Helsingin yliopisto
Elintarvike- ja ympäristötieteiden laitos

University of Helsinki
Department of Food and Environmental Sciences

ETK-sarja 1674
ETK-series 1674

Oxidative stability of solid foods with dispersed lipids

Annelie Damerau

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Agriculture and Forestry, University of Helsinki, for public examination in Auditorium XIV, University Main Building, on March 28th, 2015, at 12 o'clock noon.

Helsinki 2015

Custos: Professor Vieno Piironen
Department of Food and Environmental Sciences
University of Helsinki
Helsinki, Finland

Supervisors: Professor Vieno Piironen
Department of Food and Environmental Sciences
University of Helsinki
Helsinki, Finland

Docent Anna-Maija Lampi
Department of Food and Environmental Sciences
University of Helsinki
Helsinki, Finland

Reviewers: Professor Afaf Kamal-Eldin
Department of Food Sciences
Faculty of Food and Agriculture
United Arab Emirates University
Al-Ain, United Arab Emirates

Dr Pekka Lehtinen
Senson Oy
Lahti, Finland

Opponent: Professor Karin Schwarz
Department of Food Technology
Faculty of Agricultural and Nutritional Science
Christian-Albrechts-University of Kiel

ISBN 978-951-51-0873-9 (paperback)
ISBN 978-951-51-0874-6 (pdf; http://ethesis.helsinki.fi)
ISSN 0355-1180

Unigrafia
Helsinki 2015

Damerau, A. 2015. Oxidative stability of solid foods with dispersed lipids (dissertation). ETK-series 1674. University of Helsinki, Department of Food and Environmental Sciences. 96 + 37 pp.

ABSTRACT

The consumption of whole grain foods high in fibre is of interest because of the health-promoting effects associated with dietary fibre. Therefore, there is interest in developing new fibre-rich cereal foods. However, these kinds of foods also contain polyunsaturated lipids, which are prone to oxidation. Further, lipids are dispersed in a heterogeneous matrix of starch, proteins and fibre, which increases their tendency to oxidize because of a large surface area and possible contact with prooxidants. The oxidation of lipids decreases nutritional quality and causes the formation of undesirable flavours. Knowledge of the oxidation behaviour of dispersed lipids in solid cereal foods, and of how factors like process parameters, structural features of the products and storage conditions affect lipid oxidation, is limited.

In this thesis, the oxidative behaviour of foods with dispersed lipids was studied using two model systems. The first model system was a spray-dried emulsion containing sunflower oil encapsulated in a Na-caseinate-maltodextrin matrix, with either non-cross-linked or cross-linked proteins. The stability of the total and surface lipid fractions was determined during storage under different relative humidities (RHs). Further, the effect of RH on the amount of volatiles released from oxidized spray-dried emulsions was studied. The second model system consisted of extruded cereals produced from either whole grain oats or rye bran (coarse or fine) using different extrusion parameters. Their oxidative stability was studied during storage at 40 ºC, after milling and standardization to RH 33%. The primary oxidation was measured by peroxide values in the spray-dried emulsions and by losses of tocopherols and tocotrienols in the spray-dried emulsions and rye bran extrudates. Secondary oxidation was determined based on volatile secondary lipid oxidation products analysed by static head space (SHS-GC-FID) in the spray-dried emulsions and by head space solid-phase micro extraction (HS-SPME-GC-MS) in the extruded cereals. In addition to the oxidation parameters, enzymatic hydrolysis of lipids in the oat extrudates and the fatty acid composition of all models were studied by measuring the neutral lipid and fatty acid profiles, respectively.

Increasing the RH improved the oxidative stability of both the total and surface lipid fractions of the stored spray-dried emulsions. This behaviour was mainly linked to the loss of individual powder particles upon caking and collapsing of the matrix at RH 75%. In addition, excess protein may have delayed oxidation via its radical scavenging activity. At RH 54%, cross-linking of the protein slightly improved the oxidative stability. The profiles of the volatile oxidation products from the spray-dried emulsions analysed by HS-SPME were also influenced by the RH. The effect was related to water-induced changes in hydrophilicity, structure and binding ability of the matrix, and to partitioning and solubility of the volatiles. The highest overall amount of volatiles released was obtained at water contents of 3.1% and 5.2% (RH 11% and 33%).

The enzymatic hydrolysis of lipids in oats was effectively prevented by extrusion, even at the lowest temperature of 70 °C. The extrusion temperature could be increased to 110 °C without subjecting the lipids to non-enzymatic oxidation. However, by increasing the temperature to 130 °C, lipid oxidation was promoted, which also yielded losses of neutral lipids over time. In the case of the rye bran, the low water content (13% or 16%) in the extrusion of coarse or fine bran led to the most stable lipids during storage. The improved oxidative stability at low water contents in extrusion was connected with the higher formation of Maillard reaction products, which could have acted as antioxidants. The grinding of rye bran prior to extrusion caused a loss of tocols and increased the amounts of Maillard reaction products formed.

The oxidative stability of the dispersed lipids was shown to be highly related to water induced physical changes in the matrix structure, which makes controlling the RH in the surrounding atmosphere an important factor in storage. Further, the RH affected the amount of volatile lipid oxidation products released, and this needs to be considered in determining lipid oxidation by HS-SPME. Extrusion was shown to inactivate lipases in oats. For the lipid stability in cereal extrudates, low temperature and low water content during extrusion were shown to be beneficial.

PREFACE

I started to study food chemistry at University of Hamburg in 2003. During my studies I gained knowledge about the different aspects of food chemistry and learned how research worked during my diploma work on food allergies in wine. After a short detour in world of heavy metals and ICP-MS at Evira (Finnish Food Safety Authority) in 2008/2009, I found myself in beginning of 2010 at the University of Helsinki started to work on a new topic. Lipid oxidation chemistry was in theory not new for me, but extraction and analysis of the often quiet sensitive lipids were challenging. Also being part of a project combining different field of food science was an interesting challenge, I enjoined taking.

This study was part of a joined project between Division of Food Chemistry at the Department of Food and Environmental Sciences of the University of Helsinki (oxidation mechanisms and chemical analysis) and VTT Technical Research Centre of Finland (biomaterial science, bio- and thermomechanical processing) from 01.01.2010 to 31.03.2014. The project was carried out with financial support from the Finnish Funding Agency for Technology and Innovation (Project nos: 40500/10 and 40499/10). The last year of my PhD study was funded by the Doctoral Programme in Food Chain and Health. Their financial support is gratefully acknowledged.

I own my sincerest gratitude to my supervisors Professor Vieno Piironen and Docent Anna-Maija Lampi for giving me the opportunity of working in such an interesting project and supporting my work in last five years. Vieno, thank you for your advice, guidance and encouragement! Annukka, your help and patience apropos of lab work and writing was invaluable for me to reach so far. Thank you for this! I warmly thank also my project partners and co-authors at VTT, Dr Pirkko Forssell, Dr Riitta Partanen and MSc Timo Moisio, for their cooperation and for sharing their knowledge on food structures and processes with me. I wish to acknowledge Professor Laura Nyström for giving me the opportunity to visit her research group at ETH Zurich twice during the project to learn new techniques. Further, I want to thank the students I had the pleasure to work with during their master theses, MSc Pimwalee Kamlang-ek, MSc Marjo Pulkkinen, MSc Jia Li and MSc Xiaoxue Qin.

I wish to express my appreciation to Professor Afaf Kamal-Eldin and Dr Pekka Lehtinen for careful pre-examination of this thesis. I am thankful for your constructive comments and suggestions towards my thesis. I also wish to express my gratitude to my monitoring group Professor Maija Tenkanen and Docent Kirsi Jouppila.

I want to thank all my present and former colleagues in Viikki D-bulding for making working here so enjoyable over the years. Special thanks go to Dr Mari Lehtonen, Dr Tanja Nurmi, Dr Minnamari Edelmann, Docent Susanna Kariluoto and Miikka Olin for their help and advice especially in the beginning. I want to thank Mari, Tanja and Minnamari also for sharing their

experiences on how to write, publish and defend your thesis with me and encouraging me on my way. Further, I want to mention the current doctoral students: Bahawani Chamlagain, Tuuli Koivumäki, Marjo, Göker Gürbüz and Bei Wang. Thank you all for the joyful moments in the lab and outside of it.

Ich möchte meinen Freunden hier und Hamburg danken. Besonderer Dank geht dabei an Jenny und Martina. Jenny, du hast schon seit meiner Schulzeit mich in allem unterstützt und bist wahrlich eine meiner besten Freundinnen. Martina, wir haben uns bei Evira in 2008 kennengelernt. Du hast mir vom ersten Tag an geholfen in Finnland zurechtzukommen und wir haben uns schnell angefreundet. Ich danke dir für die vielen Gespräche in denen du mir stets aufmerksam zugehört und mir Mut zugesprochen hast.

Mein tiefster Dank geht an meine Eltern, Irma und Hans-Jürgen, und meinen Bruder, Kai, die mich stets liebevoll in allen meinen Entscheidungen unterstützt haben und immer für mich da waren, wenn ich sie gebraucht habe. Besonderer Dank geht Kai für seine Hilfe mit den Titelbild und weiteren Grafiken. Weiterhin möchte ich Raimo und Liane danken. Ihr seid für mich wie meine zweiten Eltern. Eure Hilfe und Unterstützung besonders am Anfang als ich erstmals nach Finnland kam und kaum Finnisch sprach war unschätzbar wertvoll für mich. Am Ende möchte ich allen Familienmitgliedern in Deutschland, besonders Oma und Waldi, und in Finnland danken für ihre Unterstützung.

Helsinki, February 2015

Annelie Damerau

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on following original publications, which are referred to in the text by Roman numerals **I-IV**:

I Damerau A, Moisio T, Partanen R, Forssell P, Lampi A-M, Piironen V. 2014. Interfacial protein engineering for spray-dried emulsions – Part II: Oxidative stability. Food Chem 144:57-64.

II Damerau A, Kamlang-ek P, Moisio T, Lampi A-M, Piironen V. 2014. Effect of SPME extraction conditions and humidity on the release of volatile lipid oxidation products from spray-dried emulsions. Food Chem 157:1-9.

III Lampi AM, Damerau A, Li J, Moisio T, Partanen R, Forssell P, Piironen V. 2015. Changes in lipids and volatile compounds of oat flours and extrudates during processing and storage. J Cereal Sci 62:102-109.

IV Moisio T, Damerau A, Lampi A-M, Partanen R, Forssell P, Piironen, V. 2015. Effect of extrusion processing on lipid stability of rye bran. Eur Food Res Technol. Published online. DOI:10.1007/s00217-015-2433-y.

Contribution of the author to papers I to IV:

I Annelie Damerau planned the study with the other authors and carried out the experimental work. She had the main responsibility for interpreting the results and for preparing the manuscript. She acted as the corresponding author of the paper.

II Annelie Damerau planned the study with the other authors. The experimental work was conducted partly as a Master's Thesis (P. K.). Annelie Damerau had the main responsibility in the supervision of the experimental work, in the result interpretation and in preparing the manuscript. She acted as the corresponding author of the paper.

III Annelie Damerau planned the study with the other authors. The experimental work was conducted partly as a Master's Thesis (J. L.). Annelie Damerau was co-responsible for the supervision of the experimental work in general, and had the main responsibility in the volatile research and in interpreting its results. She participated in the manuscript preparation.

IV Annelie Damerau planned the study with the other authors. She was responsible for conducting the storage test. She had the main responsibility for interpreting the results on lipid stability and writing that part of the manuscript.

ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
a_w	Water activity
ASE	Accelerated solvent extraction
CAR	Carboxen
CL	Spray-dried emulsion with cross-linked protein
DAGs	Diacylglycerols
DE	Dextrose equivalent
DHS	Dynamic headspace
DSC	Differential scanning calorimetry
DVB	Divinylbenzene
ELSD	Evaporating light scattering detector
FID	Flame-ionization detector
FFAs	Free fatty acids
FLD	Fluorescence detection
GC	Gas chromatography
HPLC	High-performance liquid chromatography
HPSEC	High-performance size exclusion chromatography
HS-SPME	Headspace solid-phase micro extraction
HT	Heat-treated
MAGs	Monoacylglycerols
MDA	Malondialdehyde
MS	Mass spectrometry
NCL	Spray-dried emulsion with non-cross-linked protein
na	Not analysed
NHT	Non-heat-treated
NP	Normal-phase
PA	Polyacrylate
PCA	Principal component analysis
PDMS	Polydimethyl siloxane
PV	Peroxide value
AnV	*Para*-anisidine value
RH	Relative humidity
SHS	Static headspace
SME	Specific mechanical energy input
TAGs	Triacylglycerols
TBA	Thiobarbituric acid

TBARS	Thiobarbituric acid reactive substance
tocols	Tocopherols and tocotrienols
UV	Ultraviolet
WSI	Water solubility index

1 INTRODUCTION

Whole grain cereal products are high in fibre and thus beneficial for health, because dietary fibre-rich diets contribute to a decreased risk of diseases like diabetes, cardiovascular diseases, colorectal cancer and obesity (Anderson et al. 2009). In recent years, consumer studies have shown that consumers are more aware of the health benefits of dietary fibre, and that their buying behaviour is influenced by this knowledge (Black and Lewis 2009). Therefore, there is interest from the food industry to develop new fibre-rich cereal foods to provide the consumer with healthy alternatives for, for example, conventional snack-products high in starch, sugar and saturated fat (Brennan et al. 2013). However, fibre-rich cereal foods often contain polyunsaturated lipids present in as dispersed lipids, which make them prone to oxidation and reduce their shelf life.

Lipid oxidation is a major chemical reaction that leads to the deterioration of foods containing polyunsaturated lipids. Oxidation can decrease nutritional quality and cause the formation of undesirable flavours, as well as compounds with possible adverse health effects (Schaich et al. 2013). This can cause challenges in the product development of foods with dispersed polyunsaturated lipids. One approach used to limit the oxidation of oils containing polyunsaturated lipids is to microencapsulate them in a carbohydrate and/or protein matrix; this can be accomplished by spray-drying or freeze-drying of oil-in-water emulsions. The matrix surrounding the oil droplets in the dried emulsions restricts contact between the oil and atmospheric oxygen and therefore may decrease oxidation (Márquez-Ruiz et al. 2003).

Process parameters, encapsulation material (wall material) and storage conditions are known to affect the oxidative stability of the dried microencapsulated oils (Velasco et al. 2003). The choice of wall material determines the behaviour during the drying process and storage. The most common encapsulation materials are carbohydrates (such as maltodextrins, starches and corn syrup solids), proteins (mainly milk proteins and gelatine) and gums (like gum arabic). They are used alone or in mixtures (Gharsallaoui et al. 2007). Several approaches like enzymatic cross-linking of proteins (Bao et al. 2011) or using bilayered interfaces (Klinkesorn et al. 2005) have been applied to modify the interfacial layer between oil and wall material, which may improve the stability of the oil. Although, there are several studies concerning the oxidative stability of dried emulsions with different matrices (Velasco et al. 2003), further knowledge on the effects of matrix-related factors on the oxidation of the dispersed oils is needed to fully understand the impact of wall material, interfacial layer and, process and storage conditions. Dried emulsions can be used as models for solid foods containing dispersed lipids.

Fibre-rich cereal products can be produced by several processes using a variety of raw materials. The difficulty in the process is to create a biopolymer network out of the main cereal ingredients,

starch and proteins, which hold (encapsulate) the lipids and prevent their oxidation by limiting the contact between the unsaturated lipids and oxygen. Such networks can be formed by extrusion of cereals (Ho and Izzo 1992). Extruded cereals are foods with dispersed lipids imbedded in a puffed structure of starch, proteins and fibre. Process parameters like heat, water content and screw speed are crucial for the final structure of the product (Moraru and Kokini 2003).

The extrusion of maize and wheat is widely studied (Moraru and Kokini 2003; Singh et al. 2007). However, the extrusion of oats and rye, both traditionally used cereals in Finland with a high acceptance by Finnish consumers (Prättälä et al. 2001), are studied less. Further, most studies focus on the process and physical attributes like expansion, crispiness and hardness. Only in a few studies was the lipid stability of the product determined directly after extrusion (Guth and Grosch 1993; Zadernowski et al. 1997; Parker et al. 2000), and even fewer papers exist on the lipid stability of cereal extrudates during storage (Rao and Artz 1989; Sjövall et al. 1997; Gutkoski and El-Dash 1998).

Oats (*Avena sativa*) is considered to be a health beneficial cereal mainly due to its high β-glucan content, which has been shown to help lower serum cholesterol, reduce glucose and insulin levels and improve satiety (Xu 2012). However, oats contains lipolytic and oxidative enzymes, which cause hydrolytic and oxidative reactions when the grain structure is broken (Lehtinen and Kaukovirta-Norja 2011). This and, in general, the higher lipid content of oats compared to other cereals could decrease the oxidative stability. Extrusion was shown to inactivate the lipolytic enzymes; however, it also initiated non-enzymatic oxidation of lipids (Lehtinen et al. 2003). Several other studies observed off-flavour formation caused by lipid oxidation in extruded oats (Guth and Grosch 1993; Sjövall et al. 1997; Parker et al. 2000).

Rye (*Secale cereale* L.) bran, the outer part of the rye grain, contains high amounts of dietary fibre and is widely produced as a by-product in rye flour manufacturing (Kamal-Eldin et al. 2009), making it appealing as a raw material in the development of fibre-rich foods. Until now a wider usage of rye bran in foods has been limited because of its bitter taste caused by specific phenolic compounds and small peptides (Heiniö et al. 2008). However, extrusion was found to be an effective processing technique in masking the intense rye-like flavour (Heiniö et al. 2003a). Nevertheless, applying extrusion to produce bran-enriched cereal products is challenging because the high fibre content leads to reduced expansion, high density, a hard and less crispy texture and thus, poor sensory perception by consumers (Robin et al. 2012). One recent study focusing on the physical attributes of the extrudates, however, showed the potential of using rye bran in extrusion (Alam et al. 2013).

Besides the raw material, process parameters and structure of the product, storage parameters can be crucial for the stability of dispersed lipids. Water sorption, desorption or diffusion may occur in products via changes in the relative humidity (RH) in the surrounding atmosphere, or because of the meta-stable nature of the produced biopolymer-water network. This can cause unwanted changes in the food matrix like the loss of crispness, caking and crystallisation, which also affect the stability of lipids in the product (Nelson and Labuza 1992).

In this thesis, the literature review gives an overview of the lipid degradation reactions, the spray-drying and the extrusion process as examples of producing solid foods with dispersed lipids, and the methods used to determine lipid degradation in spray-dried emulsions and extruded cereals. Factors affecting the oxidative stability of spray-dried emulsions during storage are reviewed, and an overview of the lipid stability of cereal extrudates with a special focus on oat and rye bran extrudates is given. The experimental part of this thesis summarizes the data published in the attached papers, I-IV. First, the volatile analyses are presented with a focus on the suitability of headspace solid-phase micro extraction (HS-SPME) for the determination of volatile secondary lipid oxidation products. Further, the effect of RH on the oxidative stability of spray-dried emulsions was studied. The storage stability of oat extrudates was determined in comparison to the storage stability of oat flours, and the oxidative stability of rye bran extrudates produced at different process parameters was studied under storage. The significance of the results is discussed, conclusions are made and further research needs are indicated.

2 REVIEW OF THE LITERATURE

2.1 Degradation of lipids

The degradation of lipids in foods mainly occurs via non-enzymatic and enzymatic oxidation processes. These processes can lead to off-flavours, unwanted textural changes and the formation of compounds with adverse health effects (Bartosz and Kołakowska 2011). Many studies and reviews have been published considering lipid oxidation mechanisms and the formation of lipid oxidation products in foods (Labuza 1971; Frankel 1980, 1998; Min and Boff 2002; Choe and Min 2006; Kiokias et al. 2009; Bartosz and Kołakowska 2011; Schaich et al. 2013). Although lipid oxidation has been studied for more than 70 years, all possible reaction pathways and factors affecting it are still not known. This shows the complexity of lipid oxidation, especially in foods where lipids are commonly accompanied by other compounds, which can have catalytic or inhibitory effects on oxidation reactions (Schaich et al. 2013).

2.1.1 Chemical lipid oxidation

The two main oxidation reactions of lipids in foods are autoxidation and photooxidation.

Autoxidation

Autoxidation is a free radical chain reaction with three main stages (initiation, propagation and termination) (Frankel 1980). In the initiation step, a hydrogen is removed from an unsaturated fatty acid in a lipid molecule, resulting in the formation of a lipid alkyl radical. Allylic hydrogens (bound to carbons next to double bonds) are preferably removed, based on the low energy levels of the corresponding C-H bonds. Therefore, the location for the abstraction of hydrogen is dependent on the chemical structure of the fatty acid (Choe and Min 2006). During the alkyl radical formation, the double bond shifts to the next carbon, creating conjugated dienes in 1,4-diene systems. Further, the shifted double bond converts from the *cis* to *trans* configuration. The formation of the first lipid alkyl radicals requires some kind of initiator; for example, excited photosensitizers (photosensitization type 1), preformed radicals or metals (Schaich et al. 2013). For many foods, an induction period can be established in which lipid oxidation may already have started by the formation of the first radicals in the initiation step, but still, no oxidation products can be detected. The length of the induction period depends on the reactivity of lipids, oxygen availability, antioxidants and catalysts present (Labuza 1971).

In the propagation step, lipid alkyl radicals react with oxygen to form peroxyl radicals, which are more reactive than the initially formed alkyl radicals. Then, the peroxyl radicals remove hydrogen again from lipid molecules causing the formation of a new lipid alkyl radicals and a hydroperoxides. This reaction establishes the radical chain (Choe and Min 2006; Schaich et al.

2013). At this point, the oxidation proceeds at a monomolecular rate with respect to hydroperoxides where peroxyl radicals are the chain carriers. Based on the oxygen availability, either the formation of peroxyl radicals (low oxygen level) or the abstraction of hydrogen from lipids by peroxyl radicals (high oxygen level) is the rate limiting step for the oxidation (Labuza 1971). In this slow early state of lipid oxidation, hydroperoxides can accumulate. They decompose either in the presence of metals, heat or ultraviolet (UV) light, or by the interaction of two hydroperoxides (bimolecular mechanism) at a high concentration of hydroperoxides to peroxyl radicals (oxidizing metals and bimolecular mechanism), alkoxyl radicals (reducing metals, heat, UV and bimolecular mechanism) and hydroxyl radicals (heat and UV). The formed alkoxyl and hydroxyl radicals are more reactive and less selective than the peroxyl radicals (Schaich et al. 2013). At this stage, oxidation proceeds at a bimolecular rate (Labuza 1971), and alkoxyl radicals become the main chain carrier. This initiates the next step of propagation (branching). In the branching step, new radical chains are created, which increase the oxidation rate. The newly created secondary chains amplify and broaden the oxidative reaction (Schaich et al. 2013).

In the termination step, stable secondary products are formed by radical recombinations, β-scission of alkoxyl radicals, co-oxidation of non-lipid molecules or group eliminations. The radical recombinations follow distinctive schemes causing the formation of dimers and polymers of alkanes, alcohols, ketones, ethers and alkyl peroxides (Schaich et al. 2013). When the alkoxyl radicals undergo β-scission, the C-C bond on either side of the alkoxyl group is cleaved. This leads to oxo-compounds and saturated or unsaturated alkyl radicals, which react further and form a complex mixture of secondary oxidation products (Choe and Min 2006). In co-oxidation, the radical is transferred to a non-lipid molecule leaving a stable compound behind. Most common radical receptors in foods are proteins or phenolic compounds (can be antioxidants). Group eliminations are a less important type of termination resulting in ketones or unsaturated compounds with an extra double bond, depending on the eliminators (HO^- and HOO^-). All of these reactions slow down lipid oxidation by terminating certain radical chains. However, oxidation continues since always some radicals are left behind (Schaich et al. 2013).

