (JT14), 30 (JT30) and 90 (JT90) days). Vertical error bars represent standard deviations (n=6). Different letters indicate statistically significant differences ( $p < 0.05$ ). Letters a and b denote differences throughout processing (MT and JnT). K and l are used for differences during thermal treatment (JnT and JT) and x, y and z denote differences throughout storage (JT, JT14, JT30 and JT90).

After thermal treatment, the AA content increased significantly to  $15 \pm 0.2 \text{ mg.100 g}^{-1} \text{ FW}$ . This contrasts with the well-known effect of speeding the oxidation process of AA when heated, leading to numerous examples of AA degradation during thermal processing of tomato products, ranging from 10 % to 80 % degradation ( $88^\circ\text{C} - 100^\circ\text{C}$ ) (Abushita et al., 2000; Dewanto et al., 2002; Gahler et al., 2003; Georgé et al., 2011; Koh et al., 2012). The thermal treatment could have inhibited the AA degrading enzymes, leading to retention of AA. This phenomenon has already been shown in broccoli at temperatures above  $70^\circ\text{C}$  (15 min) and in other fruits and vegetables such as carrots, apricots and cherries at  $98^\circ\text{C}$  (10 min) (Leong & Oey, 2012; Munyaka et al., 2010).

Storage of the juice for 14 days led to a significant decrease in AA of 37 %. Longer storage up to three months led to an additional decrease of approximately 20 %. These decreases could be ascribed to the rather high storage temperature leading to accelerated oxidation rates (Jayathunge et al., 2015; Kalt, 2005; Phillips et al., 2016), either in the presence of residual dissolved oxygen in the juice, leading to non-enzymatic oxidation of AA (García-Torres et al., 2009; Odriozola-Serrano et al., 2008) or under anaerobic conditions leading to the slow degradation of AA (Zerdin et al., 2003). As the measurements of JT were performed immediately after the thermal treatment, it can be hypothesized that these factors did not immediately result in any AA degradation in JT but they did after storage.

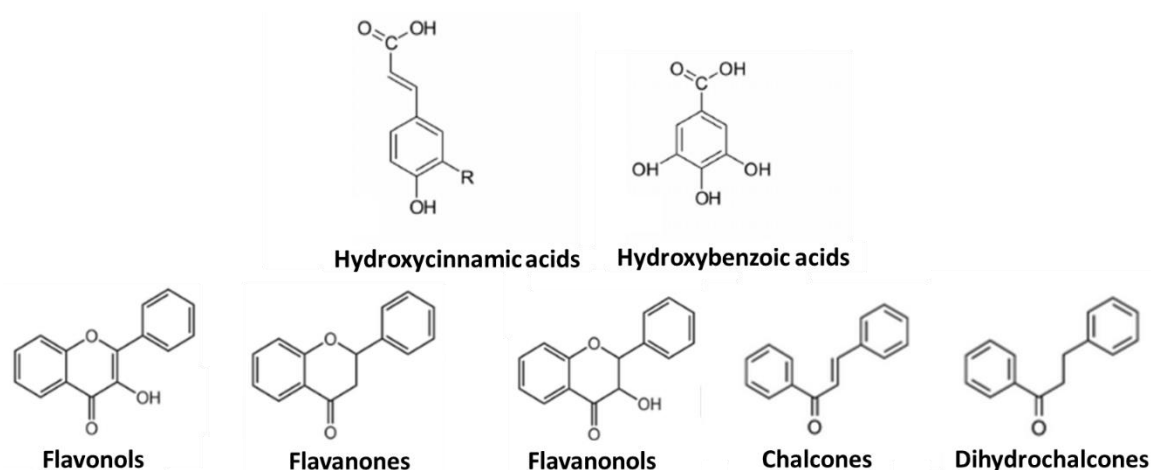


### 4.4.3 Phenolic compounds

Besides AA, phenolic compounds are well-known as the major contributors to the total hydrophilic antioxidative capacity of tomato (Martínez-Valverde et al., 2002; Takeoka et al., 2001; Toor & Savage, 2005). These compounds have been found to be susceptible to enzymatic and non-enzymatic oxidation. These effects can be induced by factors such as enzymes, oxygen, heat and metallic cations (Le Bourvellec & Renard, 2012; Manach et al., 2004; Tomás-Barberán & Espín, 2001; van der Sluis et al., 2002), but results reported are not consistent (Dewanto et al., 2002; Gahler et al., 2003; Georgé et al., 2011; Nayak et al., 2015). In this article, the impact of the proposed process is evaluated based on the fate of these compounds.

#### 4.4.3.1 Absolute concentrations

The phenolic compounds quantified in the tomato samples are shown in Table 4.3. The basic structural formulas of the corresponding phenolic classes are displayed in Figure 4.3. The sum of the measured phenolic compounds on a fresh weight basis was  $18,116 \pm 1,602 \text{ ng.g}^{-1} \text{ FW}$ ,  $10,168 \pm 129 \text{ ng.g}^{-1} \text{ FW}$  and  $58,213 \pm 3,869 \text{ ng.g}^{-1} \text{ FW}$  in MT, JnT and PR, respectively. Chlorogenic acid was the most abundant hydroxycinnamic acid, whereas naringenin chalcone and rutin were the major flavonoids (as also shown by Gómez-Romero et al. (2010)). The order of magnitude of the predominantly present compounds is generally in agreement with previous research (Chanforan et al., 2012; Gómez-Romero et al., 2010; Martínez-Valverde et al., 2002; Moco et al., 2006; Slimestad & Verheul, 2009; Vallverdú-Queralt et al., 2012).



**Figure 4.3** Subclasses of phenolic acids and flavonoids and their basic chemical structures. Figure based on Amarowicz et al. (2009) and Gurung et al. (2015).

**Table 4.3** Content of phenolic compounds (ng.g<sup>-1</sup> FW) in the analyzed sample, i.e. mashed tomato (MT), press residue (PR), juice not thermally treated (JnT), thermally treated juice (JT), thermally treated juice stored for 14 (JT14), 30 (JT30) and 90 (JT90) days. Different letters indicate statistically significant differences (p<0.05). Letters a and b denote differences throughout processing (MT, PR and JnT). K and l are used for differences during thermal treatment (JnT and JT) and w, x, y and z denote differences throughout storage (JT, JT14, JT30 and JT90). Concentrations lower than LOQ but higher than LOD are denoted by < LOQ.

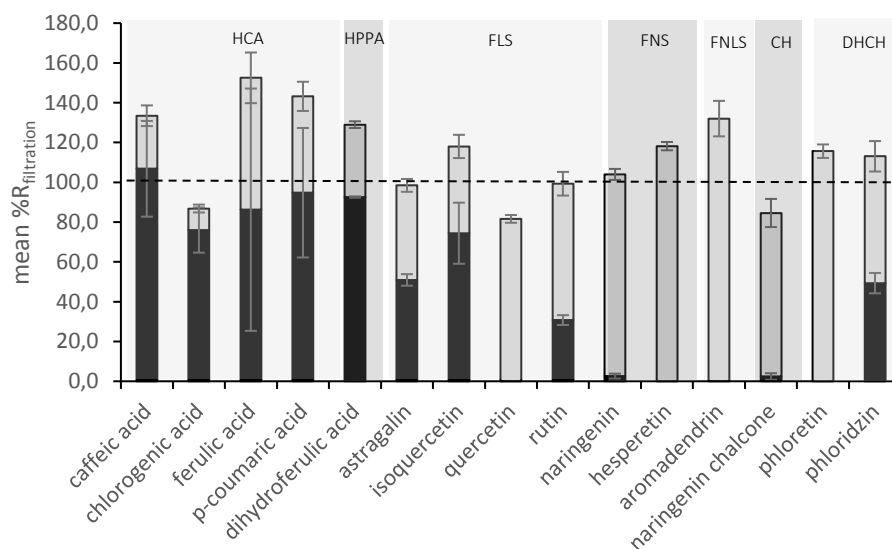
Phenolic class	Phenolic compound	MT	PR	JnT	JT	JT14	JT30	JT90
<b>Hydroxybenzoic acids</b>	<b>Protocatechuic acid</b>	<25	<55	<25	60 ± 2 <sup>w</sup>	85 ± 1 <sup>x</sup>	102 ± 4 <sup>y</sup>	126 ± 5 <sup>z</sup>
<b>Hydroxycinnamic acids</b>	<b>Caffeic acid</b>	98 ± 0.1 <sup>a</sup>	215 ± 42 <sup>b</sup>	119 ± 27 <sup>a,k</sup>	164 ± 7 <sup>l,w</sup>	155 ± 18 <sup>w</sup>	164 ± 1 <sup>w</sup>	172 ± 11 <sup>w</sup>
	<b>Chlorogenic acid (3-caffeoylquinic acid)</b>	10,242 ± 1594 <sup>a</sup>	8,943 ± 250 <sup>a</sup>	8,766 ± 54 <sup>a,k</sup>	8,334 ± 418 <sup>k,x</sup>	5,710 ± 58 <sup>w</sup>	6,012 ± 32 <sup>w</sup>	5,601 ± 242 <sup>w</sup>
	<b>Ferulic acid</b>	46 ± 3 <sup>a</sup>	248 ± 32 <sup>b</sup>	44 ± 29 <sup>a,k</sup>	62 ± 5 <sup>k,w</sup>	60 ± 8 <sup>w</sup>	63 ± 3 <sup>w</sup>	62 ± 5 <sup>w</sup>
	<b>p-coumaric acid</b>	9.4 ± 0.3 <sup>a</sup>	37 ± 5 <sup>b</sup>	10 ± 3 <sup>a,k</sup>	13 ± 1 <sup>l,w</sup>	14 ± 2 <sup>w,x</sup>	15 ± 0.2 <sup>x</sup>	15 ± 1 <sup>x</sup>
<b>Hydroxyphenyl propanoic acid</b>	<b>Dihydroferulic acid</b>	11 ± 2 <sup>a</sup>	32 ± 6 <sup>b</sup>	11 ± 2 <sup>a,k</sup>	12 ± 1 <sup>k,w</sup>	12 ± 1 <sup>w</sup>	12 ± 1 <sup>w</sup>	14 ± 1 <sup>w</sup>
<b>Flavonoids</b>	<b>Flavonols</b>							
	<b>Astragalín (Kaempferol 3-O-glucoside)</b>	1.6 ± 0.03 <sup>b</sup>	6.1 ± 0.6 <sup>c</sup>	0.91 ± 0.03 <sup>a,l</sup>	0.81 ± 0.02 <sup>k,w</sup>	0.80 ± 0.08 <sup>w</sup>	0.85 ± 0.03 <sup>w</sup>	0.82 ± 0.04 <sup>w</sup>
	<b>Isoquercitrín (Quercetin 3-O-glucoside)</b>	4.7 ± 0.6 <sup>a</sup>	17 ± 0.03 <sup>b</sup>	4.0 ± 0.3 <sup>a,l</sup>	3.4 ± 0.04 <sup>k,w</sup>	3.5 ± 0.1 <sup>w,x</sup>	3.8 ± 0.1 <sup>x</sup>	3.8 ± 0.2 <sup>x</sup>
	<b>Quercetin</b>	0.97 ± 0.11 <sup>a</sup>	6.5 ± 0.6 <sup>b</sup>	<0.5	<0.5	<0.5	<0.5	<0.5
	<b>Rutin (Quercetin 3-O-rutinoside)</b>	3105 ± 30 <sup>b</sup>	17,429 ± 1342 <sup>c</sup>	1088 ± 77 <sup>a,k</sup>	990 ± 26 <sup>k,x</sup>	823 ± 14 <sup>w</sup>	898 ± 23 <sup>w,x</sup>	965 ± 18 <sup>x</sup>
	<b>Flavanones</b>							
	<b>Naringenin</b>	145 ± 21 <sup>b</sup>	1,205 ± 140 <sup>c</sup>	4.2 ± 1.4 <sup>a,k</sup>	61 ± 14 <sup>l,x</sup>	44 ± 5 <sup>w</sup>	48 ± 1 <sup>w</sup>	46 ± 4 <sup>w</sup>
	<b>Hesperetin</b>	3.1 ± 0.4 <sup>a</sup>	29 ± 3 <sup>b</sup>	<0.5	<0.5	<0.5	<0.5	<0.5
	<b>Flavanonols</b>							
	<b>Aromadendrin (Dihydrokaempferol)</b>	1.6 ± 0.3 <sup>a</sup>	17 ± 2 <sup>b</sup>	<0.5	<0.5	<0.5	<0.5	<0.5
	<b>Chalcones</b>							
	<b>Naringenin chalcone</b>	4439 ± 155 <sup>b</sup>	29,943 ± 3617 <sup>c</sup>	120 ± 79 <sup>a,l</sup>	30 ± 35 <sup>k,w</sup>	0.66 ± 0.001 <sup>w</sup>	<0.5	<0.5
	<b>Dihydrochalcones</b>							
	<b>Phloretin</b>	8.3 ± 1.2 <sup>a</sup>	79 ± 14 <sup>b</sup>	<0.5	<0.5	<0.5	<0.5	<0.5
	<b>Phloridzin (Phloretin-2'-O-glucoside)</b>	1.4 ± 0.03 <sup>a</sup>	7.4 ± 0.7 <sup>b</sup>	0.80 ± 0.10 <sup>a,k</sup>	0.81 ± 0.02 <sup>k</sup>	<0.5	<0.5	<0.5
	<b>Sum</b>	<b>18,116 ± 1,602</b>	<b>58,213 ± 3,869</b>	<b>10,168 ± 129</b>	<b>9,731 ± 420</b>	<b>6,910 ± 63</b>	<b>7,319 ± 40</b>	<b>7,006 ± 243</b>

#### 4.4.3.2 Retention efficiencies

The main goal of the study in Chapter 4 was to evaluate the different unit process performances. Investigation of the process impact of a certain technology or process is often done by comparing the phytochemicals' concentration before and after processing, either on a fresh or dry weight basis. This was done for the thermal treatment and storage and resulting values are expressed as retention efficiencies. For the spiral-filter processing, processing of one fraction (MT) results in two fractions (PR and JnT). Conventionally, only the end product of interest is taken into account. In case of tomato juice processing, juice is the desired product and press residue is considered as waste. In contrast, in this dissertation, both juice and press residue fractions were considered as valuable end products and thus both fractions were taken into account in the calculation of the retention efficiency of the spiral-filter processing to evaluate the actual process impact.

##### a/ Spiral-filter processing

Considering all measured phenolic compounds and accounting for the obtained juice yield of  $87.8\% \pm 0.3\%$ , an average retention efficiency (% R) of  $114\% \pm 22\%$  was achieved for the filtration process with values for the individual phenolic compounds varying between 85% and 153% (Figure 4.4). However, the retention efficiencies during filtration with the spiral-filter press for each compound were never significantly lower than 100% (except for quercetin).



**Figure 4.4** Retention efficiencies (% R) of phenolic compounds throughout the filtration process. Light grey columns represent the extraction efficiency in the press residue (% E<sub>PR</sub>), whereas black columns represent the extraction efficiency in the juice (% E<sub>JnT</sub>). The vertical error bars represent standard deviations (n=2). The sum of both extraction efficiencies is the retention efficiency of the compound. The retention efficiencies have only been calculated for the compounds present in concentrations larger than the quantification limit in the MT fraction. The phenolic classes displayed are hydroxycinnamic acids (HCA), hydroxylphenyl propanoic acids (HPPA), flavonols (FLS), flavanones (FNS), flavanonols (FNLS), chalcones (CH) and dihydrochalcones (DHCH).

These values indicate that the phenolic compounds that are present in the mash were conserved (and enriched) in either JnT or PR and were not degraded during filtration. This is in contrast with literature, where rupture of the cell structures as a result of grinding, pressing or maceration can break the integrity of the plant cell, leading to oxidative enzymes that catalyze the transformation and degradation of phenolic compounds in the presence of oxygen, resulting in brown pigments (Le Bourvellec & Renard, 2012; Manach et al., 2004; Tomás-Barberán & Espín, 2001; van der Sluis et al., 2002). However in this study, no general reduction was observed, which implies that the fast spiral-filter processing was able to reduce the enzymatic activity and limit the presence of oxygen.

The small differences in retention efficiencies for the individual compounds can be partly explained by differences in susceptibility of the individual phenolic compounds to degradation. For example, chlorogenic acid is known to be a good substrate for polyphenoloxidases (PPO), making it more susceptible to enzymatic degradation (De Paepe et al., 2015b; Turk et al., 2012). Another example is rutin, a quercetin glycoside. Sugar moieties shield the hydroxyl group, which leads to higher stability against oxidation compared to their aglycons such as quercetin, as also observed in Figure 4.4. The extent of polyphenol degradation thus not only depends on the oxidation conditions but also on the phenolic structure (Georgé et al., 2011; Vallverdú-Queralt et al., 2014; Tomas et al., 2017). These individual differences in susceptibility underline the added value of weighing the retention efficiencies of the individual compounds by the magnitude of their absolute concentrations, thus avoiding that retention efficiencies of compounds present in small concentrations (e.g. quercetin) affect the interpretation. Weighed for the mass fraction of the different phenolic compounds, this % R becomes  $88 \% \pm 8 \%$ . This smaller value originates predominantly from the large share of chlorogenic acid.

In absolute concentration, it was found that PR contains about five times more phenolic compounds compared to JnT, in accordance with prior observations (Toor & Savage, 2005; Slimestad & Verheul, 2009). Physiologically, this can be explained by the role of the dermal tissues of plants in protection against oxidative damage caused by external stress conditions (Toor & Savage, 2005). This tissue location determines the matrix destination of the compounds as some compounds predominantly end up in the PR fraction and others do in the JnT fraction. Evaluating the tendency of each individual compound for migration to juice or press residue was performed by using the extraction efficiencies of JnT ( $\% E_{JnT}$ ) and PR ( $\% E_{PR}$ ). Whereas % R could be considered as an evaluation of the process used, % E can be interpreted as an evaluation of the end products. Both parameters are shown in Figure 4.4, where the sum of  $\% E_{PR}$  and  $\% E_{JnT}$  corresponds to the % R. It can be seen that the compounds retained predominantly in the press residue are flavonoids. In contrast, the phenolic acids (hydroxycinnamic acids and hydroxyphenylpropanoic acids) were mainly present in the juice. Accordingly, the mean juice extraction efficiencies (weighed for the mass fraction of the different phenolic compounds) for the

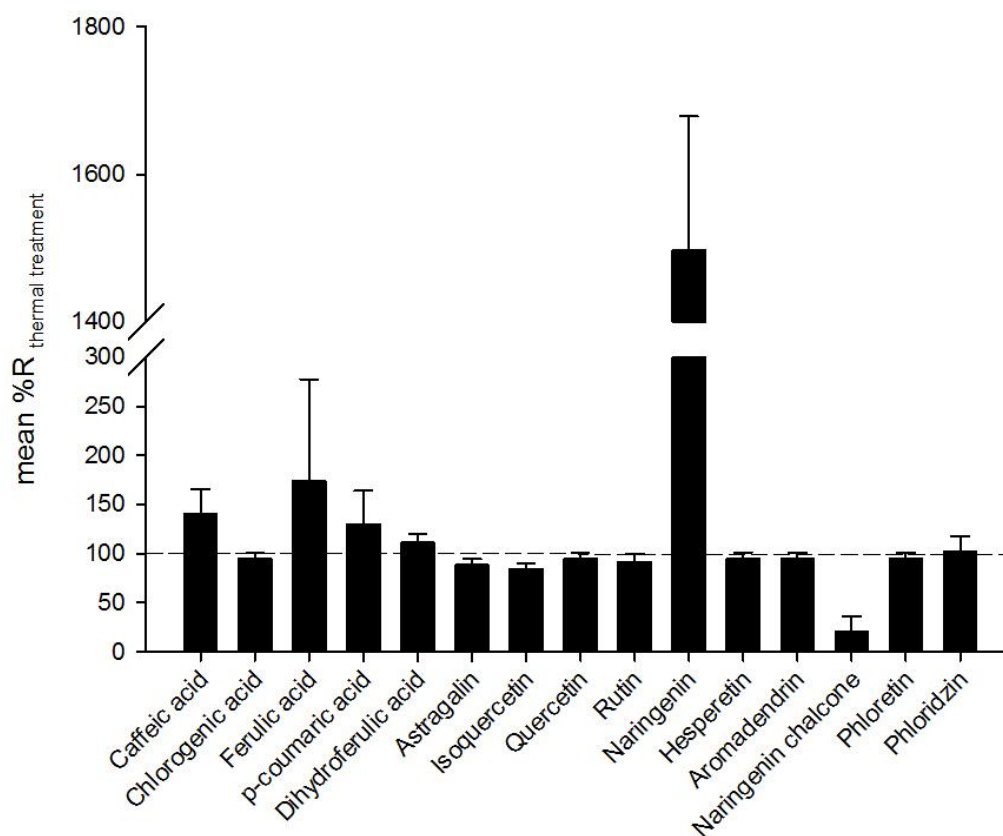
phenolic acids and flavonoids were  $76 \% \pm 12 \%$  and  $14 \% \pm 1 \%$ , respectively. These values are generally in accordance with the hydrophobic or hydrophilic nature of the compounds as expressed by their partitioning coefficient in octan-1-ol or olive oil and water ( $K_{part}$ ). Hydroxycinnamic acids are characterized by a lower  $K_{part}$ , implying a higher solubility in aqueous environment. Accordingly, they are more present in the juice fraction. Flavanones, and to a lesser extent the flavonols, are reported to have a higher  $K_{part}$ , which is reflected by a predominant presence in the press residue fraction (Choudhury et al., 1999; van Dijk et al., 2000). These findings are in accordance with current literature data (Kalogeropoulos et al., 2012; Kalt, 2005; Slimestad & Verheul, 2009; van der Sluis et al., 2002).

Although at first sight it appears that PR contains more phenolic compounds than JnT, the mean phenolic juice extraction efficiency was higher than the press residue extraction efficiency. This can be ascribed to the small mass fraction of press residue (12.2 %). The calculations have been weighed with the mass fraction thus even though PR has a higher absolute phenolic concentration, the larger mass of the juice fraction leads to a higher phenolic juice efficiency. Hence, although characterized by a smaller absolute concentration compared to PR, the resulting JnT fraction contains 49 % of the initial phenolics present in MT. We believe that the ratio of compounds in JnT and PR can be slightly tuned using other milling technologies in combination with other filter pore sizes, thereby reducing the particle size and allowing more particles in the juice.

#### **b/ Thermal processing**

For the thermal process, a mean phenolic retention efficiency of  $107 \% \pm 26 \%$  was achieved (excluding naringenin and naringenin chalcone) with values varying from 85 % to 175 % (Figure 4.5). Weighed for their mass fraction, this value becomes  $96 \% \pm 4 \%$ . In literature, conflicting results of thermal processing on the fate of phenolic compounds have been reported. Heating of tomato juice (92°C, 10 min followed by 100°C, 10 min) has been suggested to lead to disruption of cell walls, releasing oxidative and hydrolytic enzymes, destroying phenolic compounds in tomatoes (Georgé et al., 2011). Besides this enzymatic degradation, Nayak et al. (2015) report chemical oxidation of phenolic compounds to quinones and their polymers after processing at high temperatures (90 – 120 °C). Negative thermal effects on the fate of phenolic compounds have also been reported by Koh et al. (2012) and Vallverdú-Queralt et al. (2012; 2014). But if the temperature is high enough, the enzymatic activity can be deactivated, conserving phenolic compounds, as demonstrated by Dewanto et al. (2002) for thermal processing of mashed tomato at 88°C. Increases in phenolic content upon thermal tomato processing have also been reported (Capanoglu et al., 2008; Chanforan et al., 2012; Tomas et al., 2007). Gahler et al. (2003) found that phenolic compounds are released from the cellular constituents upon thermal treatment (121°C, 2 min followed by 80°C, 20 min) leading to higher concentrations measured, which has been confirmed by Vallverdú-Queralt et al. (2014). Kalt et al. (2000) also report a higher

extraction yield upon thermal treatment, which they linked to the increase of fruit skin permeability. Sometimes, the effect of increased phenolic compounds is attributed to dehydration, leading to concentration during processing (Nayak et al., 2015). The high mean thermal retention efficiency of phenolic compounds observed in this study indicates no overall degradation and thus an adequate thermal process that inactivates the enzymes but does not destroy heat labile compounds.



**Figure 4.5** Retention efficiencies (% R) of phenolic compounds throughout the thermal treatment. The vertical error bars represent standard deviations (n=2).

The individual retention efficiencies per compound are in accordance with previous studies, where the hydroxycinnamic acids (especially chlorogenic acid) and dihydrochalcones (especially phloridzin) have been identified as heat resistant phenolic compounds. The flavonols have also been reported to remain unaffected by thermal processing. Isoquercetin and rutin are identified as being more thermolabile, however (Chanforan et al., 2012; De Paepe et al., 2014; van der Sluis et al., 2005). For naringenin a strong enrichment was observed during the thermal process (1,497 %) whereas naringenin chalcone levels were depleted by heat (20 %). This can be ascribed to a cyclization of the unstable naringenin chalcone to the robust cyclic naringenin (Amarowicz et al., 2009). Correspondingly, Chanforan et al. (2012) found a 18-fold increase of naringenin upon filtration and thermal treatment of tomato. Similar results have been found by others (Capanoglu et al., 2008; 2010; Tomas et al., 2017). However, in this

study, the degradation of naringenin chalcone only took place during thermal treatment, proving the mild operating conditions of the spiral-filter press.

#### c/ Storage

Finally, during storage up to three months, the majority of the phenolic compounds showed no significant decrease (Table 4.3). The mean retention of phenolic compounds after storage was  $98 \% \pm 20 \%$ . Some compounds appeared to be more susceptible to degradation upon storage such as chlorogenic acid (-33 %) and naringenin (-25 %). Also the concentrations of naringenin chalcone and phloridzin decreased strongly after storage. Due to the large mass fraction of chlorogenic acid and its decrease upon storage, the weighed retention efficiency was  $72 \% \pm 4 \%$ . The conservation of the predominant part of the phenolic compounds during storage as observed here is in accordance with the results of storing apple and pear juice produced by using the spiral-filter press (De Paepe et al., 2015a; 2015b). In literature, decreases in phenolic compounds upon storage are often reported (Nayak et al., 2015; Odriozola-Serrano et al., 2009). The retention of phenolic compounds observed here during storage can be explained by a sufficient inactivation of oxidative enzymes and the limited presence of oxygen (Dewanto et al., 2002; Vallverdú-Queralt et al., 2012).

### 4.4.4 Carotenoids

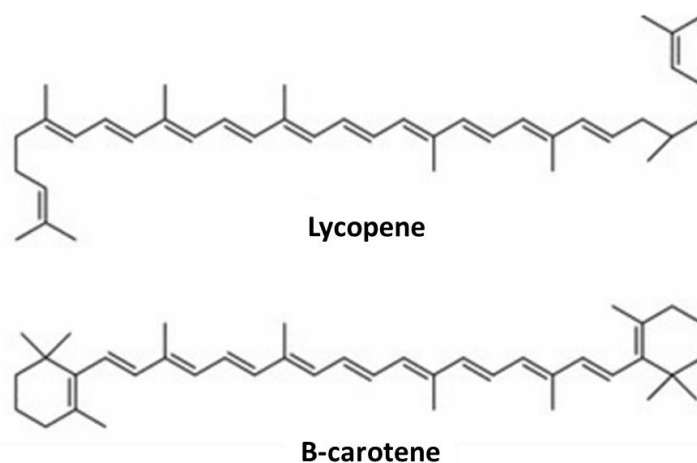
Carotenoids are highly unsaturated compounds with an extensive conjugated double-bound system, which makes them susceptible to oxidation and isomerization during processing and storage (Boon et al., 2010; Martínez-Hernández et al., 2016; Xianquan et al., 2008). These processes are influenced by the presence of light, oxygen and heat (Xianquan et al., 2008).

#### 4.4.4.1 Absolute concentrations

The concentrations of lycopene and  $\beta$ -carotene for the different samples are shown in Table 4.4 and their chemical structures in Figure 4.6.

**Table 4.4** Content of carotenoids ( $\mu\text{g}\cdot\text{g}^{-1}$  FW). The total sum also includes the *cis*-forms of lycopene (data not shown). Different letters indicate statistically significant differences ( $p < 0.05$ ). Letters a and b denote differences throughout processing (mashed tomato (MT), press residue (PR) and juice not thermally treated (JnT)). K and l are used for differences through thermal treatment (JnT and thermally treated juice (JT)) and x, y and z denote differences throughout storage (JT, thermally treated juice stored for 14 (JT14), 30 (JT30) and 90 (JT90) days).

Sample	MT	PR	JnT	JT	JT14	JT30	JT90
<i>Trans</i> -lycopene	$15 \pm 2^a$	$41 \pm 3^b$	$16 \pm 1^{b,k}$	$8.9 \pm 2.3^{l,x,y}$	$10 \pm 1^y$	$7.8 \pm 0.4^x$	$8.2 \pm 1.0^{x,y}$
B-carotene	$1.9 \pm 0.2^a$	$4.5 \pm 0.2^a$	$1.8 \pm 0.01^{a,l}$	$0.90 \pm 0.33^{k,x}$	$0.89 \pm 0.10^x$	$0.87 \pm 0.05^x$	$0.98 \pm 0.18^x$
Sum	$18 \pm 2$	$46 \pm 3$	$19 \pm 1$	$10 \pm 2$	$11 \pm 1$	$9.0 \pm 0.4$	$9.2 \pm 1.0$



**Figure 4.6** Chemical structures of lycopene and  $\beta$ -carotene. Figure based on Gülçin (2012).

The sum of the absolute amounts of the measured carotenoids was the highest in the PR fraction ( $4.6 \pm 0.3 \text{ mg}\cdot 100 \text{ g}^{-1} \text{ FW}$ ), followed by JnT ( $1.9 \pm 0.1 \text{ mg}\cdot 100 \text{ g}^{-1} \text{ FW}$ ) and MT ( $1.8 \pm 0.2 \text{ mg}\cdot 100 \text{ g}^{-1} \text{ FW}$ ). Approximately 89 % of the measured carotenoid content consisted of lycopene, whereby 96 % was *trans*-lycopene. The concentrations of the *cis*-lycopene isomers are not presented but showed the same trend as for *trans*-lycopene, with values in MT of  $0.15 \pm 0.01$ ,  $0.24 \pm 0.002$  and  $0.30 \pm 0.03 \mu\text{g}\cdot \text{g}^{-1} \text{ FW}$  for 15-*cis*, 13-*cis* and 9-*cis* lycopene, respectively.

The absolute carotenoid content of tomatoes can vary widely. The reported concentrations of lycopene in raw tomato range from 2.5 mg to 670 mg  $\cdot 100 \text{ g}^{-1} \text{ FW}$  (Martínez-Hernández et al. 2016). The  $\beta$ -carotene content has been reported to vary from 0.1 mg  $\cdot 100 \text{ g}^{-1} \text{ FW}$  (Daood et al., 2014) to 1 mg  $\cdot 100 \text{ g}^{-1} \text{ FW}$  (Georgé et al., 2011). Both the lycopene and the  $\beta$ -carotene concentrations in MT reported in this study ( $1.5 \text{ mg}\cdot 100 \text{ g}^{-1} \text{ FW}$  and  $0.19 \text{ mg}\cdot 100 \text{ g}^{-1} \text{ FW}$ , respectively) are thus rather low compared to the concentration ranges reported in literature. Various factors such as genetics and maturity, environmental factors and postharvest handling are known to markedly affect the biosynthesis of carotenoids, which may explain these low concentrations (Abushita et al., 2000; Kalt, 2005). Typically, carotenoids are relatively stable during processing, however the degree of degradation varies with the matrix constitution, i.e. the presence of vitamin C, oil, water and other phytochemicals. Especially water has been reported to have a protective effect on the carotenoid degradation. Therefore, dehydrated tomato products, such as commercial lycopene standards are sensitive to lycopene isomerization and degradation which can complicate their quantification by chemical analysis. Besides the chemical standards, also the tomato samples have been freeze-dried in this experiment, which could have resulted in a decrease of carotenoid concentration. Georgé et al. (2011) showed that lyophilization could lead to a decrease of 14 % and 47 % in  $\beta$ -carotene and lycopene, respectively due to increased porosity and thus greater exposure to oxygen. It has also been

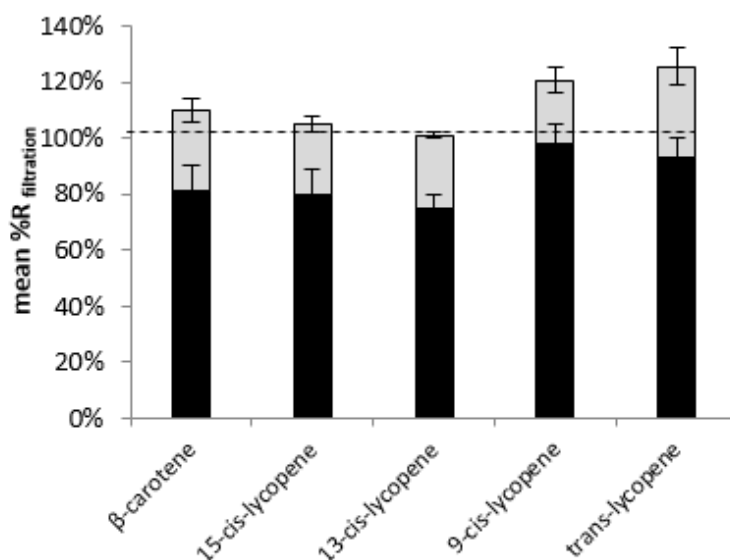


stated that the extractability of carotenoids is reduced in freeze-dried material (Martínez-Hernández et al., 2016). Interestingly, the total polyphenol concentration was reported to be unaffected by lyophilization (Georgé et al., 2011). These absolute concentrations are only used to benchmark the obtained results with the reported literature data, however, as the primary goal was to compare relative concentration changes within the process.