Photooxidation

Compared to autoxidation, in photooxidation hydroperoxides can be formed directly by the reaction of singlet oxygen with the carbon of the C-C double bond of the unsaturated fatty acid (photosensitization type 2). Singlet oxygen is formed by excitation of triplet molecular oxygen under light in the presence of photosensitizers like chlorophyll, heme proteins (for example, haemoglobin) and erythrosine. By light excitement, triplet photosensitizers can also react directly with lipid molecules by abstraction of hydrogen (photosensitization type 1). The reaction based on photosensitization type 1 results in the formation of an alkyl radical, which can start a radical chain as described for autoxidation. However, in photooxidation, based on photosensitization type 2, it is an ene and not a radical chain reaction which initiates the oxidation. During the

formation of hydroperoxides, double bonds shift and *trans* fatty acids are formed (Min and Boff 2002). Hydroperoxides with more than one double bond formed by photooxidation can be either conjugated or not, whereas in autoxidation only conjugated compounds have been observed. When hydroperoxides start to decompose, radicals are formed similar to autoxidation initiating free radical chain reactions, and the oxidation products are also similar to autoxidation (Choe and Min 2006). However, they are not identical, because of differences in structure and quantities of hydroperoxides formed by photooxidation compared to autoxidation. For example, the primary reaction products in the autoxidation of linoleic acid are 9- and 13-hydroperoxides, while in photooxidation, large quantities of 10- and 12-hydroperoxides are found in addition to the 9- and 13-hydroperoxides. Further, photooxidation is quicker than autoxidation and has, therefore, a higher potential to cause degradation of lipids in foods (Min and Boff 2002).

Off-flavour formation (β-scissions of alkoxyl radicals)

Further reaction products of the β-scissions of alkoxyl radicals, like low-molecular-weight aldehydes, ketones, alcohols and alkanes or alkenes, are mainly responsible for the off-flavour of oxidized lipids (Ho and Chen 1994). The type and amount of products formed depend highly on the structure of the hydroperoxides cleaved in the scission.

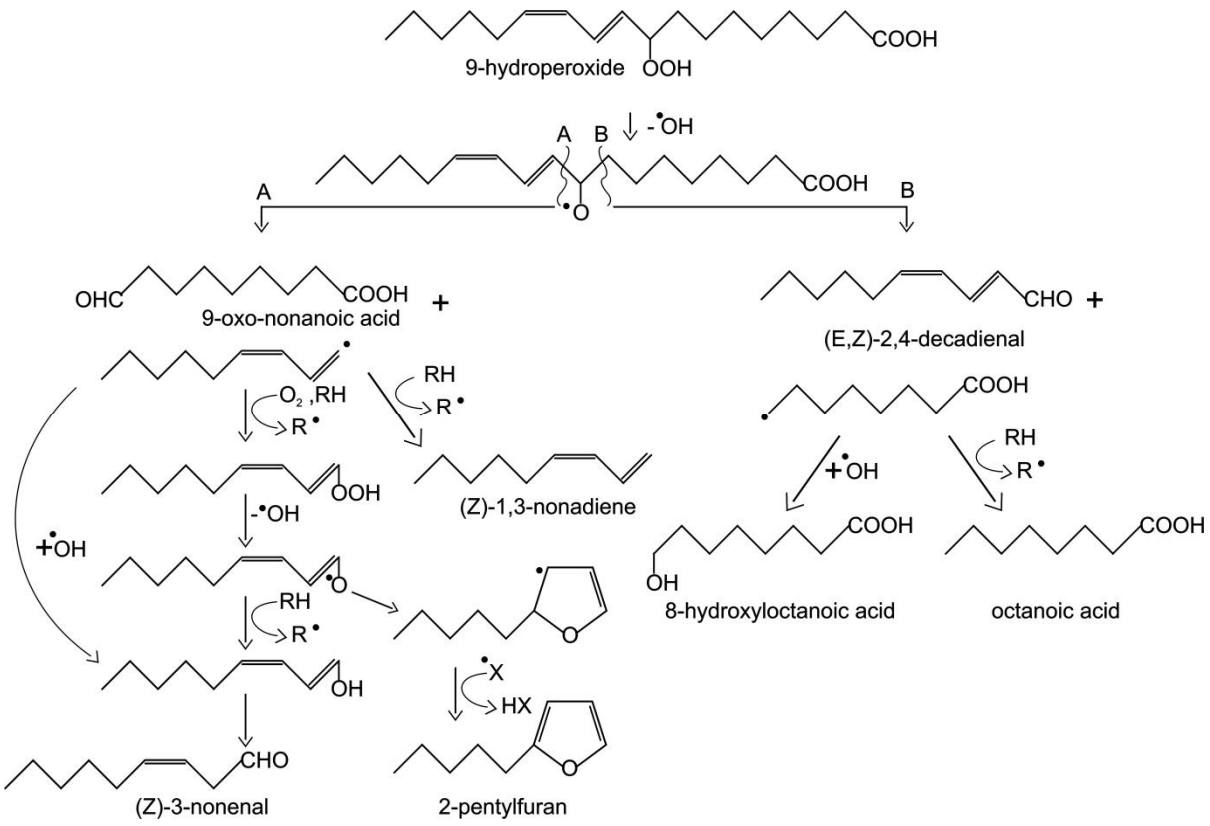

Figure 1. Scheme of β-scissions of 9-hydroperoxide of linoleic acid and formation of first stable compounds. The scheme is combined from Jeleń and Wąsowicz (2012) and Schaich et al. (2013).

From the 9-hydroperoxide of linoleic acid, an alkoxyl radical is formed by the elimination of the hydroxyl radical (Figure 1). Then, the alkoxyl radical undergoes β-scission, and 9-oxo-nonanoic acid (route A), 2,4-decadienal (route B) and alkyl radicals are formed. The formed alkyl radicals can react further. In route A, for example, 1,3-nonadiene and 3-nonenal are formed. 3-Nonenal can be formed either by the addition of a hydroxyl radical following the rearrangement from the enol form to the aldehyde, or by the formation of a new hydroperoxide followed by the formation of a newly formed alkoxyl radical, which reacts further with a lipid molecule resulting in an alkyl radical and an enol. This again undergoes the rearrangement from the enol form to the aldehyde. An alternative route for the 3-nonenal formation via the formation of a new hydroperoxide is the reaction to 2-pentylfuran by cyclisation of the newly formed alkoxyl radical following the elimination of hydrogen for the stabilisation of the aromatic heterocyclic structure. Following the breakdown route B, octanoic acid and 8-hydroxyloctanoic acid can be formed.

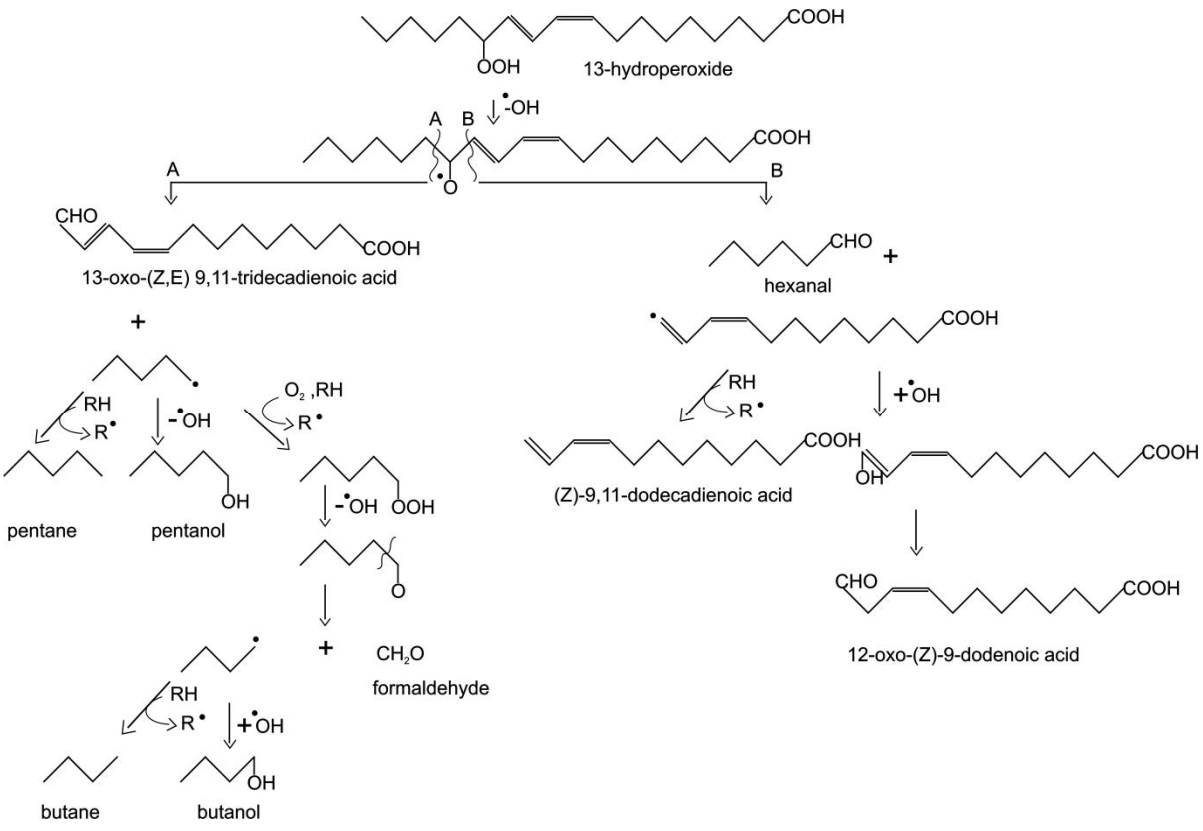

Figure 2. Scheme of β-scissions of 13-hydroperoxide of linoleic acid and formation of first stable compounds. The scheme is combined from Jeleń and Wąsowicz (2012) and Schaich et al. (2013).

The alkoxyl radical formed from the 13-hydroperoxide of linoleic acid reacts by β-scission to 13-oxo-9,11-tridecandienoic acid, hexanal and alkyl radicals, which can react further to form stable compounds (Figure 2). Following the reaction route A, pentane and pentanol are formed, but butane, butanol and formaldehyde can also be formed after the formation of a new hydroperoxide and the β-scission of the corresponding alkoxyl radical. In route B, 9,11-dodecadienoic acid and 12-oxo-9-dodenoic acid can be formed.

However, these are not the only possible compounds formed from the 9- and 13-hydroperoxides of linoleic acid. Other compounds could be created by the formation of new hydroperoxides, following the formation of new alkoxy radicals and their β-scission. Further, double bonds can convert during the formation of stable compounds (Schaich et al. 2013). Therefore, compounds with different stereochemistry, as shown in Figure 1 and 2, can be formed during lipid oxidation.

Influencing factors

Lipid oxidation in foods is influenced by the food composition (including water content, water activity (a_w) and pH), food structure and production/storage conditions. In the food composition, the chemical nature of lipids (for example, more unsaturated, more likely to oxidize) and the presence of anti- and prooxidative factors play important roles in the severity of lipid oxidation (Labuza 1971). Prooxidative factors, which initiate and/or catalyse oxidation, are preformed radicals, metals, photosensitizers and enzymes (see 2.1.2). Compounds which either quench radicals (primary antioxidants), such as phenolic compounds by the formation of stable radicals, or prevent initiation (secondary antioxidants), like chelators by the formation of complexes with metals, can be antioxidants in foods. These kinds of compounds are either naturally present in foods or can be added. Some antioxidants can also act as prooxidants depending on the concentration and conditions (like ascorbic acid in aqueous systems containing metals) (Kiokias et al. 2009). In the case of the food structure, an increased surface area of the lipids , in general, increases the degree of lipid oxidation. This impacts the stability, especially in emulsion systems, which have a high surface area (Waraho et al. 2011). High temperature (heat), high oxygen pressure and the presence of light are known to induce and accelerate lipid oxidation, while low temperature, low oxygen pressure and the absence of light help to inhibit and control lipid oxidation during production and storage (Schaich et al. 2013). The effect of the RH on lipid oxidation during production and storage depends on its effect on the a_w of the foods (see 2.3.1) (Labuza 1980). Therefore, production and storage conditions need to be controlled to enhance the stability of lipids in foods, for instance, during storage by intelligent packaging (Schaich et al. 2013).

2.1.2 Enzymatic degradation of lipids

Lipids in foods can be degraded by enzymes naturally present in the raw material. Lipases (EC 3.1.1.3) can hydrolyse the ester bonds between the acyl group and glycerol of triacylglycerols

(TAGs), diacylglycerols (DAGs), monoacylglycerols (MAGs) and phospholipids, resulting in the formation of free fatty acids (FFAs) and different acylglycerols (like DAGs, MAGs, lysophospholipids), depending on the functionality and selectivity of the enzyme. They are active at the lipid-water interface, which makes them most active in emulsion food systems like milk. Lipases cause a lipolysed off-flavour in milk and dairy products based on the release of short-chain fatty acids and further reaction products from FFAs (He et al. 2013). Lipases are also present in cereals, legumes and certain fruits and vegetables. In cereals, lipases are activated during germination (Zhou et al. 2013). However, some cereals, like oats, already show high lipase activity before germination. Molteberg et al. (1996) found an accumulation of FFAs accompanied by a paint-like flavour in stored non-heat-treated oat flour.

Lipoxygenases (EC 1.13.11.12) is another enzyme group present in cereals, legumes, fruits and vegetables causing the degradation of lipids. It catalyses the oxidation of fatty acids with a *cis*-1,4-pentadiene structure forming conjugated hydroperoxides without free radical involvement (enzyme-catalysed lipid oxidation). Free linoleic acid is the preferred substrate of most lipoxygenases, and it is oxidized to 9- and 13-hydroperoxides (Kiokias et al. 2009). The formed hydroperoxides can decompose as described earlier in autoxidation, or enzymatically by hydroperoxide lyase (EC 4.1.2.-). Some hydroperoxidase activity was observed for lipoxygenases at pH ~6, catalysing the reaction of hydroperoxides to hydroxy acids (Schaich et al. 2013). The formation of hydroxy and epoxy acids, catalysed again by another enzyme, peroxygenase (EC 1.11.1.-), from hydroperoxides and non-oxidized linoleic acid was proposed by Hamberg and Hamberg (1996). Further, they proposed the formation of a trihydroxy acid from hydroperoxides catalysed by peroxygenase and epoxide hydrolase (EC 3.3.2.-) activity. Oats present a lipoperoxidase-type activity, which combines a peroxygenase and epoxide hydrolase activity (Lehtinen and Kaukovirta-Norja 2011). Similar hydroxy and epoxy acids, as found by Hamberg and Hamberg (1996), were also detected in oat groats and flour during storage (Doehlert et al. 2010). The identified hydroxy acids were connected with the bitter flavour of oxidized oats (Biermann et al. 1980; Doehlert et al. 2010). In many foods, enzymes are inactivated during production to prevent or reduce the above mentioned enzymatic catalyst reactions of lipids, causing off-flavours in foods.

2.2 Solid food systems with dispersed lipids

In foods, lipids are often present as a dispersed phase surrounded by a continuous food matrix consisting most commonly of water, carbohydrates and proteins. Common sources of lipids in our diet are oil-in-water emulsions typical of liquid and semi-liquid foods like milk, dressings, yogurt, mayonnaise and ice cream. Dispersed lipids are present, in addition to liquid and semi-liquid foods, in solid foods such as cereal products like bread, breakfast cereals and snacks. Dispersed lipids in foods are important for the texture and flavour of the foods (Berton-Carabin et al. 2014).

Dispersed lipids are prone to oxidation based on their large surface area and potential contact with prooxidants in the continuous matrix, and oxygen from the surrounding air (Waraho et al. 2011). In recent years, intensive research has been performed to understand lipid oxidation in liquid emulsion systems. One goal of this work was to enable the use of more polyunsaturated lipids in foods to fulfil the consumer demand for healthier products (Kiokias et al. 2009; Berton-Carabin et al. 2014). However, the knowledge about lipid oxidation in oil-in-water emulsion systems cannot be transferred one-to-one to solid food systems with the dispersed lipids. The minimized water content, the common porous structure with many air cells and the higher amount of starch and fibre in these kinds of products influence the lipid oxidation mechanisms and rate (Artz and Rao 1994).

2.2.1 Spray-dried oil emulsions

One example of solid food systems with dispersed lipids is dried microencapsulated oil. There are several microencapsulation techniques available for food ingredients, like spray-drying, freeze-drying, spray-chilling, extrusion, inclusion complexation and co-crystallization (Jackson and Lee 1991). Microencapsulation processes are often used to protect the encapsulated core (often sensitive compounds like oxidatively unstable oils or flavours) from oxygen and pro-oxidants. Commonly used techniques for oils are spray-drying and freeze-drying (Márquez-Ruiz et al. 2003), which are both mechanical encapsulation processes (Madene et al. 2006). Of these two microencapsulation processes, spray-drying is the more commonly used microencapsulation technique in the food industry, based on being more economical than freeze-drying (Gharsallaoui et al. 2007). The focus here will be on spray-drying as the more common technique. The product obtained by spray-drying has an amorphous and glassy matrix because of the rapid evaporation of water from the emulsion droplets (Ré 1998).

Spray-drying process

In microencapsulation by spray-drying, an oil-in-water emulsion is fed into the spray-dryer, and the atomizer disperses the emulsion to small droplets to increase the surface area. The droplets are dried in a hot co-current or counter-current air stream, depending on the position of the atomizer towards the hot air spreader. During the drying process, water evaporates and the previously dissolved matrix compounds form a solid wall surrounding the oil droplets in the particles. Besides the process conditions, the choice of wall material is crucial for the physical structure and stability of the dried particles. In general, the wall material must have good solubility in water; thereby it should maintain the low viscosity of the solution, even at high concentrations. Further, it should have effective emulsification, film forming and drying properties (Ré 1998; Gharsallaoui et al. 2007).

Wall materials

Most of the available wall materials for spray-drying fulfil only part of the above mentioned requirements. Therefore, combinations of different materials are often used to achieve desirable properties. Carbohydrates, like starches, maltodextrins and maize syrup solids, rapidly develop a dense shell during spray-drying, but they have insufficient interfacial properties. This lowers the encapsulation efficiency for oils. These kinds of carbohydrates, therefore, require the addition of compounds with higher emulsification characteristics, like proteins, gums or other emulsifiers, or chemical modification to increase their emulsification properties (Gharsallaoui et al. 2007). Grattard et al. (2002) studied the effects of the maltodextrins dextrose equivalent (DE) 2, DE 21 and DE 40 on the oxidation rates of freeze-dried flaxseed oil emulsions. They concluded that maltodextrin DE 21 was the best suited as wall material. However, they also noted in their discussion that previously high and low DE maltodextrins were used successfully as wall material. Maltodextrins with high DE have, in general, the advantage over low DE maltodextrins in that they can be used in higher concentrations (Gharsallaoui et al. 2007).

Some proteins like gelatine show wall-forming abilities, but many others, like casein or whey proteins, need the addition of other materials, such as lactose or maltodextrin, to improve their drying and coating properties (Vega and Roos 2006; Gharsallaoui et al. 2007). Further, using protein on its own increases the risk of denaturation during the drying process, this can have a negative effect on the wall stability (Gharsallaoui et al. 2007). Proteins used in wall material were shown to have radical scavenging activity, which improved the oxidative stability of the encapsulated oil (Park et al. 2005). Some studies indicated that the cross-linking of proteins (e.g. casein and bovine) by microbial transglutaminase could enhance the oxidative stability of spray-dried oil emulsions by improving the structure of the interfacial layer formed by the proteins around oil droplets in the dried emulsions (Bao et al. 2011; Mora-Gutierrez et al. 2014). Gums such as gum arabic are used for their surface activity and film forming properties. They can be used on their own but, based on their permeability to oxygen; they are not suitable as the only microencapsulation agent for oxidatively sensitive oils (Gharsallaoui et al. 2007).

Physical structure and state of powder particles

The shape and size of powder particles is dependent on the process conditions and materials used. For example, both increasing feed rate and higher viscosity of feed emulsion can increase the particle size. The size of the particles can range from 10-15 μm up to 2-3 mm. However, the most common particle sizes are less than 100 μm (Ré 1998; Gharsallaoui et al. 2007). The location of the oil globule in the particle is important for its stability. If spray-drying is used as the encapsulation method, the core is typically distributed uniformly as microdroplets throughout the matrix of the wall material. However, the formed particles are not always one solid sphere; often the particles show air (gas) inclusions in the middle. Cracks and channels in the shell can expose the encapsulated material to oxygen, which can lead to oxidation. Diffusion is an important factor for the release (in the case of flavours) and oxidation of the core. The diffusion factor for the

particle matrix depends (besides on the material and structure of the particle) strongly on the physical state of the matrix. Transitions from a glassy to a rubber state can increase the diffusion of gases and solutes, and can cause leakage of the core material (Nelson and Labuza 1992; Ré 1998).

Encapsulation efficiency

The encapsulation efficiency plays a role in the stability of the material by expressing how much of the oil is encapsulated by the wall material (Gharsallaoui et al. 2007). Oil covering the outer layer of the particle or located in the cracks and channels of the outer layer is easily extractable and often referred to as surface oil (Márquez-Ruiz et al. 2003). The surface (free) oil content of a powder can differ based on the determination method used (no standardized method for extraction) but, in general, surface oil is considered to be non-encapsulated oil only inefficiently protected by the wall material. With the optimization of the composition of the feed emulsion and process parameters, the encapsulation efficiency can be improved to reduce the surface oil to a minimum (Vega and Roos 2006).

2.2.2 Extruded cereals

Another example of solid food systems with dispersed lipids is extruded cereals, like snack foods and breakfast cereals. Extrusion cooking is widely used to produce porous, crispy and expanded cereal products, mainly from high starch cereal materials like maize and wheat flour (Brennan et al. 2013). The puffed structure is formed by thin-walled air cells (Moraru and Kokini 2003). Most common extruded products have nutritionally poor chemical compositions, being high in energy and low in bioactive compounds like dietary fibre. In recent years, the consumer attitude has changed towards a higher awareness of health promoting foods. This is challenging the food industry to revise the composition of extruded foods to fulfil the nutritional expectations set by the consumers (Brennan et al. 2013). In the case of extruded cereals, the addition of dietary fibre or a change towards cereal material naturally high in dietary fibre might be the key to the quality improvement. Diets high in dietary fibre contribute to a decreased risk of diseases like diabetes, cardiovascular diseases, colorectal cancer and obesity (Anderson et al. 2009). The focus in this chapter will be on oat and rye bran extrudates, both high in dietary fibre.

Extrusion process

Extrusion cooking is a short-time, low-moisture and high-temperature process. In the extrusion process, cereal flour and water are fed into a single or twin screw extruder. The water addition is commonly adjusted to reach a final water content of 12-20% (Delcour and Hoseney 2010). The extruder barrel applies heat, shear and pressure on the water-cereal mixture, leading to the transformation into a viscoelastic melt. The process parameters and composition of the cereal flour determine the degree of transformation. Within the melt, nucleation of the bubbles takes place at sites where air or impurities were entrapped. After exiting the extruder through the die,

the pressure on the melt releases, the bubbles grow, flash evaporation of water occurs and the melt expands. The evaporation causes rapid cooling of the material and the viscoelastic matrix becomes glassy. In this step the expansion stops. The rate of expansion is mainly dependent on the starch content and amylopectin-amylose ratio. A higher starch content, in general, and higher amylopectin content, specifically, increase the expansion of the cereal extrudates (Moraru and Kokini 2003). Although the extrudates lose water during expansion, they often need to be dried to keep their crispiness and shape, and to prevent microbial spoilage during storage (Delcour and Hoseney 2010).

Physical and chemical changes during extrusion

During extrusion, a variety of chemical reactions and physical changes are known to take place, affecting the structure, flavour, colour and nutritional value of the extrudates. Starch gelatinization and protein denaturation occur during extrusion and lead to the formation of a viscoelastic melt inside the extruder (Moraru and Kokini 2003). Starch molecules (amylose and amylopectin) may also decrease in size by shear forces during extrusion (Singh et al. 2007). The water solubility of dietary fibre is often increased by extrusion, while the total dietary fibre content can increase or decrease, depending on the material and conditions (Singh et al. 2007; Robin et al. 2012). Ralet et al. (1990) reported an increase in soluble dietary fibre for wheat bran extrudates, and related that to the degradation of xylose, glucose and arabinose polymers. While Vasanthan et al. (2002) described an increase in insoluble dietary fibre for extruded barley flour. They explained the increase with the formation of resistant starch during extrusion.