#### 4.4.4.2 Retention efficiencies

##### a/ Spiral-filter processing

The retention efficiencies of the measured carotenoids in the filtration process are shown as the sum of the stacked bars in Figure 4.7. A mean overall carotenoid retention efficiency of  $112\% \pm 10\%$  is obtained with compound specific values ranging from  $101\%$  to  $125\%$ . Weighed for their mass fraction, this value was  $122\% \pm 15\%$ . Despite their susceptibility to degradation, the carotenoids were thus conserved throughout processing with the spiral-filter press. In conventional tomato processing, the straining process is often associated with a reduction of carotenoids due to oxidation. The high rotation speeds in the straining equipment generate large amounts of dissolved air in the tomato juice that can quickly destroy substantial amounts of lycopene. In addition, the presence of light and use of fine metal screens are reported to promote lycopene oxidation (Shi & Le Maguer, 2000).



**Figure 4.7** Retention efficiencies of carotenoids through the filtration process. Light grey columns represent the extraction efficiency in the press residue, whereas black columns represent the extraction efficiency in the juice. The vertical error bars represent standard deviations ( $n=2$ ). The sum of both extraction efficiencies is the retention efficiency of the compound. The retention efficiencies have only been calculated for the compounds present in concentrations larger than the quantification limit in the mashed tomato fraction.

The conservation of all carotenoids as observed in this study can thus be attributed to the fast processing of the spiral-filter press in a low-oxygen atmosphere, which diminishes carotenoid

oxidation and conserves the carotenoids in their original concentration and form. This is the first time that the fate of the carotenoids has been described throughout processing with the spiral-filter press.

Similar to the phenolic compounds, the carotenoids measured (lycopene and  $\beta$ -carotene) were predominantly present in the press residue. In absolute amounts, the sum of all carotenoids was about 2.5 times higher in the PR compared to the JnT fraction. This illustrates that the most lycopene is attached to the insoluble fiber portion of tomatoes, as confirmed by Toor & Savage (2005). The extraction efficiencies make it possible to evaluate the tendency of each individual compound to migrate into the juice or the press residue as shown by the black and grey bars, respectively, in Figure 4.7. In parallel to the phenolic compounds, it can be concluded that the juice extraction efficiencies were higher than those of the press residue due to the larger mass fraction of the juice, despite the larger absolute concentrations in the press residue. The mean juice extraction efficiency of the measured carotenoids was  $85 \% \pm 10 \%$ , which became  $91 \% \pm 11 \%$  when weighed for the mass fractions of the individual compounds.

#### **b/ Thermal processing**

During thermal treatment, the carotenoids underwent an average decrease in concentration of approximately  $46 \% \pm 13 \%$ , with individual values ranging from 32 % to 50 % (data not shown). In contrast to the minor effects on phenolic compounds, thermal treatment thus led to a significant decrease in carotenoid concentration. In literature, conflicting results concerning the effect of thermal treatment on total carotenoid concentration have been described (Seybold et al., 2004; Xianquan et al., 2008). Decrease (Capanoglu et al., 2008; Koh et al., 2012; Sharma & Le Maguer, 1996; Shi et al., 2003; Takeoka et al., 2001), increase (Abushita et al., 2000; Dewanto et al., 2002) and conservation (Arjmandi et al., 2017; Georgé et al., 2011) of carotenoids upon thermal processing have been reported. Here, the decrease in all-*trans*-lycopene was not accompanied by an increase in *cis*-lycopene concentration which suggests that degradation through oxidation of lycopene was the predominant mechanism (in contrast to isomerization). This is in accordance with the findings of Shi et al. (2002) who reported lycopene degradation to be the main mechanism of lycopene loss at temperatures above 100 °C.

The thermal treatment that is used to inactivate microorganisms and enzymes and extend the shelf life of juice products thus had adverse effects on the nutritive quality of tomato. Possible improvements to the proposed setup could consist of different temperature and duration settings of the thermal treatment, as both parameters have been shown to affect the carotenoid concentration (Lin & Chen, 2005a; Odriozola-Serrano et al., 2009; Shi & Le Maguer, 2000; Xianquan et al., 2008). Another possibility could be to use other treatments such as radiation treatments, electrical

treatments, ultrasound treatments, high hydrostatic pressure treatments and combinations thereof instead of traditional thermal processing (Barrett & Lloyd, 2012; Jiménez-Sánchez et al., 2017a; 2017b; Pereira & Vicente, 2010; Turk et al., 2012). These novel, alternative techniques have been widely studied for their effects on the bioactive content and quality attributes of the processed products (Barrett & Lloyd, 2012; Jiménez-Sánchez et al., 2017b). The combination of the spiral-filter press with these novel preservation techniques opens new perspectives and should be the subject of further research.

#### **c/ Storage**

After thermal treatment, a three-month storage period showed no additional consistent decrease in carotenoids, which can be attributed to the dark storage of tomato juices with minimal oxygen presence (Xianquan et al., 2008) (Table 4.4). Analogous to the phenolic compounds, decreases in carotenoids by oxidation upon storage are often reported, however (Jayathunge et al., 2015; Lin & Chen, 2005b; Odriozola-Serrano et al., 2009; Sharma & Le Maguer, 1996; Xianquan et al., 2008).

## 4.5 Conclusion

These results clearly show that the spiral-filter press led to a conservation in AA, phenolic compounds and carotenoids in the resulting juice and press residue fractions. The thermal treatment conserved the phenolic compounds and the AA content, but resulted in a significant decrease in carotenoid content. Three months of storage led to a significant decrease in AA, while the predominant part of the other compounds remained conserved. These results indicate a minimal process impact of the novel spiral-filter press on tomato, which can be attributed to fast processing in a low-oxygen atmosphere. Earlier studies on apple and pear processed with the spiral-filter press support these findings, making it a promising technology for other fruit and vegetable matrices as well. The results also show that the process conditions of the subsequent thermal stabilization step should be adjusted or that the thermal treatment should be substituted by another technology with a lower impact on the phytochemical content.

One should however keep in mind that several factors can influence the concentration and/or behavior of phytochemicals under different conditions. Factors such as the type of fruit and vegetable, ripening stage and firmness as well as processing parameters (e.g. temperature, treatment duration) and the presence of other compounds can alter the behavior of phenolic compounds and carotenoids upon thermal treatment (Capanoglu et al., 2010; Nayak et al., 2015; Tomas et al., 2017; Vallverdú-Queralt et al., 2014; Xianquan et al., 2008). For example, for carotenoids a cultivar-dependent thermal effect was shown by Seybold et al. (2004) where a decrease of lycopene and  $\beta$ -carotene was observed when heating Dutch tomatoes. The same experiment, when performed with Spanish tomatoes, led to a significant rise in lycopene during the first 30 minutes. It could be hypothesized that this difference in behavior is related to the genetic differences between fresh market tomatoes and processing tomatoes, assuming that the Dutch tomatoes were predominantly fresh market tomatoes and Spanish tomatoes predominantly processing tomatoes. Moreover, sample treatment can influence the total measured concentration, as shown for freeze-dried samples (Georgé et al., 2011; Martínez-Hernández et al., 2016). Another factor that can lead to differences and discrepancies between studies is the method of analysis. An example is the instability of commercial standards such as lycopene used in chemical analysis. In the case of phenolic compounds, the total phenolic content is often measured spectrophotometrically using the Folin-Ciocalteu method or is deduced from the antioxidative capacity. Both methods are less specific compared to methods such as LC-MS analysis and therefore do not always correctly reflect the actual changes induced by the processing (Capanoglu et al., 2008; Georgé et al., 2011; Martínez-Valverde et al., 2002; Tomas et al., 2017; Vallverdú-Queralt et al., 2012).

All of these influencing factors make sound conclusions about the process impact of a certain technology and its comparison with other technologies challenging. This study did not aim to compare or rate the different unit processes with other processes, rather it aimed to understand and evaluate the observed changes throughout the process line relative to the raw feedstock. A further evaluation of the proposed process could consist of comparing the different unit processes with other technologies and investigating the fate of different phytochemicals. To minimize the impact of the factors mentioned above, it is important to account for aspects such as using the same feedstock tomatoes, performing parallel experiments on the same scale and using the same sample treatment in combination with correct measurement methods.



## **Chapter 5**

Detailed profiling of bioactive compounds in Belgian endive (*Cichorium intybus* L. var. *foliosum*): determination of sesquiterpene lactones, phenolic compounds, antioxidative capacity and elemental composition





## **Chapter 5: Detailed profiling of bioactive compounds in Belgian endive (*Cichorium intybus* L. var. *foliosum*): determination of sesquiterpene lactones, phenolic compounds, antioxidative capacity and elemental composition**

### **Redrafted from**

Kips, L., Annaratone, C., Hertog, M., Van Ceulebroeck, C., Van Poucke, C., Van Pamel, E., Raes, K. & Van Droogenbroeck, B. (2017). Detailed profiling of bioactive compounds in Belgian endive (*Cichorium intybus* L. var. *foliosum*). *Manuscript submitted for publication in Journal of agricultural and food chemistry*.

### **5.1 Abstract**

Bitter Belgian endive chicons, consumed as leafy vegetables, are grown by ‘forcing’ roots. In Belgium, yearly 36,000 tonnes of these forced roots are byproducts and used to feed cattle. However, they could have a higher added value in food, pharma or as biocide. This chapter investigates in detail the sesquiterpene lactone content, the phenolic content, the elemental composition and the antioxidative capacity of forced roots. Their constitution was compared to industrial chicory, different cultivars of fresh Belgian endive roots, chicons and non-forced roots, both stored and non-stored. Results showed that for the sesquiterpene lactones, the lactucin forms were significantly enriched in the forced roots. Also their chlorogenic acid content (main phenolic compound) and antioxidative capacity were higher compared to non-forced roots. With very little information available in literature, this knowledge is crucial for further evaluation of the valorization opportunities for forced Belgian endive roots towards bioactive products.

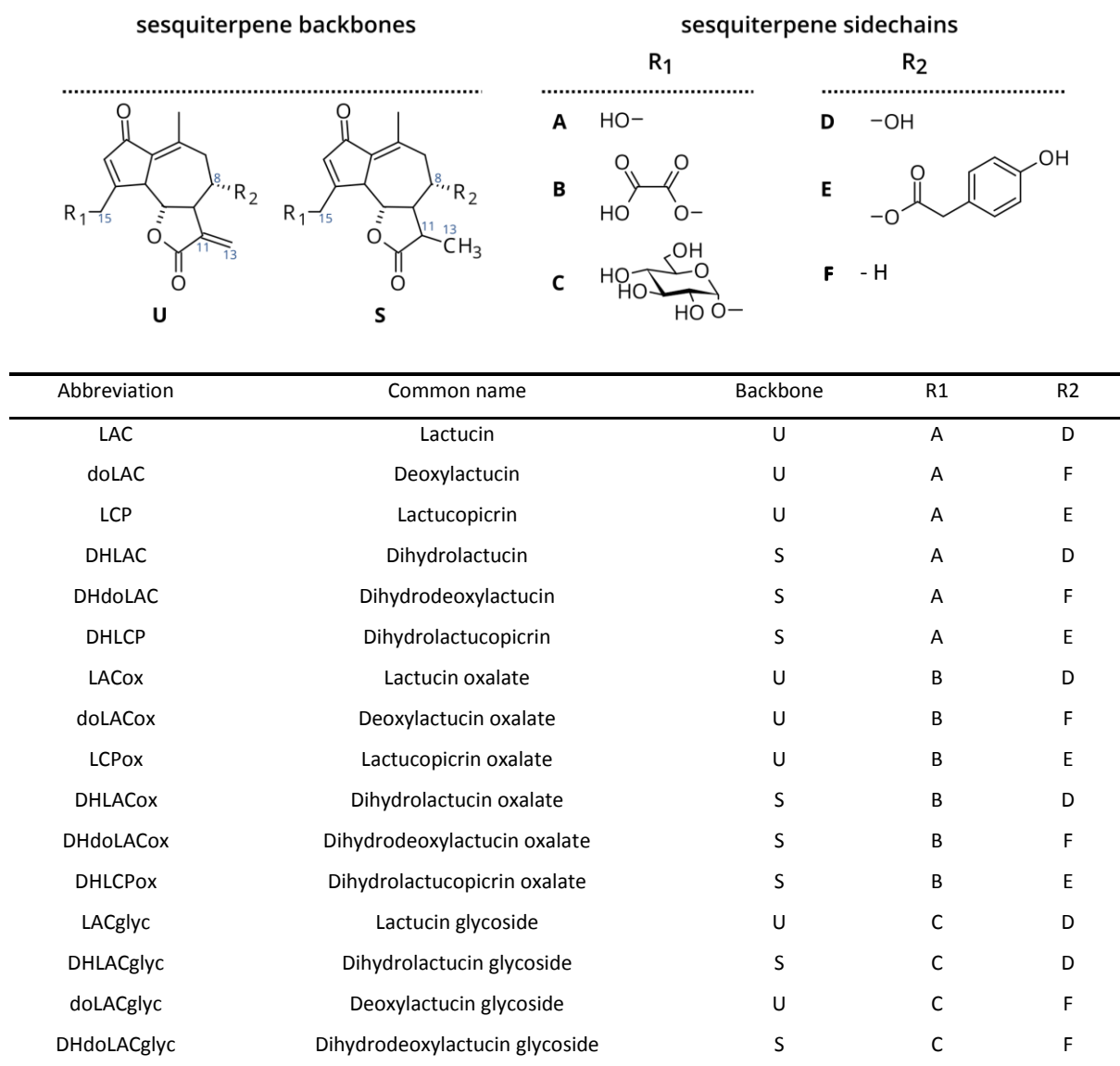
## 5.2 Introduction

In the fruit and vegetable processing sector, 40 % to 50 % of the biomass is lost during the production, handling, storage, processing, distribution and consumption stages, leading to huge losses of potentially health-beneficial and high-value biomass (FAO, 2011). Consequently, the European Union legislation supports the use of these byproducts in the framework of a circular economy (European Commission, 2015c). Such valorization can be achieved for example by the use of high-value compounds such as proteins, dietary fibers, flavor compounds and phytochemicals present in vegetal byproducts that have the potential to be used as functional food ingredients or pharmaceutical ingredients (Baiano, 2014).

The white and bitter tasting Belgian endive chicon (*Cichorium intybus* L. var. *foliosum*) is an important Belgian vegetable that belongs to the *Asteraceae* family (Wulfkuehler et al., 2013) with an annual Belgian production volume of approximately 40,000 tonnes (Department of Agriculture and Fisheries, 2014). These chicons are characterized by a bitter taste caused by sesquiterpene lactones (SLs) of the guaianolide family (de Kraker, 2002). These SLs are terpenoid compounds prevalent in the *Asteraceae* and are known to exert a variety of biological and pharmacological activities (Bischoff et al., 2004; Chadwick et al., 2013; Chaturvedi, 2011) (Figure 5.1).

To produce chicons, Belgian endive roots are forced in the absence of light. Non-forced roots are harvested on the field and stored cold (-2 °C) for up to several months, depending on cultivar. Subsequently, they are forced to produce edible chicons, which are compact heads of white to pale yellow leaves sitting on suppressed floral stems. The forcing process can take place in two different ways: (i) hydroculture or (ii) soil-based production (about 5 %). The 36,000 tonnes of forced roots, remaining after forcing of the chicons are not used for human consumption and are currently predominantly used as feed for local cattle (Department of Agriculture and Fisheries, 2014). However, as these roots are also bitter, they are expected to be major sources of SLs and could thus be of great value in various sectors such as food, pharma or biocides (Chadwick et al., 2013; Ghantous et al., 2010).

To date, rather limited research has been performed on the composition of Belgian endive chicons and roots. Whereas the genus *Cichorium* in general has been the subject of some research, a general misclassification leads to results that are difficult to allocate to specific varieties and cultivars. Several common names are circulating such as Belgian endive, French endive, coffee chicory, succory, witloof, chicon, industrial chicory and root chicory. Those terms are freely used as synonyms for various species of the *Cichorium* genus, leading to a confusing terminology, which is rooted in the presence of a great amount of varieties and cultivars (Innocenti et al., 2005; Lucchin et al., 2008). Generally, the species



**Figure 5.1** The 16 measured sesquiterpene lactone structures, differing in type of backbone and sidechain.

*Cichorium intybus* species can be split into three main cultivar groups: (i) the root chicory known as *Cichorium intybus* L. var. *sativum*, also called industrial chicory as these roots are used for industrial inulin extraction and coffee substitutes, (ii) the witloof chicory known as *Cichorium intybus* L. var. *foliosum*, and (iii) the leafy chicory, sub-classified into Sugarloaf (var. *porphyreum*), Radicchio (var. *latifolium*) and Catalogne (var. *sylvestre*) (Barcaccia et al., 2016). Despite this classification, often only the general term *Cichorium intybus* L. is mentioned in literature, hence it is difficult to gain insight in the composition of specific varieties. Another aspect that impedes valorization of roots, is the scarce analysis of the roots of the *foliosum* variety, as leaves have been the predominant focus, probably due to their commercial use as vegetables (Bahri et al., 2012; Carazzone et al., 2013; Heimler et al., 2009; Innocenti et al., 2005; Peters & van Amerongen, 1996; Sinkovič et al., 2014; Sinkovič et al., 2015). Additionally, for a long time it has been thought that the free, nonconjugated forms were the

predominant SLs, hence most analytical methods only focused on these free forms (Annaratone et al., 2016). However, by the end of the 20<sup>th</sup> century, the glycosides were discovered and recently Sessa et al. (2000) discovered that SLs in the latex

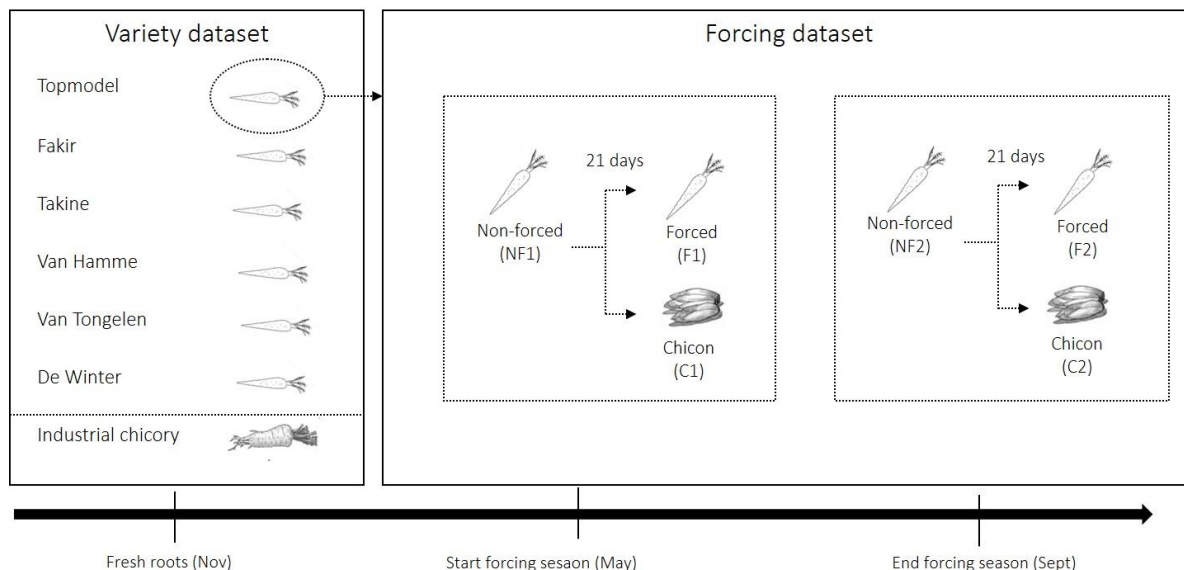
The objective of this chapter was in the first place to investigate the composition of the forced Belgian endive roots as a basis for their further valorization. In order to gain more insight in the effect of variety and cultivar on the one hand and the distribution between forced roots and the commercially valuable chicons on the other hand, various additional samples were taken, enabling the comparison of variety and cultivar (industrial chicory versus six Belgian endive cultivars), matrix (root vs. chicon), forcing (non-forced vs. forced root) and storage of the non-forced roots (four-month storage). These different samples were subjected to an investigation of (i) the SLs, both free and bound forms, (ii) the phenolic compounds, (iii) the antioxidative capacity and (iv) the elemental composition. The latter was investigated from a nutritional and food safety point of view to obtain a broad compositional fingerprint of the scarcely investigated forced Belgian endive roots. However, to allow for possible future valorization pathways, other than food, all elements that were present above the limit of quantification were reported.

With these data, a contribution was made to the composition of Belgian endive and its influencing factors, which has never been reported to the best of our knowledge. These insights can be used to further exploit the potential of forced Belgian endive roots.

## 5.3 Materials and methods

### 5.3.1 Sampling

The data in this article were organized in two different groups, the variety dataset and the forcing dataset (Figure 5.2). The first dataset contained freshly harvested Topmodel (Hoquet, hybrid) roots which were collected in November 2015 (Affligem, Belgium). In parallel, the roots of five other Belgian endive cultivars were sampled from another location (November 2015; Herent, Belgium): Van Hamme (landrace), Van Tongelen (landrace), De Winter (landrace), Takine (Vilmorin, hybrid) and Fakir (Hoquet, hybrid). The latter two, like Topmodel, can be forced both in soil as hydroponically, whereas Van Hamme, Van Tongelen and De Winter are soil-forcing varieties. Finally, roots of the closely related chicory crop (*Cichorium intybus* L. var. *sativum*) were added to the dataset (November 2015; Chic 1331, Melle, Belgium). These will be denoted as industrial chicory, in order to make a clear distinction with the Belgian endive roots. In this variety dataset, the effects of variety and cultivar were thus investigated. The Topmodel variety was followed further throughout the forcing process, which was included in the forcing dataset. These Topmodel roots were harvested in November 2014 (Affligem, Belgium) and further stored at -2 °C. Two samples were taken from these stored, non-forced roots: one after 6 months (non-forced - NF1) and one after 10 months (non-forced - NF2) (Figure 5.2).



**Figure 5.2** Sampling scheme for the Belgian endive profiling, split into two subsets which were analyzed separately i.e. the variety dataset (left panel) and the forcing dataset (right panel). In the variety dataset, non-forced roots from different variety and cultivar were sampled in November. In the forcing dataset, both roots (non-forced and forced) and chicons were sampled at the start (1 – not stored - May) and at the end (2 – stored - September) of the forcing season.

Subsequently they were forced in hydroculture during 21 days to produce chicons (C1, C2) and forced roots (forced - F1, forced - F2). This forcing dataset thus investigates the effects of storage (1 vs. 2), matrix (root vs. chicon) and forcing (NF vs. F).

From each cultivar, about 20 roots were washed under cold tap water and cut into 1 cm<sup>3</sup> cubes (CL 50 Ultra, Robot coupe, Mont-Sainte-Geneviève, Belgium). An analogous procedure was used to prepare the chicons. These samples were immediately freeze-dried (Epsilon 2-10 D LSC, Martin Christ, Osterode am Harz, Germany) and milled to a fine powder (Ultra centrifugal mill ZM 200, Retsch, Haan, Germany). The resulting dry powders were stored at -80 °C in plastic bottles (Nalgene™, Thermo Fisher Scientific, Waltham, USA) under nitrogen atmosphere until analysis.

### 5.3.2 Characterization

#### 5.3.2.1 Reagentia

The following analytical standards were purchased from Sigma (Diegem, Belgium): 3,4,5-trimethoxycinnamic acid, 4-p-hydroxyphenyl acetic acid, apigenin, avicularin, caffeic acid, chicoric acid, chlorogenic acid, cyanidin chloride, daidzein, epicatechin, dihydrocaffeic acid, dihydroferulic acid, ferulic acid, gallic acid, gentisic acid, hesperetin, hesperidin, kaempferol, luteolin, miquelianin, naringenin, o-coumaric acid, p-coumaric acid, phloretin, propyl gallate, quercetin, salicylic acid, santonin, sinapinic acid and vanillic acid. Aromadendrin, catechin, cynaroside, phloridzin, procyanidin B2, protocatechuic acid, taxifolin and naringenin chalcone were purchased from Phytolab GmbH & Co (Vestenbergsgreuth, Germany). Apigenin, astragalin, galangin, naringin, quercitrin, rutin, lactucin, 11 $\beta$ ,13-dihydrolactucin, lactucopicrin and 11 $\beta$ ,13-dihydrolactucopicrin were purchased from Extrasynthese (Genay, France). Isoquercitrin and isorhamnetin were provided by Carl Roth GmbH (Karsruhe, Germany).

#### 5.3.2.2 Determination of the sesquiterpene lactones

SLs were extracted from the freeze-dried samples in triplicate. Both extraction and separation were based on the method developed by Annaratone et al. (2016) with minor modifications. Briefly, extraction of 50 mg powdered sample was performed using 1.480 mL H<sub>2</sub>O + 0.1 % formic acid. After addition of the internal standard santonin (74  $\mu$ L, 10 ppm), the samples were shaken for 15 minutes at 30 °C at a speed of 1,300 rpm (Eppendorf thermomix comfort, Eppendorf, Rotselaar, Belgium) and centrifuged (15 min, 20817 g). The supernatans was filtered over a PVDF filter (0.22  $\mu$ M, Millipore, Overijse, Belgium) and transferred to a vial for analysis. In order to separate the SLs present, ultra-high performance liquid chromatography (UHPLC) by means of an Acquity™ UPLC (Waters, Manchester, UK) was performed. A BEH C<sub>18</sub> column (150 mm x 2.1 mm, 1.7  $\mu$ m) was used for chromatographic separation (Waters). The mobile phase consisted of H<sub>2</sub>O + 0.1 % formic acid (solvent A) and ACN + 0.1 % formic acid (solvent B). The gradient was initiated at 5 % B for 5 min, then linearly increased from 5 % to 53 % B in 20 min, held constant at 53 % for 1 min and finally set at 100 % B for 3 min. Afterwards,

the initial conditions of 5 % B were re-equilibrated for 4 min prior to the next injection. The column temperature was set at 40 °C and the flow rate was 0.350 mL min<sup>-1</sup>. The injection volume was 5 µL. Detection of the SLs was performed by means of a Synapt G2-S (Waters) high resolution mass spectrometer (HRMS). The MS detector was operated in positive electrospray (ESI+) mode with a capillary potential of 1.5 kV. Source and desolvation temperatures were 120 °C and 500 °C, respectively. Gas flows were 800 L.h<sup>-1</sup> and 20 L.h<sup>-1</sup> for desolvation and cone gas, respectively. Data were acquired in MS<sup>E</sup> mode with the collision energy at 4 eV in the low energy mode to determine the accurate mass, fragmentation spectra were obtained in the high energy mode using a collision energy ramp (8 – 40 eV). Four compounds were quantified with reference standards: lactucin (LAC), lactucopicrin (LCP), dihydrolactucin (DHLAC) and dihydrolactucopicrin (DHLCP). As no other standards were available, the other compounds (doLAC: 8-deoxylactucin; doLACglyc: 8-deoxylactucin glycoside; doLACox: 8-deoxylactucin oxalate; DHdoLAC: dihydro-8-deoxylactucin; DHdoLACglyc: dihydrodeoxylactucin glycoside; DHdoLACox: dihydro-8-deoxylactucin oxalate; DHLACglyc: dihydrolactucin glycoside; DHLACox: dihydrolactucin oxalate; DHLCPox: dihydrolactucopicrin oxalate; LACglyc: lactucin glycoside; LACox: lactucin oxalate; LCPox: lactucopicrin oxalate) were identified based on the accurate mass and fragmentation pattern and reported as relative peak areas (area compound/area internal standard). Data recording was achieved with MassLynx<sup>TM</sup> (v.4.1) while the integration was performed with TargetLynx<sup>TM</sup> (v. 4.1) (Waters).

### 5.3.2.3 Determination of the phenolic profile

Phenolic compounds were determined following the procedure described in Chapter 4 (section 4.3.2.3). Due to the presence of high amounts of chlorogenic acid, quantification of this compound was performed using 1/100 dilution (MeOH:water, 60/40, v/v) of the extracts and on an absolute basis (i.e. without using the signal of the internal standard as this was too diluted).

### 5.3.2.4 Determination of the antioxidative capacity

In order to take into account the multiple antioxidant structures and reaction mechanisms, two different assays were used: the oxygen radical absorbance capacity (ORAC) assay and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Prior et al., 2005). The ORAC-assay is based on a hydrogen atom transfer (HAT) mechanism, whereas the DPPH assay is characterized by a single electron transfer (SET) mechanism. Both analyses were performed as described by Bernaert et al. (2013). Analyses were performed in triplicate (n=3). Results were expressed in µmoles of Trolox equivalents per gram of dry weight (µmol TE.g<sup>-1</sup> DW).

### 5.3.2.5 Determination of the elemental composition

The elemental composition was determined using XRF (X-Ray fluorescence) spectrometry (Epsilon3XLE, PANalytical, Brussels, Belgium). The dry powders were placed in a cup (P1 with 4µm prolene film), manually compressed and subsequently analyzed in triplicate (n=3). Mineral elements detected in concentrations above the quantification limit were quantified: Na, Mg, Si, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Br, Rb and Sr. Data analysis was performed with PANalytical Omnia Solution precalibrated for an Epsilon3XLE spectrophotometer.

### 5.3.2.6 Statistical analysis

The data were split into two subsets: the variety dataset and the forcing dataset. Statistical analysis was carried out using SPSS Statistics 22. Treatments were compared using one-way analysis of variance (ANOVA) followed by a Scheffé post-hoc test. The dependent variables were the relative peak areas of the 16 SLs obtained by HRMS analysis, the concentrations of the four quantified SLs (LAC, DHLAC, LCP, DHLCP), the most abundant phenolic compounds (chlorogenic acid, caffeic acid, rutin, quercetin-3-O-glucuronide & isoquercetin), the antioxidative capacities (ORAC, DPPH) and the concentration of 15 mineral compounds. A significance level of  $p < 0.05$  was used. Sigmaplot 12.5 was used to visualize the data.

### 5.3.2.7 Data processing and visualization of the sesquiterpene lactones

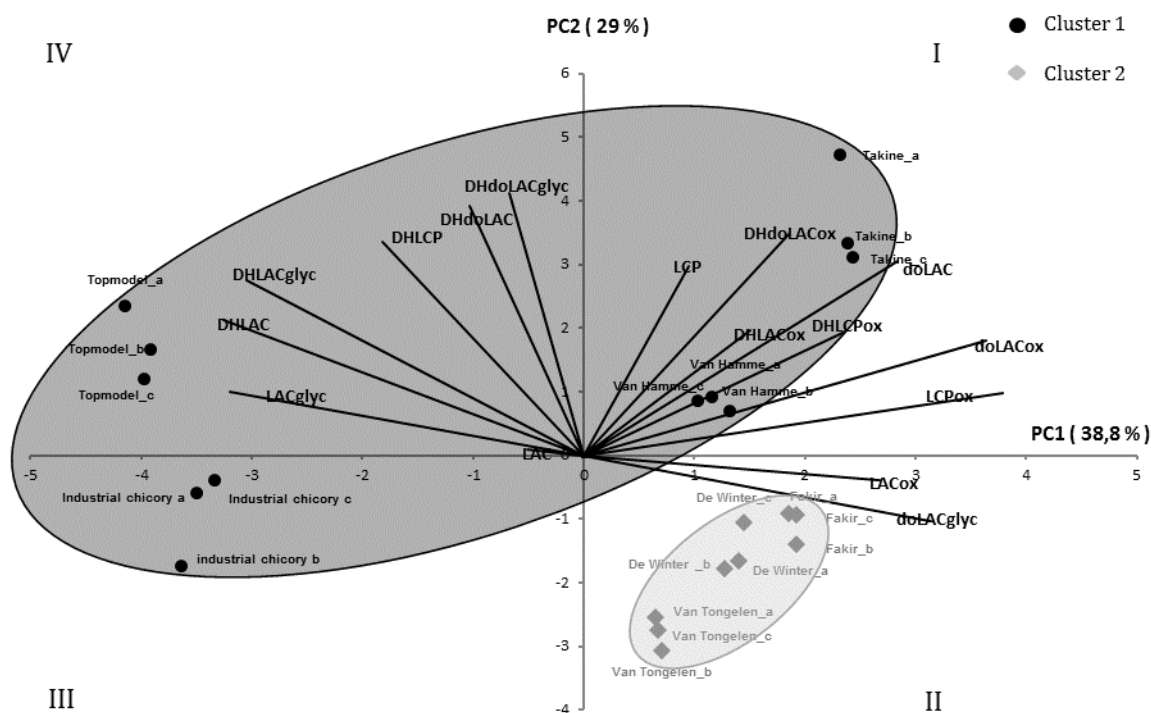
The relative peak areas of the SLs obtained by HRMS analysis were used for data visualization. The variety dataset contained the seven freshly harvested roots, in triplicate, as rows and the 16 SLs as columns (21 x 16 matrix). The second dataset contained the six Topmodel samples, in triplicate, as rows and the 16 SLs as columns (18 x 16 matrix). In the first subset, variation was mainly induced by variety and cultivar. In the second subset, variation arose from matrix, forcing treatment and storage. In both datasets the technical variance or the sum of the analytical variance (errors arising from differences during instrumental analysis) and sample work-up variance (errors arising from differences during sample work-up) were estimated from triplicate measurements. Before performing an exploratory data analysis, the data were subjected to a power transformation in order to remove the heteroscedastic error structure in the dataset, followed by a column-wise autoscaling. Subsequently, the principal component analysis (PCA) was performed for these transformed and scaled datasets. Finally, in order to discover clusters in the data, k-means clustering was performed. The number of clusters was determined using the 'Elbow' method (De Paepe et al., 2015c).



## 5.4 Results

### 5.4.1 Sesquiterpene lactone profile

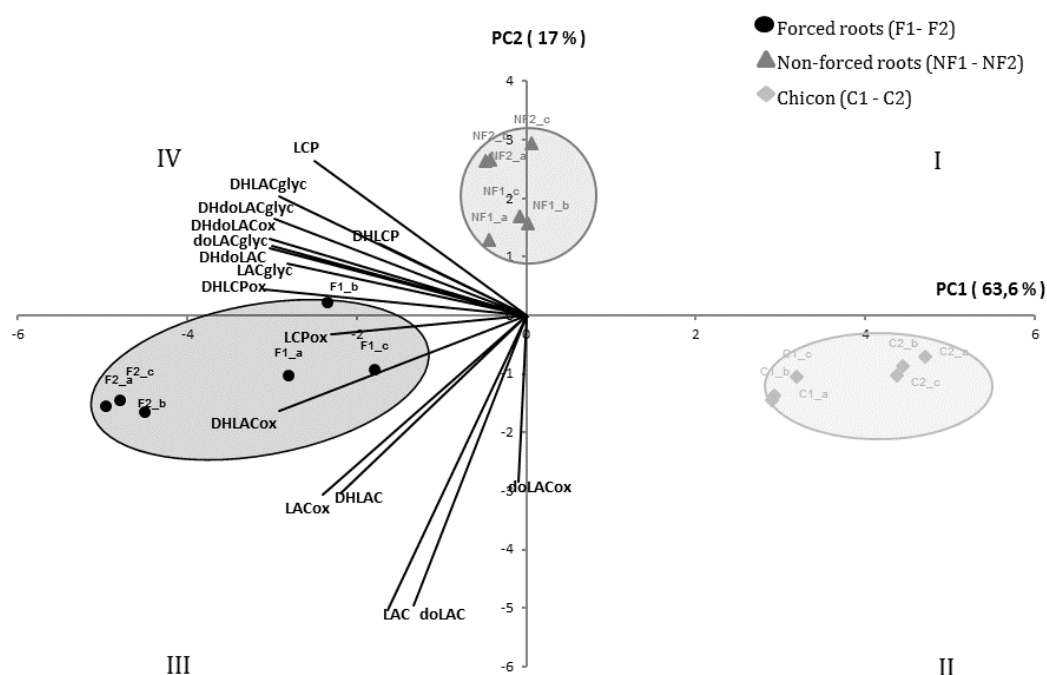
The results of the SL-analysis of the variety dataset are shown in Figure 5.3. The fresh roots of the different Belgian endive varieties and industrial chicory clustered together in two groups based on their SL-profile. In general, compounds in the fourth and second quadrant that were aligned close to the y-axis and x-axis, could be linked to the variation between cluster 1 and 2, respectively, which was illustrated by significantly higher levels of DHdoLACglyc, DHdoLAC, DHLCP and DHLACglyc in most of the cultivars of cluster 1 compared to cluster 2 (Table A1 in Appendix). LACox and doLACglyc were on average significantly more present in cluster 2 (De Winter, Fakir and Van Tongelen), the latter compound in very low levels. Within cluster 1, the compounds of the fourth quadrant that were closely located to the x-axis were significantly more present in industrial chicory and Topmodel (LAC glyc, DHLAC and DHLACglyc) whereas those in the first and second quadrant were more characteristic for the varieties Takine and Van Hamme (DHdoLACox, doLAC, DHLCPox, doLAXox, LCPox and doLACglyc).



**Figure 5.3** K-means clustered PCA-plot of the 16 measured sesquiterpene lactones in the variety subset which was power transformed and autoscaled. The total variance captured by the first two PCs is 67.8 %. The loadings were multiplied by factor 10 for visual purposes.

The more vertical the compound, the less variation it captured within cluster 1. LAC did not contribute much to the observed variation. From all measured SLs, the Belgian endive and industrial chicory roots predominantly contained oxalates. In industrial chicory however, these oxalates were less present, leading to a lower total SL-level in industrial chicory compared to Belgian endive roots.

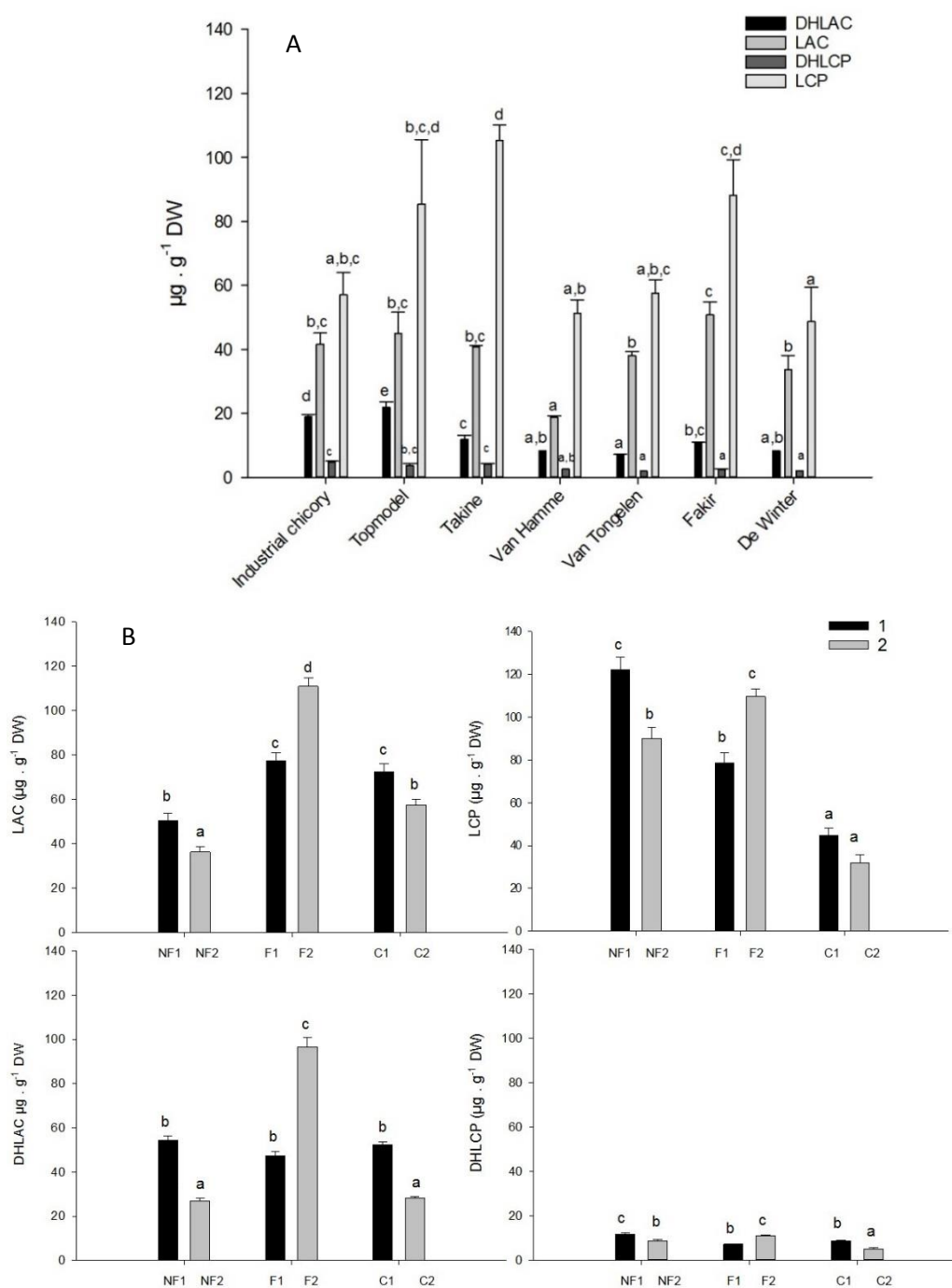
A PCA-plot of the relative SL-peak areas of the HRMS profile in the forcing dataset reveals a more pronounced clustering, where most of the variation in the SL-content of Belgian endive was related to the matrix (root and chicon) and to the forcing treatment, as samples clustered together in three distinct groups: forced roots, non-forced roots and chicons (Figure 5.4). It can be observed that the Belgian endive roots (NF and F) were significantly enriched in almost all dihydro-forms of LAC and in all glycosides compared to the chicons (Table A2 in Appendix). This resulted in chicons with overall less SLs compared to the roots, containing predominantly oxalate SLs. The roots contained predominantly oxalate and glycosidic SL-forms. Forcing the Belgian endive roots significantly increased all levels of LAC-SLs (except for DHLAC). This increase can be predominantly attributed to an increase in oxalates. After storage, even the LCP-forms significantly increased upon forcing (except LCPox). Accordingly, almost all SLs in F2-roots were thus present in significantly larger amounts compared to F1-roots (Table A2 in Appendix).



**Figure 5.4** K-means clustered PCA-plot of the 16 measured sesquiterpene lactones in the forcing subset which was power transformed and autoscaled. The total variance captured by the first two PCs is 80.6 %. The loadings were multiplied by factor 10 for visual purposes. The different samples are non-forced roots (NF), forced roots (F) and chicons (C) both not stored and stored (1 and 2).

Interestingly, when focusing only on the roots of the forcing dataset, another clustering becomes apparent (Figure A2 in Appendix). The distinct clustering between forced and non-forced roots vanished as NF2 and F1 clustered together. This indicates that both storage and forcing of NF1-roots induced a similar, increasing effect on the amount of SLs. The impact of four-month storage on the NF-roots (NF1 vs. NF2) could predominantly be attributed to an increase in the compounds located in the second quadrant (DHdoLACox, DHLACglyc, LACox, doLACglyc, DHdoLACglyc, DHLACox, DHdoLAC, LACglyc). The NF1-roots on the other hand were characterized by significantly higher levels of the compounds in the first and fourth quadrant (LCP, DHLCP, DHLAC, doLAc, LAC). Storage of the NF-roots thus showed a significant increase in all bound LAC SLs (except doLACox) and a corresponding significant decrease in the free LAC and LCP SLs (except DHdoLAC). Upon forcing (NF1 vs. F1), these effects were similar yet more pronounced and were manifested even more during forcing after storage (NF2 vs. F2). Indeed, besides the bound LAC SLs, the free LAC-forms also significantly increased upon forcing, which were complemented with the free LCP-forms upon forcing after storage. These similarities may explain the observed clustering.

Four SLs (LAC, DHLAC, LCP, DHLCP) were quantified with an external calibration curve and results are shown on a dry weight basis in Figure 5.5. This quantification could be used to distinguish the contribution of these commonly investigated SLs to the observed SL-clustering and are thus discussed accordingly. Generally, in both the variety and the forcing dataset, LAC and LCP were present in higher concentrations ( $20 - 120 \mu\text{g}\cdot\text{g}^{-1}$  DW) than their DH-counterpart ( $2 - 95 \mu\text{g}\cdot\text{g}^{-1}$  DW). Within the variety samples (Figure 5.5A), the trends observed for the four quantified compounds corresponded to the ones described in the PCA-plots. That is, a significantly larger concentration of DHLAC and DHLCP in industrial chicory, Topmodel and Takine (cluster 1 without Van Hamme). This resemblance between the PCA-plot and measured concentrations was not visible for the non-DH-forms (LAC and LCP), which could indicate that the latter two compounds were less responsible for the PCA-clustering in the variety dataset. In the forcing dataset (Figure 5.5B), the four quantified compounds did not contribute much to the distinction between the chicon and the root matrix as seen in the PCA-plot, as only the LCP-concentration was significantly lower in the chicons. This was in accordance with the trend in the PCA-plot where the distinction between chicons and roots was predominantly related to the glycosides and the DH-forms of LAC (except DHLAC). The effects of forcing and storage on the SLs observed here, corresponded to the ones seen in the PCA-plot.



**Figure 5.5** Four quantified sesquiterpene lactones (lactucin (LAC), dihydrolactucin (DHLAC), lactucopicrin (LCP) and dihydrolactucopicrin (DHLCP)) ( $\mu\text{g} \cdot \text{g}^{-1} \text{DW}$ ) A) in the variety dataset, B) in the forcing dataset. The different samples in the forcing dataset are non-forced roots (NF), forced roots (F) and chicons (C) both not stored and stored (1 and 2). Different letters indicate significant differences ( $p < 0.05$ ).

## 5.4.2 Phenolic profile

The major phenolic compounds are shown in Table 5.1. Chlorogenic acid (CHA) constituted more than 99 % of the detected phenolic compounds in both Belgian endive roots and industrial chicory roots.

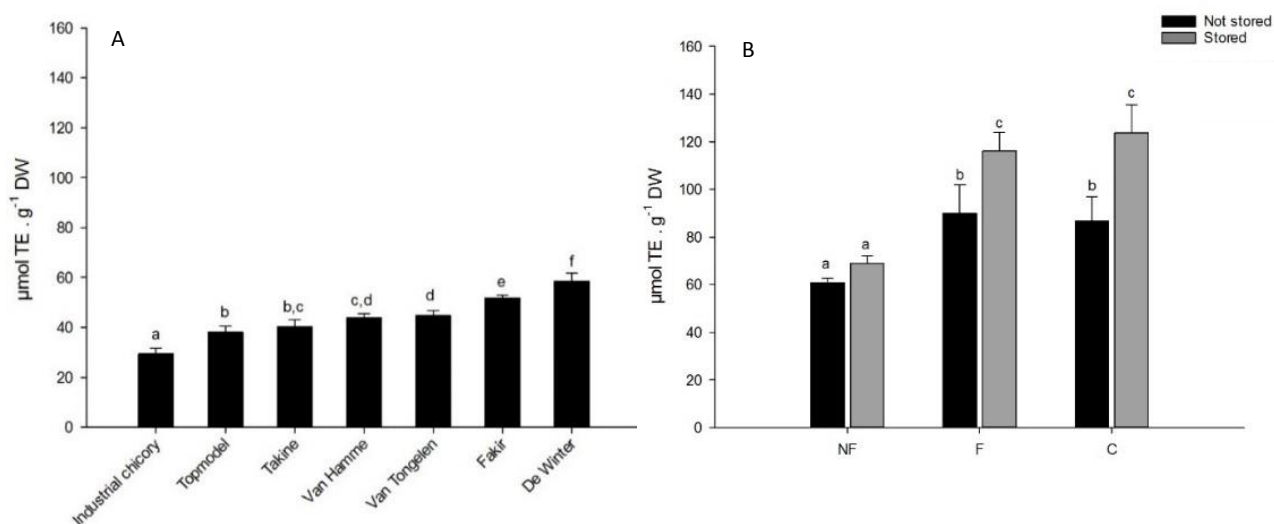
**Table 5.1** Content of phenolic compounds ( $\mu\text{g.g DW}^{-1}$ ). Different letters indicate statistically significant differences ( $p < 0.05$ ). Letters a – e denote differences in the forcing dataset. W - z are used for differences within the variety dataset. The different samples in the forcing dataset are non-forced roots (NF), forced roots (F) and chicons (C) both not stored and stored (1 and 2).

		Hydroxycinnamic acids		Flavonoids (flavonols)		
		Chlorogenic acid	Caffeic acid	Rutin	Quercetin-3-O-glucuronide	isoquercetin
Variety dataset	Industrial chicory	927 $\pm$ 45 <sup>w</sup>	2.4 $\pm$ 0.1 <sup>z</sup>	1.9 $\pm$ 0.1 <sup>x</sup>	0.37 $\pm$ 0.02 <sup>w</sup>	0.1 $\pm$ 0.01 <sup>x</sup>
	Topmodel	1,251 $\pm$ 54 <sup>x</sup>	2.2 $\pm$ 0.04 <sup>z</sup>	0.02 $\pm$ 0.0001 <sup>w</sup>	< 0.1	< 0.01
	Takine	1,473 $\pm$ 27 <sup>x</sup>	0.68 $\pm$ 0.05 <sup>w</sup>	0.02 $\pm$ 0.001 <sup>w</sup>	< 0.1	< 0.01
	Van Hamme	2,503 $\pm$ 145 <sup>y</sup>	1.0 $\pm$ 0.1 <sup>x</sup>	0.10 $\pm$ 0.01 <sup>w</sup>	< 0.1	< 0.01
	Van Tongelen	1,375 $\pm$ 95 <sup>x</sup>	0.66 $\pm$ 0.02 <sup>w</sup>	0.02 $\pm$ 0.0002 <sup>w</sup>	< 0.1	< 0.01
	Fakir	2,374 $\pm$ 90 <sup>y</sup>	1.6 $\pm$ 0.1 <sup>y</sup>	0.04 $\pm$ 0.02 <sup>w</sup>	< 0.1	< 0.01
	De Winter	2,227 $\pm$ 105 <sup>y</sup>	1.1 $\pm$ 0.04 <sup>x</sup>	0.05 $\pm$ 0.01 <sup>w</sup>	< 0.1	0.02 $\pm$ 0.007 <sup>w</sup>
	<b>p-value</b>	< 0.001	< 0.001	< 0.001	-	0.002
Forcing dataset	NF1	4,515 $\pm$ 293 <sup>b</sup>	5.3 $\pm$ 0.2 <sup>b</sup>	0.05 $\pm$ 0.004 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>a</sup>	0.01 $\pm$ 0.001 <sup>a</sup>
	F1	5,949 $\pm$ 180 <sup>c</sup>	1.8 $\pm$ 0.02 <sup>a</sup>	0.07 $\pm$ 0.01 <sup>a</sup>	0.16 $\pm$ 0.02 <sup>a</sup>	0.04 $\pm$ 0.003 <sup>a</sup>
	C1	2,358 $\pm$ 180 <sup>a</sup>	20 $\pm$ 1 <sup>c</sup>	4.7 $\pm$ 0.2 <sup>b</sup>	7.9 $\pm$ 0.4 <sup>b</sup>	1.2 $\pm$ 0.04 <sup>b</sup>
	NF2	4,233 $\pm$ 113 <sup>b</sup>	1.8 $\pm$ 0.02 <sup>a</sup>	0.08 $\pm$ 0.002 <sup>a</sup>	0.18 $\pm$ 0.01 <sup>a</sup>	0.02 $\pm$ 0.004 <sup>a</sup>
	F2	4,415 $\pm$ 126 <sup>b</sup>	5.3 $\pm$ 0.3 <sup>b</sup>	0.07 $\pm$ 0.01 <sup>a</sup>	0.15 $\pm$ 0.03 <sup>a</sup>	< 0.01
	C2	2,281 $\pm$ 67 <sup>a</sup>	25 $\pm$ 2 <sup>d</sup>	5.3 $\pm$ 0.5 <sup>b</sup>	10 $\pm$ 1 <sup>c</sup>	1.3 $\pm$ 0.2 <sup>b</sup>
	<b>p-value</b>	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

In the variety dataset, industrial chicory was characterized by a significantly lower CHA-concentration compared to the Belgian endive roots. Van Hamme, Fakir and De Winter contained the highest CHA levels, about twice as high as the level in industrial chicory. Caffeic acid levels did not follow this CHA trend, showing significantly higher concentrations in industrial chicory and Topmodel compared to the other varieties. Rutin, a flavonoid present in the fresh roots also showed a completely different distribution compared to CHA, with a significantly higher concentration (~factor 2) in industrial chicory roots compared to the Belgian endive roots. In the forcing dataset, the roots contained significantly more CHA compared to the chicons (~factor 2). Forcing significantly increased the level of CHA whereas this effect was less visible in forcing after storage. The other phenolic compounds, present in much lower concentrations showed a different profile. These were much more present in the chicons compared to the roots varying from a factor 6 in caffeic acid to 65 in the flavonoids. No consistent effects of storage were observed. Chicoric acid was identified in this dataset, but the obtained results did not allow for a correct quantification due to suboptimal peak shape.

### 5.4.3 Antioxidative capacity (AOC)

The AOC measured by the ORAC-assay on freshly harvested roots from different Belgian endive varieties and the closely related industrial chicory root are shown in Figure 5.6A. Industrial chicory and Topmodel had the lowest AOC whereas Fakir and De Winter were varieties with the largest AOC. From Figure 5.6B, it can be seen that the chicons of Belgian endive had a comparable AOC compared to the F-roots. Forcing of the NF-roots significantly increased the AOC. Furthermore, the forced roots and chicons were characterized by a significantly higher AOC when produced from stored non-forced roots. This effect was less clear in the NF-roots indicating that storage of NF at -2 °C had little impact on the AOC.



**Figure 5.6** Antioxidative capacity as determined by the ORAC-assay ( $\mu\text{mol TE} \cdot 100\text{g}^{-1} \text{DW}$ ). A) ORAC-values for the variety dataset and B) ORAC-values for the forcing dataset. Different letters indicate statistically significant differences ( $p < 0.05$ ). Vertical bars represent standard deviations. The different samples in the forcing dataset are non-forced roots (NF), forced roots (F) and chicons (C) both stored and not stored.

The DPPH-profile of the variety dataset (Figure A3A in Appendix) showed no significant differences between the different samples (except for a significantly higher AOC of Van Hamme). In the DPPH-profile of the forcing dataset (Figure A3B in Appendix), the AOC of the chicons was similar to that of the forced roots whereas the AOC of the NF-roots was significantly lower, analogous to the ORAC-assay. No other pronounced effects were observed here.

### 5.4.4 Elemental composition

The elements predominantly present in both datasets were K ( $2,500 - 4,500 \mu\text{g} \cdot \text{g}^{-1} \text{FW}$ ), P ( $300 - 450 \mu\text{g} \cdot \text{g}^{-1} \text{FW}$ ), Cl ( $250 - 600 \mu\text{g} \cdot \text{g}^{-1} \text{FW}$ ) and Ca ( $200 - 600 \mu\text{g} \cdot \text{g}^{-1} \text{FW}$ ) (Tables A3 and A4 in Appendix). A large variation was found between the varieties and cultivars (factor 1.2 – 4.9). A PCA-plot of this

elemental composition shows two clusters with the industrial chicory roots in one cluster and all Belgian endive roots in the other cluster (Figure A4 in Appendix). The elemental composition of industrial chicory roots was thus distinctly different compared to the Belgian endive roots with a significant enrichment of Cl and Mn in the former. The Belgian endive cluster was characterized by a significantly higher concentration of P, K and S. However, within the Belgian endive cluster, the composition of the Topmodel roots differed slightly from the others as these were significantly enriched in Mn, Zn, Cu and P levels compared to the other cluster members. Cl and Br on the other hand were present in significantly lower concentrations in the Topmodel roots compared to the other Belgian endive roots. In the forcing dataset, a greater amount of variation was found (factor 1.2 – 17). Here, the same three groups were found as discerned by the PCA on SLs, i.e. chicons, forced roots and non-forced roots (Figures A5 and A6 in Appendix). The chicons were characterized by significantly higher concentrations of Cl, P, K, S and Mg whereas Na, Fe and Sr were present in significantly lower amounts compared to the roots. Forcing Topmodel roots led to a significant increase in the levels of Cl, Ca, Na and Sr.

## 5.5 Discussion

The goal of this chapter was to gain insight in the composition of the currently underutilized forced Belgian endive roots which could have potential for valorization towards food, pharma or biocidal products. The analyses on the forced roots were complemented with other samples to identify the effect of variety, cultivar, matrix, forcing and storage on the constituents.

### 5.5.1 Sesquiterpene lactones

The major presence of oxalates in industrial chicory roots, Belgian endive roots and chicons is in accordance with the results of Sessa et al. (2000) who investigated the latex of *C. intybus*. It also agrees with the results of Annaratone et al. (2016) who investigated the latex of Belgian endive chicons and found a ratio of free to bound SLs close to 0.1.

Although not clustered separately in the PCA-plot, the total SL-level in chicory roots (*var. sativum*) was lower compared to the level in the Belgian endive roots (*var. foliosum*), which could be related to either variety or other factors like growing conditions, growing locations, etc. The Belgian endive cultivars tested in this study (other than Topmodel) have been grown on the same location, under the same conditions and were harvested at the same time, thus it is very likely that the variation observed in their SL-profile (factor 1.3 – 4.9) was in part related to the cultivar. The effects of both variety and cultivar have already been demonstrated for *Cichorium intybus*, affecting the total amount of SLs as well as the relative proportions (Foster et al., 2011; Peters et al., 1996; Peters et al., 1997; Van Steenkiste et al., 2013). Interestingly, the genetic background of the different cultivars (open-pollinated varieties vs. classical or CMS hybrids) nor location and growing conditions (Topmodel roots vs. other Belgian endive roots) were clearly reflected in the clustering. The elemental composition did seem to reflect this difference in variety as well as location, as industrial chicory was clustered separately from the Belgian endive roots and as the Topmodel roots were located distinctly further away from the other cluster members. Previously, location as well as location-cultivar interaction and P and N availability in the soil have also been linked to the SL-composition of chicory foliage and chicons (Foster et al., 2006; Peters et al., 1996, Peters et al., 1997). More data are thus necessary to determine the exact effect of location and soil mineral composition on the SL-profile.

Leaving out geography, variety and cultivar, the forcing dataset allowed to identify the effects of various other parameters (matrix, forcing and storage). In the PCA-plot of the forcing dataset, the samples clustered closely together in three categories: (i) chicons, (ii) NF-roots and (iii) F-roots. This demonstrates that the sesquiterpene lactone profile was linked to matrix and forcing treatment and



varied distinctively within these treatments. Compared to chicons, roots contained more SLs. Forcing these roots led to a significant increase in most SLs. Four month storage of the NF-roots seemed to significantly increase the bound SL-forms and significantly decrease the free SL-forms, which may indicate a partial conversion of free to bound forms upon storage. These stored NF-roots, enriched in bound SLs, in their turn gave rise to F-roots with an increased SL-content compared to the forced roots that resulted from the non-stored NF-roots.

In literature, very little information is available on the effect of matrix, storage and forcing the free SL-content in *Cichorium intybus* L. var. *foliosum*, leave alone data on bound SLs, thus it is difficult to make a reasonable comparison of the data obtained. Dolezal (1976 as cited in Leclerq, 1992) also reported an increase of the bitter compounds in the Belgian endive roots during forcing, however using a rather inaccurate method, including all compounds reacting with KCN. Aerial parts of other cultivars of the *foliosum* variety have been analyzed for their SL-content and the same trend was found, namely that dihydroforms are present in smaller amounts compared to their non-dihydro counterpart (Graziani et al., 2015). Analogous to the results found here, de Kraker (2002) found the SL-concentration in the roots (0.11 % – 0.81 % dry weight) to be higher compared to the leaves (not specified which kind of leaves) (0.06 % - 0.45 % dry weight).

### 5.5.2 Phenolic composition and antioxidative capacity

The hydroxycinnamic acids predominate in the *Cichorium intybus* species, represented by mono- (mainly chlorogenic acid) and dicaffeoylquinic acids and chicoric acid (Jurgoński et al., 2011; Milala et al., 2009; Sinkovič et al., 2014; 2015). This is confirmed in this study as chlorogenic acid was by far the main phenol present, constituting 99 % of the detected phenolics in all matrices sampled. Three minor compounds, identified as quercetin derivatives (flavonols) were quantified as well namely rutin, quercetin-3-O-glucuronide and isoquercetin. All of these compounds have been reported in *Cichorium intybus* species before, either in free or bound form (Carazzone et al., 2013; Ferioli & D'Antuono, 2012; Mascherpa et al., 2012).

Differences within these compounds were predominantly found between different varieties and cultivars (factor 2 difference in CHA-levels). The industrial chicory roots have been investigated previously for their phenolic content. CHA-concentration in industrial chicory roots was reported to be  $91.9 \pm 5.7 \mu\text{g}\cdot\text{g}^{-1}$  DM (Willeman et al., 2014), which is a factor 10 lower compared to the values obtained here. The large phenolic concentration ranges reported here however, highlight the large within-variety variability (factor 10), which has also been confirmed earlier by Sinkovič et al. (2014) and Annaratone et al. (2016). Besides variety and cultivar, external conditions can also exert an influence

on the phenolic composition such as crop management (Sinkovič et al., 2015) as well as biotic and abiotic factors.

Besides variety and cultivar, also the effect of matrix was large, with a 2 times higher level of CHA in the roots compared to the chicons and an up to 65 times lower concentration for the minor compounds. Whereas almost no research has been conducted in the phenolic composition of the Belgian endive roots, the edible leaves of Belgian endive have been investigated to a small extent. Recently Annaratone et al. (2016) have identified that Belgian endive chicons contain mostly simple phenylpropanoids derived from caffeic acid such as CHA (48  $\mu\text{g}\cdot\text{g}^{-1}\text{FW}$ ), neochlorogenic acid (4.2  $\mu\text{g}\cdot\text{g}^{-1}\text{FW}$ ), cynarin (11  $\mu\text{g}\cdot\text{g}^{-1}\text{FW}$ ), chicoric acid, caftaric acid and caffeic acid itself. Innocenti et al. (2005) reported CHA-concentrations in Belgian endive chicons of 16 – 74  $\mu\text{g}\cdot\text{g}^{-1}\text{FW}$ . About three times higher levels were measured in the chicons (139  $\mu\text{g}\cdot\text{g}^{-1}\text{FW}$ ) in this study. Ferioli et al. (2015) analyzed the phenolic constitution of 10 different Belgian endive chicon cultivars and found hydroxycinnamic acids to be present in levels of 1,565 – 26,055  $\mu\text{g}\cdot\text{g}^{-1}\text{DW}$ , from which the lower limit is in line with our results. It has to be noted that only a limited set of hydroxycinnamic acids has been measured in this study, which can in part explain the lower concentration compared to the often reported total sum of hydroxycinnamic acids. The effects of forcing and storage on the phenolic content in this study were found to be minor compared to the differences between matrix and variety.

Phenolic acids are widely considered as natural antioxidants with potential health benefits for humans (Innocenti et al., 2005; Sinkovič et al., 2014). In that way, a correlation is to be expected between the phenolic concentration and the AOC. Interestingly, due to their structure, no AOC is attributed to SLs (Chadwick et al., 2013). The AOC has been determined by two assays, which are based on different working principles, as no single assay can accurately reflect all of the radical sources or antioxidants in a complex system (Prior et al., 2005). This implies that the results of any two AOC-methods will not automatically follow the same trend (U.S. Department of Agriculture, 2010). The trends of the predominantly present phenolic compound CHA in the variety dataset were to some extent reflected by the ORAC-assay as industrial chicory and Topmodel were characterized by half the AOC of De Winter, Fakir and Van Hamme. The AOC measured by the DPPH assay did not detect major differences within these varieties, however the order of magnitude of the AOC was similar compared to that reported in literature (Milala et al., 2009). The observed correspondence of the CHA-profile and the AOC in the roots could be traced back to CHA being the major phenolic compound in *C. intybus* roots (Jurgoński et al., 2011; Milala et al., 2009). In the forcing dataset, no pronounced correspondance of the AOC (both ORAC and DPPH) with the phenolic profile was found. These findings suggest that there are other compounds contributing to the AOC or at least that the compounds, present in the largest concentration, do not necessarily have the largest AOC. Another explanation could be the lack of chicoric acid data in this study, as this is reported to be the major phenolic compound in chicons

(Innocenti et al., 2005). Fraisse et al. (2011) highlighted the important role of caffeoyl derivatives in antioxidative activity, with chicoric acid contributing 59 % and CHA only 4 % the total AOC in the aerial parts of *Cichorium intybus* L. Forcing the NF-roots seemed to significantly increase the AOC, leading to a rather high value compared to other vegetables, comparable with broccoli, green lettuce and spinach (U.S. Department of Agriculture, 2010). It has to be remarked though that AOC-values are currently under debate as (i) they are not considered to directly reflect the effect of specific bioactive compounds, including phenolic compounds, and (ii) the values cannot be directly extrapolated to in vivo human effects (U.S. Department of agriculture, 2016).