For both proteins and carbohydrates, interactions with lipids have been demonstrated. They bind lipids either by physical interactions like entrapment or encapsulation, or by chemical interactions such as hydrogen bonding. The type and extent of bonding depend on the molecules present; for example, polar lipids are more likely to form amylose-lipid complexes than non-polar lipids (Ho and Izzo 1992). Thachil et al. (2014) found that saturated lipids more efficiently create amylose-lipid complexes than unsaturated lipids. The binding or encapsulation of lipids during extrusion can help to prohibit lipid oxidation (Artz and Rao 1994). Further, extrusion may stabilize lipids by the denaturation of lipolytic and oxidative enzymes (Singh et al. 2007). However, high temperature in extrusion and metals originating from the extruder screw can promote lipid oxidation, which can cause off-flavours in the extrudates (Artz and Rao 1994).

Other compounds which may be subjected to oxidation by extrusion are vitamins (Singh et al. 2007). In the case of tocopherols, losses of 63 to 94% were reported for extruded cereals (wheat, barley, rye and oats) (Zieliński et al. 2001). The Maillard reaction (reactions between amino groups of amino acids, peptides or proteins and carbonyl groups of reducing sugars) can occur in the extrusion process, causing browning and flavour production. The formation of flavour active volatile compounds by the Maillard reaction depends on various factors, like the structure and amount of substrate, pH, temperature, reaction time and a_w (Jousse et al. 2002). Bredie et al.

(1998) detected pyrroles, furans, pyrazines and sulphur-containing heterocyclic compounds derived from the Maillard reaction in extruded maize flour. They observed an increase in the volatile Maillard reaction products at increasing barrel temperatures and decreasing water content during extrusion. The Maillard reaction products formed during extrusion may inhibit lipid oxidation by acting as antioxidants in cereal extrudates (Singh et al. 2007). Volatile Maillard reaction products have been shown to improve the oxidative stability of soybean oil in a model system (Elizalde et al. 1991). Further, Maillard reaction products can react with lipid degradation products during extrusion and form flavour active compounds (Ho and Chen 1994). The formation of these compounds is studied mainly in models (Whitfield 1992).

Process parameters affecting the structure of the extrudates

Extruder type, screw configuration, screw speed, barrel temperature profile, die profile, feed rate and feed moisture are known to influence the texture of the final product, and thus, need to be optimized (Ding et al. 2006; González et al. 2006; Kasprzak et al. 2013). Shear and temperature reduce the viscosity of the melt and facilitate starch gelatinization. However, above a critical temperature, expansion decreases associated with the structural degradation of the matrix. Feed moisture is an important factor, because water is the main plasticizer and enables the cereal material to undergo glass transition during extrusion. Further, moisture affects the rheological properties of the melt, like viscosity. Too low of a viscosity decreases expansion by causing a collapse of the matrix under high vapour pressure (Moraru and Kokini 2003). Ding et al. (2006) studied the effect of the extrusion conditions on the properties of wheat-based expanded snacks using a twin-screw extruder. In their study, the barrel temperature and feed moisture had the highest influence on the product characteristics. They concluded that a decrease in feed moisture and an increase in temperature favoured starch gelatinization, expansion and bubble growth, resulting in products with a low density. Higher expansion can accelerate lipid oxidation by increasing the surface area of the product and, therefore, the contact to oxygen (Artz and Rao 1994). One calculated descriptor used to describe the effect of different extrusion conditions (torque, screw speed, number of screws and mass feed rate) combined is the specific mechanical energy input (SME). The amount of SME used affects the starch conversion and rheological properties of the melt (Moraru and Kokini 2003).

Extruded oats

Oats (*Avena sativa*) is mainly consumed as whole meal oat products, like porridges, oat bread or snack biscuits. In comparison to other cereals, oats has higher fat (5-9%), soluble dietary fibre (4-6%) and protein (10-17%) contents, and high lipase activity (Delcour and Hoseney 2010). Oats also contains enzymes with lipoxygenase and lipoperoxidase activity. These enzymes, together with lipases, are responsible for the quick degradation of lipids in oats after the breakage of the grain structure (e.g. during milling) (Lehtinen and Kaukovirta-Norja 2011). The activity of the lipolytic and oxidative enzymes is related to the formation of a bitter taste; therefore, commercial oat products are heat treated during processing to inactivate endogenous enzymes (Delcour and

Hoseney 2010; Lehtinen and Kaukovirta-Norja 2011). The soluble dietary fibre in oats consists mainly of (1→3)(1→4)-β-D-glucan (β-glucan) (3% to 7% in dehulled oats). β-Glucan is a high molecular weight, linear polysaccharide with the ability to form high viscous solutions (Wood 2007).

Oats is a challenging material for extrusion because of the high lipid content. In general, a high lipid content in the melt has negative effects on the expansion volume of the extrudates. Lipids reduce the friction during extrusion and, therefore, the mechanical energy input. In addition to the lubrication effect, lipids can form a hydrophobic layer on starch granules, which reduces the moisture absorption of the granules, resulting in decreased starch gelatinization (Moraru and Kokini 2003). Further, oat lipids contain about 80% unsaturated lipids, which makes them prone to oxidation during extrusion (Lehtinen and Kaukovirta-Norja 2011). However, the production of extrudates from whole meal oat flour is of interest, because the intake of soluble oat fibre, especially β-glucan, has been shown to be beneficial to health by lowering serum cholesterol, reducing glucose and insulin levels and improving satiety (Xu 2012). Furthermore, extrusion can inactivate enzymes; therefore it could replace other heat-treatments used to stabilize oat products, which would be an economical advantage for the production. The improved shelf life of cereal brans (wheat, rice, barley and oat bran), with respect to the formation of free fatty acids, was best achieved by extrusion, in comparison to other heating technologies, like microwave heating or wet heating (Sharma et al. 2014). The lipid stability of extruded oats has been studied from certain aspects (see 2.3.2); however, the extrusion of oats is still not as widely studied as other cereals like wheat and maize.

Extruded rye bran

Rye (*Secale cereale* L.) is mainly consumed as bread (sourdough, rye-wheat and crisp bread) produced from rye flour (Bushuk 2001). One by-product of rye flour production is rye bran, and it contains the pericarp, testa and aleurone layers (outer layer) of the rye kernel (Delcour and Hoseney 2010). Commercial rye bran can also contain parts of the starchy endosperm and the germ. Rye bran is high in dietary fibre (41-48%) and low in starch (13-28%), compared to whole gain rye flour. Arabinoxylans (21-25%) and fructans (6.6-7.2%) are found to be the main dietary fibre compounds (Kamal-Eldin et al. 2009). Nordlund et al. (2013) found four-fold higher amounts of insoluble dietary fibre than soluble dietary fibre in the rye bran fraction. In their sensory analysis, rye bran was found to have a more bitter taste than the other rye grain fractions. A strong and bitter flavour for rye bran was also described earlier. This flavour was linked to the phenolic compounds and small peptides concentrated in the bran layer of the rye kernel (Heiniö et al. 2003b, 2008).

The high dietary fibre content of rye bran makes it a suitable material to develop extruded products with a high nutritional value and low energy level. Further, extrusion could be used to alter the flavour and texture of the rye bran, to increase the appeal of rye bran as source of dietary

fibre for the consumer. The extrusion of rye flour was shown to be effective in converting the intense rye-like flavour into a mild, slightly sweet flavour (Heiniö et al. 2003a). However, the high fibre content of rye bran can cause challenges for the extrusion process. The extrusion properties of products high in insoluble fibre can be improved by increasing the solubility of the fibre and/or decreasing the particle size of the material (Robin et al. 2012). The expansion properties of rye flour and wheat bran mixture were improved by reducing the particle size of the wheat bran by grinding (Santala et al. 2014). Further, a conversion from insoluble to soluble fibre was noted by the ultrafine grinding of cereal brans (Zhu et al. 2010; Alam et al. 2013).

So far, the extrusion of rye bran has not been widely studied. However, the study by Alam et al. (2013) showed the potential of rye bran for the development of extruded cereal snacks. They studied the effect of the particle size reduction of rye bran upon expansion. They extruded coarse (440 µm), medium (143 µm) and fine (28 µm) rye bran at two screw speeds (300 and 500 rpm), with an extrusion temperature of 130 °C (highest temperature of the barrel profile) at a water content of 17%, adjusted by in-barrel-water feed or preconditioning. The authors argued that the high starch content (39-44%) of rye bran allowed them to obtain expanded products without the addition of starch. The hydration process had no significant effect on the expansion, but the particle size and screw speed affected it greatly. The most expanded extrudate was achieved with fine rye bran at 500 rpm (expansion of 223-228%), while for the medium rye bran extruded at 300 rpm, the lowest expansion (141-150%) was obtained. A higher screw speed resulted in better expanded and less hard products, regardless of the particle size of the bran. The better expansion of the fine rye bran extrudates compared to the coarse and medium ones was explained by the better incorporation of the finer fibre particles with the starch matrix, leading to less disruption during the bubble development. Until now, no study could be found on the lipid stability of extruded rye bran.

2.3 Oxidative stability of dispersed lipids during storage

Solid foods with dispersed lipids, like breakfast cereals, dried soups or snack foods, are typical foods with a low a_w, allowing a long shelf life if stored under the right conditions. In general, the oxidative stability of the lipids depends on the saturation level of lipids, oxygen availability, anti-oxidative and pro-oxidative factors, light, a_w, pH and temperature (Schaich et al. 2013). With the exception of the saturation level, all other factors are either influenced by the matrix or by the storage conditions. Further, the matrix itself can undergo changes during storage, affecting the stability of the lipids.

2.3.1 Spray-dried oil emulsions

Spray-dried emulsions can differ greatly in their composition, based on the wall-materials used and encapsulated oils. Therefore, no general behaviour for the oxidative stability of spray-dried

emulsions can be described. Instead, several factors have been found to be important in the oxidative stability of spray-dried emulsions during storage. These factors include wall material composition, particle and oil globule size, the properties of the oil-matrix interface, and storage conditions (Velasco et al. 2003). The wall material composition in the encapsulation of oils influences microencapsulation efficiency and microcapsule stability during storage. Both are important for the oxidative stability of the oil (Gharsallaoui et al. 2007).

Microencapsulation efficiency

Microencapsulation efficiency is important for the oxidative stability of encapsulated oils, because surface (non-encapsulated) lipids are theoretically more susceptible to oxidation than encapsulated lipids (Márquez-Ruiz et al. 2003). An important factor in improving the encapsulation efficiency is the emulsifying capacity of the material (Gharsallaoui et al. 2007) (The emulsifying capacity of different wall material was discussed in 2.2.1.). Studies, which determined the oxidation state of encapsulated and non-encapsulated lipids separately, showed that the surface lipids were more prone to oxidation than the encapsulated lipids during storage. Baik et al. (2004) found a 10-fold higher oxidation level for the surface oil fraction (around 12% of the whole oil content) than for the encapsulated oil fraction in spray-dried fish oil emulsions encapsulated with a mixture of maize syrup solids DE 36 and sodium caseinate during storage at 30 °C and RH 11%. In the case of encapsulated milk fat, Hardas et al. (2002) determined that, independently from the storage condition, the surface fat (2.4% of the whole fat content) was always more oxidized than the encapsulated milk fat. The wall material in this study was again a mixture of maize syrup solids DE 36 and sodium caseinate. Partanen et al. (2002) found similar results for sea buckthorn kernel oil encapsulated in modified starches during storage. The lower oxidative stability of the surface lipids compared to the encapsulated lipids is thought to be caused by higher oxygen availability in the case of the surface lipids (Velasco et al. 2003).

Although the surface lipid fraction is thought to be less protected than the encapsulated fraction, it has been suggested that the surface lipids can be more stable than the encapsulated lipids. In a freeze-dried emulsion of sunflower oil in a lactose-casein matrix, the surface oil fraction oxidized slower than the encapsulated fraction (Márquez-Ruiz et al. 2003). The authors suggested that the oil droplets in the encapsulated dispersed lipids are separated from each other and, therefore, could show different oxidation rates based on the distribution of pro-oxidative and anti-oxidative factors inside the particle. To determine the oxidative state of a single oil droplet is difficult, because for most analytical methods the encapsulated lipids are first extracted and then analysed as one continuous lipid phase.

Microcapsule stability during storage

The stability of the microcapsule during storage describes the ability of a capsule formed by wall material to retain the lipids and to minimize the diffusion of oxygen.

Table 1. Summary of effect of relative humidity (RH) or water activity (a_w) on the oxidative stability of spray-dried emulsions.

Encapsulated lipids (% db)	Wall material (% db)	Measured oxidation indicator	Effect of RH on lipid stability	Reference
milk fat (40%)	maize syrup solids DE 36 (49.6%), sodium caseinate (7.5%), lecithin (2%)	peroxide value (PV), 18:2 and 18:3 fatty acid content, hexanal content	encapsulate lipids most stable at RH 52% (25 °C) compared to RH 14% and 44% without UV light (at RH 52% the powders showed signs of plasticization); with UV light most stable at RH 14%	Hardas et al. 2002
sea buckthorn seed oil (10-40%)	maltodextrin DE 18.5 and gum arabic (1:7) or maize starch sodium octenyl succinate derivate HiCap 100	PV	higher lipid stability at RH 50% (20 °C) in glassy than at RH 70% (20 °C) in rubbery state	Partanen et al. 2002
fish oil (40%)	maize syrup solids DE 36 (49.65%), sodium caseinate (7.5%), lecithin (2%), potassium phosphate (0.85%)	thiobarbituric acid reactive substance (TBARS)	best storage stability for encapsulated fish oil with added α-tocopherol at RH 11% and 33% (30 °C) compared to RH 0% and 43%	Baik et al. 2004
tuna oil (19.1%)	maize syrup solids (DE36) (76.3%), lecithin (3.8%), chitosan (0.8%) (two-layered interfacial membranes of lecithin and chitosan)	PV, TBARS	lipid stability better at RH 52% than RH 11% and 33%	Klinkesorn et al. 2005
sea buckthorn seed oil (30%)	maltodextrin DE 18.5 and gum arabic (1:7) or maize starch sodium octenyl succinate derivate DE 32 - 37	PV, *para*-anisidine value (AnV)	in glassy state (RH 11%, 20 °C) the encapsulated oil showed a prolonged oxidative stability compared to rubbery state (RH 54%, 20 °C)	Partanen et al. 2005
fish oil (40%)	glucose syrup DE 38 (50%), n-octenylsuccinate-derivated starch (10%)	PV, conjugated dienes, propanal content	highest oxidation rate at RH 54% (20 °C, rubbery state), stable in glassy state (RH 11% and 33%)	Drusch et al. 2006
flaxseed oil (40%)	whey protein isolate	PV	oxidation was increased at RH ~ 0% and 91% (37 °C) compared to at RH ~11 to 75%	Partanen et al. 2008
DHASCO single cell oil (40% docosahexa-enoic acid) (30%)	maltodextrin DE 28 (67%) and pea protein isolate (3%)	PV, TBRAS	lowest oxidation rate at RH 75% (20 ºC, rubbery state) followed by RH 11%, highest oxidation rates at RH 33% and 57% (glassy state)	Aberkane et al. 2014

Wall materials with good film forming properties are known to provide good microcapsule stability (Gharsallaoui et al. 2007) (The film forming properties of different wall materials were discussed in 2.2.1.). However, storage conditions can affect the properties of the wall material and, therefore, microcapsule stability. One storage condition highly affecting the properties of the wall material is the RH. The RH may change during transport and storage, and influences the a_w and physical state of the spray-dried oil emulsions. Both the a_w and the physical state control lipid oxidation rates in foods (Nelson and Labuza 1992). Labuza's stability map suggests that lipid oxidation is the lowest at the monolayer water content of the system (a_w of 0.2 to 0.3 for most foods). Below the water monolayer, oxidation may accelerate by the higher activity of metal catalysts (less hydration of metals) and higher hydroperoxide decomposition (less hydrogen bonding to water). Above the monolayer, the improved mobility of the oxygen and catalysts at the increased water content and the exposure of more catalytic sites through the swelling of the matrix are thought to accelerate oxidation (Labuza 1980). Further, lipid oxidation is influenced by moisture-related changes in the physical state of the matrix. Lipid oxidation is expected to proceed slowly in an amorphous glassy state. The uptake of sufficient moisture lowers the glass transition temperature of the matrix and can lead to an amorphous or crystalline rubbery state depending on the matrix composition. In a rubbery state, the lipid oxidation is thought to be accelerated by higher diffusion rates caused by higher free volume in this state than in the glassy state (Nelson and Labuza 1992; Roos and Karel 1991).

Several studies have determined the effect of RH on the oxidative stability of spray-dried oil emulsions during storage (Table 1). In the cases of Partanen et al. (2002), Baik et al. (2004), Partanen et al. (2005) and Drusch et al. (2006) the relationship between the oxidation rate and RH was in line with the above discussed concepts. However, the studies of Hardas et al. (2002), Klinkesorn et al. (2005), Partanen et al. (2008) and Aberkane et al. (2014) presented partly contrary results. In their cases, the oxidative stability was shown to be the best for storage at high RH in a rubbery state. Similar behaviour was seen by Ponginebbi et al. (2000) for freeze dried emulsions of linoleic acid. They concluded that the structural changes, which caused decreased porosity, re-encapsulation of the surface lipids and coalescence of the droplets, were responsible for better stability in a rubbery state. Klinkesorn et al. (2005), Partanen et al. (2008) and Aberkane et al. (2014) also argued that the structural collapse of the matrix decreased micropores and, therefore, reduced oxygen availability. Klinkesorn et al. (2005) further argued that Maillard reaction products (noted by a colour change of powder) formed at high RH may have acted as antioxidants in the spray-dried emulsion. Aberkane et al. (2014) also observed formation of brown-coloured polymers in powders stored at high RH (75%). They argued that the polymerisation caused termination of certain radical chains, which decreased the oxidation rate.

Therefore, a_w and the physical state do not control the oxidation rate alone in spray-dried oil emulsions, the structural changes of the wall material also need to be considered. Different wall materials react differently with an increase in RH during storage. Low molecular weight carbohydrates can undergo caking, structural collapse or crystallization (Gharsallaoui et al.

2007). Crystallization can increase oxygen permeability and, therefore, lipid oxidation (Vega and Roos 2006). However, if the structural collapse of a rubbery matrix entraps lipids and, consequently, decreases porosity and oxygen diffusion, oxidation can be decreased (Nelson and Labuza, 1992). Proteins are less affected by changes in RH, but their wall-forming ability is lower, which increases oxygen diffusion (Gharsallaoui et al. 2007). Similar structural changes that occur due to increased RH can occur when the storage temperature increases (Vega and Roos 2006). Therefore, the structural response of the wall material to changing storage conditions is important for the stability of the dispersed lipids of the spray-dried emulsions.

Particle and oil globule size

In general, an increase in particle and oil globule size seems to decrease lipid oxidation by decreasing the surface area. Both particle and oil globule size depend on the operation conditions of the spray-dryer and the wall material used for encapsulation (Gharsallaoui et al. 2007). However, in most studies, a smaller oil globule size resulted in better encapsulation efficiency, which can be preferable in the case of lipid oxidation (Velasco et al. 2003).

Oil-matrix interface

In the oil-matrix interface, reactions between the wall-material compounds and oil may occur, affecting the oxidative stability of the oil. Park et al. (2005) showed that the addition of soy protein, soy peptides or gelatine peptides inhibited the oxidation of the eicosapentaenoic acid ethyl ester encapsulated in the maltodextrin. They suggested that proteins and peptides surrounding the oil droplets improved the lipid stability by acting as radical scavengers. Further, the oil-matrix interface layer could function as an oxygen barrier, as shown by Klinkesorn et al. (2005), by using two-layered interfacial membranes to increase the oxidative stability of the spray-dried tuna oil emulsions.

2.3.2 Extruded cereals

The susceptibility of extruded cereals to lipid oxidation during storage is increased by the high surface area and low a_w of the extrudates, and metal catalysts introduced during extrusion by the barrel. Furthermore, certain extrusion conditions (like high temperature and shear) can induce lipid oxidation, which is then accelerated during storage (Artz and Rao 1994). However, the binding of lipids (Ho and Izzo 1992), anti-oxidative compounds (formed during extrusion or added) (Artz and Rao 1994) and the inactivation of lipolytic and oxidative enzymes during extrusion (Singh et al. 2007) may inhibit oxidation during storage. The main focus will be on the oxidative stability of oat extrudates. However, the stability of extruded oats is not widely studied and the stability of extruded rye bran is not yet studied; therefore, studies on other cereals will be reviewed to report the most important factors.

Process conditions

An increase in lipid oxidation for milled oat extrudate, produced from an oat flour fraction with granularity under 532 μm and with 79.20% unsaturated fatty acids in the lipid fraction,

was described with increasing extrusion temperature (Gutkoski and El-Dash 1998). The authors concluded that the extrudates were quite oxidatively stable during storage at 25 °C in the dark if the extrusion temperature was below 120 °C (highest temperature of the barrel profile). No effect of the moisture of the melt on the stability was found in this study. Sjövall et al. (1997) also reported that oat extrudates produced at higher temperatures had lower oxidative stability based on volatile secondary oxidation products. The formation of hexanal, nonanal and 2-pentylfuran was greater for the extrudate produced at 180 °C than for the extrudate produced at 140 °C during 18 weeks of storage at 32 °C. The formation of volatile lipid oxidation products causing off-flavour of the extruded oats was also reported earlier in extrudates produced from oat flour (7% lipids) at 120 ºC with 9% moisture (Guth and Grosch 1993).

Parker et al. (2000) studied volatile formation during the extrusion of four different commercial oat flours at different temperatures (150 or 180 °C) and moisture levels (14.5% or 18%) and its impact on the aroma of the extrudates. At the most severe process conditions (180 °C and 18% water), high levels of Maillard reaction products, like pyrazines and sulphur-containing alicyclic compounds, were detected in the extrudates. These compounds contributed to a toasted cereal aroma of these extrudates. One of the tested oat flours (debranned, 9.6% protein, 2.4% fibre, 7.6% lipids, 1.7% free fatty acids, some lipase activity) had lower scores for the desirable toasted aroma and higher scores for stale oil attributes, even at the more severe extrusion conditions. The results of the sensory analysis were in line with the volatile analysis. This extrudate had lower levels of Maillard reaction products and higher levels of volatiles originating from lipid oxidation, such as hexanal and pentanal, than the other extrudates produced at the same conditions. They suggested that the lower formation of Maillard reaction products was caused by a lower protein content of debranned oat flour than that of the other oat flours (13.4-15.5% protein) and/or by the interaction between the Maillard reaction precursors and aldehydes from lipid oxidation, facilitated by the lipase activity in the debranned flour. The second suggestion was tested and confirmed by the addition of linoleic acid to one of the oat flours without lipase activity, before extrusion. They concluded that residual lipase activity in the flour decreased the volatile formation by the Maillard reaction and increased the formation of volatile lipid derived compounds. In this study, no significant differences in the formation of volatile secondary lipid oxidation products were found at the different extrusion conditions if the same flour was extruded. The volatile Maillard reaction products detected in all extrudates in this study could have acted as antioxidants (Elizalde et al. 1991).

Lehtinen et al. (2003) reported that extrusion at 25% water contentand 130 °C was effective to inactivate lipolytic enzymes in oat bran. However, the extrusion increased the oxidation of polar lipids determined by a decrease in unsaturated polar lipids and an increase in hexanal in their study. An increase in lipid oxidation was also found for the free and bound lipids in extruded oat flour (extrusion temperature: 120 to 180 °C; moisture: 25% to 30%) compared to the raw material (Zadernowski et al. 1997). Zieliński et al. (2001) reported that the extrusion of whole grain oat flour at 120 °C and at 200 °C degraded 40% and 90% of the tocopherols,

respectively, compared to the whole grain oat flour with a total tocol content of 11.6 µg/g (d.m.). Dramatic losses of tocopherols can decrease the stability of lipids by decreasing the amounts of natural antioxidants in oat extrudates. Also, for other extruded cereals with added lipids, a high extrusion temperature accelerated lipid oxidation during storage. Rao and Artz (1989) found that lipid stability decreased with increasing extrusion temperature (temperature range of 115 to 175 °C; screw speed 200 rpm; moisture content 29%) in milled maize meal or maize starch extrudates with 5% added soybean oil stored at 37 °C. They explained the increase in the lipid oxidation products found at a higher temperature not only with the temperature itself, but also with an increased transition metal concentration. The higher concentration of metals was introduced by higher shear forces in the twin-screw extruder used at higher extrusion temperatures. The effects of temperature and transition metal concentration could not be separated. There is strong evidence that high temperature during the extrusion of oats induces lipid oxidation during storage. However, the effect of other process conditions, like water content and screw speed and the effect of formed Maillard reaction products, is still quiet unclear.

Physical state and RH

Lipid oxidation occurred in the glassy (T_g 172.2 ºC) and rubbery (T_g -10 °C) states of extrudates produced from a mixture of waxy maize starch, water (30%, w/w) and free fatty acid (4%, w/w, 60% linoleic acid) at 145 °C and 200 rpm (Gray et al. 2008). The initial oxidation rate was higher in the glassy (a_w 0.3) than in the rubbery (a_w 0.95) state. Gray et al. (2008) suggested this was caused by micro-cracks in the glassy surface. After the elimination of cracks and surface lipids, a better stability was obtained in the glassy than the rubbery state, as generally postulated for amorphous glassy material. In the study from Bowen et al. (2006), waxy maize starch with the addition of 4% lipids was extruded at 145 °C with a water feed rate of 1.43 L/h and a starch feed rate of 5 kg/h. The initial rate of oxidation was higher in the glassy than in the rubbery state. However, the focus of this study was on the amylopectin molecular weight changes during storage rather than lipid oxidation during the storage of the maize extrudates. Maize-based extrudates with the addition of amaranth, quinoa or kañiwa (20% of solids) were prepared (extrusion condition: water content 15-19%, screw speed 200-500 rpm, temperature 150-170 °C). The extrudates were stored at 11% and 76% RH. The formation of hexanal was lower at 76% RH than at 11% RH (Ramos Diaz et al. 2013). Therefore, there are indications for that storage in rubbery state and/or at higher RH could improve oxidative stability of extruded cereals by water plasticisation. However, to retain the crispy structure of cereal extrudates such storage conditions are not of interest.

Amylose-lipid complex

Extrusion at 120 to 150 ºC with a moisture level of 25% to 30% was shown to increase the amount of bound lipids in the extruded oat flour. The bound lipid fraction was slightly more oxidatively stable than the free lipid fraction, however, the difference was small (Zadernowski et al. 1997). A better stability for bound/complexed lipids was also shown by Thachil et al. (2014). They produced high amylose maize extrudates (45% amylose in the flour) and native maize extrudates (25% amylose in the flour), both with the addition of 1.5% fish oil at 18%

moisture, 350 rpm screw speed and 105 ºC, and stored them at room temperature for 90 days. The extrudates with higher amylose levels had a better oxidative stability than the extrudates with native amylose levels, although the extrudates with the increased amylose content had a higher surface area because of higher expansion. The better oxidative stability was awarded to the increased formation of lipid-amylose complexes when more amylose was present during extrusion.

Addition of compounds with antioxidant properties

Butylated hydroxyanisole (BHA; 50 µg/g oil weight basis) reduced lipid oxidation in milled maize starch extrudates with 5% added soybean oil stored at 37 °C (Rao and Artz 1989). The lipid oxidation stability of extrudates during storage was enhanced by the addition of phenolic compounds. Camire and Dougherty (1998) added butylated hydroxytoluene (BHT), cinnamic acid or vanillin (200 or 1000 ppm d.b.) to maizemeal for the production of fried maizemeal extrudates. All of the extrudates (except the one with 200 ppm BHT added) had higher lipid stabilities during storage for 12 weeks at 35 ºC than the control without any additions. Viscidi et al. (2004) produced extruded oat cereals from rolled oats and sucrose (10% by mass) with the addition of benzoin, catechin, chlorogenic acid, ferulic acid and quercetin (1g/kg). Benzoin, chlorogenic acid and quercetin showed the greatest effect in reducing lipid oxidation during storage at 35 ºC for 24 weeks, based on both lipid oxidation measurements and sensory evaluation. Besides the addition of selected compounds, the addition of fruit powders (blueberry, cranberry, Concord grape and raspberry) high in anthocyanins was also shown to have an inhibitory effect on lipid oxidation in extruded maize breakfast cereals produced from white maizemeal and sucrose (Camire et al. 2007). The addition of phenolic compounds or material naturally high in phenolics could also help to compensate for a possible loss of natural cereal phenolics by extrusion. Zadernowski et al. (1999) reported an approximately 50% decrease in phenolic compounds and a decrease in antioxidant properties of the oat extrudates (extrusion temperature: 120 to 180 °C; moisture: 25% to 30%) compared to oat flour. Gumul et al. (2007) also observed a loss of phenolic compounds accompanied by a decrease in antiradical activity in rye extrudates compared to the raw material.

2.4 Analysis of lipid stability in spray-dried emulsions and extruded cereals

There are multiple analytical methods available to determine the lipid oxidation status in foods recognizing/measuring primary or secondary lipid oxidation products. The choice of method depends on the lipid composition and on the food matrix. Most measured oxidation compounds are susceptible to further degradation, which also needs to be taken into account (Barriuso et al. 2013). In the case of solid food systems with dispersed lipids , such as dried oil emulsions, a variety of methods have been used to determine lipid oxidation, like peroxide value (PV), measurements of secondary volatile oxidation products or losses of tocopherols (Márquez-Ruiz et al. 2003; Velasco et al. 2003). The most common methods used to determine the degradation of lipids in spray-dried emulsions and extruded cereals are reviewed below.

2.4.1 Extraction of lipids and determination of lipid content and fatty acid composition

Many of the later described methods, such as PV and para-anisidine value (AnV), require the extraction of the lipid fraction prior to analysis. Extraction methods must be efficient; however, the extraction method should not accelerate the oxidation or degrade the oxidation products to be measured. In most cases, classical acid or alkaline based hydrolysis methods to determine the fat content of foods are not suitable due to their harsh conditions. For spray-dried emulsions, methods to extract total, encapsulated and surface lipids have been used. The surface lipids of spray-dried fish oil emulsions were extracted with 15 mL of hexane from 2.5 g of powder by vortexing at room temperature for 2 min. The hexane phase was decanted and dried under a nitrogen gas flow. The remaining powder was used for the extraction of the encapsulated lipids by dispersing it in water and extracting the lipids with a hexane/isopropanol (3:1, v/v) mixture. The extraction was repeated three times and the combined organic phases were dried under a nitrogen flow. The lipid content of the extract of the surface and encapsulated lipids was determined gravimetrically (Baik et al. 2004). A similar method with different amounts was also used by Hardas et al. (2002) for encapsulated milk fat. Drusch et al. (2006) used petrol ether instead of hexane for the extraction of the surface lipids of spray-dried fish oil emulsions. In their case, the sample was also redissolved in water for the extraction of the encapsulated lipids and then extracted with a mixture of ethanol, petrol ether and hexane. The total lipids of the spray-dried flaxseed oil emulsions were extracted by suspending 0.5 g of powder in 5 mL of water and then shaking the mixture for 30 min. A portion of the mixture was vortexed after the addition of an iso-octane/isopropanol (2:1, v/v) mixture. The organic phase was separated by centrifugation (Partanen et al. 2008). In most studies to redissolve powder in water to regain a liquid emulsion was the key element to be able to extract the encapsulated lipids.

Even without extrusion, the extraction of lipids from cereal matrices is challenging. However, during extrusion some lipids are bound by starch and proteins, complicating the quantitative extraction of lipids from extruded cereals. In several studies, extruded cereals were milled, after which the lipids were extracted with petrol ether followed by the evaporation of the solvent under nitrogen (Rao and Artz 1989; Gutkoski and El-Dash 1998; Viscidi et al. 2004). Sjövall et al. (1997) and Zadernowski et al. (1997) used a chloroform/methanol (2:1, v/v) mixture to extract the total lipids from the milled oat extrudates. Zadernowski et al. (1997) also extracted free lipids from the oat extrudates by washing the milled extrudates six times with hexane. From the extrudate residue, the bound lipids were extracted similarly as the total lipids. They determined the lipid content of each fraction gravimetrically, after the evaporation of the solvent. Thachil et al. (2014) used similar methods for the extraction of free lipids and lipids weakly bound to amylose from maize extrudates with added oil, as Zadernowski et al. (1997) used for free and bound lipids. For the extraction of the lipid complex with amylose, they used α-amylase to digest the amylose prior to lipid extraction. In addition to the determination of the lipid content, several studies also analysed the fatty acid composition of the lipid extracts or of different lipid fractions as fatty acid methyl esters by

gas chromatography (GC) with a flame-ionization detector (FID) from extruded cereals or spray-dried emulsions (Sjövall et al. 1997; Zadernowski et al. 1997; Hardas et al. 2002).

2.4.2 Primary oxidation products

Primary lipid oxidation products are hydroperoxides, which are the first semi-stable products formed during lipid oxidation.

Hydroperoxides

One of the most commonly measured parameters to determine lipid oxidation in foods is the PV stated as milliequivalents of oxygen per kg of fat or oil. It is based on the ability of the hydroperoxide group of hydroperoxides to oxidize other compounds and be reduced itself to a hydroxy group. Two well know methods using this ability are iodometry and the ferric thiocyante method. Both methods can be used for oils directly and, in the case of other foods, lipids are extracted prior to the PV measurement.

In iodometry, iodide ions are oxidized by hydroperoxides to iodine, which can be measured by titration with sodium thiosulphate using starch as an indicator (Kiokias et al. 2009), or by other end point detection methods (Dobarganes and Velasco 2002). The PV of the spray-dried emulsions of conjugated linoleic acid (Jimenez et al. 2004), spray-dried sea buckthorn oil emulsions (Partanen et al. 2002, 2005) and freeze-dried sunflower oil emulsions (Velasco et al. 2009a) were determined using this method. It was also used for extruded products: maize meal or maize starch extrudates with 5% added soybean oil (Rao and Artz 1989) and extruded oats (Zadernowski et al. 1997; Gutkoski and El-Dash 1998). Iodometry has its drawbacks, mainly because iodide can also be oxidized by the oxygen present. This reaction is catalysed by light (Barriuso et al. 2013).

The ferric thiocyanate method is based on the oxidation of Fe^{2+} to Fe^{3+} by hydroperoxides. Fe^{3+} forms, with ammonium thiocyanate, a red ferric thiocyanate complex, which can be measured photometrically at 500 nm (Kiokias et al. 2009). This method was used for the determination of the PV of spray-dried fish oil emulsions (Baik et al. 2004; Drusch et al. 2006), encapsulated milk fat (Hardas et al. 2002), spray-dried tuna oil emulsions (Klinkesorn et al. 2005), eicosapentaenoic acid ethylester encapsulate in maltodextrin (Park et al. 2005), spray-dried flaxseed emulsions (Partanen et al. 2008) and extruded oat cereals (Viscidi et al. 2004). The ferric thiocyante method is more robust than the iodometry method based on the lower susceptibility of Fe^{2+} to be oxidized by oxygen than iodide (Barriuso et al. 2013).

However, hydroperoxides are semi-stable. They are formed and/or decomposed easily during sample pre-treatment like extraction of lipids (see 2.4.1). This can cause over- or underestimation of lipid oxidation. In addition, secondary oxidation products formed from hydroperoxides in sample and/or during sample treatment are not detected by any PV measurement or direct hydroperoxide measurement method using high-performance liquid chromatography (HPLC) (Dobarganes and Velasco 2002). Therefore, in addition to

hydroperoxides, secondary oxidation products should be measured to avoid false interpretation of the oxidation. In many of the above mentioned studies this approach was followed.

Conjugated dienes

Conjugated dienes are formed from polyunsaturated fatty acid during oxidation. They absorb UV light at 235 nm, and the absorption can be measured with a spectrophotometer and be used to assess the oxidative state of lipids in foods. The method contains a risk of overestimating the oxidation if the sample contains other compounds (like carbonyl compounds) which absorb in the same region. Further, oxidation might be underestimated in oils rich in monosaturated fatty acids, which do not form conjugated dienes (Barriuso et al. 2013). Conjugated dienes were used as oxidation indicators in spray-dried fish oil emulsions (Drusch et al. 2006), freeze-dried emulsions of linoleic acid (Ponginebbi et al. 2000), extruded oats (Zadernowski et al. 1997) and extruded oat cereals (Viscidi et al. 2004). All products contained only or mainly polyunsaturated fatty acid in the lipid fraction.

2.4.3 Secondary oxidation products

Secondary oxidation products can be monomers, oligomers and polymers containing different functional groups. The differences in polarity, volatility and molecular weight make it difficult to analyse all kinds of formed products. In most studies, one compound or a group of compounds are chosen as oxidation indicators.

Aldehydes

Para-anisidine value (AnV) is based on the reaction of aldehydes (mainly 2-alenals and 2,4-alkadienals) formed during lipid oxidation with *p*-anisidine to a Schiff base, with an absorption maximum at 350 nm. The AnV is defined as 100 times the absorbance of a solution containing 1 g of fat or oil in 100 mL of solvent. Often, AnV is combined with PV measurements (Kiokias et al. 2009). The AnV has several drawbacks. First, the absorbance intensity is dependent on the unsaturation level of the aldehyde. Secondly the *p*-anisidine reacts with all kinds of aldehydes present. Therefore, the presence of aldehydes, not originated from lipid oxidation, should be considered (Barriuso et al. 2013). The method was used in combination with the PV measurement to determine the lipid oxidation in spray-dried emulsions of conjugated linoleic acid (Jimenez et al. 2004) and spray-dried sea buckthorn seed oil emulsions (Partanen et al. 2002, 2005).

Malondialdehyde (MDA) is formed by multiple scissions of cyclic internal hydroperoxides originating from fatty acids with three or more double bonds during lipid oxidation (Schaich et al. 2013). The common method to use MDA as an oxidation indicator in foods is the thiobarbituric acid reactive substance (TBARS) method. It is based on the reaction of MDA with thiobarbituric acid (TBA) at low pH and high temperature, resulting in the formation of a pink complex with an absorption maximum at 532 nm. This method can be used for certain foods without the prior extraction of lipids, which saves time and costs. However, the TBA is

not selective to MDA; additionally, other aldehydes, carbohydrates, amino acids and nucleic acids can react with TBA. Further, MDA can form Schiff bases or bonds with lysine and arginine, and is therefore no longer available for the reaction with TBA. The harsh reaction conditions (high temperature and low pH) may cause unwanted oxidation reactions, which can cause overestimation. In addition, MDA is only a minor oxidation product and is formed only from specific fatty acids (Barriuso et al. 2013). Thus, the TBARS assay is, only in selective cases, a good choice for lipid oxidation analysis. It is used mainly to determine the lipid oxidation of products containing fish oil, which is high in fatty acids with three or more double bonds. TBARS was measured from reconstituted spray-dried fish oil emulsions (Baik et al. 2004) and spray-dried tuna oil emulsions (Klinkesorn et al. 2005), and from maize extrudates with added coconut oil, fish oil and MaxEPA using distillation for extraction (Thachil et al. 2014).

Volatile secondary oxidation products

The whole volatile profile of foods can be used to estimate the oxidative status mainly related to off-flavour formation. However, often only certain volatiles are used as oxidation indicators, such as propanal or hexanal. Because these volatiles are formed from certain fatty acids during oxidation, and because they can react further, this approach without using other parameters may be unreliable. Volatile secondary oxidation products are commonly extracted from the headspace of a sample and then identified and quantified by GC. Often, mass spectrometry (MS) is used in detection, allowing the identification of compounds based on recorded mass spectra, which can be compared to compound libraries. For the headspace analysis, static headspace (SHS), dynamic headspace (DHS) and HS-SPME are used.

In SHS, the sample is placed in an airtight vial and heated. When the equilibrium between the gas phase and the sample is reached, an aliquot of the headspace gas is injected into the GC. The method is rapid and inexpensive. However, only a representative fraction of headspace is analysed for its volatile content, which reduces the sensitivity of the method (Barriuso et al. 2013). SHS was used to measure propanal from a spray-dried fish emulsion (Drusch et al. 2006) and hexanal from encapsulated milk fat (Hardas et al. 2002). In both studies, the SHS data was combined with the PV data, and in the case of Drusch et al. (2006), with the data on conjugated dienes. In the DHS technique, the volatiles are extracted continually (no equilibrium needed) by purging the sample with inert gas. Then, the gas flows through a porous polymer trap that collects the volatiles. The collected volatiles are analysed by GC. This method is more sensitive than the SHS, but slower, more complex and expensive (Barriuso et al. 2013). Sjövall et al. (1997) used the DHS technique to determine the volatile profile of oats extruded at different temperatures. The main volatile secondary oxidation products detected were hexanal, decane, 2-pentylfuran and nonanal.

HS-SPME is based on the adsorption and absorption of volatile analytes from the headspace onto a polymer-coated silica fibre. Desorption of compounds takes place in a hot injector port of the GC. In HS-SPME, two equilibria need to be established: one between the sample and headspace and another one between the headspace and fibre. The second equilibrium depends

on the fibre vs. the type of volatiles analysed. This allows the adjusting of the method towards the analytes of interest (Wardencki et al. 2004). The most common fibre coatings are polydimethyl siloxane (PDMS), divinylbenzene (DVB), carboxen (CAR) and polyacrylate (PA). PDMS is used for the extraction of non-polar volatiles, while PA is used predominantly for polar compounds. Another coating used for polar compounds is DVB. CAR has a microporous structure and, therefore, this coating absorbs low molecular weight compounds well, while DVB has a macroporous structure and is, therefore, suited for the extraction of semi-volatile compounds with a higher molecular weight. Often, a mixture of different coating materials is used, as in the case of bipolar compounds (e.g. alcohols and aldehydes). PDMS is often combined with CAR to enlarge the surface area for extraction, and DVB to increase the polarity (Balasubramanian and Panigrahi 2011). HS-SPME has the advantages of SHS (short time, relatively low costs, can be automated) without the drawback in sensitivity (Wardencki et al. 2004). Further, lower temperatures can be used in HS-SPME than are commonly used for SHS. This reduces the risk of further oxidation during extraction (Jeleń et al. 2012). Paradiso et al. (2008) measured the volatile profile of extruded maize based breakfast cereals by HS-SPME-GC-MS with DVB/CAR/PDMS fibre to determine the effect of the addition of tocopherol on the oxidative stability during storage.

One factor which needs to be considered when any of the above headspace methods are used to measure volatiles from foods is the release of these compounds from the food matrix. The release of volatiles is controlled by the volatility of the compounds, which forces the compounds out of the matrix, and by chemical-physical binding forces, which withhold the compounds in the matrix. These factors depend on the chemical and physical properties of the volatiles and the composition and structure of the surrounding food matrix (Guichard, 2002). The release of certain volatile compounds can be improved by the addition of water and/or salt (Wardencki et al. 2004). Most studies on the release and binding of volatiles were done using simplified model systems (Meynier et al. 2004; Jouquand et al. 2006; Kühn et al. 2006). However, most foods like cereal extrudates have a more complex composition. Data on the effects of structure and storage condition on the release of volatiles in real food systems is limited.

Oligomers and polymers

Oligomers and polymers are formed during extensive lipid oxidation. The formation of oligomers and polymers can affect the texture of foods, for example, in oils a rise in viscosity can be seen. High-performance size exclusion chromatography (HPSEC) was used to analyse fatty acid polymers formed by the advanced oxidation of sunflower oils (Morales et al. 2010). HPSEC is based on the separation of compounds according to their molecular weight. It is most commonly performed on the polar lipid fraction, which must be extracted and purified first (Barriuso et al. 2013). The polar lipid fractions of freeze-dried sunflower oil and fish oil emulsions were analysed by HPSEC. The obtained data was used, together with the data on α-tocopherol losses (see 2.4.4), to determine the oxidative stability of the freeze-dried oil emulsions (Velasco et al. 2006, 2009a, 2009b). In addition to volatile secondary oxidation

products, Paradiso et al. (2008) measured triacylglycerol polymers by HPSEC from stored extruded maize based breakfast cereals.

2.4.4 Analysis of loss of tocopherols and tocotrienols

Tocopherols (saturated side chains) and tocotrienols (unsaturated side chains) are lipid-soluble antioxidants naturally occurring in most foods. They can be combined under the term tocols. They are consumed during lipid oxidation. Four vitamers (α, β, γ and δ) of tocols exist. The content and type of tocols can be analysed by normal-phase (NP) HPLC using fluorescence detection (FLD) with an excitation wavelength of 292 nm and an emission wavelength of 325 nm (Lampi and Piironen 2009). This method has been used to determine tocopherol losses in the freeze-dried oil emulsions (Velasco et al. 2006, 2009a, 2009b) and in extruded cereals (Zieliński et al. 2001).

2.4.5 Analysis of enzymatic hydrolysis of lipids

The analysis of free fatty acids is only of interest in spray-dried emulsions and extruded cereals if the materials used exhibit lipase activity, such as non-heat-treated oats. Sharma et al. (2014) measured the free fatty acid content of cereal brans before and after extrusion, and during storage using alkaline titration. Lehtinen et al. (2003) compared the effect of different heat treatments (including extrusion) on the free fatty acid content of oats. They separated the extracted lipids by thin layer chromatography and analysed the fatty acid composition of the different lipid classes as fatty acid methyl esters by GC.

3 OBJECTIVES OF THE STUDY

The development of new whole grain foods high in fibre is of interest because of the health-promoting effects which are associated with the high consumption of dietary fibre. The stability of fibre-rich cereal foods is quite often challenging, because they are heterogeneous systems with dispersed lipids prone to oxidation. The aim of this thesis was to study the oxidative behaviour of foods with dispersed lipids based on model systems (spray-dried emulsions and extruded cereals), and to link oxidative stability to the structural features of the products and to the process parameters.

More specifically the objectives were:

- To develop, apply and characterize analytical methods to study the stability of lipids in food models with dispersed lipids using primary and secondary lipid oxidation products, losses of tocols and neutral lipid profiles with a special focus on volatile compounds (**I-IV**).

- To study the oxidative stability during the storage of spray-dried emulsions used as models for foods with dispersed lipids (**I**).

- To determine changes in the lipids of whole grain oat extrudates during storage and to relate them to changes occurring in oat flours (**III**).

- To investigate the oxidative stability of extruded rye bran in correlation to extrusion parameters (**IV**).

4 MATERIALS AND METHODS

This section summarises the materials and method used in this study. More detailed information is presented in the original papers (**I-IV**).

4.1 Materials

4.1.1 Spray-dried sunflower oil emulsions

Two model spray-dried emulsions (studies **I-II**) containing sunflower oil (30%) from Bunge Finland Oy (Raisio, Finland), maltodextrin DE 22.2 (67%) from Grain Processing Corporation (Muscatine, Iowa, USA) and either non-cross-linked Na-caseinate (3%) from Kaslink Foods (Koria, Finland) or cross-linked Na-caseinate (3%) were produced as described by Moisio et al. (2014). The cross-linked Na-caseinate was prepared by enzymatic cross-linking with transglutaminase prior to emulsification (Moisio et al. 2014). The prepared dried emulsions were referred to as a spray-dried emulsion with non-cross-linked protein (NCL) and a spray-dried emulsion with cross-linked protein (CL).