### 5.5.3 Valorization of forced Belgian endive roots

Yearly approximately 36,000 tonnes of forced Belgian endive roots are fed to local cattle, which could be used otherwise to serve a higher added value in food, pharma or as biocide. In order to investigate in which manner they can serve another purpose, investigation of the vitamins (not measured) and minerals is valuable, specifically regarding food valorization. Calcium and potassium are for example considered nutrients of U.S. public health concern because low intakes are associated with health concerns (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). Additional to their beneficial impact for consumers, their presence can be translated into nutrition claims, which is important for marketing of the resulting food products. Particularly interesting in this regard are the claims “source of” and “rich in”, which may be used solely in conjunction with permitted vitamins and minerals (EC 1169/2011). Using the first claim is permitted if 15 % of the nutrient daily reference intake of the vitamin or mineral is supplied by 100 g of the product, whereas the second claim requires at least twice this value. Based on the results from this study, the K, Fe and Cu-levels in forced Belgian endive roots seem to meet the “source of” criterion, however more research is necessary to establish this.

Besides minerals, an investigation of the bioactive compounds was made in order to facilitate further valorization of the forced Belgian endive roots into products with higher added value. Indeed, the related *Cichorium intybus* species have been widely used in herbal preparations with multiple health beneficial effects, often ascribed to the presence of the phenolic compounds (hydroxycinnamic acids and flavonoids) and SLs (Das et al., 2016; Ferioli et al., 2015; Milala et al., 2009). The specific phenolic compounds (CHA, caffeic acid, chicoric acid and derivatives of quercetin), found in the Belgian endive roots have been reported to express a wide range of activities such as antihyperglycemic, anti-inflammatory, antibacterial and anti-carcinogenic (Azay-Milhau et al., 2013; Milala et al., 2009). Also the bioactivity of the sesquiterpene lactones has been subject of numerous investigations, although

often not specifically for the structures found in this study or more generally related to an extract containing SLs (Ghantous et al., 2010; Picman, 1986; Prakash & Gaikwad, 2012). Historically, SLs have been the active ingredient in folk medicines for many treatments such as diarrhea, burns, influenza and neurodegradation (Chadwick et al., 2013; Ghantous et al., 2010). They have gained interest for treating human diseases due to their potential for the treatment of inflammation, cardiovascular diseases, cancer and more. Some SL-derived drugs from thapsigargin, artemisinin and parthenolide even reached cancer clinical trials (Ghantous et al., 2010). Due to their hypothesized evolutionary significance in plants as deterrents against herbivores and anti-fungal, anti-bacterial allelopathic agents, they could also be used in crop production (Chadwick et al., 2013).

Although the above mentioned properties imply promising industrial valorization opportunities, several steps need to be undertaken before valorization of the currently low-value, forced Belgian endive roots into food, pharmaceutical products or herbicides can be realized. Firstly, due to their recent discovery, the SL-oxalates, shown here to be the predominantly present form of SLs, have not yet been investigated for their stability nor bioactivity. Although the net bioactive effects may to some extent be assumed, based on the knowledge of related compounds of the same class in related matrices (Chadwick et al., 2013), these novel compounds should be the subject of specific research before considering their utility in a biological context. Secondly, related to the bioactivity of novel compounds, a matrix effect should be taken into account. The role of compounds in the plant matrix is complex and often cannot be assumed to be allocated to a single compound. Thus, even when the bioactivity of a certain present compound is known, this knowledge has to be interpreted cautiously, as the matrix in other *Cichorium* varieties may suppress or enhance the activity of a specific compound or compound class, leading to a different activity. Therefore, actual valorization of Belgian endive forced roots will specifically require the obtained extracts to be bio assayed. Thirdly, if extraction of SLs and phenolic compounds on industrial scale is desirable, the green extraction method used for SL-extraction as proposed here, using only water and formic acid is an environmentally friendly option. Furthermore, it can be used for the simultaneous extraction of phenolic compounds (Annaratone et al., 2016). This upscaling however requires rigorous testing of the extraction efficiency, performance and economic viability on a pilot and industrial scale.

Besides serving as a basis for the identification of potential valorization pathways of forced Belgian endive roots, the obtained SL-data can also be valuable in regard to the taste of the currently commercially valuable chicons. This is gaining interest as growers often want to offer Belgian endive chicons of diverse bitterness levels. Therefore, the link between bitterness and SLs should be reassessed (including both bound and free forms), in order to identify the most bitter-tasting compounds. Further investigating the effect of manipulation of the roots (cultivation conditions ,

storage conditions, forcing conditions) on the SL-profile in chicons and linking this to the bitter taste may enable us in the future to influence and customize the final taste of the chicon. In effect however, this control might not be as straightforward, taking into account different aspects such as a SL-dependent disease resistance, cultivar-location interaction effect on the SLs and the occurrence of other bitter compounds (D'Antuono et al., 2016; Leclerq, 1992; Peters et al., 1997; Peters & van Amerongen, 1998; van Beek et al., 1990; Van Steenkiste et al., 2013). More research is thus necessary in order to gain more insight into the factors influencing the expression of bitterness in chicons in relation to the SLs.



## **Chapter 6**

Reflective discussion





## Chapter 6: Reflective discussion

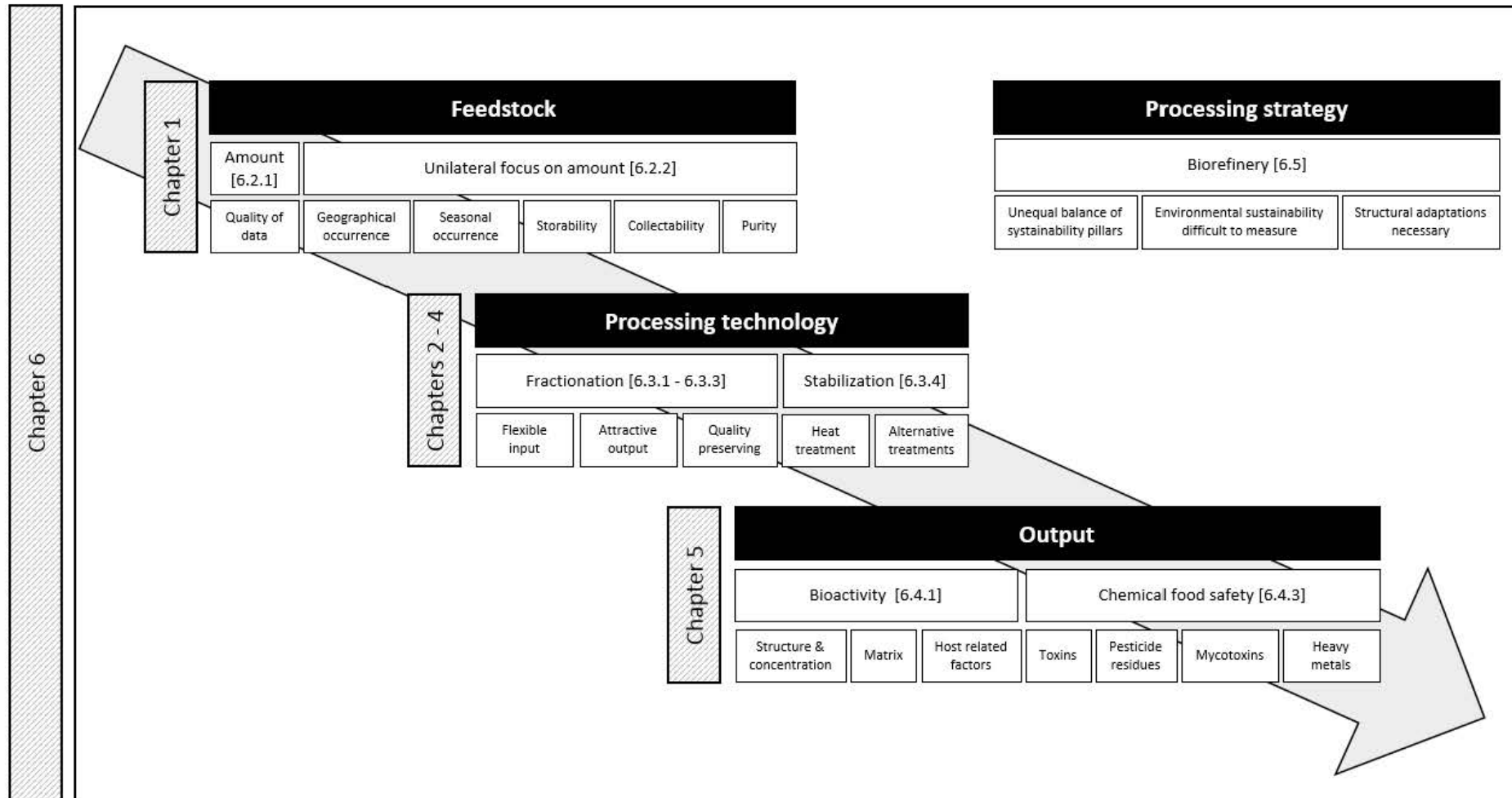
### 6.1 General framework

In this dissertation, the valorization of horticultural byproducts has been assessed from a broad perspective, thereby applying an open and transdisciplinary approach (Chapter 1). From the explorative idea development phase, it was established that horticultural byproducts represent a rich feedstock with ample potential valorization applications. Furthermore, continuing technological developments have enabled the translation of a large part of these innovative ideas into lab-scale experiments and further conversion into a proof-of-concept, reaching pilot scale in some cases. However, this is currently often the terminus. As a consequence, further development and subsequent translation towards commercialization remains scarce. Throughout our research, we have identified a number of aspects that can have an influence on this apparent difficulty of translating ideas for valorization of horticultural byproducts into commercialized concepts. These aspects are depicted in Figure 6.1 and will be discussed in more detail in this chapter.

The remainder of this discussion is divided into four main parts. Firstly, in section 6.2, the facilitating or obstructing impact on the valorization pathway of six traits related to the horticultural byproduct feedstock are discussed (labeled *Feedstock* in Figure 6.1). Secondly, the extent to which these traits are manifested often depends on the type of byproduct. However, two of these traits are inherently present in almost all horticultural byproducts (i.e. high moisture content and seasonal occurrence) and can obstruct their valorization. Consequently, the processing technology must be tuned to be able to cope with these constraints (labeled *Processing technologies* in Figure 6.1). These constraints are used to explain, discuss and evaluate the suitability of the spiral-filter press as one of the possible options for the valorization of horticultural byproducts in section 6.3. Thirdly, a number of feedstock-related traits (e.g. variation in type and concentration of phytochemicals) and processing-related aspects (e.g. amount of refining, process impact) determine the characteristics of the derived products and thus the created added value (labeled *Output* in Figure 6.1). These aspects and their repercussions on the valorization process are discussed in section 6.4, specifically focused on food products. Finally, the biorefinery processing strategy, in which the adopted processing technology fits, is critically discussed. This strategy is increasingly being advocated for its use in the valorization of biomass, however its adoption and diffusion as a strategy for valorizing horticultural byproducts is limited today. Throughout our research, we have identified three main aspects that can be experienced as barriers, which are discussed in 6.5, together with potential measures to stimulate biorefineries.

Figure 6.1 thus serves as a framework for this reflective discussion and covers aspects related to the different stages of the supply chain, from horticultural byproduct feedstock, to processing and (predominantly food) output products. Different aspects are critically reflected upon and specifically linked to the feasibility of valorizing horticultural byproducts. The framework includes aspects investigated in the previous chapters of this dissertation, complemented with additional important aspects related to the topic of the valorization of horticultural byproducts. Hence, the reflective discussion (i) provides more insight into how the different research chapters of this dissertation relate to each other, and (ii) puts the performed research into a broader perspective of valorizing horticultural byproducts within the bioeconomy.

Although the framework structures and relates a large number of important aspects that can affect the feasibility of a potential valorization trajectory, it cannot however be viewed as a completely comprehensive guide. It is primarily intended as a schematic representation of the structure of this research and the topics addressed in this general discussion.



**Figure 6.1** General framework of the reflective discussion tackling aspects related to the valorization of horticultural byproducts throughout the supply chain.

## **6.2. Feedstock: is the available amount of byproduct the major criterion to determine its suitability for valorization?**

A constantly available and sufficient amount of feedstock often facilitates the valorization of byproducts. Therefore, a profound insight into the yearly available amount of byproducts and the variation of supply throughout the year are crucial for the viability of the valorization pathway (Matharu et al., 2016; Mirabella et al., 2014; OVAM, 2014; OVAM, 2015b).

In the introduction of this dissertation (Chapter 1), an overview was given of the amount of byproducts occurring in Flanders throughout various parts of the food supply chain. This entailed an overview of the different parts (both edible and inedible) of different crops occurring at different stages (primary production and harvesting, processing and auction stages). From this, information regarding crop specific amounts could be deduced, as well as general information on the relative share of the different crop classes and production stages. From the Flemish data, it became clear that the predominant part of inedible byproducts is created during harvesting. The amount of edible byproducts on the other hand is largest during harvesting and processing and predominantly arise from outdoor vegetables and fruits. This trend is confirmed by the European data available (Table 1.4 in Chapter 1) with the amount varying in function of the type of processing.

The overview provided in this dissertation and other quantitative spreadsheets on food waste and byproducts (e.g. EU-FP7 projects FUSIONS<sup>13</sup>, NOSHAN<sup>14</sup> and REFRESH<sup>15</sup>, Cost action EUBIS<sup>16</sup>) are valuable from some specific points of view. For example, they can provide a general insight in the amount of byproducts occurring in a certain region and allow to draw overall conclusions about the relative amount of the different types of byproducts. However, two aspects should be taken into consideration when interpreting the data from such databases, namely (i) the reliability of the data and (ii) the unilateral focus on the amount of byproducts.

### **6.2.1 Lack of reliable data on the amount of available byproducts can limit their usefulness**

Even though the issue of waste and byproducts is increasingly being conceived, there is still a scarcity on detailed and qualitative statistics on their amounts (Ekman et al., 2013). This can be mainly

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<sup>13</sup> 'Food Use for Social Innovation by Optimizing Waste Prevention Strategies' - <http://www.eu-fusions.org/>

<sup>14</sup> <http://www.noshan.eu/index.php/en/>

<sup>15</sup> 'Resource Efficient Food and dRink for the Entire Supply cHain' - <http://eu-refresh.org/>

<sup>16</sup> <http://costeubis.org/>

allocated to three different factors: (i) lack of consistent definitions, (ii) data based on estimations, and (iii) the so-called circular referencing.

The first factor lying at the basis of this limited data quality is the different terminology for byproducts and waste, as described in the introduction of this dissertation. Adopting different criteria or not clearly pinpointing the criteria used, can lead to using different definitions and classifications of waste and byproducts (Ekman et al., 2013; Hennig et al., 2016; Stenmark et al., 2016). This is for example very clear in case of inclusion or exclusion of inedible byproducts. These differences in terminology lead to figures that are hard to interpret and compare, thus detrimental for the data quality.

Secondly, the amounts are often based on estimations or were measured using different methods (Hennig et al., 2016; Stenmark et al., 2016). Absent data are often extrapolated using waste percentages from similar biomasses or industries. For example, in the European database (Table 1.4, Chapter 1), the byproducts occurring at the cultivation stage were predominantly fixed at 20 %, whereas the transport stages were assumed to generate about 15 % of byproducts. Also for quantifying the share of the processing industry, waste percentages derived from the processing of similar crops or industries are often used. For example, the volume of waste generated during production of various types of fruit juices has been calculated based on a waste percentage, reported for the production of orange juice (EUBIS Cost action<sup>17</sup>), while it is evident that different juice yields will be obtained using different feedstocks and different technologies. Other examples are the amount of byproducts resulting from the peeled tomato industry which are assumed to be equal to the amounts generated during juice production or the byproducts of frozen cauliflower and broccoli which were assumed to be the same as for other vegetables such as chards (EU datasheet in Chapter 1). Even though these educated guesses can help to answer to society's hunger for quantitative data, they are not sensitive to crop specific differences nor to the effect of small changes in practices (e.g. small-scale initiatives).

Thirdly, the data available are frequently reused, leading to 'circular referencing', thereby jeopardizing the quality of the data. In projects aiming for byproduct valorization, data collection of the available amounts of byproducts is often the first step. This has led to an increased amount of available databases during the past decade. However, these often consist of citing previous estimations, without performing additional research for data collection or quality checks. This may lead (i) to confusion regarding the prevalent databases, not clearly defining the hierarchy within the circulating databases,

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<sup>17</sup> <http://costeubis.org/pdf/waste-mapping.pdf>

the source document, the year of data collection, the region sampled and the measuring methodology adopted and (ii) to confusion as to the causes of differences between databases.

Consequently, the data available are thus rather based on rough estimations that are being continuously reused but that are not systematically monitored nor uniformly defined, hence causing a lack of data specificity. This makes these data unsuitable as a base for drafting a specific byproduct valorization business plan as well as for a detailed monitoring of the effects of changes in policy or management. Consequently, the added value of these general databases can be considered as rather limited. An improvement could be to consistently perform actual measurements on these byproducts, using a standardized protocol for different crop types. These should be accompanied by a detailed description of the parameters such as the year, variety, location, cultivation and harvesting method. The adopted terminology as to which biomass parts are included should also be formalized and explicitly stated. Furthermore, these measurements should be organized and verified by a centralized authority in order to guarantee the uniformity and quality of the data, thereby providing a solid base to make comparisons and spot trends. This might convert the estimations into reliable and transparent figures and could avoid circular referencing in the future. These detailed, systematically collected data would also allow to measure the effect of certain actions or policy measures and monitor progress. This rationale is increasingly being recognized by the European Commission (European Commission, 2016b) as for example demonstrated by the FUSIONS project, proposing a practical guideline for a harmonized approach for EU member states on how to determine and quantify food waste in different stages of the food supply chain (Stenmarck et al., 2016). Also the assignment of one central instance (Institute of Agricultural, Fisheries and Food research) responsible for the collection of projects related to byproducts has been introduced under the form of the Agrocycle database. In the future, this could be complemented by the collection of quantitative data related to the occurrence of byproducts.

### 6.2.2 More than just amounts as criterion for evaluating the feasibility for valorization of a certain byproduct

A second issue that merits attention in light of data collection is the unilateral focus on the amount of byproduct as a basis to estimate the valorization potential of horticultural byproducts. The byproducts occurring from the food industry are characterized by specific characteristics such as regional and seasonal availability and rapid product quality decay (Jonkman et al., 2017; Russ & Mayer-Pitroff, 2004). These characteristics can lead to specific requirements regarding transport, storage and processing and can influence the quality of the derived end product(s). Hence, they can determine the viability of a certain valorization and thus provide additional criteria to determine whether or not a

byproduct is suitable for valorization (Jonkman et al., 2017; Sweet et al., 2016; Tsolakis et al., 2014). Although the importance of logistics has been widely recognized in the light of valorizing byproducts and the development of the bio(based) economy (Kusch et al., 2014; OVAM, 2014), the repercussions of some logistic traits on the feasibility of valorization are often not specifically addressed nor documented. In addition to the amounts of byproducts, five traits were identified for their effect on the feasibility of an envisaged valorization pathway: (i) geographical spread, (ii) seasonal occurrence, (iii) ability for storage, (iv) collectability and (v) purity.

#### 6.2.2.1 Geographical spread

The figures available frequently refer to amounts collected in a widespread geographical region, not discriminating for their often scattered or concentrated occurrence. This geographical occurrence is a first trait that influences the practical feasibility, performance and scale of a specific processing strategy (Budzianowski & Postawa, 2016; Ghatak, 2011; Hennig et al., 2016; OVAM, 2014; Poltronieri & D'Urso, 2016; Sweet et al., 2016). When focusing on one particular feedstock, processors may need to source scattered occurring byproducts from different regions to operate the processing equipment throughout the year, (Jonkman et al., 2017). Byproducts occurring in a rather concentrated manner can facilitate the collection and reduce transport costs. Also the proximity of the source of byproducts and their subsequent processors influence the feasibility of a valorization route (Mirabella, 2014). Completely excluding this specific geographical information and only including large-scale data, can limit their usefulness for practical translation into case studies and for crafting specific valorization strategies. In general, byproducts generated at large processing facilities and surplus products at the produce auctions can be considered to suffer least from this geographical spread, as they occur centralized and in substantial amounts in the production chain. Clusters of greenhouses also provide opportunities for the collection of horticultural byproducts, however these are still predominantly under development (OVAM, 2014). In the Netherlands, a large greenhouse cluster area has been developed and in parallel an *Interreg 2 Seas* project (BioBoost) has been initiated to valorize the hereby occurring byproducts. Mobile processing units can also be used in case byproducts are not available together on one location. Examples are mobile fruit juice presses<sup>18</sup> driving around during fruit harvesting and storage seasons and the Dutch mobile grass biorefinery, refining grass into fibers, proteins, phosphate and juice (GRASSA<sup>19</sup>).

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<sup>18</sup> <http://www.appelpom.com/index-nl.html>

<sup>19</sup> <http://grassa.nl/>

#### 6.2.2.2 Seasonal occurrence

Besides a geographical occurrence, the byproducts also often occur concentrated in time. This seasonality is a second aspect that may influence the economic feasibility of a valorization route due to ineffective use of equipment and discontinuous supply of outputs to the market (Budzianowski & Postawa, 2016; Fava et al., 2015; Hennig et al., 2016; Jonkman et al., 2017; Kasapidou et al., 2015; Sweet et al., 2016; Tsolakis et al., 2014). Often, peak volumes are generated in a certain time frame as described for tomato in the introduction (Chapter 1). This can be obviated for example by using a flexible processing technology, able to process a wide range of byproducts throughout the year into a range of related end products (e.g. vegetable juices, soups, powders, etc.), whereby each byproduct guarantees a sufficient supply during the season in which it is available (Jonkman et al., 2017). Investing in such flexible processing technologies can thus lead to a profitable business case, for example at a large produce auction, where different byproducts are centralized.

#### 6.2.2.3 Ability for storage

In case of scattered and seasonally available byproducts, processors can also temporarily store the byproducts, averaging the peak volumes (Jonkman et al., 2017). Cooled and frozen storage are promising options, however large storage capacities are often absent. Furthermore, chilled storage or storage at ambient temperatures is often impeded by the high moisture content and corresponding fast spoilage of the byproducts. Depending on the crop, this can be manifested to a greater (e.g. perishable leafy crops such as lettuce) or lesser (e.g. tuber or root crops such as beets and carrots) extent. This ability for storage is a third trait that puts constraints on further processing in order to preserve the quality of the byproducts, for example through demanding cold temperature during storage and transport or limiting the duration of storage and processing conditions (Poltonieri & D'Urso, 2016). These can exert a distinct influence on the economic feasibility of the valorization as well (Jonkman et al., 2017; Sweet et al., 2016). This is also the case for primary products and can be illustrated by the sugar beet campaign. This only lasts three to four months per year and long storage of the beets is impossible due to quality degradation. This limits the time available for processing, which in its turn increases the size of the processing facilities. This increased capacity comes at a cost, even though the equipment cannot be operated to process beet throughout the year (Jonkman et al., 2017).

Rapid pretreatments to remove unwanted water before transport (e.g. drying, pressing) have been reported to be a good option to overcome this issue of storage. In case of sugar beets for example, decentrally pressing the beets to a thick juice and subsequent transport of this juice to a central processing facility can be a viable alternative for current practices, as the quality of the liquid is less



perishable. These can lead to a reduced transport cost and less dependency on the seasonal production, which in its turn can reduce the fixed cost of the processing facility by allowing it to be smaller (Bruins & Sanders, 2012; de Jong et al., 2005; Jonkman et al., 2017; OVAM, 2014).

#### 6.2.2.4 Collectability

The byproducts must not only be present, they must also be easily collectable. In this regard, their ability to allow for selective collection is a fourth trait, determining to a large extent the technical requirements and the economic feasibility of the valorization. Byproducts occurring during harvesting are predominantly left on the field. Allowing for example the co-collection or on-field sorting of crops and crop byproducts would avoid the need for two separate passes over one field and could also improve the quality of the byproducts (see 6.2.2.5 Purity) (KTN, 2016). In most cases, an adaptation or completely novel design of current harvesting machines would be necessary (Fava et al., 2015). This additional cost can impact the economic feasibility of the total valorization pathway. However, in the long run, the separate, dedicated collection will improve the homogeneity and purity of the byproducts, thus limiting the following necessary pretreatment(s) and related extra costs. Stimulating the selective collection throughout the entire logistic production chain is adopted as one of the action programs by the Flemish policy (OVAM, 2015b).

#### 6.2.2.5 Purity

A final trait is related to the purity of the byproduct. For example, the presence of undesired subjects may pose a problem, as is the case in greenhouse vegetables, where plastic clips and ropes often remain in the biomass after collection. Also, surplus products at the auctions as well as food waste at the supermarkets contain impurities, such as labels and cardboard or plastic packaging material. De-packaging before upcycling is a laborious and costly extra pretreatment (KTN, 2016). Besides physical impurities, also chemical and microbial contamination with for example pesticides, mycotoxins and soil residues can impede subsequent valorization by compromising the chemical and microbiological safety. To avoid the presence of soil contamination on harvesting byproducts for example, an adaptation of the harvesting equipment may be required, impacting the economic feasibility of the valorization (Agneessens et al., 2014; OVAM, 2015b; Van Buggenhout et al., 2016). For example, pumpkins grown for their seeds, are harvested and the seeds are automatically separated. The residual pumpkin biomass is thrown back on the field, complicating or even prohibiting collection of pure pumpkin biomass due to the presence of soil and stones (personal communication Van Droogenbroeck, 2016). Surplus products occurring at the produce auctions will be less susceptible to this chemical and microbial contamination, as the products have been approved for sale and have to comply with the food standards regarding chemical and microbiological safety. Also the byproducts

resulting after food processing generally score better on this form of impurity as their lack of purity is often only of an esthetic nature (e.g. freezing, canning). In many cases, this is not an issue when subsequent processing is applied. The purity of the harvesting byproducts on the other hand can be more obstructing for some derived products. For example, valorization into food products will be subjected to a more stringent regulation regarding purity compared to valorization into compost. This stresses the importance of tuning the targeted end product on the achievable degree of purity of the horticultural byproduct (KTN, 2016; OVAM, 2014).

### 6.2.3 Application of the above mentioned criteria on the choices made in this dissertation

For the choices of the two crops investigated in this dissertation, namely tomato and Belgian endive, the logistic traits mentioned above were taken into account to the best of our ability.

A class of byproducts, namely the surplus products occurring at the produce auctions, was selected in Chapters 2, 3 and 4 to evaluate the suitability of the spiral-filter press for processing horticultural byproducts.

- **Geographical occurrence & collectability:** centralized.
- **Seasonal occurrence:** present but constant supply of different crops throughout the year.
- **Purity:** products meet commercial criteria.

Within this class of byproducts occurring at the auctions, tomato was chosen as an illustrative case.

- **Amount:** tomatoes are one of the major byproducts occurring at the auctions (3.1 10<sup>3</sup> tonnes in 2015).
- **Ability for storage:** rather than choosing a crop that scores high on the ability for storage, a perishable crop, susceptible to a quick deterioration was chosen to demonstrate the potential of the processing technology.

In contrast to the choice of a class of byproducts in Chapters 2, 3 and 4, Belgian endive roots were selected as biomass feedstock for the study in Chapter 5.

- **Amount and seasonal occurrence:** yearly approximately 40,000 tonnes in Flanders, arising consistently throughout the year.
- **Geographical occurrence:** concentrated in Flemish Brabant (about 40 %) and West-Flanders (about 40 %) (personal communication National chicory research center, 2013).

- **Purity and collectability:** predominantly hydroculture (about 95 %) after which the forced roots are free of soil residues and easy to collect.
- **Ability for storage:** firm root structure making the roots less susceptible to microbial degradation during storage.

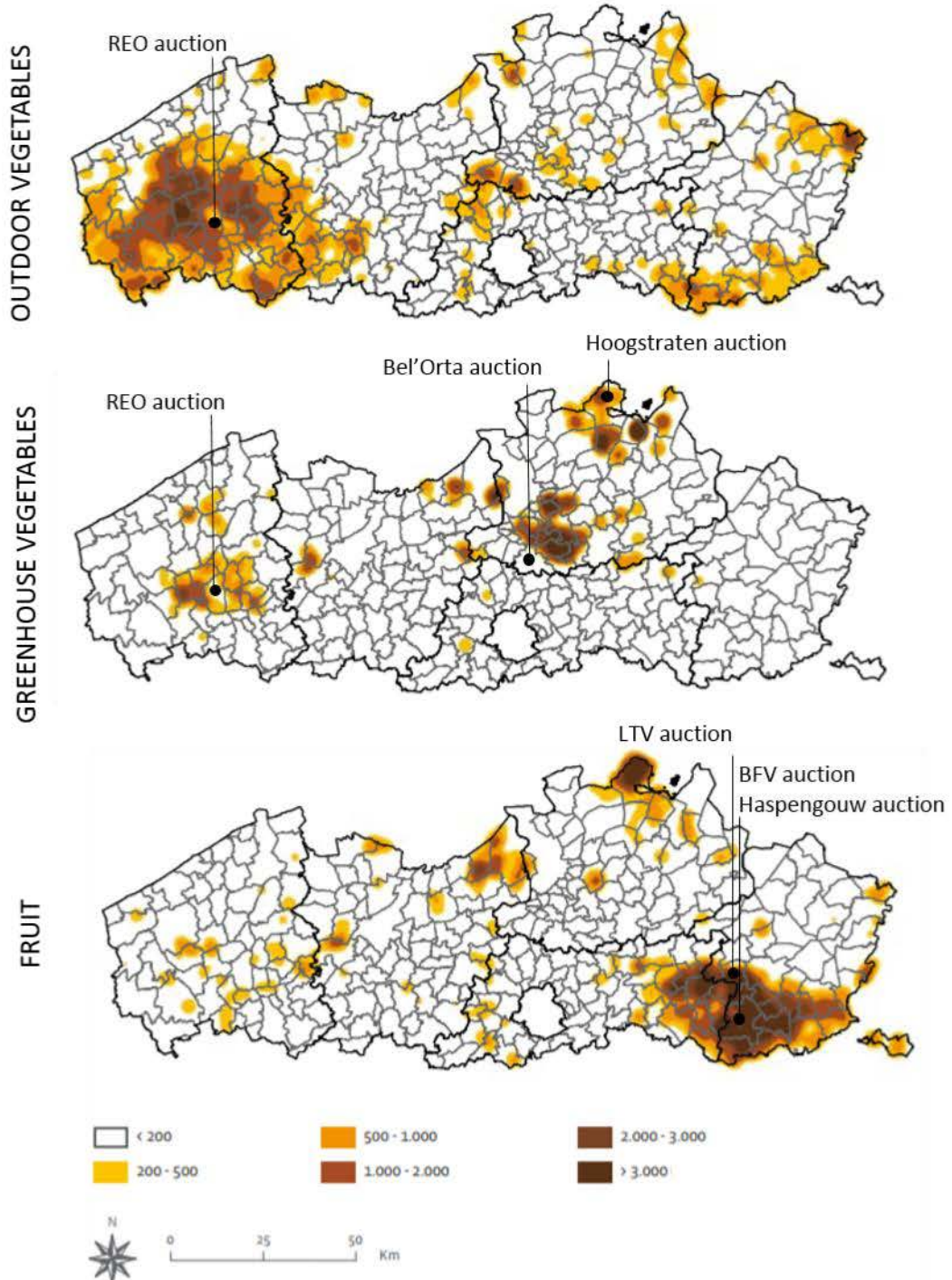
Thus, in conclusion, the quality of the data on the amount of byproducts is limited and in need of amelioration. In addition to the amounts, the impact of five other traits was discussed regarding their effect on the feasibility of the valorization. Some of these aspects appear to be less impeding for byproducts generated at the produce auctions and during food processing, compared to harvesting byproducts (e.g. geographical occurrence, collection, purity). Other aspects are inherently present in almost all horticultural byproducts (e.g. small amounts, difficult storage due to high moisture content, seasonal occurrence), which requires a processing strategy appropriately tuned on these traits. Depending on the targeted end product, the degree of manifestation of these aspects differs. End products with a higher added value (e.g. bioactive compounds) may impose stricter quality-related conditions on the feedstock, whereas lower-value end products may be more depending on the available amounts and geographical occurrence (e.g. anaerobic digestion and composting).

### **6.3 Processing: is the spiral-filter press suitable to valorize horticultural byproducts?**

Byproducts occurring at the auctions suffer less from geographical spread, difficult collectability and lack of purity, which may increase the feasibility of their valorization (Figure 6.2). Other aspects such as difficult storage and seasonal occurrence are however inherently present in predominantly all horticultural byproducts, including those at the auctions. Therefore, they require a processing technology appropriately tuned on these traits.