4.1.2 Cereal extrudates

Four oat extrudates (**III**) were prepared with a twin screw extruder from prior milled (a Fritsch cutting mill using 4 mm screen) non-heat-treated (NHT) dehulled oat grains (63% starch, 16% protein and 6% lipids) from Raisio Group (Nokia, Finland) (Moisio et al. submitted). The initial water content during extrusion was 19.4% in all extrusion trials. The extrusion temperature and screw speeds were adjusted in each trial (Table 2). The physical and chemical properties of the oat extrudates were described by Moisio et al. (submitted). The extrudates were homogenized by a knife mill prior to the storage experiment. In addition to the extrudates, two oat flours were prepared for comparison (**III**). The flours were produced from either NHT or from industrially heat-treated (HT) dehulled oat grains from the Raisio Group (Nokia, Finland) by milling the grains to a particle size of 0.5 mm. The produced flours were referred to as HT flour (water content 5.2% at RH 33%) and NHT flour (water content 6.2% at RH 33%).

Table 2. Extrusion parameters and water content of the oat extrudates after stabilization at relative humidity (RH) 33%.

Extrudate	Extrusion temp. (°C)	Screw speed (rpm)	Water content at RH 33% (%)
A	70	200	5.4
B	130	200	4.7
C	110	100	4.8
D	110	400	6.4

Twelve rye bran extrudates were produced either from coarse (633 μm) commercial rye bran (38% starch, 26% total dietary fibre, 12% water, 2% lipids) from Fazer Mill and Mixes (Lahti,

Finland), or from fine rye bran (15 μm), prepared by grinding the coarse bran (**IV**). The feed rate (2.4 kg/h) and screw speed (300 rpm) were kept constant in all extrusion trials with a twin screw extruder, while either the extrusion temperature, water content or material (coarse or fine rye bran) were altered (Table 3). This resulted in three different extrusion series (temperature series, coarse bran water content series and fine bran water content series) containing four different extrudates. After extrusion, the extrudates were dried at 70 °C for 15 hours and milled before storage and analysis. In addition to the chemical and physical properties of the milled rye bran extrudates, the properties of both brans were analysed (**IV**).

Table 3. Extrusion parameters and water content of the rye bran extrudates after stabilization at relative humidity (RH) 33%.

Extrudate	Bran particle size	Extrusion temp. (°C)	Initial water content (%)	Water content at RH 33% (%)
coarse 80 °C	coarse	80	22	7.3
coarse 100 °C	coarse	100	22	6.6
coarse 120 °C	coarse	120	22	6.2
coarse 140 °C	coarse	140	22	6.7
coarse 13%	coarse	120	13	6.0
coarse 16%	coarse	120	16	5.7
coarse 22%	coarse	120	22	6.1
coarse 30%	coarse	120	30	6.0
fine 13%	fine	120	13	5.2
fine 16%	fine	120	16	5.6
fine 22%	fine	120	22	6.8
fine 30%	fine	120	30	6.7

4.1.3 Reagents, standards and reference materials

To obtain the selected RHs (**I-IV**), phosphorous pentoxide (RH ~0%), lithium chloride (RH 11%), magnesium chloride hexahydrate (RH 33%), magnesium nitrate hexahydrate (RH 54%) and sodium chloride (RH 75%) were purchased from Sigma-Aldrich (Steinheim, Germany).

A GLC-63 mixture of fatty acid methyl esters and C19:0 methyl ester (Nu-Check Prep, Elysian, MN, USA) were used for identification and as the internal standard for fatty acid analysis, respectively (**I-IV**). Dipalmityl- (≥ 99%) and monopalmityl- (≥ 99%) glycerols, and palmitic (≥ 99%) and oleic (≥ 99%) acids were also obtained from Nu-Check-Prep (Elysian, MN, USA), whereas tripalmitylglycerol (>85%) was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) to be used as standards in the neutral lipid analysis (**III**). Hexanal (> 98%), 1-penten-3-ol, 2-decanone and decanal (≥ 98%) were purchased from Merk (Darmstadt, Germany) to be used for the identification of the volatiles (**II-IV**). Further, nonane (99%), dodecane (99%), propanal, isobutyraldehyde (≥ 99%), 2-pentylfuran (≥ 97%), butanal, 1-undecenal, and diethyl phthalate (99%) were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) to also be used for the volatile identification (**III-IV**). The α-, β-, γ- and δ-tocopherols were acquired as an isomer kit from Merck (Art. 15496) (**I and IV**).

Tocomin® for the identification of the tocotrienols was purchased from Carotech Inc. (Talmadge Village, Edison, NJ, USA).

Milled blueberry oatmeal cookies (Kantolan, Helsinki, Finland) (**II-III**) or blueberry and blackberry oat cookies (Jyväshyvä Paussi Karhunvatukka ja Mustikka, Kraft Foods Finland, Helsinki, Finland) (**IV**) were used as reference materials in the HS-SPME-GC-MS analysis. A mixture of oleyls (TLC-reference standard 18-6 A) from Nu-Check-Prep (Elysian, MN, USA) was analysed in each HPLC sequence in the analysis of the neutral lipid classes to show the stability of the HPLC method (**III**). For the tocol analysis of the rye bran extrudates, a control rye bran extrudate (extrusion parameter: 120 °C and 22% water) was used as a reference material to verify the quality of the extraction and HPLC analysis (**IV**).

4.2 Storage experiments

4.2.1 Storage experiment of spray-dried sunflower oil emulsions at different RHs

The NCL and the CL were stored at five RHs: ~0%, 11%, 33%, 54%, and 75% at 22 °C in the dark over a period of 29 weeks (**I**). Powder samples were stored either as 2-3 mm layers of 3 g of powder in open Petri dishes for chemical analyses or as a 2-3 mm layer of 0.5 g of powder (three replicates) in open 20-mL headspace vials for the analysis of hexanal for each time point. Chemical analyses, including the determination of the fatty acid composition, PV and α-tocopherol content, were conducted separately from the surface and total lipid extracts, whereas hexanal was measured directly from the headspace of the sample, after one week of storage and then every four weeks. All results for the surface and total lipids are given based on the oil content of the lipid extracts.

4.2.2 Oxidation experiment of spray-dried emulsions for volatile release studies

Three batches of 20 g of each spray-dried emulsion (NCL and CL) were oxidized at 40 °C for 4 weeks (**I-II**). The repeatability of the oxidation was confirmed by measuring the PV of each batch. Five 0.5 g replicates of the oxidized NCL and CL were weighed in 20-mL headspace vials, and the open vials were stabilized at five different RHs (~0%, 11%, 33%, 54% and 75%). After one week of stabilization, the vials were closed and the secondary volatile oxidation products were measured either by SHS (**I)** or HS-SPME (**II**). The comparability between the powders stabilized at different RHs was confirmed by measuring the PVs.

4.2.3 Storage experiment of oat extrudates and flours

The oat extrudates and the flours were stored for 15 weeks at 40 °C after an one-week standardization at RH 33% at 22 °C (**III**). The degradation (hydrolysis and oxidation) of lipids was analysed by measuring the neutral lipid profile and volatile secondary oxidation products after the one-week standardization period (zero week time point), and every three weeks during the storage period. For the neutral lipid profile analysis, 10 g portions of the oat

flours and 8 g portions of the oat extrudates were placed in 100 ml glass bottles for each time point. For the volatile compound analysis, samples of 1.00 g were placed in 20 ml headspace vials in triplicate for each time point. After the standardization, the bottles and vials were tightly sealed and placed in an oven.

4.2.4 Storage experiment of rye bran extrudates

The oxidative stability of the rye bran extrudates during storage at 40 °C for 10 weeks was determined based on the losses of tocols, and the formation of the volatile secondary oxidation products measured after a one-week standardization period (zero week time point) and every two weeks (**IV**). Similar to the oat extrudates, the samples were divided (10 g in 100 ml glass bottles for tocol analysis and 1.00 g in 20 ml headspace vials for volatile analysis), standardized at RH 33%, sealed and placed in an oven.

4.3 Analytical methods

4.3.1 Lipid extraction methods

Surface lipids
The extraction of the surface lipids of the spray-dried emulsions was based on the method of Baik et al. (2004), after modifications. The sample (0.3 g) was washed with 5 mL of heptane by mildly shaking for 15 min and then centrifuged (3000 rpm for 2 min). The organic phase was separated from the solid sample (**I**).

Total lipids
The total lipids of the spray-dried emulsions were extracted using the method of Baik et al. (2004), after small modifications. The sample (0.3 g) was resuspended in 3 mL of water (40 °C) and vortexed. The lipids were extracted by shaking with 10 mL of a heptane/2-propanal mixture (3:1, v/v). After shaking, the mixture was centrifuged (3000 rpm for 2 min) and the organic phase was collected (**I-II**). The total lipids of the extruded cereals were extracted after HCl hydrolysis of the milled samples with petroleum ether and diethyl ether according to the AOAC official method 996.06 (AOAC 2001) (**III-IV**).

ASE extractable lipids
ASE extractable lipids (free and most of the bound lipids) were extracted from the cereal extrudates, oat flours and rye brans (1.0 g) by accelerated solvent extraction (ASE, Dionex ASE-200, Dionex Corporation, Sunnyvale, CA, USA) with acetone. The extracts were evaporated to dryness and the residues were dissolved in heptane (**III-IV**).

4.3.2 Lipid content and fatty acid analysis

The lipid content and fatty acid compositions of the extracts (**I-IV**) were analysed by fatty acid analysis according to Soupas et al. (2005). The fatty acid methyl esters were identified by

comparison to a standard GLC-63 mixture and quantified by the internal standard method, using C19:0 methyl ester as the internal standard. The lipid content was calculated as a sum of the fatty acid methyl esters.

4.3.3 Analysis of neutral lipid classes

Neutral lipid classes, TAGs, DAGs, MAGs and FFAs were analysed by normal-phase HPLC (Agilent 1200 HPLC system, Agilent Technologies, Santa Clara, CA, USA) with an evaporating light scattering detector (ELSD) (Waters 2420 ELSD, Waters®, Milford, MA, USA) (**III**). For separation, a LiChrosorb diol column (5 µm, 3×100 mm, VDS optilab Chromatographie Technik GmbH, Berlin, Germany) with a linear gradient elution consisting of a mixture of heptane and 0.1% acetic acid, and an increasing proportion of isopropanol (from 0.06% isopropanol at 0-8 min, to 2% during 8-25 min, and at 2% at 25-40 min) with a flow rate of 0.5 ml/min at 25 °C, was used. The ELSD was set to a drift tube temperature of 60 °C, nebuliser temperature of 42 °C and gain of 10. Nebulisation was performed with filtered air at a flow rate of 1.4 l/min. The results are presented in mg per g sample.

4.3.4 Peroxide value

Hydroperoxides in the surface (**I**) and total (**I-II**) lipid extracts of the spray-dried emulsions were measured by PV using a ferric thiocyanate method (Lehtonen et al. 2011). The results were calculated in meq/kg of extracted oil.

4.3.5 Tocol analysis

Tocols were measured from the lipid extracts (surface and total lipid extracts, **I**; lipid extracts obtained by ASE, **IV)** by NP-HPLC-FLD as described by Schwartz et al. (2008). The results are presented in µg per g sample.

4.3.6 Hexanal content

The hexanal content of the spray-dried emulsions was analysed by SHS-GC-FID as described by Rey et al. (2005). The thermostatic time at 80 °C was adjusted to 18 min (**I**). The results are given as peak areas.

4.3.7 Analysis of volatile profiles by HS-SPME-GC-MS

The HS-SPME-GC-MS method was developed based on a previously reported method by Paradiso et al. (2008). The volatile compounds were analysed using an HS-SPME injector (combiPAL, CTC Analytics, USA) with a DVB/CAR/PDMS fibre (50/30 µm film thickness; Supelco, USA) (**II-IV**). The SPME was coupled to a GC (HP 6890 series, Agilent Technologies Inc., Wilmington, DE, USA) with an MS detector (Agilent 5973 Network,

Agilent Technologies Inc., Wilmington, DE, USA). The GC was equipped with a capillary column SPB-624 (30 m × 0.25 mm i.d., 1.4 μm film thickness; Supelco, USA).

Three SPME incubation and extraction step conditions (condition 1, 40 °C and 250 rpm, **II**; condition 2, 50 °C and 250 rpm, **II-IV**; condition 3, 40 °C and 500 rpm, **II**) were used. The incubation and extraction times were 20 min and 30 min, respectively. The fibre was desorbed for 10 min at 250 °C in the injection port of the GC, which was operated in splitless mode. The GC operation conditions were the following: helium flow 0.7 mL/min; oven temperature 40 °C for 5 min, then increased by 5 °C/min to 200 °C and held at 200 °C for 10 min. The ionisation energy of the MS was 70 eV and the scan range was from 50 to 300 amu. The results were given as peak areas. Identification of the compounds was performed by matching their mass spectra with the database Wiley 7N (Wiley RegistryTM of Mass Spectral Data, 7th Edition, USA) and by comparing the retention times and mass spectra with those of the standards.

4.4 Data analysis

All measurements were carried out in triplicate (if not stated otherwise), and the results are expressed as mean values (± standard deviations). In study **I**, a pairwise signed rank test was used for the PV and a pairwise t-test for the α-tocopherol losses and hexanal amounts in the comparison of the spray-dried emulsions using STATGRAPHICS® Centurion XVI (StatPoint Technologies, Inc., 2010, USA). A pairwise signed rank test was also applied in comparison of the peak areas of the NCL and the CL in study **II**. A value of $p \leq 0.05$ was considered to be statistically significant. The SPME-GC-MS data (**II-III**) were analysed by the principal component analysis (PCA) with the Unscrambler® X (v.10.1; CAMO Software AS, 2011, Norway). The peak areas were area normalized and mean centred before the PCA. The PCA was also used in determining the effect of the rye bran grinding and extrusion on the stability of the extrudates, based on the tocols data and SPME-GC-MS data of the indicator compounds (**IV**).

5 RESULTS

5.1 Volatile analysis from solid foods with dispersed lipids

5.1.1 Detection of volatiles by HS-SPME-GC-MS (II-IV)

The developed HS-SPME-GC-MS method was able to detect from the oxidized spray-dried emulsions a total of 70 volatiles (45 identified, **II**), from the stored extruded oats a maximum of 150 volatiles (62, **III)** and from the stored rye bran extrudates a total of 88 volatiles (63, **VI**). The identified volatiles were mainly secondary lipid oxidation products in dried emulsion and oat extrudates, and secondary lipid oxidation and Maillard reaction products in rye bran extrudates (Appendix 1). The most abundant group of volatiles was aldehydes from the lipid oxidation and Strecker degradation, followed by ketones in the spray-dried emulsion and oat extrudates, and by furans and pyrazines and then ketones in the rye bran extrudates. Hydrocarbons, alcohols, acids and furans were found in all of the three studied models, while esters were observed only in the cereal extrudates and lactones only in the dried emulsion and oat extrudates. Pyridines, pyrazines and sulphur-containing volatiles were only detected in rye bran extrudates produced at a low water content (13% and 16%) or high temperature (140 ºC).

5.1.2 Effect of RH on the amount of volatiles released (I-II)

To study the suitability of using hexanal measured by SHS as an oxidation indicator for the dried emulsions stored at different RHs, the effect of the RH on the amount of hexanal released was studied (**I**). For an equally oxidized spray-dried emulsion, a dissimilar amount of hexanal was measured after the stabilization at different RHs (Table 4). The hexanal amount measured was nearly 5-fold higher at RH 54% than at RH ~0%. The amount of hexanal released was thus strongly dependent on RH. A dependency of the released amount on the RH was also seen when the experiment was repeated using HS-SPME as the extraction method for hexanal (**II**). The effect was, however, less pronounced with HS-SPME (extraction temperature 40 °C) than with SHS (80 °C) as the extraction method (Table 4).

Table 4. Relative hexanal amounts from the oxidized non-cross-linked dried emulsion (NCL) stabilized at five relative humidities and measured with static headspace (SHS) (n = 10) and headspace solid-phase micro extraction (HS-SPME) (40 °C at 250 rpm, n = 5). Results presented as percentages of hexanal peak areas, compared to those at relative humidity (RH) ~0%.

Method	RH ~0%	RH 11%	RH 33%	RH 54%	RH 75%
SHS	100 ± 3	206 ± 5	318 ± 14	483 ± 27	310 ± 15
HS-SPME	100 ± 12	179 ± 10	179 ± 8	151 ± 6	172 ± 5

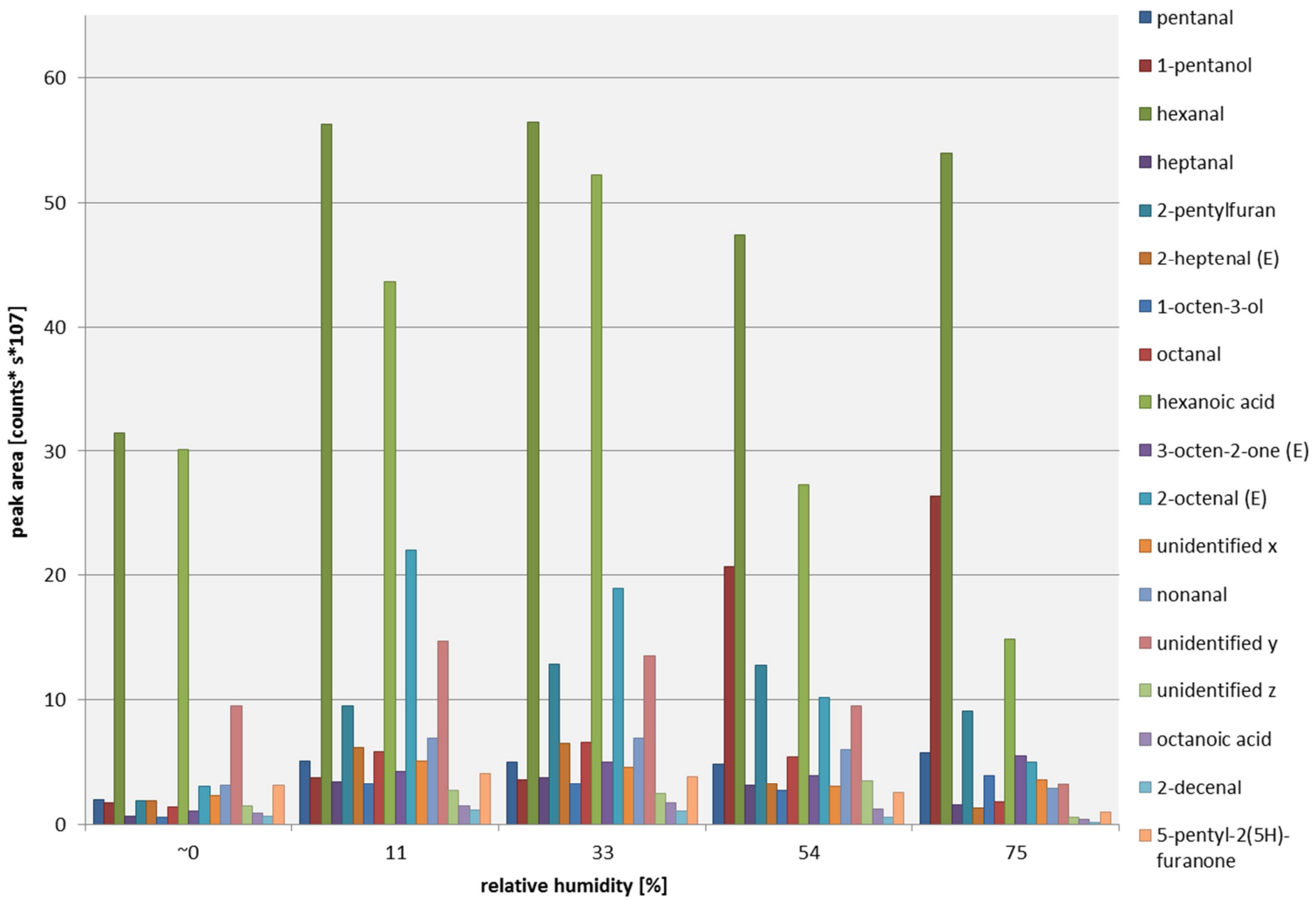

Figure 3. Mean peak areas in counts per second of 18 indicator compounds (indicator compound patterns) of the spray-dried emulsion with non-cross-linked protein (NCL) stabilized at five different relative humidities (RHs). Measurement (n = 5) was done with headspace solid-phase micro extraction (HS-SPME) (40 °C and 250 rpm).

The effect of the RH on the profiles of the volatile secondary lipid oxidation products of the oxidized spray-dried emulsions was determined by HS-SPME, based on 18 indicator compounds consisting of 15 identified (Appendix 1) and 3 unidentified (x, y, z) volatiles, which combined, represented over 90% of the amount of all detected compounds. Except between RH 11% and 33%, clear differences between the indicator compound patterns at different RHs were seen (Figure 3). The highest amount of all indicator compounds released from the NCL was determined at RH 33%, closely followed by RH 11%. At RH ~0% the amount of volatiles released was the lowest (ca. 50% of that at RH 33%) followed by RH 75% and 54%.

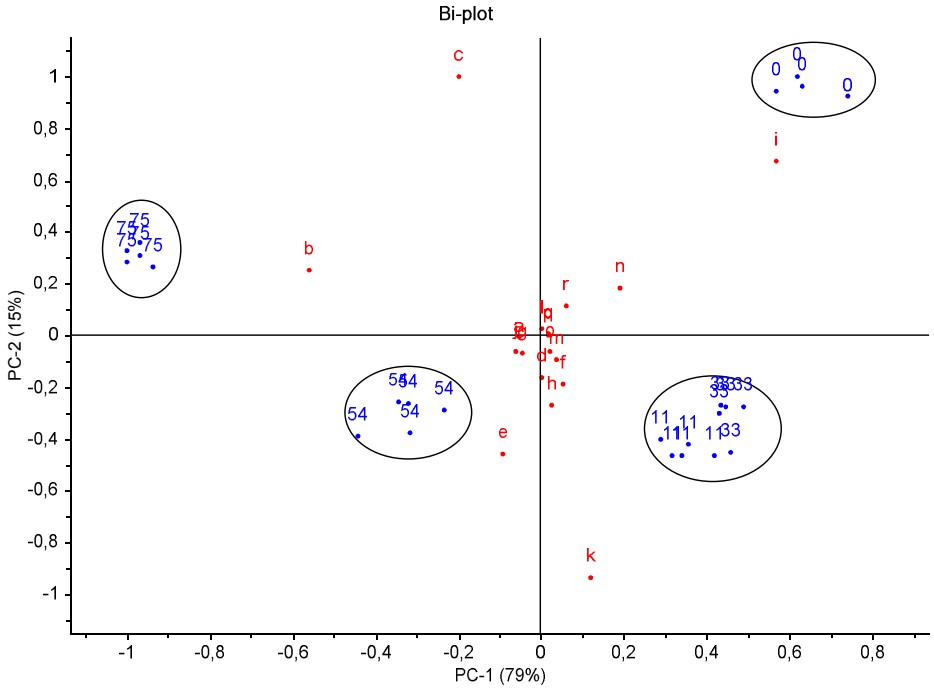

Figure 4. PCA bi-plot of the 18 indicator compounds (peak areas shown in Fig. 3) of the spray-dried emulsion with non-cross-linked protein (NCL) stabilized at five different relative humidities (RHs); objective symbols (blue) = RH (0, 11, 33, 54, 75); variable symbols (red) = indicator compounds (a - r); (a. pentanal, b. 1-pentanol, c. hexanal, d. heptanal, e. 2-pentylfuran, f. 2-heptenal (E), g. 1-octen-3-ol, h. octanal, i. hexanoic acid, j. 3-octen-2-one (E), k. 2-octenal (E), l. unidentified x, m. nonanal, n. unidentified y, o. unidentified z, p. octanoic acid, q. 2-decenal, r. 5-pentyl-2(5H)-furanone). Measurements (n = 5) were done with headspace solid-phase micro extraction (HS-SPME) (40 °C and 250 rpm).