The high moisture content is the first common denominator of almost all horticultural byproducts, leading to a difficult storage. Therefore, a pressing technology was selected which is able to perform a separation of liquid and solid fractions, aiming at minimal waste and optimal utilization of all fractions. Furthermore, three additional constraints were imposed on this pressing technology in order to increase the chances of successful valorization towards food, which was primarily targeted, in line with the cascade principle (OVAM, 2012b; 2015). Firstly, the relatively small and geographically dispersed volumes of byproducts and the seasonality of their production can obstruct the feasibility of the valorization process. This can be increased by choosing a technology flexible in type of feedstock and able to process different inputs (Budzianowski & Postawa, 2016; Fava et al., 2015; Lin et al., 2014;

Matharu et al., 2016). Secondly, consumers increasingly demand attractive products (i.e. products with attractive color, appearance and taste) which thirdly, maximally retain the naturalness of the fruits and vegetables. Hence, a technology that is able to meet these demands is expected to generate products that are competitive and may be able to lead to a successful valorization of horticultural byproducts.



**Figure 6.2** Intensity map of the horticultural sector in Flanders, expressed in euro standard output per ha in 2011 (Platteau et al., 2012).

These three targeted technology characteristics (flexibility towards feedstock, attractive output products and limited process impact) are used to evaluate the suitability of the spiral-filter press for valorizing horticultural byproducts, drawing further on the results obtained in Chapters 2, 3 and 4 (part 6.3.1 – 6.3.3). This fractionation in liquid and solid products can be seen as a pretreatment to be complemented either with further refining and conversion (in line with the biorefinery concept) or with stabilization of the derived homogeneous fractions, depending on the desired amount of refining and the envisaged end products. In this dissertation, a thermal processing step has been included to stabilize the derived products, which is also critically assessed hereafter (part 6.3.4) (Figure 6.3).

### 6.3.1 Can the spiral-filter press be used to process different matrices?

In Chapters 2, 3 and 4, the processing of tomato was described. Besides tomatoes, also apples and pears have been processed with the spiral-filter press, as described by De Paepe (2015a, 2015b). Furthermore, a range of other matrices including banana, strawberry, beans, carrot, cauliflower, celeriac, celery, leek, peas, red beet, salsify but also corn and nuts have been successfully processed recently with this spiral-filter press (data not included in this dissertation, personal communication De Paepe, 2016). This clearly confirms its flexibility towards different types of feedstock.

In Chapter 2, more insight into the working principle of the spiral-filter press was gained. This was based on tomato, but the insights can be extrapolated to the processing of different feedstocks. The general working principle consists of a combination of (i) compression forces, exerted by both the feed pump and the spiral rotation and (ii) underpressure acting on the mash through the filter element, which lead to an effective separation of solid and liquid fractions. Conventional pressing technologies are often only based on one driving force. Juice extraction with a horizontal rotary press or belt press for example is based on the application of compression forces whereas the working principle of the decanter is based on centrifugal forces only (Lozano, 2006; Rombaut et al., 2014).

For soft, berry-like matrices such as tomato, a high spiral and vacuum frequency appeared to be crucial in order to obtain a high juice yield. In contrast, this spiral frequency was found to be negatively correlated to the juice yield when processing harder products such as apples and pears. Indeed, as the latter type of matrix did not lead to a blocking of the filter pores, the lower spiral frequency increased the residence time in the extraction cell, thereby raising the juice yield (De Paepe et al., 2015a; 2015b). Furthermore, also the processing of more liquid products, which may result from a thermal or enzymatic pretreatment, was investigated. It was found that this type of input requires processing with less steep spiral channels. This can be explained by the fact that softer, more liquid products are less susceptible to compressing forces, which consequently implies that an increased steepness does not

generate any extra driving force. Moreover, less steep channels lead to an increased residence time of the liquid input product, thereby improving the juice extraction. Processing of very fluid pulps, derived from soft fruits such as raspberries and strawberries are often difficult to squeeze during pressing with conventional pressing technologies, such as horizontal and belt presses (Beveridge & Rao, 1997). Furthermore, soft tissue can collapse under compression forces, leading to particles that clog the escape channels (Beveridge & Rao, 1997; Roberts et al., 2004). In contrast, the rotating spiral in the spiral-filter press scrapes the surface of the filter which clears the pores and assures a continuous juice extraction.



**Figure 6.3** Tomato processing: A) washing, B) milling, C) pressing with spiral-filter press into D) press residue and E) juice, F) thermal pasteurization and G) resulting products, i.e. mashed tomato, press residue, non thermally treated tomato juice, thermally treated tomato juice.

### 6.3.2 Can the spiral-filter press produce attractive and natural products?

A technology able to produce tasty and fresh products with an attractive color and without the use of additives or preservatives can increase the market value of the byproduct-derived food products.

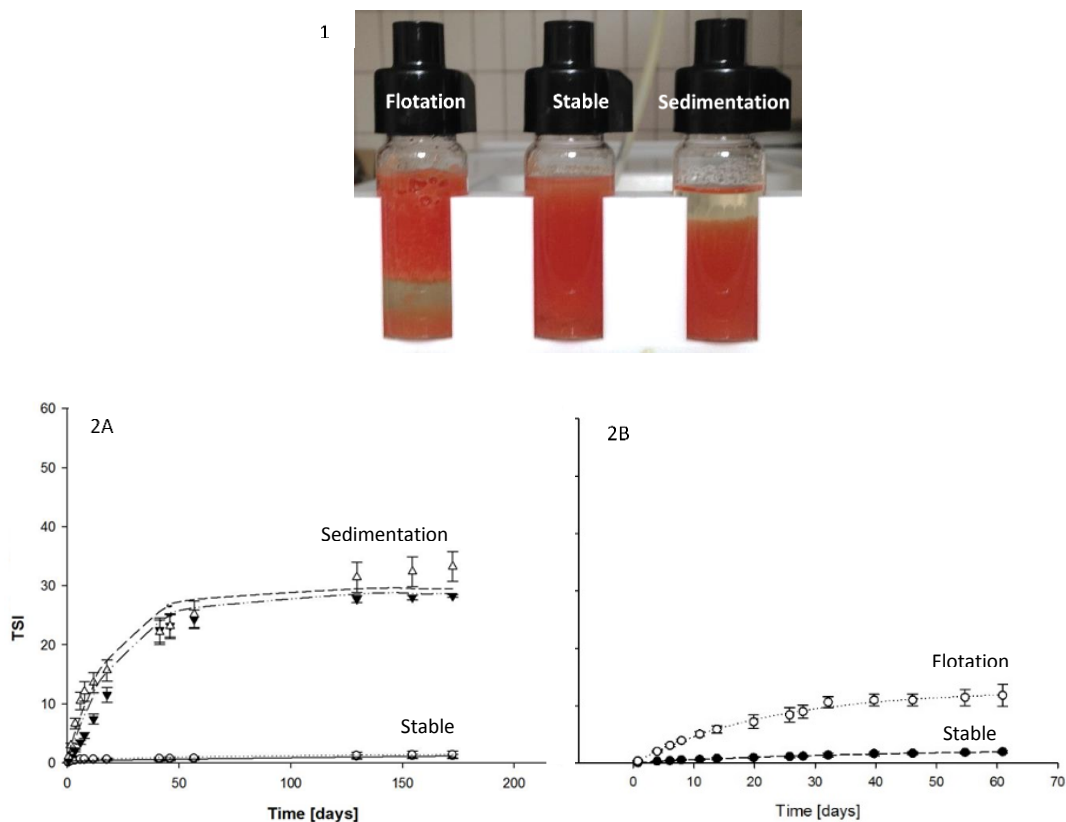
#### 6.3.2.1 Liquid end products

In Chapter 2, the processing conditions were tuned to (i) achieve optimal juice yield without using enzymes and (ii) to refine the tomato in different fractions. Hence, insight was gained into the effect of changing parameters on the process performance and juice characteristics. Even though these insights are crucial to understand the working principle of the spiral-filter press, the optimization needs

to be performed case per case, for each novel matrix. For example, changing the process conditions gave rise to fractions of different turbidity (juices and purees). It was found that the filter pore size primordially influenced the turbidity of the juice and the amount of insoluble particles. As expected, a larger filter pore size gave rise to a thicker, more turbid juice comparable to a soup or a passata (Figure 6.3). However, processing of peas with the 5-channel spiral instead of a 4-channel spiral changed the end product from a juice to a puree while the same filter was used (data not included in this dissertation). Thus, only by changing the spiral and keeping the filter constant, the turbidity and viscosity of the juice was changed. This was caused by the fact that the channels were not completely filled when the 4-channel spiral was used. Using the 5-channel spiral, they were completely filled, which led to a deeper vacuum and thus to a greater driving force for particles to travel to the juice fraction, leading to a more turbid juice. This knowledge could not be derived from our experiments on tomato, as in all experiments the channels were completely filled with tomato mash. It is thus of vital importance to generate a broad knowledge on the working principle of the spiral-filter press, by processing a wide variety of raw materials in order to be able to identify and fully understand the combined effect of the different process parameters on the output characteristics. This knowledge is also necessary for the daily operation of the spiral-filter press. Especially when processing byproducts, variation in feedstock characteristics within one type of biomass often occurs. Variation in color or texture will for example exist due to variation in moment of harvesting or temperature of storage. This variation can generate differences in the optimal processing conditions. For example, cold storage of tomatoes can lead to discoloration, chilling injury, decreased softening, decreased weight loss and altered aroma profiles (Farneti et al., 2015; Tadesse et al., 2015). Different tomato cultivars are known to have a different tolerance to chilling stress and their processing can thus affect the process performance and resulting products. For example, a change in firmness can change the effect of milling and the compressibility of the matrix in the spiral-filter press, influencing the juice yield and the juice characteristics (e.g. particle size distribution). Such a change in firmness can be the result of a difference in enzymatic activity which could in turn also affect the stability of the resulting tomato juice. Therefore, no rigid, unique protocol per type of biomass can be provided. Rather some guiding principles and an *ad hoc* finetuning of the process parameters is necessary which requires a good understanding of the working principles of the press. The work performed in this dissertation subscribes to this, but it is clear that a larger range of experiments on additional feedstocks should be performed in the future to further increase this knowledge.

In Chapter 3, the physical stability of the resulting tomato juice was investigated, as the visual appearance of a cloudy drink is a decisive factor for consumer acceptance (i.e. homogeneous distribution without sedimentation or flotation) (Beveridge, 2002). We were able to produce a stable

tomato juice without sedimentation (Growdena JFO). Quick processing with a large filter size and maximum vacuum were process parameters found to beneficially affect the stability of tomato juice. However, using exactly the same process conditions on another variety (Merlice JFO) generated an unstable juice subjected to sedimentation (Figure 6.4). This illustrates that changing the process parameters alone is often not sufficient to control the juice stability. This effect of variety on stability can partly be attributed to the difference in inherent water insoluble solids (WIS) characteristics (Barrett et al., 1998; Beveridge, 2002; Kaur et al., 2007; Kubo et al., 2013) as described in Chapter 3. However, we believe that the effect of variety on the stability is predominantly due to a different enzymatic activity, inherent to different varieties (Aghajanzadeh et al., 2016; Laratta et al., 1995; Moelants et al., 2014). As this has not been investigated, we cannot make an unequivocal assessment of the effect of process parameters on juice stability. Therefore, in future assessments of the spiral-filter press juice stability, we recommend to include additional aspects for example via measurements of the concentration and activity of the enzymes active in the cloud stability of tomato juice (pectin methylesterase and polygalacturonase) or investigation of particle interactions.



**Figure 6.4** 1) Tomato juice subjected to flotation, stable tomato juice and tomato juice subjected to sedimentation. 2A) Turbiscan Stability Index (TSI) profile of stable tomato juice and unstable tomato juice, subjected to sedimentation. 2B) TSI-profile of stable tomato juice and unstable tomato juice, subjected to flotation.



In that manner, the influence of varying process variables and different varieties on both WIS characteristics and enzymatic activity can be further elucidated and consequently be used more effectively to control the juice stability.

For the choice of the filter in Chapter 4, we used the information obtained in Chapter 2 and 3. As it was shown that larger filter pores lead (i) to an increased yield and (ii) to more and larger WIS (which contribute to a higher juice stability), a larger filter unit of 500  $\mu\text{m}$  (instead of 300  $\mu\text{m}$  or 60  $\mu\text{m}$ ) was used. Furthermore, the experiments were performed on the cultivar most susceptible to juice sedimentation (Merlice). Interestingly, another type of instability was found in this case. Instead of sedimentation, as seen in Chapter 3 for this variety, a flotation phenomenon was observed here (Figure 6.4). Due to the dense macrostructure of the tomato juice produced with a 500  $\mu\text{m}$  filter unit, the air bubbles inherently present in the tomato fruit and those introduced during milling, were not able to escape from the juice during processing (not during the production in the spiral-filter press, nor during storage in the vacuum storage tank or during pasteurization). After filling and storage in sealed bottles, these air bubbles gradually rose upwards in the juice, thereby taking part of the insoluble solids with them. This resulted in flotation, leaving a clear-colored serum at the bottom of the bottles. By including a hot vacuum degassing step (60 °C at 100 mbar for 3 sec) before the pasteurization treatment, we were able to avoid this type of instability. Briefly, increasing the temperature of the juice decreased its viscosity which allowed the trapped air bubbles to escape. Furthermore, the increasing juice temperature also decreases the gas solubility which could have contributed to the phenomenon of escaping gas bubbles. A better understanding of these different physico-chemical processes is crucial for further product development. In most cases stable juices are demanded, however transparent juices with a fruit or vegetable taste have also been shown to gain interest during this research project.

#### 6.3.2.2 Solid end products

Although this dissertation focused on the optimization of the quality and appearance of the liquid products, the press residue is another important fraction resulting from the spiral-filter processing. Its added value for the food and feed industry has been investigated extensively, predominantly on lab scale and is mainly associated with the high fibrous content and the associated phytochemicals.

Multiple studies have evaluated the potential of the press residue of tomato for use in food as functional or nutritional ingredient (e.g. O'Shea et al., 2009). Sogi et al. (2002) for example have investigated the utilization of tomato seed cake in bread, which improved loaf volume, texture and crumb quality. Another example is tomato pulp powder addition to ketchup as a thickener, thereby improving the color and texture of the product (Farahnaky et al., 2008). Dried tomato peels have been reported to be a useful food ingredient due to their fiber content and have been included in fermented

sausages to test the increase in nutritional value due to the presence of lycopene (Calvo et al., 2008; García Herrera et al., 2009). A myriad of other studies and projects have focused on the added value of similar fruit and vegetable processing byproducts such as berry pomace, defatted strawberry and blackcurrant seeds, orange pomace, lemon pomace, apple skin powder in food (Balasundram et al., 2006; Larrauri, 1999; Kamerer et al., 2014; O'Shea et al., 2012; Papoutsis et al., 2016; Rombaut et al., 2014; Šarić et al., 2016; SUSFOOD ERA-Net Berrypom project<sup>20</sup>).

Although not specifically addressed in the preceeding chapters, the press residue obtained from the spiral-filter processing of tomato (Chapters 2, 3 & 4) has been further subjected to some experiments regarding their valorization (unpublished results, Figure 6.5). The seeds and peels were separated using a flotation-sedimentation process in water. The seeds were dried in a fluidized-bed air-dryer and mechanically cold-pressed, yielding approximately 13 % oil. The oil consisted predominantly (80 %) of unsaturated fatty acids (linoleic and oleic acid) and was characterized by a low peroxide value ( $3.13 \pm 0.34$  mEq/kg) and a low amount of free fatty acids ( $0.287 \pm 0.024$  g/100g oil), which is in accordance with the findings of Zuorro et al. (2014). This tomato seed oil was found to be an interesting ingredient in cosmetic products and the commercial production is currently being investigated by an industrial partner (personal communication Schatteman, 2016; Zuorro et al., 2014). The remaining seed hulls contained a relatively large amount of fat (15 %) and protein (32 %) which indicates their potential as food and feed ingredient. This has also been shown by Sarkar & Kaul (2014). As shown by Sarkar & Kaul (2014) and confirmed in Chapter 4, the peels were characterized by a high phenolic content and antioxidative capacity which might be valuable in food- or feed-derived products. Formulation of the dried tomato peels and seed hulls in bread did not yield promising results, as they led to an increasing water absorption and a decreasing bread volume (predominantly in case of the dried tomato peels) (personal communication Mouton, 2016). These results are however preliminary and have to be complemented with additional research and development.

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<sup>20</sup> <http://berrypom.mw.tu-dresden.de/index.html>



**Figure 6.5** Experiments to separate and valorize the tomato press residue (unpublished results). A) press residue separation, B) peels, C) seeds, D) fluidized bed dryer, E) oil press, F) tomato seed oil, G) tomato seed hulls, H) dried tomato peel and I) dried fractions of seed hulls and peels incorporated in bread.

### 6.3.3 Can the spiral-filter press produce qualitative end products?

In Chapter 4, the quality-preserving character of the spiral-filter press for phenolic compounds, carotenoids and ascorbic acid was studied.

#### 6.3.3.1 Total retention efficiency

The retention efficiency of the different phenolic compounds and carotenoids during filtration was never significantly lower than 100 % (except for quercetin). The content of the highly labile ascorbic acid was also conserved upon processing with the spiral-filter press. This high retention of micronutrients is very important in regard to maintaining the native constitution and nutritional quality of the feedstock. A significant decrease in lightness however was observed (data not shown), which might be attributed to enzymatic dark color formation, as a result of polyphenol oxidase (PPO) activity. This could be related with the observation of a slight parallel decrease in the concentration of chlorogenic acid, which is reported to be a PPO-substrate in various fruits and vegetables such as tomato, eggplant, and apple (Casado-Vela et al., 2005; De Paepe et al., 2015b; Mishra & Gautam, 2016; Turk et al., 2012). Specific PPO-measurements should be performed in order to confirm this phenomenon. The highly abundant and susceptible chlorogenic acid could be used as a quality indicator in future experiments.

Processing with other presses often does not lead to a conservation of phenolic compounds. Taking the case of apple as an example, as one of the most studied juices, not all of the native phenolic compounds present in the apple mash could be found after pressing either in the juice or in the press residue when using a belt press (Turk et al., 2012) or a horizontal rotary press (van der Sluis et al., 2002). These observations can probably be attributed to enzymatic oxidation. Actually, the production of a juice with a low content of phenolic compounds is sometimes actively pursued, since phenolic compounds can contribute to undesired astringency or haze formation and inhibit the pectolytic enzymes, used to increase the juice yield (Beveridge, 2000; van der Sluis et al., 2002; Will et al., 2008). Therefore, effective polyphenol removal techniques are used such as grinding to open air, mash aeration before enzymatic incubation and pressing, mash oxidation during pressing and clarification after pressing using clarifying agents such as polyvinylpyrrolidone which lead to dramatic reduction in the phenolic content (García-Torres et al., 2009; Le Bourvellec & Renard, 2012; Oszmiański et al., 2007; Turk et al., 2012; van der Sluis et al., 2002). However, following the trend for natural, high quality and minimally processed products with clean labels (Balasundram et al., 2006; Kammerer et al., 2014; Kasapidou et al., 2015; Moure et al., 2001; Sharma et al., 2016), these practices are increasingly being avoided, focusing on the production of cloudy juices with a higher phenolic content (Markowski et al., 2015; Will et al., 2008). Production of such juices and avoiding oxidative degradation using the conventional equipment, requires specific modifications (e.g. pressing under inert atmosphere, additional degassing of dissolved oxygen) or the use of quality preserving additives (e.g. ascorbic acid addition) (García-Torres et al., 2009; Markowski et al., 2015; Will et al., 2008). A clear advantage of the spiral-filter press is that it allows juice extraction under low oxygen conditions, thereby limiting oxidation of the phenolic compounds, carotenoids and ascorbic acid. The effect of conventional pressing systems on the fate of carotenoids has been less extensively investigated. Aspects such as oxygen, light, metals, enzymes and severity of the treatment have however been reported to affect their presence (Rodríguez-Amaya, 2001). In conventional tomato-processing for example, the straining process is often associated with a reduction of carotenoids due to oxidation. The high rotation speed in the straining equipment generates large amounts of dissolved air in the tomato juice that can quickly destroy substantial amounts of lycopene. In addition, the presence of light and use of fine metal screens in this filtration process are reported to promote lycopene oxidation (Reyes-De-Corcuera et al., 2014; Shi & Le Maguer, 2000).

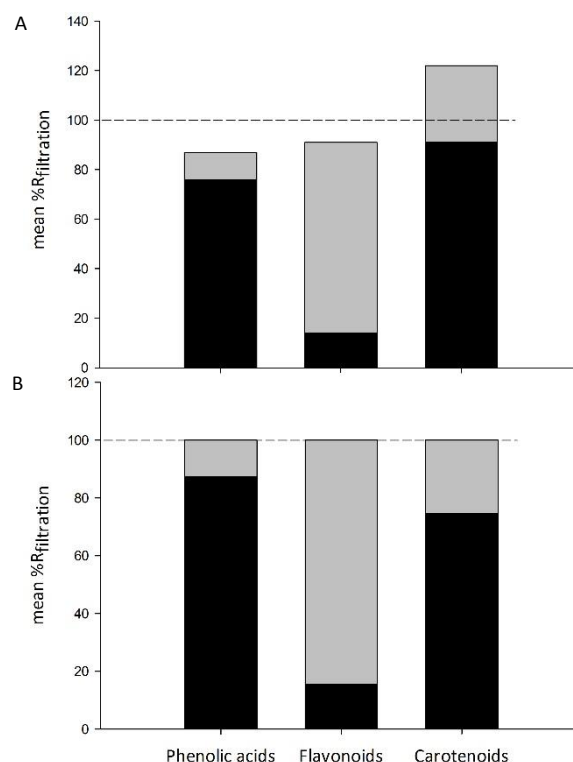
#### 6.3.3.2 Tissue localization

The resulting juice and press residue were characterized by a different composition (Chapter 4). In absolute amounts, it was found that the press residue contained more phenolic compounds and carotenoids, which has already been reported earlier (Shi & Le Maguer, 2000; Stewart et al., 2000;

George et al., 2004; Slimestad & Verheul, 2009; Toor & Savage, 2005). Physiologically, this can be explained by the role of dermal plant tissues in the protection against damage caused by external stress conditions (Toor & Savage, 2005). Thus, even though the press residue mass only comprised 12.2 % of the total processed tomato mass, its composition stresses the importance of valorizing such press residue fractions, which is in line with the idea of total biomass valorization.

Despite the higher absolute concentration in the press residue for phenolics and carotenoids, the press residue extraction efficiency was in both cases lower compared to the juice extraction efficiency. This can be attributed to the larger mass of the juice fraction. Taking into account the juice yield, about 49 % of the phenolic compounds present in the mashed tomato end up in the juice fraction after filtration. This average value might be somewhat misleading as the juice extraction efficiencies of phenolic acids and flavonoids are 76 % and 14 %, respectively. This could be explained by the more hydrophilic nature of the phenolic acids compared to the flavonoids (Choudhury et al., 1999; van Dijk et al., 2000). Furthermore, also the compound specific tissue location of the phenolic compounds can affect these findings. In contrast to phenolic acids, which were reported to be more evenly distributed in all tissues of tomato fruit, flavonoids have been found to be mainly located in the solid parts of the tomato (skin and seeds), which may complicate their extraction due to interaction with cell wall compounds (Padayachee et al., 2017; Slimestad & Verheul, 2009; Stewart et al., 2000; Toor & Savage, 2005). Analogous trends have been found in apple juice production (van der Sluis et al., 2002; Will et al., 2008). Approximately 91 % of the initial carotenoids present in the mashed tomato fraction were found in the juice. Despite their hydrophobic character, this juice extraction efficiency was thus higher compared to that of the phenolic compounds. However, the higher retention efficiency of carotenoids (122 %) compared to that of the phenolic compounds (88 %) can bias the interpretation of this figure (see Chapter 5). When this retention efficiency was scaled to 100 %, the contribution of the juice efficiency was calculated and showed a larger value for the phenolic acids (87 %) compared to carotenoids (75 %), followed by flavonols (15 %) (Figure 6.6). This trend is in accordance with the absolute concentrations in the tissues, where the largest enrichment in press residue was found for the flavonoids, followed by lycopene and phenolic acids. Other aspects can influence the final distribution such as the intracellular location. Carotenoids are for example synthesized and stored in chloro- and chromoplasts and are thus harder to extract due to interaction with proteins (Padayachee et al., 2017). Also interaction with plant cell wall compounds can occur and influence the extractability. For example, phenolic compounds present in the cell vacuoles, are released upon rupture of the cell wall, making them able to bind with proteins and polysaccharides of the cell walls (Hutzler et al., 1998; Le Bourvellec & Renard, 2012; Padayachee et al., 2017). Thus, several factors can influence the final

compound distribution and extractability such as the native tissue location, intracellular location and interaction with the cell wall compounds (Padayachee et al., 2017; Slimestad & Verheul, 2009).



**Figure 6.6** A) Measured retention efficiency during filtration, constituted out of juice extraction efficiency (black) and press residue extraction efficiency (grey). B) Retention efficiency scaled to 100 %, constituted out of juice extraction efficiency (black) and press residue extraction efficiency (grey).

The cause of the carotenoid retention efficiency larger than 100 % (122 %) and the phenolic retention efficiency smaller than 100 % (88 %) could be related to the effect of the spiral-filter treatment on the compounds extractability and on their vulnerability to oxidation. It appears that pressing under vacuum increases the extraction efficiency of carotenoids compared to maceration, without destructing them and leading to a larger concentration of carotenoids after spiral-filter processing. This particular effect has not yet been reported, however, there is clear evidence that juicing (Reboul et al., 2006; Tydeman et al., 2010a) and other processing technologies (mechanical, chemical and enzymatic) significantly enhance the carotenoid bioaccessibility and extractability (Michelon et al., 2012; Moelants et al., 2012). Shi & Le Maguer (2000) have attributed this to the dissociation of carotenoids from the plant matrix upon processing. The smaller figure of phenolic retention efficiency on the other hand could imply that these compounds are more sensitive to oxidation, compared to carotenoids (Kalt, 2005).

Thus, whereas the amount of degradation and the resulting total amount of micronutrients present in the processed products is influenced by (i) processing technology and processing conditions (oxidative environment, duration, temperature, etc.) and (ii) type of compounds (vulnerability to degradation), the composition of the resulting end products is mainly dependent on the compound characteristics (native tissue location, intracellular location, interaction with the cell wall compounds, solubility). The processing conditions might slightly influence the final composition though, for example the milling technology might increase the press-residue-related compounds in the juice fraction. Also changing the filter size might allow more press residue particles in the juice fraction.

### 6.3.4 Which are the next processing steps?

Based on the experiments performed, the spiral-filter press was found to be a suitable technology able to tackle the major hindering traits of horticultural byproducts thus leading to a generic applicability for a range of feedstocks. Also the quality-preserving characteristics regarding the phenolic compounds, carotenoids and ascorbic acid content in the end products, were shown to be positive.

The liquid and solid end products are however still susceptible to deterioration, thus further processing steps are necessary. As mentioned above, these can consist of further refining and conversion or only stabilization of the derived homogeneous fractions. The latter was performed in this dissertation for the liquid fractions. A thermal treatment was proposed in Chapters 3 and 4. From these results, a conservation of ascorbic acid and the predominant phenolic compounds was found upon processing. Furthermore, a significant decrease in redness were observed. This was in line with a significant decrease (about 46 %) in the carotenoid content. This confirms that conventional thermal treatment may negatively affect the physical, nutritional or bioactive properties of fruit and vegetable juices, as also shown in literature (Abushita et al., 2000; Capanoglu et al., 2008; Dewanto et al., 2002; Gahler et al., 2003; Georgé et al., 2011; Jiménez-Sánchez et al., 2017a; Koh et al., 2012; Nayak et al., 2015; Oms-Oliu et al., 2012). In order to preserve the benefits associated with the minimal processing impact of the spiral-filter press, a mild stabilization technology should be included in the future. In this regard, novel technologies are increasingly being investigated such as radiation treatments (UV light, high-intensity light pulses,  $\gamma$ -irradiation), electrical treatments (pulsed electric fields, radiofrequency electric fields, microwave heating, ohmic heating), ultrasound treatment, high hydrostatic pressure (HHP), inert gas treatments (supercritical carbon dioxide, ozonation) and combinations thereof (Barrett & Lloyd, 2012; Jiménez-Sánchez et al., 2017a; 2017b; Oms-Oliu et al., 2012; Pereira & Vicente, 2010; Turk et al., 2012). While some technologies are already being exploited at commercial scale (e.g. HPP, PEF, microwave), others are only tested at lab scale. Furthermore, a lack of standardization in operation

conditions make comparisons between different studies difficult. More research on their performance on pilot and industrial scale is thus necessary (Jiménez-Sánchez et al., 2017a). The application of some of these novel technologies (HHP and pulsed electric field) as pretreatment or conservation in combination with the spiral-filter press was recently the subject of investigation in the follow-up research project HighQJuice<sup>21</sup>.

#### **6.4 Output: can the chemical profile be used to explore the potential bioactivity?**

In this dissertation, the distribution of different phytochemicals in Belgian endive was mapped (Chapter 5, Figure 6.7) and the process impact of the spiral-filter press was investigated on the phenolic and carotenoid content of tomato (Chapter 4). This generates valuable information on the type and abundance of certain phytochemicals which can be used as a basis to explore potential functionality of derived products. Indeed, a range of potentially health-promoting activities have been attributed to these compounds, which has aroused interest for their use in the area of nutrition and food science such as functional foods and nutraceuticals (Bohn et al., 2015; Gowe, 2015; O'Shea et al., 2012; Porrini & Riso, 2008; Schieber et al., 2001).

Intake of foods rich in phenolic compounds and carotenoids has been associated with a reduced incidence of cardiovascular diseases, diabetes mellitus and several types of cancer (Bazzano, 2005; Bohn et al., 2015; Del Rio et al., 2013; Dillard & German, 2000; Rodriguez-Mateos et al., 2014; Williamson & Manach, 2005). Although less extensively studied, the sesquiterpene lactones have also been attributed with a range of potential activities, although often not specifically for the structures found in this study or more generally related to an extract containing SLs (Amorim et al., 2013; Ghantous et al., 2010; Picman, 1986; Prakash & Gaikwad, 2012). Pharmacological properties include antimicrobial, antiprotozoal, anticancer, antihelmintic, anti-inflammatory and analgesic activity (Bischoff et al., 2004; Chadwick et al., 2013; Chaturvedi, 2011; Ghantous et al., 2010; Picman, 1986; Padilla-Gonzalez et al., 2016; Prakash & Gaikwad, 2012). Historically, SLs have been the active ingredient in folk medicines for many treatments such as diarrhea, burns, influenza, and neurodegradation (Chadwick et al., 2013; Ghantous et al., 2010; Padilla-Gonzales et al., 2016). They have gained interest for treating human diseases due to their potential for the treatment of inflammation, cardiovascular diseases, cancer and more. Today, the WHO recommends artemisinin-based combination therapies for the treatment of malaria (WHO, 2016). Some guaianolides (a specific

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<sup>21</sup> <http://www.flandersfood.com/projecten/highqjuice>



type of SLs, particularly present in *Cichorium* genus (de Kraker, 2002)) are highly specific anti-tumor agents that inhibit aromatase and thus possess potential applications in the treatment of breast cancer (Padilla-Gonzales et al., 2016). Drugs derived from parthenolide, thapsigargin and artemisinin have reached cancer clinical trials (Ghantous et al., 2010).

However, even though a compound group may be associated with a certain functionality, there is a great difference between showing the presence of a certain compound in a given matrix, which has been associated with a certain functionality on the one hand and drawing conclusions about their biological activity *in vivo* on the other hand.

#### 6.4.1 The presence of a bioactive compound is no guarantee for its bioactivity in derived products

In order to exert a health benefit, the compound must be bioavailable (D'Archivio et al., 2010; Gonzalez et al., 2015; Padayachee et al., 2017; Rein et al., 2012). This can be defined as “the fraction of the ingested nutrient or compound that reaches the systemic circulation and the specific sites where it can exert its biological action” (Porrini & Riso, 2008). Hence, a high *in vitro* functionality does not necessarily guarantee a high bioavailability and bioactivity in the human body. Thus to establish evidence of a certain bioactivity in the human body, evaluating the bioavailability is a first crucial step (D'Archivio et al., 2010; Williamson & Manach, 2005). Several factors are known to affect this bioavailability, which can be grouped in three categories: (i) the chemical structure and concentration, (ii) the matrix and (iii) the host-related factors (D'Archivio et al., 2010; Padayachee et al., 2017). Variation in these factors can create variation in the bioavailability and thus the bioactivity. This bioavailability and the influencing factors will be discussed hereafter in the context food products, drawing further on the data generated in this dissertation.

##### 6.4.1.1 Chemical structure and concentration

The concentration and chemical structure are among the main factors influencing the bioavailability of a compound. Structural features such as the configuration of compound, the degree of molecular linkage (e.g. glycosylation, esterification, acylation), conjugation with other compounds and solubility determine their rate and extent of absorption and metabolism, and accordingly their effect in the human body (Balasundram et al., 2006; D'Archivio et al., 2010; Gonzales et al., 2015; Porrini & Riso, 2008; Rein et al., 2012). This chemical structure and concentration may be impacted by natural variation as well as processing.