The PCA was used to specify the effect of RH on the individual compounds. In the bi-plot, the drier (RH ~0%, 11%, 33%) and the wetter (RH 54% and 75%) samples were located on different sides of PC-1 (Figure 4). No differentiation between the samples stabilized at RH 11% and 33% could be made, either in the indicator compound patterns (Figure 3) or in the PCA (Figure 4). At both RHs, the samples correlated positively with the C7- to C9-aldehydes (2-octenal, heptanal, octanal, 2-heptenal (E) and nonanal) and negatively with hexanal and 1-pentanol in the bi-plot (Figure 4). Whereas samples stabilized at RH ~0% were associated with hexanoic acid and

unidentified y, and correlated negatively with 2-pentylfuran and 1-octen-3-ol. At RH 54%, the samples were related with 2-pentylfuran, 1-octen-3-ol, 3-octen-2-one and pentanal, while at RH 75% they correlated positively with hexanal and 1-pentanol and negatively with the C7- to C9 aldehydes. The effect of RH on the indicator compound pattern was nearly similar for the CL as described above for the NCL. However, the total amount of released volatile compounds was slightly higher at RH ~0% and 11% and lower at RH 75% from the CL than from the NCL.

5.1.3 Effect of HS-SPME extraction conditions on the amount of volatiles released (II)

The effect of different HS-SPME extraction conditions on the volatile profiles of the oxidized spray-dried emulsions was studied. An increase in the extraction temperature of the HS-SPME from 40 to 50 °C improved the overall liberation of the selected 18 volatile indicator compounds from the NCL at all RHs. Thereby, the increase in the overall amount of volatiles released was dependent on the RH (100% at RH ~0%; ca. 50% at RHs 11%, 33% and 54%; 15% at RH 75%). The PCA of the indicator compounds showed a shift in positions at all RHs at 50 °C compared to 40 °C (Figure 5). This indicated a change in the relative ratios of the compounds if extracted at different temperatures. The separation according to temperature was mainly based on pentanal and hexanal, which decreased, and octanal, hexanoic acid, 2-octenal (E), nonanal, unidentified y and 5-pentyl-2(5H)-furanone, which increased if extracted at 50 °C compared to 40 °C, according to the loadings of the PCA.

Doubling the agitation speed increased the overall amount of volatiles released at the RHs ~0% to 54%. However, the increase was less marked than that seen for increasing the extraction temperature. At RH 75% no increase was observed. The PCA of the indicator compounds in the case of the agitation speed comparison displayed only a separation according to the RH (Figure 5). The loadings of the PCA were comparable for the samples stabilized at the same RH and extracted at different agitation speeds.

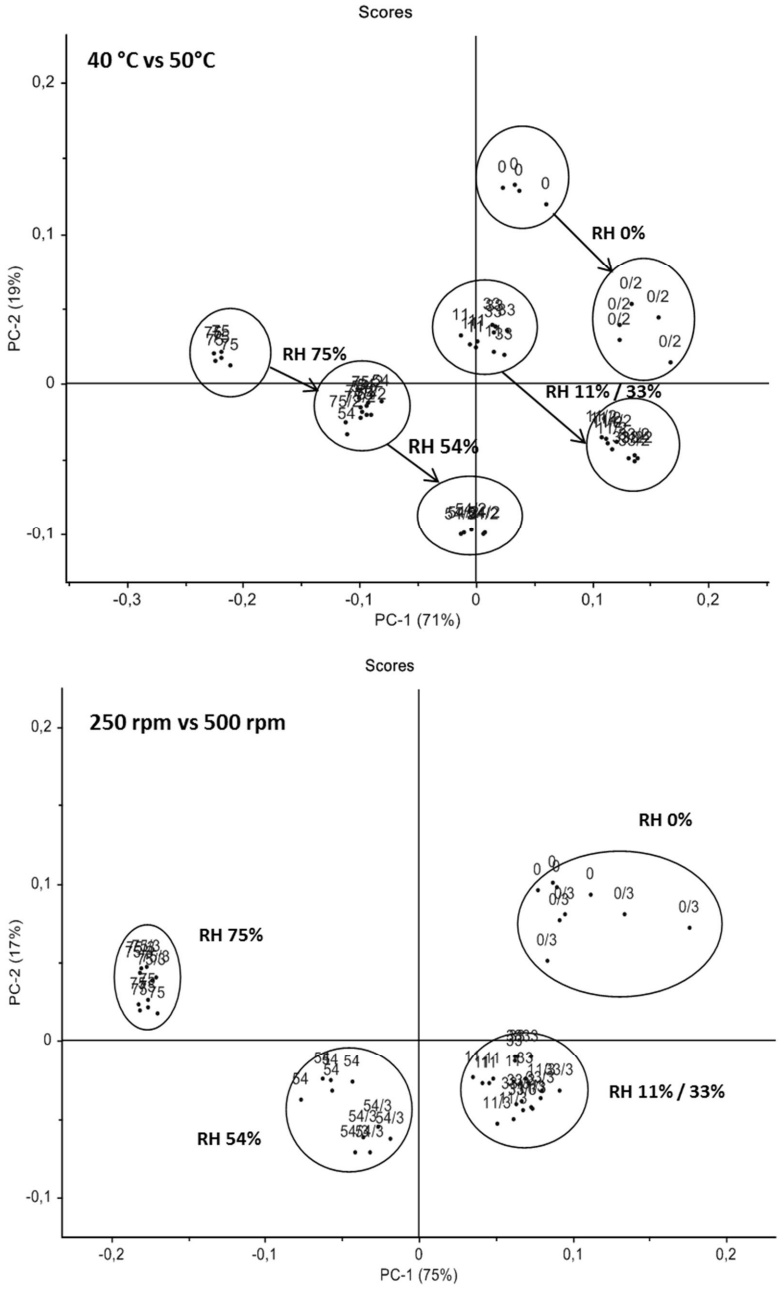

Figure 5. PCA score plots (above, temperature 40 vs. 50 °C; below, agitation speed 250 vs. 500 rpm) using the 18 indicator compounds measured with headspace solid-phase micro extraction (HS-SPME) conditions 1 (40 ºC and 250 rpm; only relative humidity (RH as symbol), 2 (50 ºC and 250 rpm; RH/2 as symbol) and 3 (40 ºC and 500 rpm; RH/3 as symbol) of the spray-dried emulsion with the non-cross-linked protein (NCL) stabilized at five different RHs (0%, 11%, 33%, 54%, 75%) ($n \leq 5$), with arrows indicating the changes in position in the PCA at different extraction temperatures.

The effects of extraction temperature and agitation speed were similar for the CL as described above for the NCL.

5.2 Lipid stability of spray-dried sunflower oil emulsions during storage

5.2.1 Characterization of lipids in spray-dried emulsions (I)

Prior to storage, the lipids of the freshly prepared spray-dried emulsions were characterized and their oxidative status determined. The fatty acid compositions of both spray-dried emulsions were 57.8% linoleic acid, 26.8% oleic acid, 6.2% palmitic acid and 3.5% steric acid. The NCL had a surface lipid content (lipid content extractable by heptane) of 5.0% and the CL of 6.0%, respectively. This meant that the total lipids (30% d.b. of the powders) were mainly (95% or 94%) made up of lipids encapsulated in a Na-caseinate-maltodextrin matrix. The initial PVs of the surface lipids were 1.8 meq/kg for the NCL and 1.5 meq/kg for the CL, respectively. For the total lipids, PVs of 3.6 meq/kg and 3.2 meq/kg were determined for the NCL and the CL, respectively. The tocol profile of the encapsulated sunflower oil was dominated by α-tocopherol. Other tocols, β-tocopherol, γ-tocopherol and β-tocotrienol were only present in minor amounts. Therefore, α-tocopherol losses were used as oxidation indicator in the study **I**. The initial α-tocopherol contents of the surface lipids were 416 and 424 ng/mg, and those of the total lipids were 483 and 500 ng/mg for the NCL and the CL, respectively.

5.2.2 Storage stability at different RHs (I)

The surface lipid contents of both dried emulsions were constant during storage for 29 weeks at RHs 11% and 33%. At RH ~0% for the NCL and CL and at RH 11% for the NCL a decrease in the surface lipid content with a coincident drop in linoleic acid was observed at the end of the storage period. At RH 75% a decrease in the extracted surface lipids without a change in the fatty acid profile was noted for both dried emulsions after week 13. The extraction efficiency of the total lipids was between 99% and 70% at RHs ~0% to 54% during storage. At RH 75%, the extraction efficiency for the CL declined to 40% at the end of the storage period. For the NCL, a decrease in the extraction efficiency was seen at RH 75%, but it was less pronounced than that for the CL.

The PVs of the surface and total lipids of both emulsions showed the same trend; the PVs were the higher the lower the RH was (Figure 6). This indicated a higher oxidative stability at a high RH than at a low RH. For the surface lipids, the formation of hydroperoxides was observed from the beginning of the storage period; therefore, no induction period could be established. After 17 weeks for the NCL and after 20 weeks for the CL, a high increase in the PV was detected at RH ~0%, followed by a decrease after 25 weeks. The PV of the NCL at RH 11% also dropped after storage for 25 weeks. For the total lipids, not such a dramatic decomposing of the hydroperoxides was observed.

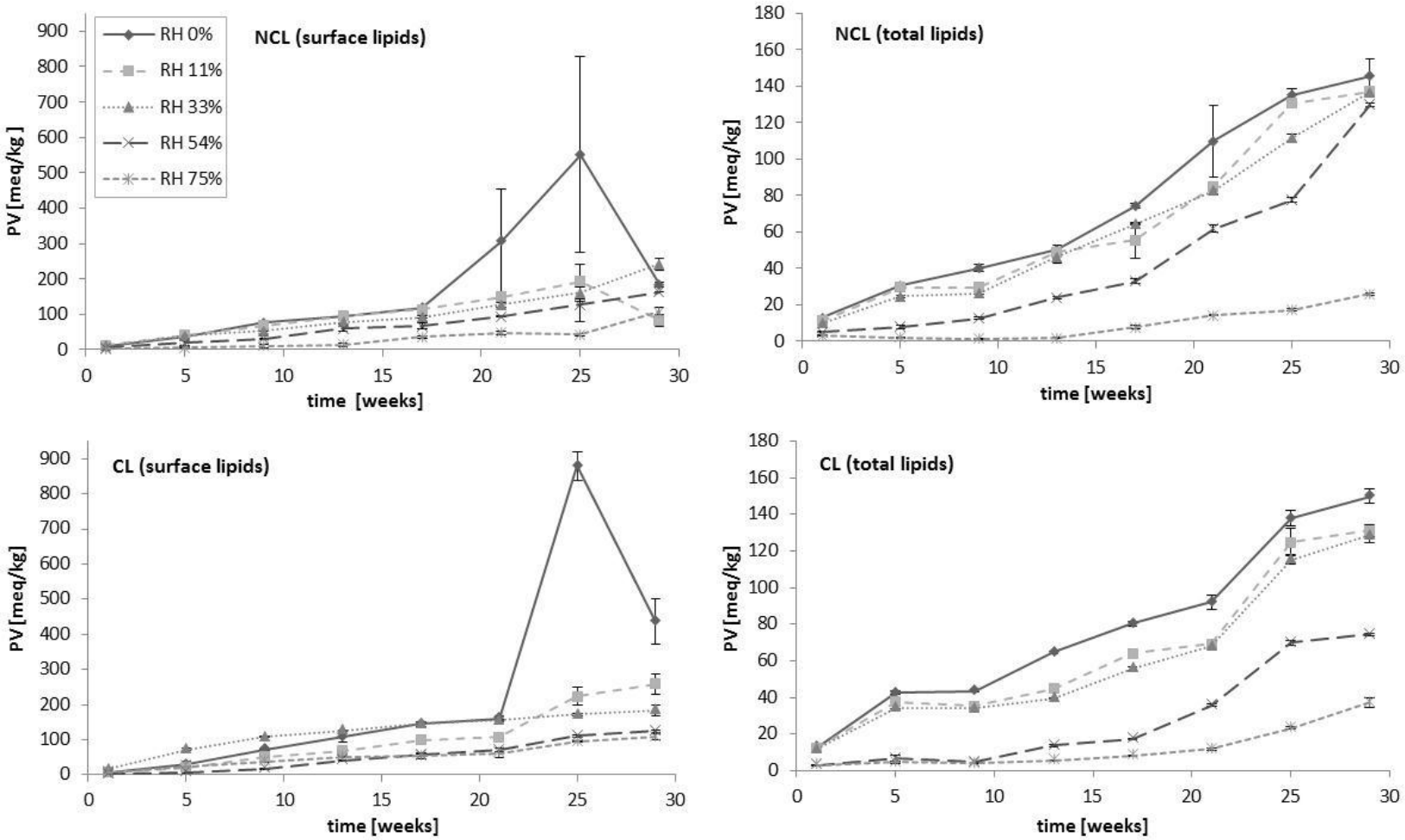

Figure 6. Peroxide values (PV) (mean ± s.d.; meq/kg of extracted oil; n = 3) of the surface (left) and total (right) lipid extracts of the spray-dried emulsion with non-cross-linked protein (NCL; above) and the spray-dried emulsion with cross-linked protein (CL; below) stored for 1 to 29 weeks at five relative humidities (RHs).

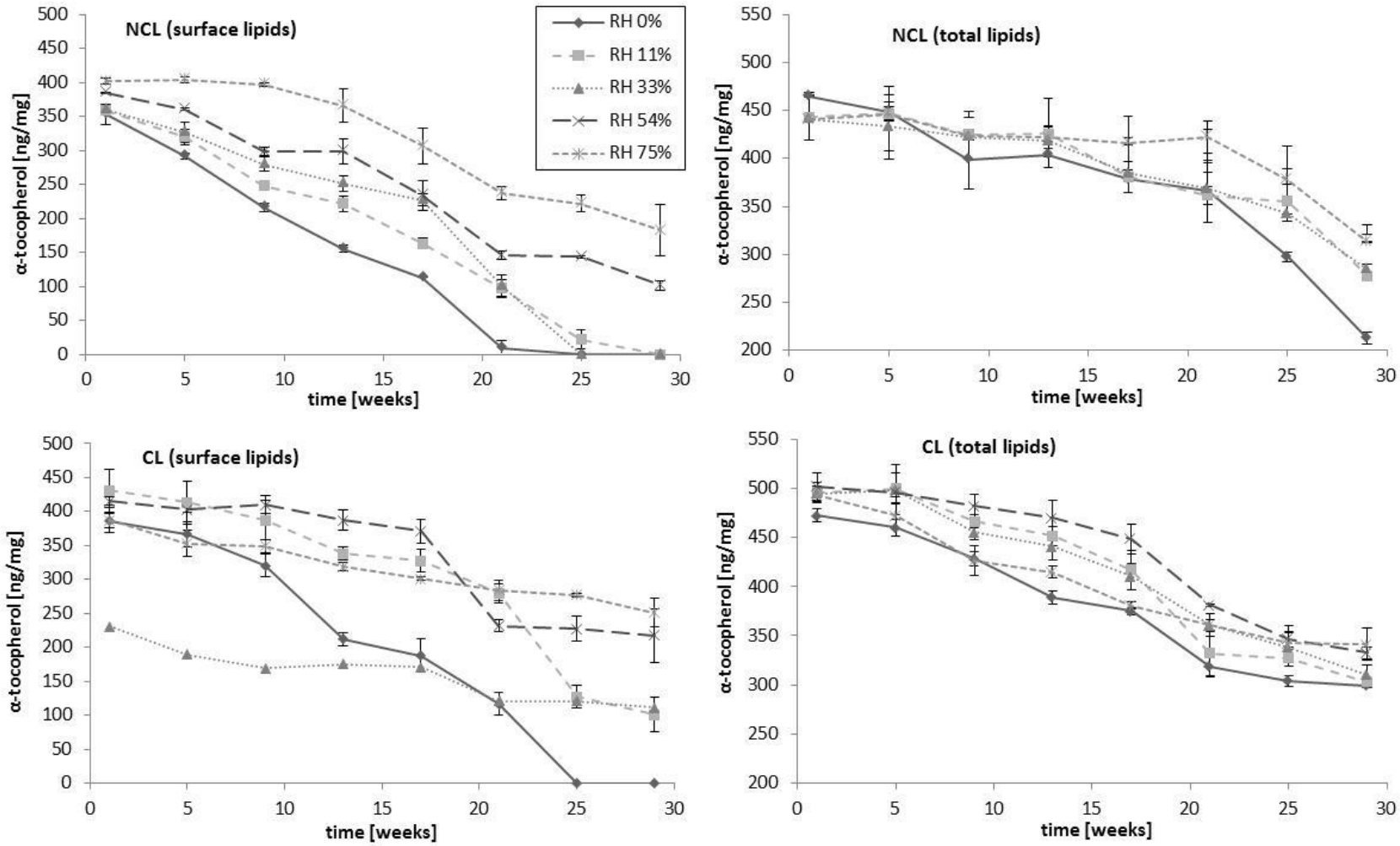

Figure 7. α-Tocopherol contents (mean ± s.d.; ng/mg of extracted oil; n = 3) of the surface (left) and total (right) lipid extracts of the spray-dried emulsion with non-cross-linked protein (NCL; above) and the spray-dried emulsion with cross-linked protein (CL; below) stored for 1 to 29 weeks at five relative humidities (RHs).

The PVs of the total lipids were, in general, lower (by twofold) than those of the surface lipids at all RHs. At RH 54% and 75% for the NCL and at RH 75% for the CL, an induction period of up to week 13 was seen (Figure 6). After 13 weeks, the PVs at all RHs increased.

The α-tocopherol losses were in line with the formation of the hydroperoxides. The higher the formation of hydroperoxides based on the PV, the greater the losses of α-tocopherol. The greatest losses over time were detected at RH ~0% followed by RH 11% and 33% for the surface and total lipids of both emulsions (Figure 7). Again, this showed higher oxidation rates at low RHs. All α-tocopherol in the surface lipids was degraded in the NCL by week 25 at RH ~0% and 33%, and by week 29 at RH 11%. In the CL, no α-tocopherol was detectable at RH ~0% for the surface lipids at 25 weeks of storage. For the surface lipids of the CL at RH 33%, marked losses of α-tocopherol were already observed at the beginning of the storage period, but when storage progressed, the degradation rate levelled off. For the total lipids, the α-tocopherol content was higher than for the surface lipids (2.7-fold in the NCL and 1.9-fold in the CL after 17 weeks) and the differences between the RHs were less pronounced than for the surface lipids.

The third measured oxidation indicator was the formation of hexanal as the main volatile secondary oxidation product. The highest amounts of hexanal were observed at the two lowest RHs (~0% and 11%), followed by RH 54% for the NCL and the CL (**I**; Figure 4). At RHs 33% and 75% no increases in the hexanal amounts were seen. In general (after considering the effect of the RH on the amount of hexanal released; see 5.1.2), the hexanal measurements suggested higher oxidation levels under dry conditions for both dried emulsions, as seen by the measurements of the PVs and α-tocopherol losses.

In summary, the total lipids were shown to be more oxidatively stable than the surface lipids at all tested RHs. Both dried emulsions showed the highest oxidative stability at the highest tested RH (75%). Although the trend in the oxidation behaviour was the same for the NCL and the CL, small but significant improvements in the oxidative stability by protein cross-linking were observed at certain RHs. The CL was more stable than the NCL at RH 54% based on the PVs and α-tocopherol content, and at RH ~0% based on the α-tocopherol content.

5.3 Lipid stability of cereal extrudates during storage

5.3.1 Initial characterization of lipids in cereal flours, brans and extrudates (III-IV)

The non-heat-treated (NHT) and heat-treated (HT) oat flours contained around 56 mg/g of lipids, of which 88% were extractable by ASE using acetone (Table 5). Whereas the extraction efficiency by ASE from the oat extrudates was lower, with 63% to 74% of the total lipids. The content of the ASE extractable lipids of the rye bran extrudates was also lower than in the rye

brans (Table 5). Extrusion had no significant effect on the fatty acid composition, either in the case of the oat extrudates or rye extrudates. In the oat flours and extrudates, both oleic and linoleic acid represented approximately 40% of the fatty acids (Table 5). In the rye brans and rye bran extrudates, linoleic acid contributed approximately 60% and oleic acid approximately 13% to the total fatty acids. In the rye brans and rye bran extrudates, the linolenic acid proportion was higher (ca. 9%) than in the oat flours and extrudates (ca. 1.5%).

Table 5. Lipid content, unsaturated fatty acid distribution and tocol content of oat flours, oat extrudates, rye brans and rye bran extrudates (mean ± s.d.; n = 3; fresh weight basis; FA-ASE = sum of fatty acids extractable by accelerated solvent extraction; NHT = non-heat treated; HT = heat-treated; extrudates A, B, C, D = oat extrudates; coarse [°C]/[%] = coarse rye bran extrudates; fine [%] = fine rye bran extrudates).

Product	Total lipids (mg/g)	FA-ASE (mg/g)	18:1 (%)*	18:2 (%)*	18:3 (%)*	Total tocols (µg/g)
NHT oat flour	56.7 ± 2.6	50.2 ± 2.0	38.5	40.6	1.7	na
HT oat flour	54.7 ± 1.6	48.3 ± 1.4	38.6	40.1	1.4	na
extrudate A	63.3 ± 1.7	42.3 ± 2.9	38.5	40.7	1.6	na
extrudate B	61.1 ± 0.4	39.0 ± 0.5	39.3	39.3	1.4	na
extrudate C	62.3 ± 0.2	46.3 ± 2.8	38.2	40.0	1.6	na
extrudate D	61.7 ± 2.1	39.1 ± 1.9	38.4	39.8	1.5	na
coarse rye bran	17.6 ± 0.8	14.5 ± 0.5	13.1	57.9	7.9	50.1 ± 1.4
fine rye bran	21.9 ± 2.2	20.9 ± 0.3	13.0	58.9	8.0	32.9 ± 0.5
coarse 80 °C	na	9.2 ± 0.1	14.0	60.6	8.8	61.2 ± 1.1
coarse 100 °C	na	9.8 ± 0.1	13.9	60.7	8.8	64.6 ± 0.9
coarse 120 °C	na	10.3 ± 1.0	13.9	60.5	8.7	67.2 ± 5.4
coarse140 °C	na	11.2 ± 0.2	13.8	60.8	8.8	65.3 ± 1.2
coarse 13%	na	11.0 ± 0.1	14.1	60.9	8.9	66.0 ± 4.1
coarse 16%	na	11.2 ± 0.2	14.0	60.7	8.8	66.0 ± 1.0
coarse 22%	na	10.9 ± 0.2	13.9	60.8	8.8	64.2 ± 2.3
coarse 30%	na	11.3 ± 0.4	14.0	60.6	8.8	64.7 ± 1.8
fine 13%	na	11.9 ± 0.5	12.9	60.9	8.8	37.4 ± 0.4
fine 16%	na	10.4 ± 0.1	13.1	60.8	8.8	30.2 ± 0.2
fine 22%	na	10.0 ± 0.3	13.2	61.1	8.9	29.4 ± 0.3
fine 30%	na	11.0 ± 0.2	13.1	61.4	9.0	30.0 ± 0.8

na = not analysed

* = fatty acid composition based on FA-ASE

A comparison of the total tocol content (sum of α- and β-tocopherols and α- and β-tocotrienols) of the coarse and fine rye bran showed that the grinding process decreased the total tocol content (Table 5). The decrease in the tocopherol content was around 50%, and that in the tocotrienols was about 28%. The amount of total tocols was higher in the coarse rye bran extrudates than in the coarse rye bran; although the extractability of lipids by ASE was lower for the extrudates than for the bran. However, the amount of extractable total tocols was slightly decreased in the fine rye bran extrudates when compared to the fine rye bran, except for the extrudate produced at 13% water content.

5.3.2 Storage stability of oat extrudates in comparison with flours (III)

The storage stability of the oat extrudates and flours was determined by measuring their neutral lipid and volatile profiles. The major neutral lipid class in all four oat extrudates was TAGs followed by FFAs. DAGs were present in low amounts, and no MAGs were detected in any of the extrudates. In extrudate B (130 °C and 200 rpm) after six weeks of storage, the content of the TAGs started to decline, and at the end of the storage period, only 1.9 mg/g of the TAGs were detectable (Table 6). The loss of the TAGs was not accompanied by the formation of FFAs. The extrudates A (70 °C and 200 rpm) and D (110 °C and 400 rpm) behaved similarly and lost approximately 20% of the TAGs and approximately 40% of the FFAs during storage. In extrudate C (110 °C and 100 rpm), the TAGs decreased after 6 weeks of storage by around 40%, but afterwards the content remained stable. The content of the TAGs and FFAs of the HT flour was stable during the whole storage period (Table 6). However, the TAGs of the NHT flour decomposed rapidly. The high decrease in the TAGs was accompanied by a high increase in the FFAs showing lipase activity in the NHT flour.

Table 6. Triacylglycerol (TAG) and free fatty acid (FFA) contents in lipids extracted by accelerated solvent extraction (ASE) of oat flours and extrudates: fresh, after standardization at relative humidity of 33% (0 weeks) and storage at 40 °C for 15 weeks (mean ± s.d.; fresh weight basis; n = 3; NHT = non-heat treated; HT = heat-treated; extrudates A, B, C, D = oat extrudates).

Lipid class	Time (weeks)	NHT flour	HT flour	extrudate A	extrudate B	extrudate C	extrudate D
	fresh	22 ± 1	24 ± 1	na	na	na	na
	0	14 ± 1	25 ± 1	27 ± 4	27 ± 3	33 ± 6	20 ± 4
TAG	6	2.0 ± 0.3	26 ± 3	29 ± 6	25 ± 3	19 ± 2	18 ± 3
(mg/g)	9	2.2 ± 0.1	25 ± 3	na	na	na	na
	12	na	na	29 ± 1	2.5 ± 0.3	22 ± 3	14 ± 1
	15	0.7 ± 0.05	26 ± 1	22 ± 1	1.9 ± 0.2	20 ± 0.8	16 ± 1
	fresh	6.0 ± 0.1	3.0 ± 0.9	na	na	na	na
	0	15 ± 0.1	2.5 ± 0.2	6.2 ± 0.6	6.2 ± 0.6	6.8 ± 2	4.0 ± 1
FFA	6	26 ± 0.1	2.7 ± 0.05	2.0 ± 0.2	4.2 ± 2	6.5 ± 0.7	4.0 ± 0.8
(mg/g)	9	20 ± 0.3	2.9 ± 0.6	na	na	na	na
	12	na	na	4.3 ± 0.2	1.3 ± 0.02	6.5 ± 0.6	5.8 ± 0.7
	15	22 ± 3	4 ± 0.3	3.9 ± 0.2	0.4 ± 0.02	3.3 ± 0.1	2.5 ± 0.1

na = not analysed

Volatile secondary oxidation products were measured to determine the oxidative stability of the oat extrudates during storage. For each extrudate, a PCA (**III**) was conducted with data from 12 indicator compounds (Appendix 1). From the PCAs, 6 key compounds with the highest impact on the PCA were selected (Figure 8).

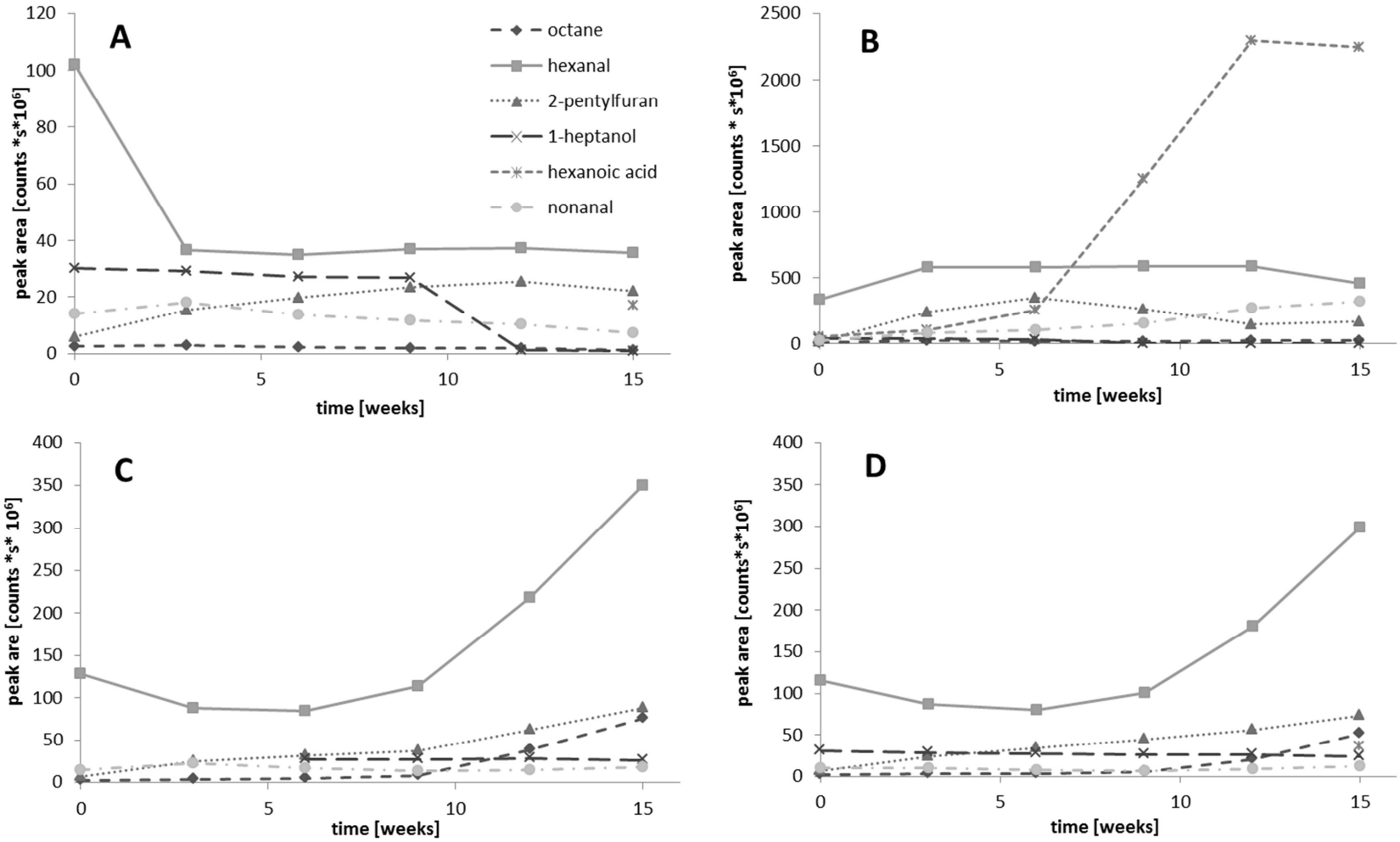

Figure 8. Mean peak areas in counts per second of 6 key compounds (octane, hexanal, 2-pentylfuran, 2-hepten-1-ol, hexanoic acid, nonanal) of the oat extrudates A (70 ºC, 200 rpm), B (130 ºC, 200 rpm), C (110 ºC, 100 rpm) and D (110º, 400 rpm) stored at 40°C for 15 weeks (n = 3).

For extrudate A produced at 70 °C, the level of hexanal had decreased by 3 weeks of storage and remained stable afterwards. All other key compounds showed lower levels than hexanal, and remained more or less stable during the whole storage period. Extrudate A was, therefore, rather stable during storage. The stability of extrudate A was comparable to the HT flour, based on the comparable levels of hexanal and 2-pentylfuran (Figures 8 and 9).

The hexanal levels of extrudate B produced at 130 °C were threefold greater than the ones of extrudate A at the beginning of the storage test (Figure 8). The hexanal and 2-pentylfuran amounts increased during the first 6 weeks of storage. Afterwards, they stabilized and decreased slightly. At 9 weeks, the hexanoic acid became the dominating compound in the volatile profile of extrudate B. In the end, the amounts of volatiles were 30 times higher in extrudate B than in extrudate A. Extrudate B could be considered highly oxidized. For the NHT flour, a similar high hexanal level to that for extrudate B was determined, but the amounts of 2-pentylfuran were higher, and hexanoic acid was only a minor compound in the NHT flour compared to extrudate B (Figures 8 and 9).

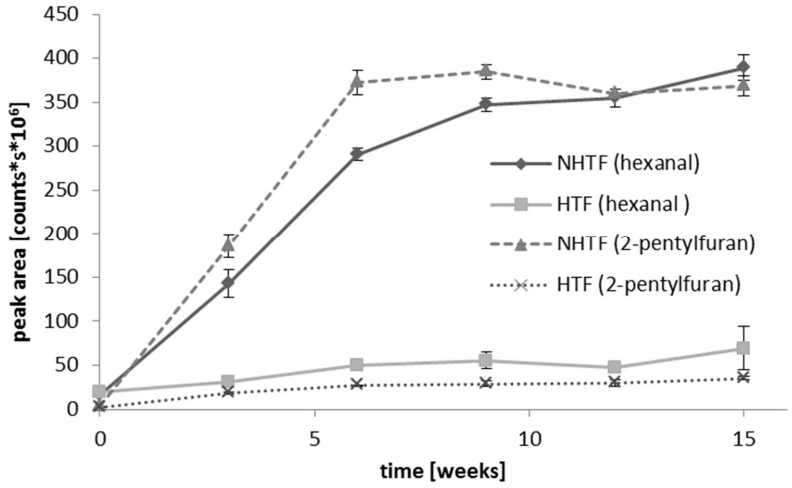

Figure 9. Mean peak areas of hexanal and 2-pentylfuran (mean ± s.d.; counts per second; n = 3) of non-heat-treated (NHT) and heat-treated (HT) flours stored at 40 °C for 15 weeks.

The amounts of the key compounds of extrudates C and D, both produced at 110 °C but at different screw speeds, behaved quiet similarly (Figure 8). However, small differences were observed. The amounts of hexanal, 2-pentylfuran and octane were slightly higher in extrudate C than D, indicating slightly more lipid oxidation in extrudate C than D. Based on the amounts of the key compounds, extrudates C and D were more oxidized than extrudate A, but less oxidized than extrudate B.

5.3.3 Storage stability of rye bran extrudates (IV)

In study **IV**, the lipid stability of the rye bran extrudates during storage was determined by analysing the remaining tocols as oxidation indicators, and by analysing the volatile profiles to monitor the development of secondary volatile oxidation products. α-Tocopherol and hexanal were chosen to display the general oxidation behaviour during storage.

Temperature series

The extrudates produced at 80, 100 and 140 °C, with a water content of 22%, had losses of 68% to 76% in α-tocopherol after 10 weeks (Figure 10, a). The extrudate produced at 120 °C had a greater loss of around 90%. This extrudate also had the highest formation of hexanal, followed by those produced at 140, 100 and 80 °C (Figure 10, a). However, none of the extrudates in the temperature series showed oxidative stability during storage. The only extrudate containing volatile Maillard reaction products (only in low amounts) in this series was the one produced at 140 °C.

Coarse bran water content series

The extrudates produced with coarse rye bran at a low water content had the smallest loss in tocols: 11% and 32% of the α-tocopherol at 13% and 16% water, respectively (Figure 10, b). The extrudates produced at 22% and 30% water had greater losses of the α-tocopherol: 84% and 78%, respectively. The extrudate produced at 22% water had the highest amount of hexanal after 10 weeks, followed by the extrudates produced at 30%, 16% and 13% water (Figure 10, b). In the extrudates produced at 13% and 16% water, the amount of hexanal even decreased between weeks 2 and 4, indicating that more hexanal was bound to the matrix than was formed through lipid oxidation. The only extrudate of the coarse bran water content series to be considered oxidatively stable was the one produced at a water content of 13%. In this series, the extrudates produced at a low water content (13% and 16%) contained considerable amounts of volatile Maillard reaction products.

Fine bran water content series

The initial tocol content was much lower in the fine bran extrudates when compared to the coarse bran extrudates (Table 5). At 13% and 16% water content, the loss in the α-tocopherol was 4% and 7%, respectively (Figure 10, c). The extrudates produced at the higher water content (22% and 30%) again had greater losses in the α-tocopherol: 25% and 65%, respectively. The formation of hexanal in the fine bran water content series was, in general, one-third, compared to the extrusion series with the coarse rye bran (Figure 10, b and c). An increase in hexanal was only seen for the extrudates produced at 22% and 30% water, whereas for the extrudates produced at 13% and 16% water, the hexanal levels decreased (Figure 10, c).

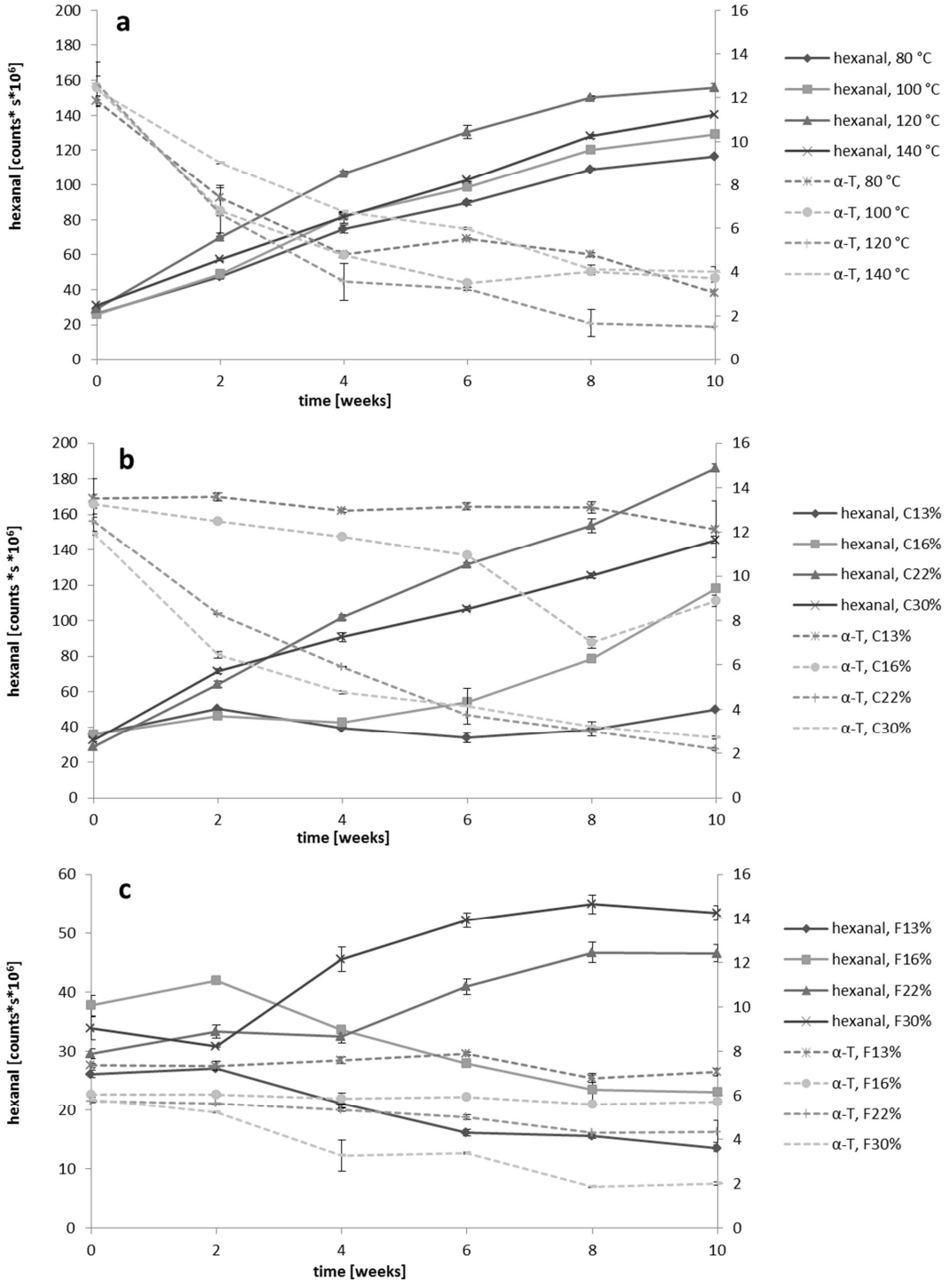

Figure 10. Hexanal peak areas (mean ±s.d.; counts per second; n = 3) and α-tocopherol content (mean; µg/g; n = 3) of the coarse rye bran extrudates of the temperature series [80, 100, 120, 140 °C] (a), of the coarse bran water content series [C13%, C16%, C22%, C30%] (b), and of the fine bran water content series [F13%, F16%, F22%, F30%] (c) stored for 10 weeks at 40 °C.

These extrudates had high levels of volatile Maillard reaction products; up to 4 times higher than the extrudates produced at the same water content with the coarse rye bran. In the fine bran water content series, both extrudates produced at low water could be considered to be stable during storage.

PCA analysis of both water content series

The most interesting extrudates based on α-tocopherol losses and hexanal levels during storage were the ones produced at a low water content containing volatile Maillard reaction products. These extrudates had a distinctive roasted flavour in comparison with the other extrudates, which had more of a plain rye flavour. The strongest roasted flavour (unpleasant, nearly burned) was noticed in the extrudate produced at 13% water with the fine rye bran. Therefore, both of the water content series were analysed by PCA to gain more detailed information on the oxidation behaviour of these extrudates. The PCA was based on the data of the four main tocols (α- and β-tocopherols and α- and β-tocotrienols) and of five volatile indicator compounds (two secondary volatile oxidation products and three volatile Maillard reaction products; see Appendix 1) during storage.

All time points of the extrudates produced at 13% water, with either the coarse or fine rye bran, and of the extrudate produced at 16% water with the fine bran were grouped together (Figure 11). This confirmed the already observed stability of these extrudates over time (Figure 10, b and c). All three extrudates displayed a negative correlation with the volatile oxidation indicators. The extrudate produced with the fine rye bran at 13% water was associated with methylpyrazine and furfural, while the extrudate produced with the coarse bran at 13% water was associated with 2,5-dimethylpyrazine. The extrudate produced with the fine rye bran at 16% water correlated positively with both pyrazines.

The extrudate produced at 16% water with the coarse bran strongly correlated with all four tocols from weeks 0 to 4 (Figure 11). After 6 weeks, this extrudate began to associate more strongly with hexanal and 2-pentylfuran, indicating that unlike the corresponding fine bran extrudate, oxidation started in this extrudate. At 0 weeks, neither the coarse nor the fine rye bran extrudates produced at a high water content (22% and 30%) were clearly associated with any variable, but the extrudates produced with the fine rye bran were already heading towards the general direction of oxidation (indicated by the arrow in Figure 11). After 2 weeks, all of the extrudates produced at high water had already started to correlate, mainly with hexanal and 2-pentylfuran. It was observed that the extrudate produced from the fine rye bran correlated more strongly with 2-pentylfuran than with hexanal (most time points were found on the left side of the arrow).

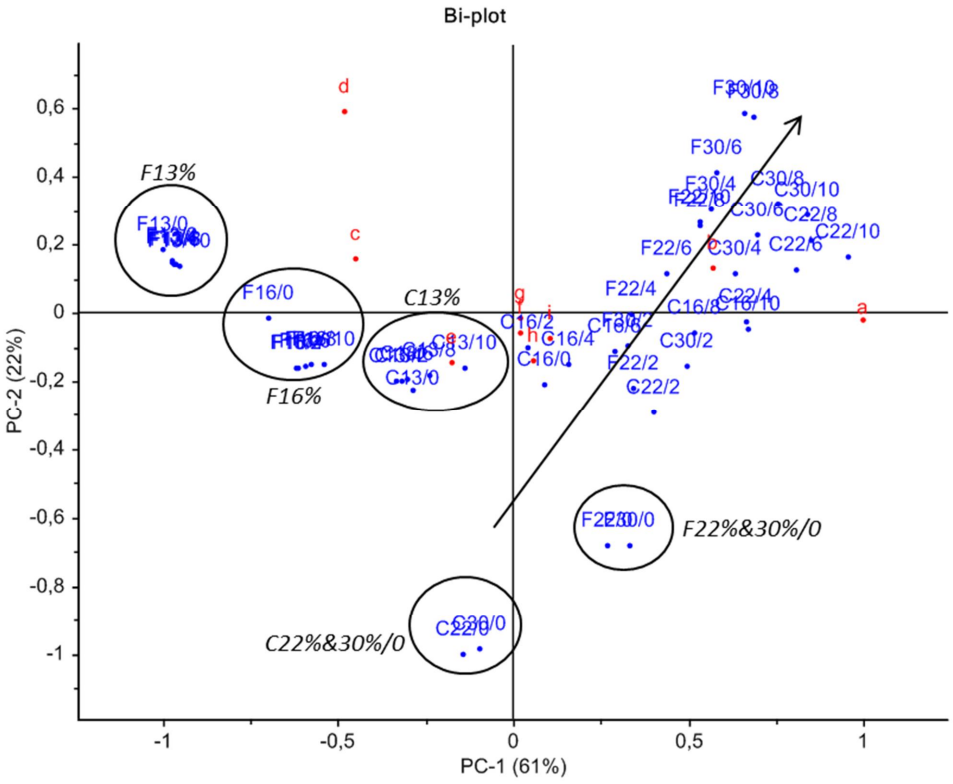

Figure 11. PCA biplots of the tocols and volatile indicator compounds of the rye bran extrudates of the water content series with the coarse rye bran (C) and the fine rye bran (F); objective symbols (blue) = C or F water content (13, 16, 22, 30)/storage time in weeks (0, 2, 4, 6, 8, 10); variable symbols (red) = a - i (a. hexanal, b. 2-pentylfuran, c. methylpyrazine, d. furfural, e. 2,5-dimethylpyrazine, f. α-tocopherol, g. β-tocopherol, h. α-tocotrienol, i. β-tocotrienol).

Based on the PCA, only both extrudates produced at 13% and the extrudate produced at 16% water, with the fine bran, were oxidatively stable throughout the storage period. All other extrudates showed the formation of volatile oxidation compounds and tocol degradation.

6 DISCUSSION

6.1 Analytical methods to study stability of dispersed lipids

6.1.1 Volatile analysis by HS-SPME-GC-MS from solid matrices with dispersed lipids

Several factors affect the selectivity and efficiency of HS-SPME; one important one is the extraction coating of the fibre (Balasubramanian and Panigrahi 2011). The HS-SPME method developed in this thesis was based on the method of Paradiso et al. (2008), which used a DVB/CAR/PDMS fibre. During the method development, different fibres (PDMS/DVB, CAR/PDMS and DVB/CAR/PDMS) were tested for the extraction of volatiles from spray-dried emulsions (data not published). Of the tested fibres, the three-compound fibre (DVB/CAR/PDMS) was able to extract the widest variety of volatile compounds with a satisfying sensitivity. Earlier, Jeleń et al. (2000) tested different fibre coatings (PA, DVB/CAR/PDMS, PDMS, carbowax/DVB) for the extraction of volatiles from refined rapeseed oil. In their study, DVB/CAR/PDMS was also the preferred coating, because of the low detection limits and satisfactory linearity for most volatiles. Mildner-Szkudlarz et al. (2003) used a CAR/PDMS/DVB fibre for the extraction of volatile oxidation products from olive, soybean, sunflower, peanut and rapeseed oil. HS-SPME-GC-MS using a DVB/CAR/PDMS fibre was also used earlier to analyse lipid oxidation in several cereal products (Klensporf and Jeleń 2005, 2008; Paradiso et al. 2008, 2009).