**a/ Natural variation**

In Chapter 5, it was shown that the types (free forms, conjugated forms, dihydroforms) and amounts of the SLs were strongly influenced by variety and cultivar. Depending on the individual SL, a variation of a factor up to 5 has been found to result from changing the cultivar while keeping the location, growing and harvesting conditions constant. Also the phenolic concentrations varied between cultivars and varieties, with differences of a factor 2 for example for chlorogenic acid (CHA) levels. Other external aspects that were not specifically investigated here, such as agricultural conditions, harvesting conditions and ripeness have also been reported to lead to different structures and concentrations of phytochemicals (D'Archivio et al., 2010; Manach et al., 2004; Tiwari & Cummins, 2013; Tomás-Barberán & Espín, 2001). Interpreting these varying results can be further complicated by the use of different extraction and analysis methods. In case of Belgian endive for example, traditional extraction methods allowed the measurement of the non-conjugated SL-forms only whereas the use of more sophisticated up-to-date methods led to the conclusion that the bound forms appear to be predominantly present (Annaratone et al., 2016; Sessa et al., 2000).

**b/ Variation induced by processing and different tissues**

The SL-profile of the Belgian endive roots was also shown to be influenced by storage and forcing treatment, as shown in Chapter 5. The four-month storage of the NF-roots significantly increased the bound SL-forms and significantly decreased the free SL-forms, which may indicate a partial conversion of free to bound forms upon storage. Forcing these roots led to a significant increase in most SLs (up to factor 3.5, depending on the individual SL). The effect of matrix was even larger, showing a higher concentration in the roots compared to the leaves (up to factor 39, depending on the individual SL). For the phenolic compounds, this effect of matrix was also significant with a two times higher level of CHA in the roots compared to the chicons and an up to 65 times lower concentration for the minor phenolic compounds. The effects of forcing and storage were smaller.

In Chapter 4, it was shown that filtration using the spiral-filter press had a minimal effect on the concentration of phenolics and carotenoids in tomato. The thermal treatment resulted in a conservation of the predominant phenolic compounds, whereas a significant decrease (about 46 %) was observed in the carotenoid content. Also a cyclization of naringenin chalcone was evoked by the thermal treatment. Finally, a three-month storage showed a 57 % decrease in ascorbic acid and no decrease in the majority of the phenolic compounds and carotenoids. The effect of a multitude of different processing technologies on the stability of a range of phytochemicals in different fruits and vegetables is widely described in literature. Both limited and strong effects on their concentration and conversion have been described, depending on factors such as matrix, phytochemicals, type of

processing, duration, temperature, pressure, presence of oxygen, etc. (Tiwari & Cummins, 2013; Tomás-Barberán, 2001).



**Figure 6.7** Belgian endive production and the resulting forced roots in hydroculture. A) Belgian endive roots grown in the field, B) Belgian endive chicons and forced roots after hydroculture, C) non-forced roots and forced roots with chicon, D-F) washed and cut forced roots.

#### c/ Possible implications of differences in chemical structure and concentration on horticultural byproduct valorization

Byproducts often have to be taken ‘as such’, rather than being produced with a predetermined goal (Lin et al., 2014; OVAM, 2014). Natural and processing-induced variation can thus lead to a feedstock characterized by a heterogeneous composition, which may obstruct the straightforward valorization of horticultural byproducts into functional products (Kasapidou et al., 2015). This may require an extra pretreatment for example by including a selective collection of certain varieties (Center of Expertise for Plant Compounds, 2016b; OVAM, 2014). Special conversion or purification steps may also be necessary to upgrade the byproducts, depending on the processing history (van der Goot et al., 2016). These aspects are less stringent when no specific bioactivity is targeted. For example, in case of processing horticultural byproducts in juices, different batches can be mixed based on their brix value in order to counter the difference in taste generated by the varying composition, as traditionally done by the beverage industry.

#### 6.4.1.2 Matrix

Besides the chemical structure and concentration, the food matrix is also a determining factor in the functionality of a certain compound. It has been shown that matrix structure and composition may positively or negatively influence the bioaccessibility and further the bioavailability (Bohn et al., 2015; Padayachee et al., 2017; Sensoy, 2014). This bioaccessibility is often seen as the first step of bioavailability and has been defined as “the fraction of a compound which is released from the food

matrix in the gastrointestinal lumen and thereby made available for intestinal absorption” (Rein et al., 2012; Saura-Calixto et al., 2007). This bioaccessibility is influenced by (i) the matrix structure and (ii) synergistic or antagonistic activity of compounds present in the matrix (Rein et al., 2012).

#### **a/ Matrix structure**

The physical state of the food matrix plays a key role in the release, accessibility and biochemical stability of food compounds (Palafox-Carlos et al., 2011). Cellular compartmentalization can physically trap phytochemicals and decrease their corresponding bioaccessibility. The binding of phytochemicals with food matrix constituents such as proteins, fibers, fat or alcohol may also affect the bioavailability of the phytochemical (Balasundram et al., 2006; D’Archivio et al., 2010; Padayachee et al., 2017; Rein et al., 2012). While dietary fibers and proteins are likely to cause detrimental effects on the phenolic and carotenoid bioaccessibility and absorption in the small intestine, the presence of dietary lipids appears to increase the bioavailability (Gonzalez et al., 2015; Lemmens et al., 2014; Palafox-Carlos et al., 2011).

Disruption of the matrix due to physical (e.g. chopping, cutting, slicing, trimming, mashing, juicing) and thermal processing steps (e.g. cooking, steaming, frying) can liberate phytochemicals and make them more extractable and bioaccessible (Bohn et al., 2015; D’Archivio et al., 2010; Sensoy, 2014). Physical processing for example decreases the particle size and thus increases the surface area for digestive enzymes. This leads to a greater release of phytochemical compounds from the matrix (Lemmens et al., 2014; Palafox-Carlos et al., 2011; Tydeman et al., 2010b). Thermal processing has been shown to break down cell constituents and thus enhance the levels of free phenolic compounds and carotenoids, probably attributed to an increased extractability (Chanforan et al., 2012; Dewanto et al., 2002; Porrini & Riso, 2008; Sensoy, 2014). The combination of mechanical and thermal treatment has also been reported to enhance the phenolic bioaccessibility, extractability and bioavailability. For example, Martínez-Huélamo et al. (2015) described an increased concentration of naringenin glucuronide in urine and plasma samples after consumption of tomato sauce compared to raw tomato. On the other hand, this cell disintegration may also lead to oxidation or degradation, which may decrease the health-beneficial effects of the compounds, as also illustrated above (6.4.1.1) (Bohn et al., 2015; Sensoy, 2014).

#### **b/ Interaction with other phytochemicals compounds**

Besides the food matrix structure, also the presence of other phytochemicals may influence the action or stability of certain phytochemicals. Combining foodstuffs can thus result in different bioaccessibilities. It is increasingly being conceived that phytochemicals do not act in isolation. They rather act together with many other compounds in the food matrix, leading to synergistic or

antagonistic effects (Jacobs & Tapsell, 2013). Several phenolic compounds have for example been shown to be better absorbed in the small intestine in the presence of additional phenolic compounds. The presence of antioxidants may also prevent oxidation of phytochemicals (Bohn, et al., 2014). The lycopene degradation vulnerability has for example been shown to be influenced by the presence of vitamin C, vitamin E, flavonoids and non-lycopene carotenoids (Capanoglu et al., 2010). Therefore, foods are increasingly considered as a whole, rather than as a sum of compounds (Dillard & German, 2000; van der Goot et al., 2016). This implies that studying the functionality of individual compounds or aiming for intense fractionation and recovery of isolated compounds may be misleading and may hamper the use of functional compounds present in the original matrix.

From this matrix effect some questions arise regarding the strategy to extensively biorefine horticultural byproducts into pure, isolated compounds or compound groups. Whereas this strategy might be beneficial to level out the differences in concentration and composition, often encountered in the byproduct feedstock, the bioactivity and thus the added value of the resulting isolated fractions might be limited.

#### 6.4.1.3 Host-related factors

Gaining insight in the bioactivity of a certain compound or byproduct may also be hampered by host-related factors leading to inter- and intraindividual variability. Inter-individual variability are the differences between individuals such as genetic make-up, exposure patterns, disease states, life stage, gender and physiological condition. Intra-individual variability are within-person fluctuations such as seasonality, time of awakening, stress, etc. (Almeida et al., 2009; Bohn et al., 2017). These aspects complicate the execution and outcome of human intervention trials. For example, two cancer prevention trials have suggested that high dosages of  $\beta$ -carotene, achieved by formulations with high bioavailability might lead to harmful effects, in contrast to the widely accepted epidemiologic evidence indicating that diets rich in carotenoid-rich fruits and vegetables are associated with a reduced risk of lung cancer (ATBC study group, 1994; Omenn et al., 1996; Dillard & German, 2000). A possible explanation could be the free-radical-rich atmosphere in the lungs of cigarette smokers that enhances the oxidation of  $\beta$ -carotene, after which the resulting oxidative metabolites might accelerate lung tumorigenesis (Dillard & German, 2000; Wang et al., 1999).

#### 6.4.2 Investigating bioactivity is not straightforward

Thus, measuring the amount of potentially bioactive compounds and gaining insight in the variation of their abundance is a first step towards elucidating the development of an effective functional end product, as performed in this research. The literature data available can be used to give an indication

of the presence or absence of a certain compound group and their potential functionality in a human body. However, a careful assessment is required, as simple extrapolation of the possible health-beneficial effects and generalizations for related products can be misleading. The putative bioactivity should thus be tested for different matrices individually, thereby investigating the stability and interaction with other food ingredients. The effect in the human body can be demonstrated by human intervention studies. However, these are very difficult to perform thus often *in vitro* or animal studies are carried out to elucidate the mechanism of action upfront (D'Archivio et al., 2010; Dillard & German, 2000; Moran et al., 2013). Great care is required when performing and interpreting the data from *in vitro* studies. Amongst others, both concentration and type of compounds used in these *in vitro* studies are important. The tested concentrations often exceed those occurring in real life. Physiological concentrations of phenolic compounds for example rarely exceed the nmol/L-level in blood plasma. Elevated *in vitro* doses may thus not necessarily be relevant for the *in vivo* situation (D'Archivio et al., 2010; Williamson & Manach, 2005). Furthermore, parent compounds are often metabolized by microbiota or undergo extensive modification. *In vitro* tests or animal models using only the native structures instead of the metabolites thus need cautious extrapolation (D'Archivio et al., 2010; Del Rio et al., 2013; Porrini & Riso, 2008; Williamson & Manach, 2005). Hence, even though these *in vitro* studies may help to shed light on the mechanisms of action, they need to be complemented by *in vivo* experiments to indicate their possible bioactivity in the human body (Fernández-García et al., 2009).

These *in vivo* tests are also required for obtaining claims related to the potential health-beneficial effect (Brookes, 2016; Dillard & German, 2000; O'Kennedy et al., 2016; Younesi & Ayseli, 2015). For example, Fruitflow® is the first European Food Safety Authority-approved product with a health claim under the European health claims regulation (1924/2006). It is a tomato based concentrate for which human volunteer studies have demonstrated the potency and bioavailability of the active compounds (polyphenols, flavonoids and nucleosides) as natural cardio-protective functional ingredients (O'Kennedy et al., 2016).

### 6.4.3 What about the chemical food safety of the byproduct-derived products?

Identifying the bioavailability of bioactive food compounds is essential for evaluating their potential health-beneficial effects but also for their toxicity (Rein et al., 2012). When ingested at high concentrations, some phytochemical compounds may exhibit a toxic activity (Balasundram et al., 2006). Furthermore, contaminants may remain or even accumulate in the byproducts, leading to potentially hazardous effects upon consumption.

#### 6.4.3.1 Plant toxins

Indeed, many plant constituents are toxic as they have been developed through evolution for the specific purpose of the plant's self-defense towards microbes, insects and other animals (Dillard & German, 2000). For example, phenolic compounds, when ingested at high concentrations, may exhibit negative activity such as carcinogenicity and genotoxicity (Mennen et al., 2005). The disappointing results from the human intervention trials with  $\beta$ -carotene supplementation, as referred to above, demonstrate the potentially adverse effects of carotenoids (ATBC study group, 1994). Also for SLs, severe toxicity and other adverse effects have been reported (Amorim et al., 2013; Padilla-Gonzalez et al., 2016). SL-containing plants for example have long been known to cause systemic allergic contact dermatitis and toxic syndromes in farm animals (Amorim et al., 2013; Paulsen, 2015). There is also a growing concern about their genotoxicity and embryotoxicity (Amorim et al., 2013). Also other phytochemicals have been attributed with a toxicity such as glycoalkaloids, most commonly found in the *Solanaceae* family (e.g. potato, tomato, eggplant) (Scherhauser et al., 2015).

Elucidating (i) which structural moieties can cause unwanted toxicity and (ii) in which dose they do, is crucial for the evaluation of the efficacy and safe use of the end products (Ghantous et al., 2010; Kasapidou et al., 2015; Mennen et al., 2005; Zhang et al., 2015a). These data are also required when applying for a health claim or a novel food (Brookes, 2016).

#### 6.4.3.2 Pesticide residues

Residues of pesticides and other agrochemicals can be concentrated in the outer layers of crops (Moncalvo et al., 2016; Scherhauser et al., 2015). These are often the byproducts (e.g. peels, press residue, etc.) and thus may pose a risk for human consumption. Performing efficient routine analysis and using quality control systems are thus crucial in this regard. This presence of pesticides is however not only an issue for byproducts, it also applies to regular fruit and vegetable consumption. Therefore, the exposure of the Belgian population to residues of plant protection products through the consumption of fruit and vegetables is continuously monitored and was recently investigated by the FASFC (Federal Agency for the Safety in the Food Chain). It was shown that on average 95 % of the 11,000 fruit and vegetable samples analyzed were compliant with the legal limits and 30 % – 40 % of the samples contained no residues. Furthermore, the estimated average exposure of adult consumers appears to be lower (even up to 100 times lower for the majority of the evaluated residues) than the toxicological reference value, namely the acceptable daily intake (ADI). Even though the resulting risk of pesticide residues will be depending on the targeted use (e.g. use of peel for application in human consumption), the dose and the form in which the byproducts are used, these observations show a strict compliance with the regulation (Scicom, 2015).

### 6.4.3.3 Mycotoxins and traces of heavy metals

Mycotoxins are toxic metabolites derived from fungi (e.g. aflatoxins, ochratoxins, fumonisins, etc.) (Scherhauser et al., 2015). A long-term exposure can affect the immune system and normal development or cause cancer (WHO, 2015b). Cereals and grains are among the main sources that are contaminated, but some also occur in horticultural products. Patulin is for example commonly found in apples and pears with brown rot whereas tomatoes are susceptible to contamination with mycotoxins from *Alternaria* species (da Cruz Cabral et al., 2016; Scherhauser et al., 2015). Furthermore, also the presence of trace levels of heavy metals can have adverse effects on health (Moncalvo et al., 2016; San Martin & Zufía, 2016). Cadmium and lead are for example known to exert detrimental effects on the kidneys and nervous system, respectively (WHO, 1996). On the other hand, some metals are essential for the human metabolism such as iron, chromium, copper and zinc (Moncalvo et al., 2016; WHO, 1996). The risk of these forms of contamination depends on the origin of the byproducts. For example, byproducts produced in the auctions and in greenhouse production in general could be expected to be less susceptible to traces of these compounds.

It thus follows that horticultural byproducts may contain a number of compounds with potentially adverse effects for humans. In order to cope with these potentially hazardous compounds, there is a need for (i) additional research on the potential adverse effects of plant phytochemicals and (ii) stringent quality control on products developed from byproducts ensuring that they are in line with the defined legal requirements.

## 6.5 Biorefineries: one recipe for guaranteed success?

The spiral-filter press technology was investigated in this study for its applicability to fractionate horticultural byproducts. It can be regarded as a pretreatment to be complemented either (i) with further refining and conversion or (ii) with stabilization of the derived homogeneous fractions, depending on the desired amount of refining and the envisaged end products. Either way, this flexible and quality-conserving pretreatment technology fits in the biorefinery concept, aiming for minimal waste and optimal valorization of all fractions.

### 6.5.1 The biorefinery concept

Within the bioeconomy, the cascade principle is increasingly advocated for optimal use of biomass and byproducts (SCAR, 2014). This guiding principle suggests a priority order with high value applications on top followed by lower value applications, entailing systematic exploitation of biomass for higher-



added-value products, before using it as an energy source (IWG BE, 2013; Keegan & Kretschmer, 2013) (Chapter 1). Whether a certain application is associated with a high or low added value is determined by its social, environmental and economic dimensions (Mourad, 2016). From a social perspective, food security is of primordial importance. Therefore, valorization of biomass towards food is most often perceived as the highest added-value application (de Besi & McKormick, 2015; Girotto et al., 2015; IWG BE, 2013; Maciulevičius, 2016; OVAM, 2012; 2015b; SCAR, 2014). The environmental added value lies in the fact that the production processes should aim at minimizing waste and limit the adverse impact on the environment. The economic dimension finally implies that the proposed biomass valorization should be economically profitable.

It is believed that this can be realized through using biorefineries (McKormick & Kautto, 2013; Mohan et al., 2016; Odegard et al., 2012). In analogy with the petroleum refineries, biorefineries convert biomass and byproducts into a range of products with a high total added value (Girotto et al., 2015; Ekman et al., 2013). The objective is to optimize the use of resources and minimize wastes, thereby maximizing benefits and profitability (WEF, 2010). The value of such a biorefinery concept is being increasingly recognized for optimal biomass utilization, since linear production models, targeting one specific end product and accordingly generating large fractions of waste, are being increasingly criticized. Although the latter is not a totally novel concept and has already been used in some traditional industries such as the paper, wood, sugar and meat industry, it is an emerging field, for which research and development are still at their initial stages (McCormick & Kautto, 2013; SCAR, 2014).

To accomplish this integral biomass use, the feedstock can be subjected to multiple sequential combinations of unit processes such as pretreatment, recovery, transformation and downstream processing (Fava et al., 2015). These can involve (but are not restricted to) physical processing (e.g. dewatering, centrifugation, size reduction, extrusion, drying), chemical processing (e.g. distillation, hydrolysis, extraction), biological processing (e.g. enzymatic treatment, fermentation) or thermal processing (e.g. pyrolysis) and combinations thereof (Ghatak, 2011; Lin et al., 2014; Rosentrater, 2005; WEF, 2010).

Feedstocks may originate from dedicated crops (e.g. corn, sugarcane). Industrially successful biorefineries nowadays are predominantly present for this so-called first generation biomass, consisting of edible biomass such as starch crops, sugar crops, oil crops and wood (IEA bioenergy, 2012; WEF, 2010). Some examples include pilot and commercial plants for biorefining sugar and starch crops into bioethanol and animal feed (Crop Energies AG, Germany; Permolex, Canada), commercial plants for biorefining rapeseed and sunflower oilseed into biodiesel, glycerine, animal feed, chemicals and

polymers (Sofiproteol, France) and lignocellulosic crops into bioethanol, chemicals, biomaterials and heat (Ensyn, Canada; Lignol, Canada; Zellstoff Stendal GmbH, Germany) (WEF, 2010). Besides primary crops, also waste and byproducts from agriculture, food and forest sectors can be used as biorefinery feedstock (Cherubini, 2010; Ekman et al., 2013; Ghatak, 2011; WEF, 2010). An example of such a second generation biorefinery plant is the pilot-installation for refining lignocellulosic residues into bioethanol, animal feed, electricity and heat (Inbicon IBUS, Denmark). Different pilot and demonstration plants based on grass have also been reported, involving the production of several combinations of products such as a feed product, grass fibers and biogas (Biowert, Germany), feed, materials, fertilizer and/or biogas (Grassa, Netherlands), insulation material and co-generation of biogas (Biorefinery, Ireland) and lactic acids, amino acids and biogas (Utzenaich, Austria) (Mandl, 2010). Despite the examples mentioned above, the majority of the biobased products are being produced in single production chains, based on one conversion technology and not on a cascading combination of technologies (Cherubini, 2010; McCormick & Kautto, 2013).

The examples mentioned above indicate that biorefineries and biofuels are currently closely related and that they are mainly associated with the conversion of agricultural crops or byproducts (Cherubini, 2010; McCormick & Kautto, 2013). Utilization of horticultural byproducts, or more generally food waste, often remains an idea or a promising strategy, leading to a scarce amount of successful industrial implementations (Fava et al., 2015; Yang et al., 2015). However, interest is growing as shown by the increasing amount of research conducted. Diverse byproducts (e.g. onion byproducts, orange peel, olive mill wastewaters, grape pomace, apple pomace, tomato and bell pepper foliage) have been assessed for their potential in biorefineries on research level (Ekman et al., 2013; Farhat et al., 2011; Federici et al., 2009; Gama et al., 2015; Martinez et al., 2016; Schuurbiers et al., 2013). They are mainly recognized for their content of dietary fibers, vitamins, natural antioxidants and mono- and di-oligosaccharides (Fava et al., 2015). More specifically, the case of citrus byproducts has been extensively investigated. Currently, citrus juice industries dry their residue and sell it either as raw material for pectin extraction or pelletized for animal feeding. Multiple studies have however investigated the conversion of different citrus byproducts into various added-value products such as essential oils, pectin, dietary fibers, enzymes, proteins, natural antioxidants, bioethanol, organic acid and/or prebiotics (Lin et al., 2013; Matharu et al., 2016; Mamma & Christakopoulos, 2013). Besides specific feedstocks such as citrus, multiple articles have reviewed the state of the art of biorefining food waste conversion in general. Kiran et al. (2014) and Yang et al. (2015) for example gave an overview of the current biochemical processes for the conversion of organic waste into valuable products, showing that these are still mostly situated in the lab-scale stage and that effective downstream processes are necessary for their adoption.

## 6.5.2 Difficulties when applying the biorefinery concept

In general, it can be concluded that specific interest in biorefining horticultural byproducts is rising in Europe. Although the industrially implemented examples of horticultural-byproduct-based biorefineries are still scarce, the idea is being incorporated in many policy texts and research projects. However, even though the biorefinery concept, and by extension the bioeconomy, sounds straightforward, it appears to be rather difficult to put into practice (de Besi & McCormick, 2015). Although not specifically investigated in this research, literature studies and discussions with stakeholders have led us to identify three main aspects that can be experienced as barriers for adoption and diffusion of the biorefinery concept. These are related with (i) the perceived primordial importance of economic feasibility, (ii) the lack of inherent environmental sustainability and its difficult measurement and (iii) the necessary structural changes accompanied by implementing the biorefinery approach in practice. These aspects are discussed from the perspective of biorefineries and can also be interpreted in the context of the bioeconomy in general.

### 6.5.2.1 Economic feasibility appears to be the major driving force

Acquiring a balanced combination of social, ecologic and economic aspects appears to be difficult in practice, both on policy level and on industrial level (Boehlje & Bröring, 2011; McCormick & Kautto, 2013; Mourad, 2016; Staffas et al., 2013).

Policy is often unable to stimulate a consistent, simultaneous adoption of these three sustainability pillars. In the translation of their policy, economic growth is often addressed first, followed only to a limited extent by aspects of environmental and social sustainability (McCormick & Kautto, 2013; Pfau et al., 2014; Staffas et al., 2013). This is for example illustrated by policy documents such as the USA bioeconomy blueprint as well as the OECD policy agenda, not specifically addressing the environmental sustainability as a driving force (Organization for Economic Cooperation and Development) (Staffas et al., 2013). In the European strategy, the environmental sustainability is more explicitly stated (European Commission, 2012). However, even in this case, the legislation remains complex and sometimes even contradictory. This can be illustrated by Flanders stimulating the use of horticultural byproducts in the food industry (OVAM, 2015b) in contrast to the non-transparent regulation associated with the waste status (Directives 2008/98/EC; Materialendecreet, VLAREMA) as well as the strict quality requirements for the valorized food products (e.g. novel food, additives, claims) (Fava et al., 2015; Mourad, 2016). Also Europe, pushing the utility of biomass towards the production of biofuels and renewable energy by binding goals and accompanying support mechanisms, is rather contradictory with the top priority of food and biomaterials (Bos-Brouwers et al., 2012; Carus et al., 2015; de Besi & McCormick, 2015; Keegan et al., 2013; OVAM, 2014; 2015b).

Besides policy, also industry struggles with addressing the three sustainability pillars in a balanced way. Even though they are increasingly concerned about environmental sustainability, this is often believed to come at the expense of economic productivity and competitiveness (Boehlje & Bröring, 2011; de Jong et al., 2005). Accordingly, in practice, profitability remains the ultimate driving force, while social and ecologic issues are considered secondary (Aramyan & Valeeva, 2016; Budzianowski & Postawa, 2016; McCormick & Kautto, 2013). The economic feasibility is thus often used as primary criterion to evaluate the biomass valorization trajectory. However, as applying the basic principles of the bioeconomy is not always economically feasible, this may have an impact on its difficult adoption.

For example, an extensive level of refining using environmentally sustainable technologies is advocated in the biorefinery approach. This idea originates from the assumption that incorporating multiple processing steps and producing several products, helps to increase the environmental sustainability by (i) using the entire biomass and thus avoiding the production of residues and (ii) providing better resource efficiency due to the maximization of the value derived from this biomass (Fava et al., 2015; Keegan et al., 2013). From an economic point of view, the different end products are expected to enter different markets and augment the total economic added value of the products (Budzianowski & Postawa, 2016; Fava et al., 2015). However, it also implies that the initially required economic investments increase and that the economic value of the derived products must be high in order to be able to justify the necessary investments (Fava et al., 2015; Keegan et al., 2013; Kusch et al., 2014; McCormick & Kautto, 2013; Mirabella et al., 2014; WEF, 2010). Therefore, this high level of refining is not always easy to accomplish, which can be illustrated by a study performed by Royal Haskoning DHV for sugar extraction from horticultural products. In a first step, C5-sugars were extracted from hemicellulose, followed by the recovery of C6-sugars in a second step. However, the enzymatic hydrolysis to recover the C6-sugars appeared to be too expensive compared to the extra revenues provided by the C6-sugars, leading to an overall negative business case, whereas excluding this step rendered the business case profitable (Koop et al., 2014).

Another example of the fact that applying the basic principles of the bioeconomy is not always economically feasible, is related to the adoption of novel, clean technologies. Even though they might be associated with clear social and environmental benefits, their adoption is often hampered by financial aspects or uncertainties (Aramyan & Valeeva, 2016; del Río Gonzalez, 2005). Aspects such as high initial investment costs which are not necessarily related with immediate increased revenues, high switching costs, uncertainty regarding regulation, required organizational adaptations, market uncertainties and technical uncertainties can overwhelm the economic and financial advantages of implementing clean technologies. Some of these issues can be expected to be alleviated when the technologies mature (Dietrich et al., 2016). Indeed, often they have only been recently developed and

are therefore still expensive or they have not reached their maximum efficiency yet. This is sometimes referred to as early adoption costs (del Río Gonzalez, 2005). By intensifying research and development on process upscaling and performance, the associated costs can thus be expected to be reduced (Fava et al., 2015; Kamm & Kamm, 2015; Matharu et al., 2016; Schieber et al., 2001; Staffas et al., 2013). Also the expected increase in oil prices could make clean technologies economically more viable in the future (Fava et al., 2015).

Finally, the stringent regulations associated with byproduct valorization towards food or materials can also render the cascade principle economically less feasible compared to other options such as conversion to energy or composting. Byproduct valorization towards food can be subjected to the novel food legislation<sup>22</sup>. This obliges the producers of 'novel' foods or ingredients to demonstrate the safety of their product before market introduction in order to protect the consumer (Baiano, 2014). Brookes (2016) states that the time necessary for completing food ingredient research (e.g. novel food) can take 4 to 10 years, divided in 2 to 5 years for research and 2 to 5 years for product development (incl. regulatory approval). The time necessary for authorizing the sale of novel foods/ingredients in the EU market is already 36 months on average. Time for approval for health claims is about 30 months (Brookes, 2016). The costs associated with the development of a novel food product with a health or nutrition claim may cost in the range of €15 million to €20 million. These are less when launching a new ingredient without a health claim. A significant part of this overall cost comes from regulatory requirements (e.g. generation of safety data, clinical trials, etc.) and can add up to 50 % of the total costs of bringing a product to the market (Brookes, 2016). Also for cosmetics<sup>23</sup>, pharmaceutical products<sup>24</sup> and chemicals<sup>25</sup>, strict regulations are present. Without questioning their importance, these strict quality requirements include a rigorous testing and administrative cost, which may be impeding for smaller companies (Lin et al., 2013; OVAM, 2014).

It can thus be concluded that economic aspects are often considered dominant and can hamper the adoption of the basic principles of the biorefinery approach.

#### 6.5.2.2 Environmental sustainability not self-evident and difficult to measure

The envisioned environmental benefits of biorefining and cascading lie in the form of minimization of residues and optimal resource efficiency (Fava et al., 2015; Keegan et al., 2013; Pfau et al., 2014).

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<sup>22</sup> Novel food is defined as food that has not been consumed to a significant degree by humans in the EU prior to 1997. It can be newly developed, innovative food or food produced using new technologies and production processes as well as food traditionally eaten outside the EU (EC 1852/2001).

<sup>23</sup> [https://ec.europa.eu/growth/sectors/cosmetics/legislation\\_en](https://ec.europa.eu/growth/sectors/cosmetics/legislation_en)

<sup>24</sup> [https://ec.europa.eu/growth/sectors/healthcare\\_en](https://ec.europa.eu/growth/sectors/healthcare_en)

<sup>25</sup> [https://ec.europa.eu/growth/sectors/chemicals\\_en](https://ec.europa.eu/growth/sectors/chemicals_en)

(Dewulf & Van Langenhove, 2006). However, this is not self-evident (Mourad, 2016; Pfau et al., 2014). Extensive biorefining itself may indeed be associated with a substantial environmental impact on different stages in the supply chain.

Starting at the beginning of the supply chain, the impact of removing biomass, which is conventionally left on the field, can influence the total environmental sustainability. Removal of these residues can on the one hand aid in minimizing the risks of N-losses during autumn (predominantly for N-rich vegetable crops such as cauliflower, cabbages, leek and celery). On the other hand, their removal also causes loss of their beneficial contribution to soil quality and fertility. Using fertilizers or compost could make up for the removed nutrient value. However, their production is also associated with a certain environmental impact, which could partly offset the benefits gained by the removal and use of the agricultural residues (Agneessens et al., 2015; Budzianowski & Postawa, 2016; De Meester et al., 2011; Wellisch et al., 2010). Using biomass residues is often automatically considered a more sustainable resource compared to fossil based feedstock, however in the longer term, it might turn out to be worse (De Meester et al., 2011). It is increasingly being argued that small-scale preprocessing at the agricultural company could help to overcome this. Instead of refining the biomass centrally and transporting products that contain predominantly water and minerals back to the farms (e.g. molasses and lime fertilizer in case of sugarbeet refining), the latter should not be removed from the farms in the first place (Bruins & Sanders, 2012). Unnecessary costs associated with their transport are accordingly avoided and they can be reused on the local field as fertilizers.

Also the necessary transport of biomass, characterized by high moisture content and low energy density, affects the environmental impact of the biorefinery (Bruins & Sanders, 2012; Budzianowski & Postawa, 2016; Keegan et al., 2013). The extent to which byproducts are transported depends on the scale of the biorefinery. Petroleum biorefineries use raw materials that do not contain water and where all transported product is used. Therefore, large petroleum biorefineries benefit from economies of scale. Biorefineries obey different rules due to the logistic aspects mentioned above (water content, seasonal and scattered occurrence) (Bruins & Sanders, 2012). Small-scale utilization of byproducts within one farm or company eliminates the need for transport, in contrast to large-scale biorefining exceeding the boundaries of one company. The optimal size of a biorefinery has to find an adequate balance between centralized and distributed performance (Budzianowski & Postawa, 2016; Bruins & Sanders, 2012). In this context, a model is proposed by McCormick & Kautto (2013) which is based on the proximity of raw materials, production and consumption sites and which strives towards many integrated, local production plants. This so-called “glocal” approach ensures that residues and wastes are fully utilized by different processes in a concentrated region. Bruins & Sanders (2012) advocate the decentralized preprocessing followed by a more capital-intensive processing at large

centralized factories. In Flanders, this concept could be realized by setting up valorization actions at the produce auctions and at large horticultural processors, located close to the horticultural production sites.

Besides the removal of byproducts from the land and transport, the processing is also associated with an environmental impact, for example by its energy consumption, use of polluting solvents and/or chemicals and the production of hazardous waste (Mirabella et al., 2014; van der Goot et al., 2016; Wellisch et al., 2010). Technological advancements on designing low-environmental-impact technologies could for example make these technologies more sustainable (Fava et al., 2015; Kamm & Kamm, 2015; Lin et al., 2014; Schieber et al., 2001). Novel thermal and non-thermal preservation technologies such as microwave heating and pulsed electric fields appear to be more environmentally friendly in terms of energy efficiency, water savings and/or reduced emissions, compared to traditional ones (Pereira & Vicente, 2010). Other examples are the Refractance Window Drying and the similar Dry-On-Water technology, which can significantly lower the energy use, normally consumed by conventional drying technologies such as freeze-drying and spray-drying (Baeghbali et al., 2015; Van Mierlo, 2016). Also a range of extraction technologies are being developed with a lower environmental impact such as supercritical fluid extraction, ultrasound-assisted extraction, microwave-assisted extraction, pulsed electric field extraction, enzyme-assisted extraction, benign solid-liquid solvent extraction and pressurized fluid extraction (Baiano, 2014; Cherubini, 2010; Matharu et al., 2016; Rombout et al., 2014). Besides the use of novel, sustainable technologies, also the processing strategy can be adapted. Van der Goot et al. (2016) argue that food products are often produced with a high purity, a defined composition and a broad applicability, which demand intensive processes. They advocate a transition from pure and highly processed ingredients to enriched fractions which are produced using mild fractionation processes and are less destructed and less pure, but show improved functional properties (e.g. more compounds present in their native state unaltered by processing, presence of impurities that can help in the final functionality, presence of natural structure beneficially affecting the bioavailability of micronutrients).

Finally, also the types of products that are manufactured and how they are used and disposed of at the end of life, influence the environmental sustainability of the biorefinery (Wellisch et al., 2010).

Thus, although aiming for environmental sustainability by minimizing residues and optimal resource efficiency, the biorefining process itself and the associated supply chain are also characterized by an environmental impact (Pfau et al., 2014). Hence, biorefining is not environmentally beneficial *per se* and the total environmental added value of the derived end products may be undermined by the created environmental impact (Dewulf & Van Langenhove, 2006; Wellisch et al., 2010). Therefore,

understanding the full consequences of a biorefinery is necessary and sustainability of the whole supply chain must be assessed (Wellisch et al., 2010). However, measuring the environmental sustainability of an entire supply chain is difficult. In this context, a system analysis approach for measuring the environmental impact such as Life Cycle Analysis (LCA) is promising (Budzianowski & Postawa, 2016; Cherubini, 2010; De Meester et al., 2011; European Commission, 2012; Fava et al., 2015; Keegan et al., 2013; Pfau et al., 2014; Unger et al., 2016).

### 6.5.2.3 The biorefinery approach calls for changes in the strategy and behavior patterns

Adopting the biorefinery approach often requires a number of changes in the strategy and organization of the industry and in the behavior of consumers, which may obstruct its implementation (Keegan et al., 2013).

In order to create integrated biorefineries, a concerted action of multiple non-traditional partners will be necessary to create knowledge from a variety of sciences and technologies and to cover all aspects of the value chain (European Commission, 2012; Fava et al., 2015; Golembiewski et al., 2015; Mourad, 2016; Pfau et al., 2014; Tsolakis et al., 2014). For example, the food industry and the cosmetic and/or pharmaceutical industries are allying, targeting the production of nutraceuticals and functional foods. The bioenergy sector is increasingly cooperating with the chemical industry for the production of biobased materials and chemicals (Boehlje & Bröring, 2011; Younesi & Ayseli, 2015). This leads to the emergence of novel supply chains cutting across the borders of the existing organizations and sectors. This might be quite challenging for the participating companies, as these require moving beyond the core business and the traditional framework of expertise (Aramyan & Valeeva, 2016; Boehlje & Bröring, 2011; Dansereau et al., 2014; Keegan et al., 2013; Kircher, 2012; McCormick & Kautto, 2013). These new intersections of previously relatively independent industries require increased cooperation and managing capabilities to facilitate the knowledge transfer among actors from different scientific backgrounds (Boehlje & Bröring, 2011; European Commission, 2012; Golembiewski et al., 2015). The European Commission is aware of this challenge and stimulates the interactive cooperation model for conducting research and innovation, for example via European Innovation Partnerships (EIPs) under the Europe Horizon 2020 Strategy. The REFRESH project, focusing on reducing food losses, has already adopted this method by working in collaboration with working platforms that provide the team with guidance on business and consumer acceptance of byproducts (Sweet et al., 2016).

Besides the cooperative approach, an integrated biorefinery is often associated with structural changes in the supply chain. These can originate from the adoption of novel technologies, which may be associated with financial changes (e.g. high investment costs, high switching costs), technical changes (e.g. lack of full control of process operational variables) and regulatory uncertainties (del Río



Gonzalez, 2005; Pereira & Vicente, 2010). Market insecurity can further aggravate the company's reluctance to produce new products. The consumers might not be willing to pay price premiums for products or processes with a lower environmental impact. Potentially adverse consumer perception towards new products may also pose uncertainties and risks for the producers. Consumers might be hesitant to embrace new products, such as those generated from byproducts or waste streams. Also the use of dedicated feedstocks produced by innovative technologies (e.g. genetic modification, novel breeding techniques) or novel production technologies (e.g. nanotechnology, irradiation) can give rise to products characterized by an insecure market (Golembiewski et al., 2015; Frewer et al., 2011; McCormick & Kautto, 2013; Pereira & Vicente, 2010). For example, the introduction of the food irradiation technology has been constrained due to a negative consumer attitude, despite the potential benefits (Aramyan & Valeeva, 2016; Pereira & Vicente, 2010). Consequently, consumer acceptance is essential for the adoption and diffusion of new technologies and derived products (Aramyan & Valeeva, 2016). Consumer acceptance is found to increase with knowledge and perceived usefulness (Golembiewski et al., 2015). This can for example be reached by using transparent certifications, quality labels and education campaigns (European Commission, 2012; Frewer et al., 2011). Also research driven by the market instead of by technology is believed to stimulate commercialization of biobased end products (Rönnlund et al., 2014).

### 6.5.3 Potential measures to stimulate biorefineries

Reconciling economic, ecologic and social aspects through the cascade biorefining system is not straightforward. It can also be deduced that the biorefinery concept is no rigid concept applicable in every situation with an ensured positive end balance. A range of different ways and levels of refinement are possible and the viability and applicability of the specific design has to be evaluated case-by-case. Consequently, no *one-size-fits-all* translation of the biorefinery concept can be made (Jonkman et al., 2017; Wellisch et al., 2010). In theory however, the concept holds strong and (i) by allowing some flexibility to account for different contextual factors and (ii) by further supporting and investing in research and development of novel technologies, the practical translation can be facilitated and become more feasible.

Even though no concrete strategy applicable for all contexts can be proposed, a balanced regulatory framework with clear priorities and ambitions regarding sustainability can help to alleviate some of the aforementioned challenges (de Besi & McCormick, 2015; European Commission, 2012; McCormick & Kautto, 2013; Pfau et al., 2014). An aspect that can be suggested in this regard is to set up a standardized systemic approach that can be used as an evaluation framework for measuring the

sustainability of a product or production system from an economic, environmental and social point of view and thus can provide guidance to industry, government and society (De Menna et al., 2016). Nowadays, some isolated methodologies exist to measure the environmental (life cycle analysis - LCA), cost (life cycle costing - LCC) or social impact (social LCA - S-LCA) of decisions and products. The results of these analyses are difficult to relate because they are often based on different assumptions or include different parts of the life cycle (De Menna et al., 2016; Valdivia et al., 2011). Therefore, a holistic approach, encompassing all sustainability pillars is necessary to 'get the whole picture' and to make informed and balanced decisions. Various efforts have been made in the past. Recently, Valdivia et al. (2011), supported by the United Nations Environment Programme (UNEP), have developed a Life Cycle Sustainability Assessment (LCSA), which is a combination of an LCA, an LCC and an S-LCA, and which evaluates all environmental, social and economic negative impacts and benefits throughout a products life cycle. They state that while the application is already feasible, more research is needed in regard to improving data acquisition, management and access, enhancing and facilitating the methodology and providing guidance for interpretation and communication of the results (Valdivia et al., 2011). The European Commission is aware of these challenges and is further stimulating integrated sustainability assessments by (i) supporting the further development of integrated assessment methodologies and (ii) requiring research proposals to include sustainability assessments (Wellisch et al., 2010).

This sustainability framework can be linked to establishing an expert cluster that can use the developed methodology for evaluating and comparing technologies for their overall sustainability (Wellisch et al., 2010). This expertise could in turn be used by policy makers and adopters to make more informed choices. Good scores can for example serve as a criterion for funding research and development of certain technologies in order to reduce their cost and increase their scale and performance. It can also be used as a base for allocating grants for the use of these technologies or providing soft loans, thereby directly stimulating their industrial adaptation. This holistic sustainability assessment could in turn be used to aid in ensuring a market value, by linking it to the development of a transparent standardization, verification and labeling system for the derived products to prove their environmental benefits and their safety (del Río Gonzalez, 2005; Maciulevičius, 2016; Wellisch et al., 2010).

Another aspect that can help to facilitate the adoption of the bioeconomy and thus the biorefinery is associated with the current lack of legislative transparency and the stringent regulation for food and feed production. Complicated legislation makes it difficult for producers of byproducts to know what kind of byproduct is allowed in which application. Even though the importance of strict regulations is widely acknowledged for guaranteeing food safety and controlling for correct consumer information, the procedures are often long and costly. Creating more transparency in the administrative procedure

(which procedure to follow, what requirements need to be met, how long the procedure will take and how much it will cost) and limiting the duration and thus the associated costs, can already mean a great difference in the business case (Brookes, 2016). The European Commission is aware of this challenge. This is demonstrated for example by the EU-funded REFRESH project, developing a mobile application for businesses to facilitate valorization of byproducts towards feed, by providing them with clear information on the applicable legislation and requirements. The EU-funded BACCHUS<sup>26</sup> project aims to alleviate the legislative complexity related to food production by providing information about the European regulation on nutrition and health claims and developing tools and resources to facilitate the generation of robust and exploitable scientific evidence to support health claims. Furthermore, the European Commission supports the clarification of the EU-legislation related to waste, food and feed. They also want to facilitate food donation and the use of byproducts in feed, without compromising the safety (European Commission, 2016b). Also Flanders is aware of the role policy can play in stimulating the bioeconomy and has founded a platform for collecting the obstructing regulations and issues related to a lack of transparency or complicated procedures<sup>27</sup>.

## 6.6 Conclusion and further perspectives

This dissertation aimed to facilitate the valorization of horticultural byproducts. Therefore, two currently hindering aspects related to (i) the need to cope with moist byproducts occurring scattered, both in time and space and (ii) the lack of knowledge on the composition of some byproducts, were tackled from a technical perspective, leading to scientific insights in the defined research areas. Additionally, these scientific insights were discussed in the bioeconomy context and critically discussed from a broader technical and socio-economic perspective. As a result, this dissertation clearly shows the possibilities of these byproducts and accordingly the opportunities for their valorization. On the other hand, it shows the complexity of valorizing horticultural byproducts and the necessary multifaceted approach. Today, we are only at the beginning and much work is to be done, but the interest keeps growing and the route is being paved.

This research has allowed us to identify some avenues for further research. These have been extensively described above but the main suggestions are summarized below.

- **Valorization of the press residue fraction**

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<sup>26</sup> <http://www.bacchus-fp7.eu/>

<sup>27</sup> <http://www.ewi-vlaanderen.be/wat-doet-ewi/ondernemende-economie/bio-economie/meldpunt-belemmerende-regelgeving>

This dissertation focused on the liquid fraction but clearly showed the enrichment of phytochemicals in the press residue. In order to optimally valorize the whole byproduct, further research should focus on the handling and product formulation of this press residue fraction. Aspects that can be investigated to allow valorization of the associated phytochemicals and fibers are for example the oxygen-free collection of the press residue after pressing, further stabilization, optimization of the pilot-scale extraction of phytochemicals, product formulation and evaluation of the final product properties such as functionality and sensory and safety aspects.

- **Investigation of novel stabilization technologies**

The thermal treatment used in this dissertation led to a significant decrease in redness and carotenoid content of the tomato juice. This was in line with the finding that conventional thermal treatment may negatively affect the physical, nutritional and/or bioactive properties of fruit and vegetable juices, as described in literature (Jiménez-Sánchez et al., 2017a). Future research should thus investigate coupling the spiral-filter press to a less impacting stabilization technology on pilot scale in order to generate a low total processing impact on the byproduct-derived products.

- **Economic assessment of the valorization process**

In order to create more insight in the economic viability of the proposed valorization strategy and to allow comparison with other scenarios, an economic assessment of the whole resulting process (biomass collection, transport, pretreatment, pressing, stabilization, downstream processing) should be performed. Subsequently, different scenarios could be tested varying in type of end product, scale, location, legislative procedures, etc.

- **Bioactivity testing**

The concentration and variability of phytochemicals in function of natural and processing related factors has been investigated in this study, showing the potential of forced Belgian endive roots. However, this is only the first step towards valorizing these byproducts. Careful assessment of a several aspects is necessary such as for example their functionality in extracts or in original root matrix, the compounds and the dose responsible for this functionality, and their stability, bioaccessibility and bioactivity in the final matrix.

- **Pilot-scale extraction and fibrous root fraction valorization**

If the functionality of the derived extracts is promising, an extraction process at pilot scale should be investigated regarding performance and economic viability. The extraction method proposed in this research for the sesquiterpene lactones is a good starting point as it is relatively simple and green. Furthermore, this also allows for the valorization of the remaining fibrous fraction.

- **Evaluation of toxicity and contaminants**

Depending on the derived product and the valorization sector, the potential toxicity of the end products should also be investigated. Elucidating which structural moieties can cause unwanted toxicity (e.g. pesticide residues, unknown effects of SLs) and in which dose they do, is crucial for the evaluation of the efficacy and safe use of the derived end products.



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## Appendix



## Appendix

### Appendix 1: Calculation of the juice yield

Consider the tomato juice production process. On each moment  $t$  during the juice extraction process, both during the transient of steady-state phase (Figure A1), the formula below applies:

$$m_m(t) = m_j(t) + m_p(t) \quad (1)$$

with  $m_j(t)$ ,  $m_p(t)$  and  $m_m(t)$  the mass flow rates on each time  $t$  of the juice, pomace and mash respectively. Integration of both sides with respect to time from the beginning (120 s) to the end (160 s) of the sampling phase (part of steady-state phase) delivers:

$$\int_0^t m_m(t)dt = \int_0^t m_j(t)dt + \int_0^t m_p dt \quad (2)$$

As  $m_m$ ,  $m_j$  and  $m_p$  can be considered constant during the sampling phase, equation (2) can be converted to:

$$m_m \int_0^t dt = m_j \int_0^t dt + m_p \int_0^t dt$$

corresponding to:

$$m_m \Delta t = m_j \Delta t + m_p \Delta t \text{ or } M_m = M_j + M_p \quad (3)$$

with  $M_m$  the mass of the mash processed during the sampling phase, and  $M_j$  and  $M_p$  the mass of the juice and pomace produced during the sampling phase respectively.

The juice yield can be defined as:

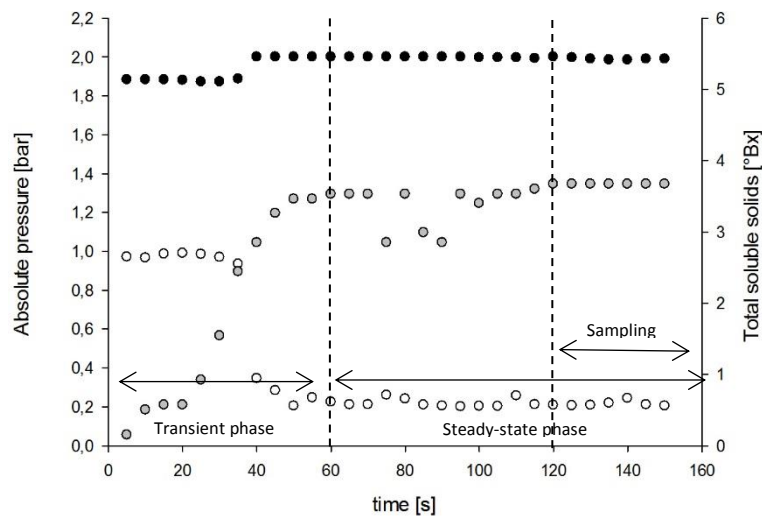
$$JY(t) = \left( \frac{m_j(t)}{m_m(t)} \right) \cdot 100 \% \quad (4)$$

With inclusion of equation (1), this becomes

$$JY(t) = \left( \frac{m_j(t)}{m_j(t) + m_p(t)} \right) \quad (5)$$

By the fact that  $m_m$ ,  $m_j$  and  $m_p$  can be considered as constant during the sampling phase (part of the steady-state phase), they can be equalized to  $M_m/t$ ,  $M_j/t$ , and  $M_p/t$  respectively. As a consequence, the percentage juice yield can be calculated from:

$$JY = \left( \frac{M_j}{M_j + M_p} \right) \cdot 100 \% \quad (6)$$



**Figure A1** Absolute underpressure in extraction cell (white), feed pump pressure (black) and total soluble solids (grey) during transient and steady-state phase.

## Appendix 2: Derivation of an expression for (i) the wet based concentration, (ii) the retention efficiency and (iii) the extraction efficiency of the filtration

### a. Wet based concentration

The material balances for the freeze-drying system (batch process) are:

Total mass balance:

$$M_{s,w} = M_{H_2O} + M_{s,d} \quad (1)$$

with  $M_{s,w}$  the mass of the sample before freeze-drying (wet sample),  $M_{H_2O}$  the mass of water removed from the sample during freeze-drying and  $M_{s,d}$  the mass of the sample after freeze-drying (dry sample).

Partial mass balance for water:

$$M_{s,w} MC_{s,w} = M_{H_2O} 100\% + M_{s,d} MC_{s,d} \quad (2)$$

with  $MC_{s,w}$  the moisture content of the sample before freeze-drying and  $MC_{s,d}$  the moisture content of the sample after freeze-drying.

Partial mass balance for compound i:

$$M_{s,w} c_w = M_{s,d} c_d \quad (3)$$

with  $c_w$  and  $c_d$  the concentration of compound i in the sample before and after freeze-drying, in other words, the concentration on wet base or dry base, respectively. From equation 1, 2 and 3 can be derived that:

$$\frac{M_{s,w}}{M_{s,d}} = \frac{AOC_d}{AOC_w} = \frac{100\% - MC_{s,d}}{100\% - MC_{s,w}} \quad (4)$$

In this manner, a linkage could be made between  $c_w$  and  $c_d$ :

For all samples

$$c_w = c_d \left( \frac{100\% - MC_w}{100\% - MC_d} \right) \quad (5)$$

### **b. Retention efficiency**

The spiral-filter press is used for filtration. The general terms *mash*, *juice* and *press residue* are used, which correspond to MT, JnT and PR.

Following material balances are valid for a control volume, which encloses the spiral-filter press:

Total mass balance:

$$m_{m,w} = m_{j,w} + m_{p,w} \quad (6)$$

with  $m_{m,w}$ ,  $m_{j,w}$  and  $m_{p,w}$  the mass flow rates of the mash, juice and press residue respectively.

Partial mass balance for water:

$$m_{m,w} MC_{m,w} = m_{j,w} MC_{j,w} + m_{p,w} MC_{p,w} \quad (7)$$

with  $MC_{m,w}$  the moisture content of the mash,  $MC_{j,w}$  the moisture content of the juice and  $MC_{p,w}$  the moisture content of the pomace on wet base.

Partial mass balance for compound i:

$$m_{m,w} c_{i,m,w} = m_{j,w} c_{i,j,w} + m_{p,w} c_{i,p,w} \quad (8)$$

The retention efficiency (%  $R_i$ ) of a specific compound during pressing can be defined as:

$$\% R = \left( \frac{m_{i,j} + m_{i,p}}{m_{i,m}} \right) \cdot 100 \% \quad (9)$$

what corresponds with:

$$\% R = \left( \frac{m_{j,w}c_{i,j,w} + m_{p,w}c_{i,p,w}}{m_{m,w}c_{i,m,w}} \right) \cdot 100 \% \quad (10)$$

Substituting  $m_{i,m}$ ,  $c_{i,p,w}$ ,  $c_{i,j,w}$  and  $c_{i,m,w}$  by their corresponding expressions (5) and (8) gives:

$$\% R = \left( \frac{m_{j,w} \left( \frac{100\% - MC_{j,w}}{100\% - MC_{j,d}} \right) c_{i,j,d} + m_{p,w} \left( \frac{100\% - MC_{p,w}}{100\% - MC_{p,d}} \right) c_{i,p,d}}{(m_{j,w} + m_{p,w}) \left( \frac{100\% - MC_{m,w}}{100\% - MC_{m,d}} \right) c_{i,m,d}} \right) \cdot 100 \% \quad (11)$$

The % juice yield (JY) can be defined as:

$$JY = \left( \frac{M_{j,w}}{M_{j,w} + M_{p,w}} \right) \quad (12)$$

Combining equation 11 and 12:

$$\% R = \left( \frac{JY \left( \frac{100\% - MC_{j,w}}{100\% - MC_{j,d}} \right) (c_{i,j,d}) + (100\% - JY) \left( \frac{100\% - MC_{p,w}}{100\% - MC_{p,d}} \right) (c_{i,p,d})}{\left( \frac{100\% - MC_{m,w}}{100\% - MC_{m,d}} \right) (c_{i,m,d})} \right) \quad (13)$$

This formula holds for %  $R_{\text{filtration},i}$

### c. Extraction efficiency

The extraction efficiency (% E) or the percentage of the amount of compound i in the mash which is found in the juice after juice extraction is defined in an analogue way as outlined for % R:

$$\% E = \left( \frac{Y\% \left( \frac{100\% - MC_{j,w}}{100\% - MC_{j,d}} \right) (c_{i,j,d})}{\left( \frac{100\% - MC_{m,w}}{100\% - MC_{m,d}} \right) (c_{i,m,d})} \right) \cdot 100 \% \quad (14)$$

The % R for the thermal treatment can be deduced from (13). This process doesn't generate two fractions, hence the total input is equal to the total output, hence the JY is equal to 100 %, simplifying the formula to:

$$\% R_i = \left( \frac{\left( \frac{100\% - MC_{\text{output},w}}{100\% - MC_{\text{output},d}} \right) (c_{i,\text{output},d})}{\left( \frac{100\% - MC_{\text{input},w}}{100\% - MC_{\text{input},d}} \right) (c_{i,\text{input},d})} \right) \quad (15)$$



With  $c_{i,input,d}$  and  $c_{i,output,d}$  the concentration of compound  $i$  in the input or output stream on a dry basis and  $MC_{input,d}$ ,  $MC_{input,w}$ ,  $MC_{output,d}$  and  $MC_{output,w}$  the moisture contents of input and output on a dry and a wet base.

This equation holds for %  $R_{thermal,i}$

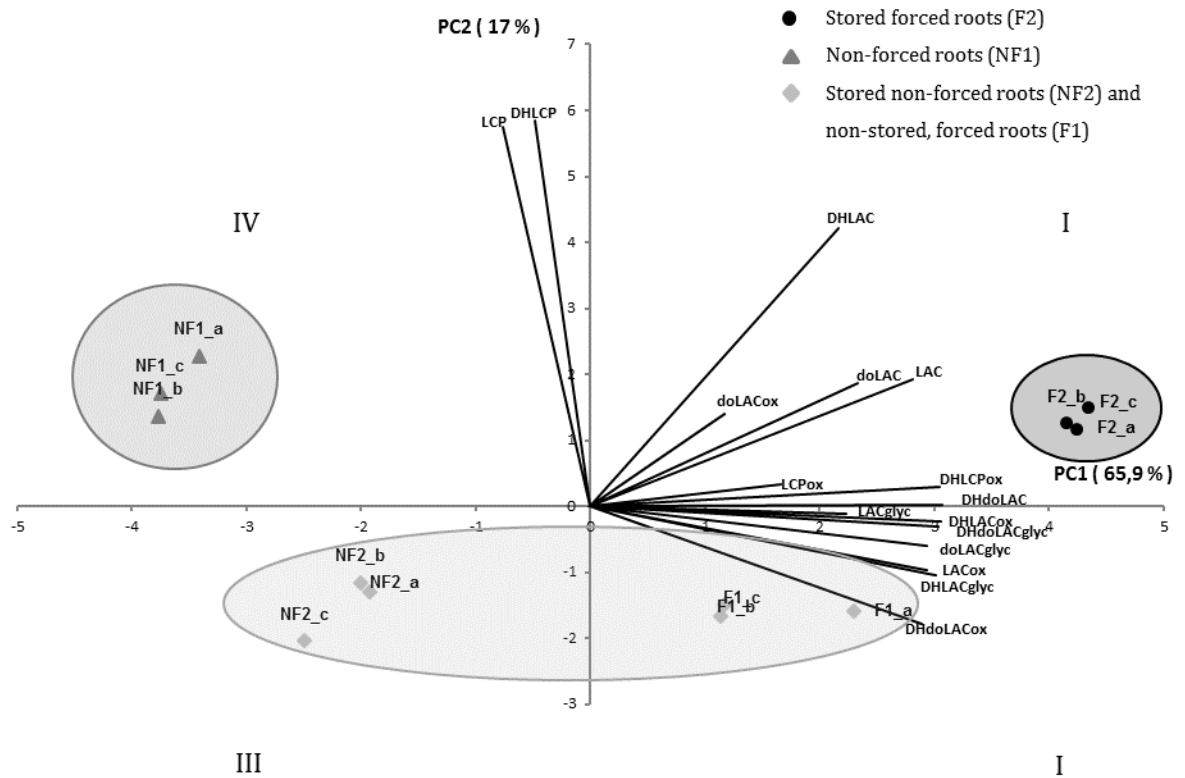
Based on De Paepe et al., 2015a.

**Table A1** Relative peak areas (area compound/area internal standard) of the 16 measured SLs in the HRMS profile of the variety dataset. Different letters indicate statistically significant differences ( $p < 0.001$ ) between different samples.

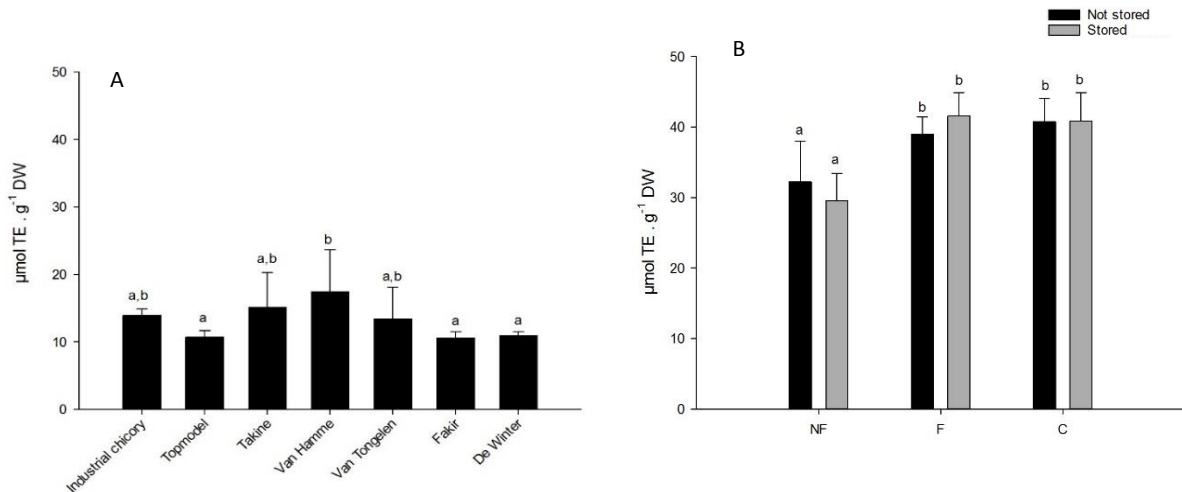
	Industrial chicory	Topmodel	De Winter	Fakir	Van Hamme	Van Tongelen	Takine
DHLACglyc	0.59 ± 0.05 <sup>c</sup>	1.1 ± 0.1 <sup>c</sup>	0.30 ± 0.01 <sup>a,b</sup>	0.30 ± 0.01 <sup>a,b</sup>	0.34 ± 0.01 <sup>b</sup>	0.19 ± 0.02 <sup>a</sup>	0.50 ± 0.07 <sup>c</sup>
DHLACox	0.38 ± 0.04 <sup>a,b</sup>	0.38 ± 0.05 <sup>a,b</sup>	0.47 ± 0.03 <sup>b</sup>	0.41 ± 0.01 <sup>b</sup>	0.30 ± 0.02 <sup>a</sup>	0.39 ± 0.03 <sup>a,b</sup>	0.60 ± 0.02 <sup>c</sup>
DHLAC	0.44 ± 0.01 <sup>d</sup>	0.59 ± 0.04 <sup>e</sup>	0.22 ± 0.01 <sup>a,b</sup>	0.28 ± 0.01 <sup>b,c</sup>	0.21 ± 0.001 <sup>a,b</sup>	0.18 ± 0.01 <sup>a</sup>	0.32 ± 0.04 <sup>c</sup>
DHdoLACglyc	0.44 ± 0.07 <sup>a,b</sup>	1.0 ± 0.02 <sup>c</sup>	0.50 ± 0.002 <sup>b</sup>	0.39 ± 0.02 <sup>a,b</sup>	0.88 ± 0.03 <sup>c</sup>	0.27 ± 0.02 <sup>a</sup>	0.88 ± 0.12 <sup>c</sup>
DHdoLACox	0.15 ± 0.01 <sup>a</sup>	0.20 ± 0.01 <sup>a</sup>	0.19 ± 0.01 <sup>a</sup>	0.20 ± 0.004 <sup>a</sup>	0.66 ± 0.01 <sup>b</sup>	0.14 ± 0.01 <sup>a</sup>	0.67 ± 0.16 <sup>b</sup>
DHdoLAC	0.49 ± 0.06 <sup>a,b</sup>	1.5 ± 0.1 <sup>d</sup>	0.58 ± 0.003 <sup>b</sup>	0.47 ± 0.02 <sup>a,b</sup>	1.1 ± 0.02 <sup>c</sup>	0.28 ± 0.03 <sup>a</sup>	1.1 ± 0.2 <sup>c</sup>
DHLCPox	0.09 ± 0.01 <sup>b,c</sup>	0.05 ± 0.003 <sup>a</sup>	0.10 ± 0.003 <sup>c,d</sup>	0.07 ± 0.002 <sup>b</sup>	0.11 ± 0.01 <sup>d,e</sup>	0.07 ± 0.003 <sup>a,b</sup>	0.13 ± 0.01 <sup>e</sup>
DHLCP	0.07 ± 0.01 <sup>c</sup>	0.06 ± 0.01 <sup>b,c</sup>	0.03 ± 0.003 <sup>a</sup>	0.04 ± 0.004 <sup>a</sup>	0.04 ± 0.002 <sup>a,b</sup>	0.03 ± 0.002 <sup>a</sup>	0.07 ± 0.01 <sup>c</sup>
LACglyc	0.12 ± 0.02 <sup>c</sup>	0.13 ± 0.003 <sup>c</sup>	0.08 ± 0.002 <sup>b</sup>	0.03 ± 0.002 <sup>a</sup>	0.05 ± 0.01 <sup>a,b</sup>	0.08 ± 0.01 <sup>b</sup>	0.08 ± 0.004 <sup>b</sup>
LACox	3.8 ± 0.3 <sup>a</sup>	5.2 ± 0.1 <sup>b</sup>	7.2 ± 0.2 <sup>c</sup>	8.8 ± 0.2 <sup>d</sup>	4.1 ± 0.03 <sup>a</sup>	7.0 ± 0.2 <sup>c</sup>	7.6 ± 0.1 <sup>c</sup>
LAC	0.50 ± 0.04 <sup>b</sup>	0.62 ± 0.09 <sup>b,c</sup>	0.46 ± 0.06 <sup>b</sup>	0.70 ± 0.05 <sup>c</sup>	0.26 ± 0.01 <sup>a</sup>	0.52 ± 0.02 <sup>b</sup>	0.56 ± 0.01 <sup>b,c</sup>
doLACglyc	0.003 ± 0.001 <sup>a</sup>	0.01 ± 0.001 <sup>b</sup>	0.03 ± 0.001 <sup>e</sup>	0.02 ± 0.002 <sup>d</sup>	0.01 ± 0.002 <sup>c</sup>	0.02 ± 0.002 <sup>c</sup>	0.01 ± 0.001 <sup>c</sup>
doLACox	3.1 ± 0.3 <sup>a</sup>	3.9 ± 0.1 <sup>b</sup>	8.6 ± 0.3 <sup>d</sup>	9.4 ± 0.2 <sup>e</sup>	12 ± 0.1 <sup>f</sup>	7.7 ± 0.3 <sup>c</sup>	16 ± 0.1 <sup>g</sup>
doLAC	0.25 ± 0.03 <sup>a</sup>	0.40 ± 0.09 <sup>a,b</sup>	0.41 ± 0.06 <sup>a,b</sup>	0.60 ± 0.05 <sup>c</sup>	0.69 ± 0.03 <sup>c</sup>	0.43 ± 0.02 <sup>b</sup>	1.0 ± 0.01 <sup>d</sup>
LCPox	0.22 ± 0.01 <sup>a</sup>	0.21 ± 0.01 <sup>a</sup>	0.31 ± 0.002 <sup>b,c</sup>	0.33 ± 0.01 <sup>c,d</sup>	0.29 ± 0.01 <sup>b</sup>	0.27 ± 0.01 <sup>b</sup>	0.37 ± 0.01 <sup>d</sup>
LCP	0.08 ± 0.01 <sup>a</sup>	0.14 ± 0.03 <sup>b,c</sup>	0.09 ± 0.02 <sup>a</sup>	0.14 ± 0.02 <sup>b,c</sup>	0.09 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a,b</sup>	0.17 ± 0.01 <sup>c</sup>

**Table A2** Relative peak areas (area compound/area internal standard) of the 16 measured SLs in the HRMS profile of the forcing dataset. Different letters indicate statistically significant differences ( $p < 0.001$ ) between different samples.

	NF1	F1	C1	NF2	F2	C2
DHLACglyc	1.5 ± 0.02 <sup>b</sup>	2.1 ± 0.1 <sup>d</sup>	0.20 ± 0.004 <sup>a</sup>	1.8 ± 0.03 <sup>c</sup>	2.3 ± 0.02 <sup>e</sup>	0.13 ± 0.003 <sup>a</sup>
DHLACox	0.97 ± 0.03 <sup>a</sup>	2.0 ± 0.2 <sup>c</sup>	0.97 ± 0.01 <sup>a</sup>	1.4 ± 0.04 <sup>b</sup>	2.7 ± 0.04 <sup>d</sup>	0.76 ± 0.03 <sup>a</sup>
DHLAC	1.3 ± 0.1 <sup>b</sup>	1.1 ± 0.02 <sup>b</sup>	1.2 ± 0.03 <sup>b</sup>	0.63 ± 0.04 <sup>a</sup>	2.2 ± 0.1 <sup>c</sup>	0.64 ± 0.02 <sup>a</sup>
DHdoLACglyc	1.9 ± 0.04 <sup>b</sup>	2.8 ± 0.02 <sup>d</sup>	0.18 ± 0.04 <sup>a</sup>	2.0 ± 0.03 <sup>c</sup>	3.1 ± 0.02 <sup>e</sup>	0.13 ± 0.03 <sup>a</sup>
DHdoLACox	0.62 ± 0.06 <sup>b</sup>	1.1 ± 0.01 <sup>d</sup>	0.23 ± 0.01 <sup>a</sup>	0.90 ± 0.06 <sup>c</sup>	1.2 ± 0.1 <sup>e</sup>	0.21 ± 0.0003 <sup>a</sup>
DHdoLAC	2.9 ± 0.1 <sup>b</sup>	4.9 ± 0.1 <sup>d</sup>	0.13 ± 0.004 <sup>a</sup>	3.4 ± 0.1 <sup>c</sup>	6.2 ± 0.2 <sup>e</sup>	0.09 ± 0.004 <sup>a</sup>
DHLCPox	0.21 ± 0.003 <sup>c</sup>	0.27 ± 0.02 <sup>d</sup>	0.16 ± 0.001 <sup>b</sup>	0.23 ± 0.01 <sup>c</sup>	0.31 ± 0.01 <sup>e</sup>	0.11 ± 0.004 <sup>a</sup>
DHLCP	0.19 ± 0.01 <sup>d</sup>	0.11 ± 0.001 <sup>b</sup>	0.14 ± 0.01 <sup>c</sup>	0.13 ± 0.01 <sup>b,c</sup>	0.17 ± 0.001 <sup>d</sup>	0.08 ± 0.003 <sup>a</sup>
LACglyc	0.07 ± 0.01 <sup>b</sup>	0.09 ± 0.003 <sup>c</sup>	0.06 ± 0.004 <sup>a</sup>	0.10 ± 0.003 <sup>d</sup>	0.13 ± 0.005 <sup>e</sup>	0.05 ± 0.002 <sup>a</sup>
LACox	4.2 ± 0.1 <sup>a</sup>	10 ± 0.4 <sup>c</sup>	5.0 ± 0.1 <sup>b</sup>	5.0 ± 0.1 <sup>b</sup>	11 ± 0.2 <sup>c</sup>	5.0 ± 0.2 <sup>b</sup>
LAC	0.60 ± 0.04 <sup>b</sup>	0.93 ± 0.04 <sup>c</sup>	0.87 ± 0.05 <sup>c</sup>	0.44 ± 0.03 <sup>a</sup>	1.3 ± 0.04 <sup>d</sup>	0.68 ± 0.03 <sup>b</sup>
doLACglyc	0.06 ± 0.002 <sup>a</sup>	0.14 ± 0.01 <sup>c</sup>	-	0.12 ± 0.002 <sup>b</sup>	0.24 ± 0.006 <sup>d</sup>	-
doLACox	3.8 ± 0.1 <sup>a</sup>	8.4 ± 0.5 <sup>d</sup>	5.4 ± 0.1 <sup>b</sup>	3.7 ± 0.1 <sup>a</sup>	7.2 ± 0.1 <sup>c</sup>	5.7 ± 0.1 <sup>b</sup>
doLAC	0.48 ± 0.01 <sup>b</sup>	0.64 ± 0.03 <sup>c</sup>	0.52 ± 0.01 <sup>b</sup>	0.32 ± 0.02 <sup>a</sup>	0.66 ± 0.05 <sup>c</sup>	0.49 ± 0.01 <sup>b</sup>
LCPox	0.18 ± 0.01 <sup>a</sup>	0.22 ± 0.09 <sup>a</sup>	0.16 ± 0.01 <sup>a</sup>	0.19 ± 0.01 <sup>a</sup>	0.25 ± 0.07 <sup>a</sup>	0.14 ± 0.01 <sup>a</sup>
LCP	0.16 ± 0.01 <sup>c</sup>	0.10 ± 0.01 <sup>b</sup>	0.06 ± 0.01 <sup>a</sup>	0.12 ± 0.01 <sup>b</sup>	0.14 ± 0.01 <sup>c</sup>	0.05 ± 0.01 <sup>a</sup>



**Figure A2** k-means clustered PCA-plot of the 16 measured sesquiterpene lactones in the forcing subset containing only the roots, which was power transformed and autoscaled. The total variance captured by the first two PCs is 82.9 %. The loadings were multiplied by factor 10 for visual purposes.



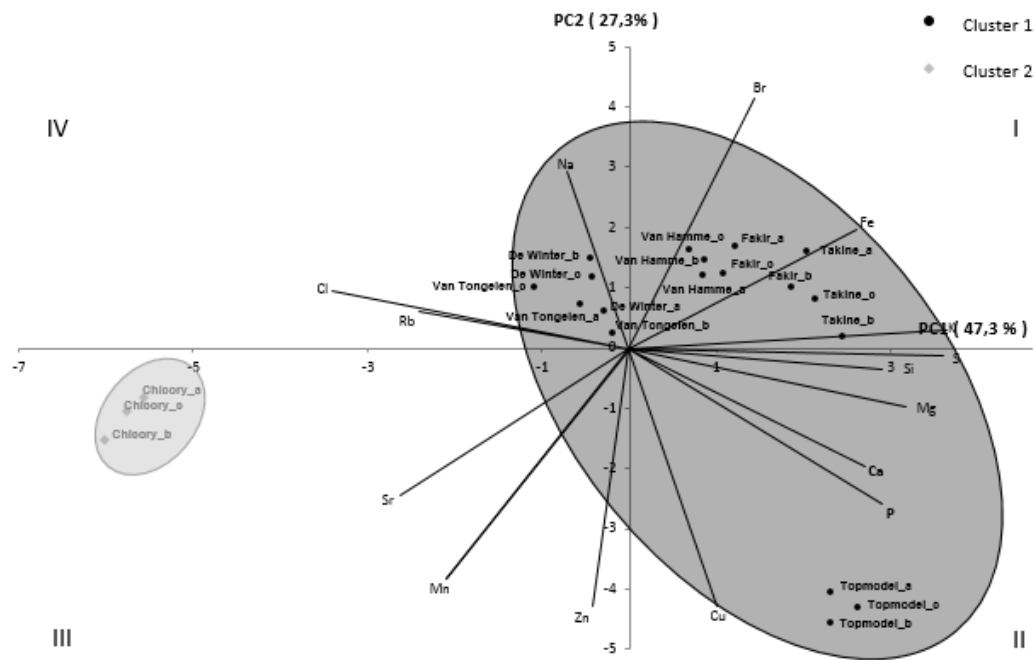
**Figure A3** Antioxidative capacity as determined by the DPPH assay ( $\mu\text{mol TE} \cdot 100\text{g}^{-1}\text{FW}$ ). A) DPPH values the variety dataset and B) DPPH values for the forcing dataset. Different letters indicate statistically significant differences ( $p < 0.05$ ). Vertical bars represent standard deviations.

**Table A3** Elemental composition of the variety dataset. Values are expressed in  $\mu\text{g}\cdot\text{g}^{-1}$  FW. Different letters indicate statistically significant differences ( $p < 0.05$ ) between different samples.

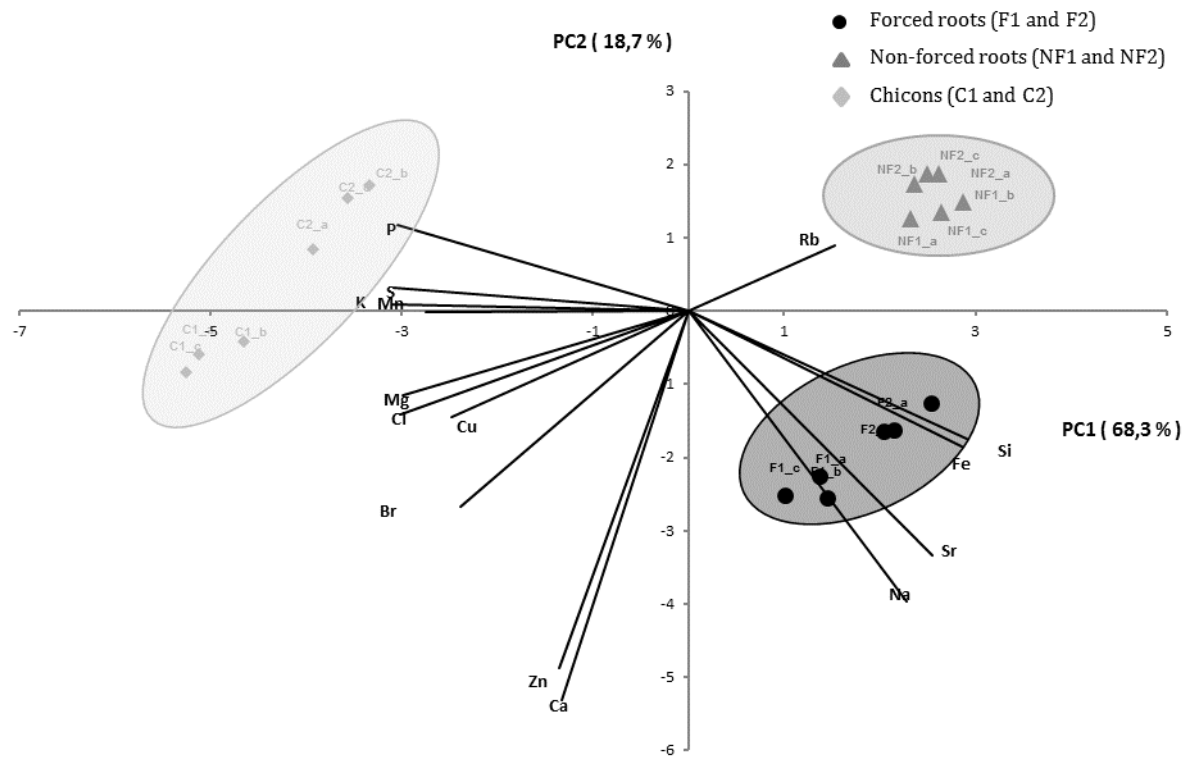
	Industrial chicory	Topmodel	Takine	Van Hamme	Van Tongelen	Fakir	De Winter
<b>Na</b>	37 ± 6 <sup>b</sup>	14 ± 3 <sup>a</sup>	27 ± 1 <sup>a,b</sup>	28 ± 6 <sup>a,b</sup>	35 ± 3 <sup>b</sup>	69 ± 2 <sup>c</sup>	31 ± 1 <sup>b</sup>
<b>Mg</b>	87 ± 1 <sup>a</sup>	136 ± 3 <sup>c</sup>	143 ± 6 <sup>c</sup>	106 ± 0.3 <sup>b</sup>	98 ± 3 <sup>a,b</sup>	130 ± 3 <sup>c</sup>	102 ± 8 <sup>b</sup>
<b>Si</b>	46 ± 1 <sup>a</sup>	58 ± 3 <sup>c</sup>	52 ± 1 <sup>a,b,c</sup>	55 ± 1 <sup>b,c</sup>	49 ± 2 <sup>a,b</sup>	58 ± 3 <sup>c</sup>	55 ± 3 <sup>b,c</sup>
<b>P</b>	289 ± 4 <sup>a</sup>	444 ± 7 <sup>d</sup>	347 ± 9 <sup>b,c</sup>	345 ± 1 <sup>b,c</sup>	368 ± 8 <sup>c</sup>	362 ± 9 <sup>b,c</sup>	342 ± 4 <sup>b</sup>
<b>S</b>	112 ± 1 <sup>a</sup>	175 ± 1 <sup>d</sup>	178 ± 5 <sup>d</sup>	156 ± 0.3 <sup>c</sup>	157 ± 4 <sup>c</sup>	168 ± 4 <sup>d</sup>	141 ± 2 <sup>b</sup>
<b>Cl</b>	498 ± 8 <sup>d</sup>	245 ± 2 <sup>a</sup>	321 ± 6 <sup>b,c</sup>	304 ± 1 <sup>b</sup>	319 ± 7 <sup>b,c</sup>	327 ± 9 <sup>b,c</sup>	339 ± 7 <sup>c</sup>
<b>K</b>	3,079 ± 42 <sup>a</sup>	4,413 ± 43 <sup>c,d</sup>	4,524 ± 133 <sup>d</sup>	4,141 ± 12 <sup>b,c</sup>	3,982 ± 97 <sup>b</sup>	4,141 ± 115 <sup>b,c</sup>	4,136 ± 36 <sup>b,c</sup>
<b>Ca</b>	310 ± 5 <sup>a</sup>	359 ± 5 <sup>c</sup>	351 ± 17 <sup>b,c</sup>	330 ± 5 <sup>a,b,c</sup>	335 ± 12 <sup>a,b,c</sup>	321 ± 11 <sup>a,b</sup>	329 ± 4 <sup>a,b,c</sup>
<b>Mn</b>	1.9 ± 0.1 <sup>d</sup>	1.5 ± 0.1 <sup>c</sup>	0.87 ± 0.1 <sup>b</sup>	0.61 ± 0.04 <sup>a</sup>	0.80 ± 0.03 <sup>a,b</sup>	0.71 ± 0.06 <sup>a,b</sup>	0.78 ± 0.06 <sup>a,b</sup>
<b>Fe</b>	5.3 ± 0.2 <sup>a</sup>	7.4 ± 0.2 <sup>b,c</sup>	7.9 ± 0.7 <sup>b,c</sup>	8.4 ± 0.1 <sup>c</sup>	6.6 ± 0.3 <sup>a,b</sup>	11 ± 1 <sup>d</sup>	8.5 ± 0.2 <sup>c</sup>
<b>Cu</b>	1.2 ± 0.1 <sup>a,b</sup>	1.8 ± 0.1 <sup>c</sup>	1.1 ± 0.1 <sup>a,b</sup>	1.1 ± 0.1 <sup>a,b</sup>	1.2 ± 0.04 <sup>a,b</sup>	1.4 ± 0.1 <sup>b</sup>	1.0 ± 0.1 <sup>a</sup>
<b>Zn</b>	2.7 ± 0.2 <sup>c</sup>	3.3 ± 0.1 <sup>d</sup>	1.7 ± 0.2 <sup>a</sup>	1.7 ± 0.02 <sup>a</sup>	1.7 ± 0.1 <sup>a</sup>	2.4 ± 0.2 <sup>b,c</sup>	2.0 ± 0.1 <sup>a,b</sup>
<b>Br</b>	0.82 ± 0.06 <sup>a</sup>	0.60 ± 0.02 <sup>a</sup>	2.3 ± 0.4 <sup>c</sup>	1.8 ± 0.1 <sup>b,c</sup>	1.5 ± 0.1 <sup>b</sup>	2.0 ± 0.2 <sup>b,c</sup>	1.5 ± 0.1 <sup>b</sup>
<b>Rb</b>	0.61 ± 0.06 <sup>b,c</sup>	0.44 ± 0.03 <sup>a,b</sup>	0.40 ± 0.07 <sup>a</sup>	0.48 ± 0.03 <sup>a,b,c</sup>	0.61 ± 0.02 <sup>b,c</sup>	0.52 ± 0.03 <sup>a,b,c</sup>	0.60 ± 0.08 <sup>c</sup>
<b>Sr</b>	1.3 ± 0.1 <sup>b</sup>	1.1 ± 0.02 <sup>a,b</sup>	0.99 ± 0.17 <sup>a</sup>	0.99 ± 0.01 <sup>a</sup>	1.1 ± 0.04 <sup>a,b</sup>	0.99 ± 0.07 <sup>a</sup>	1.0 ± 0.1 <sup>a,b</sup>

**Table A4** Elemental composition of the forcing dataset. Values are expressed in  $\mu\text{g}\cdot\text{g}^{-1}$  FW. Different letters indicate statistically significant differences ( $p < 0.05$ ) between different samples.

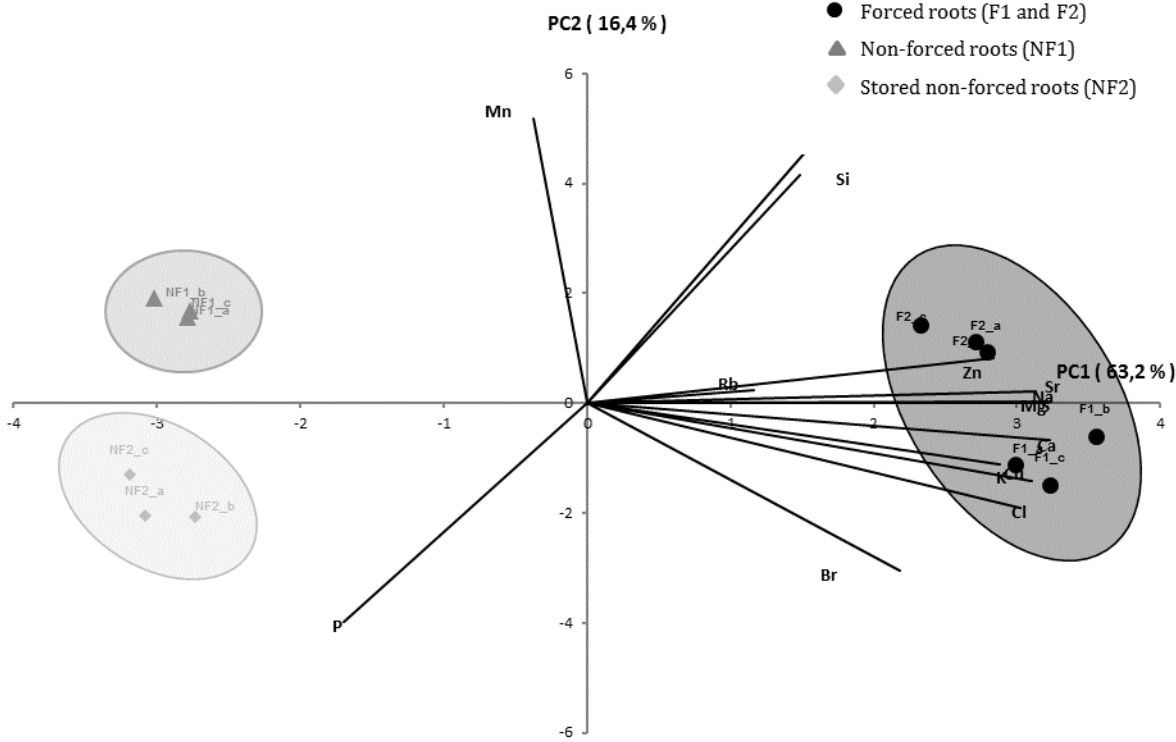
	NF1	F1	C1	NF2	F2	C2
<b>Na</b>	154 ± 4 <sup>b</sup>	288 ± 13 <sup>c</sup>	19 ± 1.2 <sup>a</sup>	166 ± 9 <sup>b</sup>	289 ± 3 <sup>c</sup>	17 ± 1 <sup>a</sup>
<b>Mg</b>	194 ± 4 <sup>b</sup>	193 ± 5 <sup>b</sup>	126 ± 1 <sup>a</sup>	203 ± 3 <sup>b</sup>	201 ± 6 <sup>b</sup>	117 ± 7 <sup>a</sup>
<b>Si</b>	159 ± 20 <sup>c</sup>	117 ± 15 <sup>b</sup>	23 ± 1 <sup>a</sup>	132 ± 4 <sup>b,c</sup>	125 ± 9 <sup>b,c</sup>	22 ± 1 <sup>a</sup>
<b>P</b>	425 ± 5 <sup>c</sup>	316 ± 1 <sup>a,b</sup>	301 ± 0.3 <sup>a</sup>	458 ± 2 <sup>d</sup>	320 ± 7 <sup>b</sup>	307 ± 7 <sup>a,b</sup>
<b>S</b>	206 ± 3 <sup>c</sup>	178 ± 1 <sup>b</sup>	155 ± 1 <sup>a</sup>	207 ± 1 <sup>c</sup>	178 ± 2 <sup>b</sup>	156 ± 6 <sup>a</sup>
<b>Cl</b>	400 ± 11 <sup>a</sup>	613 ± 4 <sup>d</sup>	544 ± 6 <sup>c</sup>	453 ± 4 <sup>b</sup>	459 ± 1 <sup>b</sup>	385 ± 8 <sup>a</sup>
<b>K</b>	3,439 ± 59 <sup>d</sup>	3,278 ± 39 <sup>c</sup>	2,876 ± 24 <sup>a,b</sup>	3,520 ± 24 <sup>d</sup>	2,985 ± 32 <sup>b</sup>	2,792 ± 79 <sup>a</sup>
<b>Ca</b>	436 ± 6 <sup>c</sup>	612 ± 10 <sup>f</sup>	246 ± 2 <sup>b</sup>	465 ± 1 <sup>d</sup>	556 ± 4 <sup>e</sup>	177 ± 8 <sup>a</sup>
<b>Mn</b>	2.0 ± 0.1 <sup>e</sup>	1.2 ± 0.03 <sup>b,c</sup>	0.97 ± 0.02 <sup>b</sup>	1.5 ± 0.1 <sup>d</sup>	1.3 ± 0.1 <sup>c,d</sup>	0.68 ± 0.04 <sup>a</sup>
<b>Fe</b>	24 ± 1 <sup>d</sup>	17 ± 1 <sup>b</sup>	2.9 ± 0.04 <sup>a</sup>	20.3 ± 0.3 <sup>c</sup>	22 ± 1 <sup>c</sup>	2.6 ± 0.1 <sup>a</sup>
<b>Cu</b>	1.9 ± 0.03 <sup>b,c</sup>	1.7 ± 0.1 <sup>b</sup>	0.75 ± 0.01 <sup>a</sup>	2.0 ± 0.2 <sup>c</sup>	1.74 ± 0.1 <sup>b</sup>	0.83 ± 0.04 <sup>a</sup>
<b>Zn</b>	5.9 ± 0.1 <sup>d</sup>	5.1 ± 0.1 <sup>c</sup>	2.0 ± 0.02 <sup>a</sup>	5.3 ± 0.02 <sup>c</sup>	4.7 ± 0.1 <sup>b</sup>	1.9 ± 0.1 <sup>a</sup>
<b>Br</b>	1.1 ± 0.01 <sup>a</sup>	4.2 ± 0.1 <sup>d</sup>	3.7 ± 0.1 <sup>c</sup>	1.4 ± 0.1 <sup>b</sup>	1.1 ± 0.04 <sup>a</sup>	0.90 ± 0.1 <sup>a</sup>
<b>Rb</b>	1.2 ± 0.03 <sup>b,c</sup>	0.95 ± 0.02 <sup>b</sup>	0.16 ± 0.28 <sup>a</sup>	1.4 ± 0.1 <sup>c</sup>	1.2 ± 0.1 <sup>b,c</sup>	0.49 ± 0.05 <sup>a</sup>
<b>Sr</b>	2.7 ± 0.04 <sup>b</sup>	3.5 ± 0.1 <sup>c</sup>	0.37 ± 0.01 <sup>a</sup>	3.0 ± 0.1 <sup>b</sup>	3.9 ± 0.1 <sup>d</sup>	0.24 ± 0.02 <sup>a</sup>



**Figure A4** k-means clustered PCA-plot of the 15 measured elements in the variety subset which was power transformed and autoscaled. The total variance captured by the first two PCs is 74.6 %. The loadings were multiplied by factor 10 for visual purposes.



**Figure A5** k-means clustered PCA-plot of the 15 measured elements in the forcing subset which was power transformed and autoscaled. The total variance captured by the first two PCs is 87 %. The loadings were multiplied by factor 10 for visual purposes.



**Figure A6** k-means clustered PCA-plot of the 15 measured elements in the forcing subset only containing roots, which was power transformed and autoscaled. The total variance captured by the first two PCs is 79.6 %. The loadings were multiplied by factor 10 for visual purposes.



## **Curriculum Vitae**



## Curriculum Vitae

### Personal information

Name: Lies Kips  
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### Education

- 2012-2016**      **PhD in Bioscience engineering**  
*Ghent University – ILVO*  
Doctoral dissertation: ‘Characterization and processing of horticultural byproducts: a case study of tomato and Belgian endive roots’  
Promotors: Dr. ir. Bart Van Droogenbroeck (ILVO), Prof. Dr. ir. Katleen Raes (Ghent University) and Dr. ir. Els Van Pamel (ILVO).
- 2010-2012**      **Master of Bioscience Engineering: Environmental Technology**  
*Ghent University*  
Master thesis: ‘Cavitation: breaking the water column in plants’  
Promotors: Prof. Dr.ir. Kathy Steppe and Dr. ir. Annelies Baert
- 2007-2010**      **Bachelor of Bioscience Engineering: Environmental Technology**  
Ghent University

## Scientific publications in journals with peer review

**Kips, L.,** De Paepe, D., Bernaert, N., Van Pamel, E., De Loose, M., Raes, K. & Van Droogenbroeck, B. (2016). Using a novel spiral-filter press technology to biorefine horticultural by-products: the case of tomato. Part I: process optimization and evaluation of the process impact on the antioxidative capacity. *Innovative Food Science and Emerging Technologies*, 38, 198 – 205.

**Kips, L.,** De Paepe, D., Bernaert, N., Van Weyenberg, S., Van Pamel, E., De Loose, M., Raes, K. & Van Droogenbroeck, B. (2016). Using a novel spiral-filter press technology to biorefine horticultural by-products: the case of tomato. Part II: evaluation of the process impact on the physical tomato juice quality. *Innovative Food Science and Emerging Technologies*, 38, 213 – 220.

**Kips, L.,** De Paepe, D., Van Meulebroeck, L., Van Poucke, C., Larbat, R., Bernaert, N., Van Pamel, E., De Loose, M., Raes, K. & Van Droogenbroeck, B. (2017). A novel spiral-filter press for tomato processing: process impact on phenolic compounds, carotenoids and ascorbic acid content. *Manuscript submitted for publication*.

**Kips, L.,** Annaratone, C., Hertog, M., Van Ceulebroeck, C., Van Poucke, C., Van Pamel, E., Raes, K. & Van Droogenbroeck, B. (2017). Detailed profiling of bioactive compounds in Belgian endive (*Cichorium intybus* L. var. *foliosum*). *Manuscript submitted for publication*.

## Conferences, symposia and trainings

### International conferences with oral presentation

Kips, L., De Paepe, D., Bernaert, N., Van Pamel, E., De Loose, M., Raes, K. & Van Droogenbroeck, B. (2016). Sesquiterpene lactone profile in the crop and root of Belgian endive (*Cichorium intybus* L. var. *Foliosum*) as determined by means of UHPLC-HRMS. RRB-12, Renewable Resources & Biorefineries, Ghent, Belgium, 30 May – 1 June.

Kips, L., De Paepe, D., Bernaert, N., Van Pamel, E., De Loose, M., Raes, K. & Van Droogenbroeck, B. (2016). Using a novel spiral-filter press technology to biorefine horticultural byproducts: process optimization and evaluation of the process impact. The case of tomato. ISEKI Food Conference, Vienna, Austria, 6 – 8 July.

Kips, L., Raes, K., De Loose, M., Van Pamel, E. & Van Droogenbroeck, B. (2014). Valorization requires stabilization: the case of horticultural by-products. Total Food, Norwich, UK, 11 – 13 November.

Kips, L., Bernaert, N., De Loose, M. & Van Droogenbroeck, B. (2013). Use of byproducts as system innovation: GeNeSys – valorization pathways for plant-derived byproducts and wastes. RRB-9, Renewable Resources & Biorefineries, Antwerp, Belgium, 5 – 7 June.

#### **International conferences with poster presentation**

Kips, L., Annaratone, C., Hertog, M., Van Ceulebroeck, C., Van Poucke, C., Van Pamel, E., Raes, K. & Van Droogenbroeck, B. (2016). Sesquiterpene lactone profile in the crop and root of Belgian endive (*Cichorium intybus* L. var. *Foliosum*) as determined by means of UHPLC-HRMS. ISEKI Food Conference, Vienna, Austria, 6 – 8 July.

Van Pamel, E., Annaratone, C., Kips, L., Van Droogenbroeck, B., Van Poucke C., De Rouck, A., Hertog, M. & Nicolai, B. (2015). Determining sesquiterpene lactones in Belgian endive roots (*Cichorium intybus* L. var. *foliosum*) by means of UHPLC-HRMS. Mass Spectrometry in Food and Feed II, Ghent, Belgium, 15 September.

Van Lancker, J., Mondelaers, K., Viaene, J., Kips, L., Hanseeuw, E. & Van Huylenbroeck, G. (2014). Supporting system innovation in the bioeconomy: illustration of an innovation feasibility evaluation instrument. ISEE Conference, Reykjavik, Iceland, 12 – 15 August.

Van Lancker, J., Hanseeuw, E., Kips, L., Viaene, J. & Mondelaers, K. (2013). A conceptual model for innovation research in the bioeconomy. ICABR – 17, Ravello, Italy, 18 – 21 June.

Kips, L., Bernaert, N., Van Pamel, E. & Van Droogenbroeck, B. (2013). GeNeSys: use of byproducts as system innovation. Knowledge for Growth, Ghent, Belgium, 30 May.

#### **Trainings**

Training school Montreux (2016). Regulations and policy in food waste valorization. COST-EUBIS Action TD1203.

Training school Lisbon (2014). Food waste processing in the frame of the biorefinery concept. COST-EUBIS Action TD1203.

Workshop Turin (2013). Biorefinery for the production of energy and bio-based products. COST-EUBIS Action TD1203. (Speaker)

Training school Dublin (2013). Designing food structures with health benefits: from concept to commercialization. COST Action FA1001

## Scientific reports

Verstraete, K., Van Droogenbroeck, B, Bernaert, N. & Kips, L. (2017). *Witloofwortels als rijke grondstof*. Flanders' FOOD Radar.

Kips, L., Van Droogenbroeck, B., Bernaert, N. & Verstraete, K. (2015). *Haal meer uit je product*. Management & Techniek.

Kips, L., Matthijs, F. & Riebbels, G. (2014). *De kers op de tomaat*. VMT Food.

Bernaert, N., Kips, L., Van Droogenbroeck, B. & De Loose, M. (2014). *ILVO-onderzoek helpt kringlopen te sluiten*. VILT.

Kips, L. & Van Droogenbroeck, B. (2014). *Valorisatie van groente- en fruitreststromen: opportuniteiten en knelpunten*. ILVO Mededeling nr. 165, 70 p.

## Supervision of master students and internships

Meylemans, W. (2016). *Waardevol afval? Karakterisatie van geforceerde witloofwortel*. Master of Science in Bioscience, Ghent University, Supervisor: Prof. Dr. Marc De Loose.

Van de Walle, J. (2015). *Valorisatie, karakterisatie en economische doorrekening van de overproductie van tomaat*. Master of Science in Bioscience, Ghent University, supervisor: Prof Dr. Marc De Loose.

Wylin, Z. (2015). Internship Ter Groene Poorte.

Hervent, E. (2014). *Stabiliseren van tuinbouwreststromen om te kunnen valoriseren*. Master of Science in Bioscience. Ghent University, Supervisor, Prof. Dr. Marc De Loose.

Nageshwar, P. (2014) Internship Ghent University.

## Specialist courses (Ghent University)

Introduction to R (2014)

Analysis of Variance (2013)

Advanced Academic English: Writing skills (2013)

Advanced Academic Conference Skills (2012)