In addition to lipid oxidation products, Maillard reaction products were detected with the developed HS-SPME method in this thesis. Earlier, Coleman III (1996, 1997) showed the potential to analyse volatile Maillard reaction products by SPME using a model of standard compounds in water. Nowadays, Maillard reaction products are commonly analysed from liquid samples of coffee or cocoa (Balasubramanian and Panigrahi 2011). Lojzova et al. (2009) developed a method to analyse substituted pyrazines and other formed flavour compounds during the Maillard reaction in potato chips. They tested different fibres (carbowax/DVB, PDMS, PDMS/DVB, DVB/CAR/PDMS) and also selected the three-compound fibre used in this study because of its high extraction efficiency for different volatiles. However, if the focus in a study is on one specific volatile or volatile group, other fibres with more specific selectivity may be used.

The identified compounds in the volatile profiles of the spray-dried emulsions analysed by HS-SPME-GC-MS were typical secondary oxidation products, mainly from linoleyls (18:2) and oleyls (18:1). This was expected based on the fatty acid composition (linoleic acid 60% and oleic acid 27%) of encapsulated sunflower oil. Similar volatiles were reported in the oxidation studies of sunflower oil and sunflower oil emulsions (Mildner-Szkudlarz et al. 2003; Villière et al. 2007). HS-SPME-GC, combined with either MS or FID, was also used to detect volatile secondary

oxidation products from vegetable oils, to use them for the differentiation of oxidized oils from non-oxidized oils (Jeleń et al. 2000; Mildner-Szkudlarz et al. 2003).

The volatile profiles of the stored oat extrudates had high similarities with the ones of the oxidized spray-dried emulsions, and consisted mainly of typical secondary oxidation products from linoleyls and oleyls. However, more volatiles were detected in the oat extrudates than in the dried emulsions, and the higher amounts of acids in the oat extrudates suggested a more progressed lipid oxidation in certain oat extrudates. Further, oat lipids contained higher amounts of oleic acid (40%) and lower amounts of linoleic acid (40%) than the sunflower oil, which affected the formation of secondary oxidation products. Similar volatile compounds derived from lipid oxidation were found in the stored oat flakes and oatcakes analysed by HS-SPME-GC-MS (Klensporf and Jeleń 2005; Cognat et al. 2012), and in oat extrudates analysed by DHS-GC-MS (Sjövall et al. 1997; Parker et al. 2000).

Volatiles found in rye bran extrudates were also derived from the oxidation of linoleyls and oleyls. However, more compounds formed from the linolenyls, like 2,4-heptadienal, were detected in the rye bran extrudates, compared to the oat extrudates, due to the higher amount of linolenic acid (9%) in the rye lipids than the oat lipids. In addition to secondary lipid oxidation products, Maillard reaction products (pyrazines and furans) and Strecker degradation products (Strecker aldehydes like 2-methylbutanal and 3-methylbutanal) were found in rye bran extrudates. Heiniö et al. (2003a) described similar alcohols, aldehydes, ketones, esters and furans to those found in this study in fresh rye extrudates produced at 140 ºC and 250 rpm with a feed moisture of 19%-20%. These volatiles were analysed by DHS-GC-MS; however, they did not detect any pyrazines, one of the dominating volatile groups in rye bran extrudates in this study. The difference might be caused by the higher moisture content used in the extrusion process. Low water content and high barrel temperature are known to promote the formation of Maillard reaction products. In this study, only the rye bran extrudates produced at a low water content (13% or 16%) showed a high formation of Maillard reaction products. Earlier, Parker et al. (2000) detected volatile Maillard reaction products in oat extrudates, and Bredie et al. (1998) in maize extrudates. In both studies, DHS-GC-MS was used to analyse the volatile compounds.

It was shown that by using the developed HS-SPME-GC-MS method, volatile secondary lipid oxidation products could be analysed from dried oil emulsions and cereal extrudates. The obtained volatile profiles were comparable to the ones found earlier for similar products. Further, it was possible to detect volatile Maillard reaction products formed during extrusion. Until now, the HS-SPME-GC-MS has not been widely used for simultaneous analysis of lipid oxidation and Maillard reaction products. The performance of the HS-SPME-GC-MS method was controlled throughout all experiments by measuring the volatile profile of a reference material in each

sequence. This approach can be recommended, for example, for determination when the fibre should be changed.

6.1.2 Effect of HS-SPME extraction conditions on the amount of volatiles released

The effect of the HS-SPME extraction conditions (incubation and extraction temperature, and agitation speed) was determined based on 18 selected indicator compounds (Appendix 1) from spray-dried sunflower oil emulsions. Pentanal, 1-pentanol, hexanal, heptanal, 2-pentylfuran, 2-heptenal (E), 1-octen-3-ol, 3-octen-2-one (E) and 2-octenal (E) belonging to the 15 identified indicator compounds are formed by the oxidation of linoleyls. While octanal, nonanal and 2-decenal (E) are oxidation products of oleyls (Choe and Min 2006). Hexanoic acid, octanoic acid and 5-pentyl-2(5H)-furanone could be formed through further reactions of hexanal, octanal and 2-pentylfuran.

Increasing the HS-SPME incubation and extraction temperature from 40 °C to 50 °C improved the liberation of the selected indicator volatiles. The impact was dependent on the RH, but the trend was similar at all tested RHs. Higher vapour pressure, increased mobility and possible decreased solubility of the volatiles in the matrix at 50 ºC, rather than at 40 ºC, could have caused the increase in the overall release at 50 °C (Voilley and Souchon 2006). In our preliminary study on the extraction of volatiles from cereal flours and extrudates, an even higher improvement in the liberation of volatiles was achieved by using 50 °C instead of 40 °C (Pulkkinen 2012). Therefore, 50 ºC was used to determine the lipid stability of cereal extrudates during storage.

The decreases in the pentanal and hexanal peak areas at 50 ºC, compared to 40 ºC, were most likely due to further oxidation to pentanoic acid and hexanoic acid, respectively (Kruse et al. 2006; Ishida and Haruta 2007). At higher extraction temperature, the peak areas of acids (pentanoic acid, hexanoic acid and octanoic acid) increased, strongly implying further oxidation of the aldehydes at 50 °C. Jeleń et al. (2000) also observed the further oxidation of volatiles at 50 °C compared to 20 °C in the SPME analysis of volatiles from refined rapeseed. However, they concluded that the advantages of using 50 °C instead of 20 °C (the improved quantities of volatiles and shorter time to reach the equilibrium) overcame the risk of further oxidation, especially because shorter incubation and extraction times are needed at 50 ºC than at 20 ºC. In our preliminary study, no further oxidation of aldehydes in cereal flours and extrudates was observed at different extraction temperatures (Pulkkinen 2012). This may be connected with the markedly lower lipid content of the cereal products, compared to the spray-dried emulsions.

The further oxidation of hexanal may also explain the differences seen in the hexanal amount measured by SHS and HS-SPME (Table 4). The extraction temperature of the SHS was distinctly higher (80 °C) than the extraction temperature of the HS-SPME. It can be expected, based on the

above discussion, that the higher temperature led to the oxidation of hexanal to hexanoic acid, which was, however, not measureable with the SHS method used. Therefore, in further oxidation studies of cereal extrudates, the whole volatile profile was measured by HS-SPME to detect possible further oxidation products. However, the incubation and extraction times were shorter for the SHS than the HS-SPME method, which should have reduced oxidation. The big differences in the relative hexanal amounts among the different RHs, when SHS was used, may be explained by even more marked water induced structural changes (see 6.1.3) and the higher water vapour pressure at 80 °C. At 80 °C, both spray-dried emulsions were in a rubbery state at RH 33% to 75% and at RH 11% near the transition region (Moisio et al. 2014).

A change in the HS-SPME agitation speed did not affect the relative ratios of the released volatiles. Additionally, in our preliminary study, a change in the agitation speed did not affect the volatile profiles of the tested products (Pulkkinen 2012). This was as expected because agitation in the SPME extraction is mainly used to shorten the time to reach the equilibrium (Balasubramanian and Panigrahi 2011).

In conclusion, based on this study and the reviewed literature, incubation and extraction temperatures of 40 ºC or 50 ºC, respectively, are recommended to be used for the extraction of lipid oxidation products by SPME. Temperatures higher than 50 ºC, or even 50 ºC, could cause further oxidation during incubation and extraction in oxidatively sensitive products. Agitation can be used to shorten the incubation and extraction times without changing the volatile profile.

6.1.3 Effect of RH on the amount of volatiles released from spray-dried emulsions

The hexanal amounts measured from oxidized spray-dried emulsions stabilized to different RHs after oxidation should be similar, in theory. However, the hexanal amounts measured by the SHS were up to 5-fold higher at certain RHs. These differences could be explained by differences in the amount of hexanal released. The release of volatiles is controlled by two main factors, the volatility of the compounds, which forces the compounds out of the matrix, and the resistance provided by the matrix to withhold the compounds. These factors depend on the chemical structure of the volatiles and the composition and physical state of the surrounding food matrix (Le Thanh et al. 1992; Madene et al. 2006). A change in the physical state can change the diffusion in solid foods. One factor which influences the physical state of solid foods is the water content (Roos and Karel 1991). Water also increases the hydrophilicity of the food matrix, which can facilitate the release of hydrophobic volatiles by changing their partitioning and solubility in the matrix (Madene et al. 2006). Therefore, depending on the food matrix, a change in RH connected with a change in water content can affect the release of volatiles, to a great extent.

The matrix in this study consisted of Na-caseinate (3% of the dry matter), which was located mainly in the interfacial layer and partly on the surface of the particles, and maltodextrin DE 22.2 (67% of the dry matter), which was the main wall material (Moisio et al. 2014). Several studies have shown that Na-caseinate can bind volatile compounds through hydrogen bonds, hydrophobic interactions and ionic bonds, or even via covalent linkages (Kühn et al. 2006). Meynier et al. (2004) determined binding of hexanal to the amino acids of Na-caseinate. Maltodextrin was also shown to bind the volatiles (Guichard 2002). Jouquand et al. (2006) determined the retention of C6 aroma compounds (including hexanal) in model starch dispersions. They demonstrated the ability of hexanal to form complexes with amylose. Therefore, the matrix of spray-dried emulsions can, in general, bind hexanal as well as other volatiles. However, this still did not explain the effect of RH. Le Thanh et al. (1992) showed that casein bound volatiles more or less independently from the water content, while the ability of the maltodextrins to bind the volatiles was highly influenced by the water content. They concluded that volatiles are bound by casein mainly through hydrophobic interactions; however, volatiles are bound by maltodextrins mainly through the hydrogen bonds between carbohydrates, water and volatile molecules.

To study the effects of RH on the amount of the volatile secondary lipid oxidation products released of oxidized spray-dried emulsions in more detail, the amounts of 18 indicator compounds were studied at 5 different RHs by HS-SPME-GC-MS. The results showed that the effect of RH was different for individual volatiles. At RH ~0% , the released amount of all volatiles was lower than at other RHs. The low amount of volatiles released could be caused by the low amount of water in the matrix (the water content was under 1% at RH ~0%; Moisio et al. 2014). The water at RH ~0% was likely bound to the maltodextrin and, therefore, not free to facilitate the release of hydrophobic volatiles from the matrix. This was also seen in the relative ratios of the indicator compounds as demonstrated in the PCA (Figure 4). Hexanoic acid, a relatively hydrophilic compound, was released in higher quantities than, for example, 2-pentylfuran and 2-octenal, which are more hydrophobic volatiles.

At RH 11% and 33%, the total amount of volatiles released was the highest, and the indicator compound patterns closely resembled each other. At these RHs, the dried emulsions were in the glassy state at the extraction temperature of 40 °C, and fully kept their powder-like structures (Moisio et al. 2014), which insured a large surface area towards the gas-phase. Therefore, the volatiles could be released easily, facilitated by the higher water content of 3.1% and 5.2% at RH 11% and 33%, respectively. The C7- to C9-aldehydes were released best at these conditions.

At RH 54% and 75%, the dried emulsions were at the extraction temperature of 40 ºC in the rubbery state and the structure was collapsed (Moisio et al. 2014), which decreased the surface towards the headspace. These marked structural changes may explain the low overall amount of

volatiles released at the RHs 54% and 75%. The observed higher proportion of low molecular weight volatile compounds (like pentanal, 1-pentanol and hexanal) in the total released volatiles under wetter conditions may be due to the hydrophobic nature of most of the volatile oxidation products. Therefore, the higher water content of 6.7% at RH 54% and 12.0% at RH 75% improved their release. Further, based on the study of Le Thanh et al. (1992), some matrix-bound volatile compounds may be excluded from the matrix when their hydrogen bonds to maltodextrin are replaced by hydrogen bonds to water molecules at higher water contents.

The NCL and CL had nearly similar volatile profiles at the same RH. Therefore, the effect of cross-linking on the amount of volatiles released was small. However, some differences in the total amounts of released volatiles were observed. The monomers of natural Na-caseinate were partially transformed to higher molecular weight oligomers in the CL by inter-molecular cross-linking (Moisio et al. 2014). The interfacial protein layer structure was perhaps altered by the increased molecular size caseins, leading to the increased permeability of the interfacial protein layer for volatiles at dry conditions. At wet conditions, the matrix of both dried emulsions was collapsed. Excess protein, found to be concentrated on the surface of the particles during drying (Moisio et al. 2014), may cause the formation of agglomerates, leading to a denser matrix under wet conditions. Cross-linking slightly increased the amount of protein on the surface of the powders (Moisio et al. 2014), which could contribute to the differences between the CL and NCL under wet conditions. Further, the binding capacity of the Na-caseinate could also be affected by the cross-linking because of the alterations in the protein conformation, which could cause the exposure and/or inclusion of the binding sites for volatiles.

In summary, the effect of RH on the amount of volatiles released was associated with water-induced changes in hydrophilicity, structure and the binding ability of the Na-caseinate-maltodextrin matrix. The effects could be mainly attributed to the maltodextrin used as the wall material. The effect of the RH was studied previously, mostly in simple models using a volatile standard. This study showed the effect in a more complex model measuring the volatile profile of naturally formed volatiles. The information gained in this study will assist in the interpretation of volatile oxidation product profiles from solid foods with dispersed lipids obtained by HS-SPME or other headspace methods. The results of the study indicate further that HS-SPME is a useful method for studying matrix-related changes in solid foods. To minimize the observed effect of the RH/water content on the amount of volatiles released in the studies of the oxidative stability of cereal extrudates, all extrudates were standardized to the same RH before storage, and the comparability of the water contents was checked.

6.1.4 Other methods to study stability of dispersed lipids

The oxidative stability of both spray-dried sunflower oil emulsions (NCL and CL) stored at different RHs was determined by measuring the PVs, α-tocopherol losses and the formation of hexanal. Additionally, the content of the total and surface lipids, and their fatty acid profiles, were analysed. Both extraction methods used (for total and surface lipids) were mild, avoiding heating or extreme pH to decrease the risk of the decomposing of hydroperoxides during extraction. All three oxidation indicators, one for primary (PV) and one for secondary (formation of hexanal) lipid oxidation, and the consumption of one antioxidant (α-tocopherol), confirmed the higher oxidation rate at a lower RH. For example, in the case of the dramatic decomposing of hydroperoxides, as seen for the surface lipids at low RH (~0% and 11%), a total loss of α-tocopherol accompanied with a decrease of linoleic acid and increased formation of hexanal was observed. Therefore, the used methods supported each other and allowed a description of the oxidation behaviour of spray-dried emulsions.

In the case of oat extrudates, the storage stability was observed by measuring the neutral lipid profile of lipids extracted by ASE, and by measuring the volatile profiles by HS-SPME-GC-MS. To measure the neutral lipid profile was of interest, because of the known lipase activity in oats (Ekstrand et al. 1992). For this measurement, the lipids had to be extracted from the complex cereal matrix. The extraction by ASE was efficient enough to extract the majority of lipids without causing much further oxidation. However, the decomposing of the hydroperoxides was still a concern with this extraction method. Therefore, the PV was not used to determine the lipid oxidation in the cereal extrudates. The lipid oxidation was determined by analysing the volatile secondary oxidation products. Compared to the spray-dried emulsion study, HS-SPME-GC-MS was used instead of SHS-GC-FID (see discussion above, 6.1.2). The data from the volatile secondary oxidation products correlated with the consumption of lipids by the oxidation measured in the neutral lipid profiles.

Although the lipid oxidation of the oat extrudates could be followed by measuring the volatile profile, it was decided in the case of the rye bran extrudates to analyse the loss of tocols as a second oxidation indicator, similar to the study of spray-dried emulsions. Both measured oxidation indicators (volatile profiles using HS-SPME-GC-MS and loss of tocols using NP-HPLC-FLD) were in line. The extrudate with the highest formation of volatile secondary oxidation products also showed the greatest loss in tocols. The neutral lipid profile was not measured because the lipase activity was lower in the rye than in the oats (Meister et al. 1994), and the oat extrusion study already showed that the extrusion was able to inactivate lipase at a lower extrusion temperature than that used in the rye bran extrusion experiment.

In general, the methods used supported each other and enabled the study of lipid oxidation in the selected model systems. The approach using several oxidation indicators for the determination can be recommended, because the lipid oxidation can be followed at the different stages (going from the formation of primary to the formation of secondary oxidation products). Further, it reduces the risk of false interpretation, which can be caused by further reactions of the measured indicator.

6.2 Oxidative stability of spray-dried sunflower oil emulsions stored at different RHs

Increasing the RH from ~0% to 75% improved the oxidative stability of the spray-dried emulsions. The lipid oxidation rates of the surface lipids were up to two-fold compared to the total lipids. The NCL and the CL presented the highest oxidation rates at low RHs (~0% to 33%) for both the surface and total lipids. At these RHs, the dried emulsions were in the glassy state and kept their powder-like structure during storage (Moisio et al. 2014). The loosely packed powder ensured fast oxygen diffusion between the particles and the high availability of oxygen at the particle surface. Further, at very low RHs, cracks and pores could occur in the surface of the powder particles, increasing oxygen transfer to the oil droplets. Additionally, less interference from the water molecules, which hydrate localized catalysts, could promote lipid oxidation at low RH (Hardas et al. 2002). Therefore, the higher oxygen availability and less hydration of the catalysts at low RHs may explain the highest oxidation rates at RH ~0% to 33%. Contrarily, the low oxidation rates of the surface and total lipids of both dried emulsions at the high RHs (54% and 75%) may be explained by the water induced structural changes, or caking. With an increasing RH, the matrix absorbed increasing amounts of water. The water contents of the powders were 0.8% at RH ~0%, 3.1% at RH 11%, 5.2% at RH 33%, 6.7% at RH 54% and 12.0% at RH 75%, respectively (Moisio et al. 2014). The increased water absorption caused the structure to collapse. At RH 75%, the dried emulsions were in a rubbery state, fully losing their powder-like structure and becoming sticky “gum-like” solids (Moisio et al. 2014). The average distance of the oil droplets from the outer surface of the particle agglomerates increased as the dried emulsions were subjected to water-induced caking, which could have decreased the availability of oxygen as the rapid gas-phase diffusion in the loosely packed matrix was replaced by slower modes in the collapsed matrix (Le Meste et al. 2002). Caking had already started to take place in the glassy state at RH 54%, which was near the transition region (Moisio et al. 2014). The caking of the matrix at RH 75% also explained the drop in the extraction efficiency of the total lipids at RH 75%.

Further, the lower surface lipid content at RH 75% indicated that the surface lipids were not excluded from the collapsed matrix, as seen by Drusch et al. (2006) for encapsulated fish oil stored at 5 °C and RH 59%; instead, they remained entrapped and were therefore protected, as suggested by Le Meste et al. (2002) and Nelson and Labuza (1992). The entrapment could be

facilitated by excess protein, which was found to be concentrated during drying on the surface of the particles (Moisio et al. 2014). Surface proteins could improve the oxidative stability by coating the surface lipids, as discussed by Vega and Roos (2006). In addition, Na-caseinate in the interfacial-protein layer around the oil droplets and on the surface of the particles could have antioxidant properties, protecting the total and surface lipids against oxidation at high RHs, as was shown by Park et al. (2005) for freeze-dried emulsions with added proteins and/or peptides. They proposed that the proteins and peptides had strong radical scavenging activity and, therefore, could suppress lipid oxidation, especially at high RHs.

Similar results on decreased lipid oxidation at high RHs have been reported previously by Ponginebbi et al. (2000) for freeze-dried emulsions of linoleic acid, by Partanen et al. (2008) for flaxseed oil encapsulated in whey protein and by Aberkane et al. (2014) for microencapsulation of oil rich in polyunsaturated fatty acids in a maltodextrin/pea protein isolate or maltodextrin/pea protein isolate/pectin matrix using spray-drying. Ponginebbi et al. (2000) and Aberkane et al. (2014) argued that entrapment by caking in a rubbery state improved the oxidative stability. The opposite results (the best oxidative stability in a glassy state near the mono layer) were found by Grattard et al. (2002), Baik et al. (2004), Partanen et al. (2005) and Drusch et al. (2006). Their results were in line with Labuza's stability map and the concept that lipid oxidation is the lowest in the amorphous glassy state (Labuza 1980; Roos and Karel 1991). However, different encapsulation materials were used in the above mentioned studies, compared to this study. Different encapsulation materials display different structural behaviours (like crystallization, caking and structural collapse) with increasing water absorption, which can affect the stability of the encapsulated oil by affecting oxygen diffusion and the content of the encapsulated lipids (e.g. lipids can be excluded or entrapped) (Vega and Roos 2006; Gharsallaoui et al. 2007).

Na-caseinate cross-linking improved the oxidative stability at RH 54%. Further, a small improvement was seen at RH ~0%. Similar results have been shown by Bao et al. (2011) for microalgae oil encapsulated with maltodextrin and partly cross-linked Na-caseinate. They attributed the effect to enhanced emulsification properties and a more compact layer formation. Partanen et al. (2013) also showed that partly cross-linked β-casein had a higher density and mechanical strength than the native β-casein layer. Additionally, a slightly greater amount of Na-casein on the surface of the powder particles of the CL (Moisio et al. 2014) could have contributed to the higher stability of the surface lipids in the CL than the NCL at higher RHs.

Testing as wide of an RH range as in this study was of scientific interest to see the oxidation behaviour of the model at extreme conditions, but in practice, spray-dried emulsions are not kept at as high or as low of an RH as those tested in this study. Further, storage conditions in industry are chosen to preserve the flow-ability of powders. Nevertheless, the spray-dried emulsions in

this study were used as models of solid food systems with dispersed lipids. The obtained data may be relevant to other food systems.

6.3 Lipid stability of oat extrudates

The storage stability of the oat extrudates was determined by analysing the neutral lipid profile of the extracted lipids to observe the hydrolysis of the TAG, and by analysing the volatile profiles to observe the formation of the volatile secondary lipid oxidation products during storage. The obtained data was compared to the data obtained for the HT and the NHT oat flour. The neutral lipid profiles of all oat extrudates showed a decrease in FFA. No hydrolysis of extrudate lipids during storage was thus observed, which meant that the lowest extrusion temperature of 70 °C was enough to inactivate lipases. Previously, the inactivation of lipases in cereal brans was obtained by extrusion at 120 °C with around 20% moisture (Meister et al. 1994), at 130 °C with 25% moisture (Lehtinen et al. 2003) and at 140 °C with 20% moisture (Sharma et al. 2014). In this study, the inactivation of lipases was thus achieved at a lower extrusion temperature with comparable moisture content (19.4%). However, the lipase activity is higher in the bran than in the whole grain of the oats (Ekstrand et al. 1992), and oat bran may require a higher extrusion temperature than whole grain flour for the inactivation of lipases.

High losses of TAGs in the extrudate produced at 130 °C after 6 weeks of storage were attributed to extensive oxidation, which caused high formation of secondary lipid oxidation products. Further, polymerisation during oxidation may have contributed to the measured high losses of TAGs by slightly decreasing the extractability of lipids. On the other hand, in the NHT flour, the losses of the TAGs were due to lipase activity. However, high oxidation levels were also observed for the NHT flour. Compared to the oxidized extrudates, the level of 2-pentylfuran was high in the volatile profile of the NHT flour. 2-Pentylfuran is proposed to be formed by singlet oxygen oxidation through 10-hydroperoxides of linoleys (Choe and Min 2006), or from 9-hydroperoxides of linoleys (Ho and Chen 1994). The 9-hydroperoxides decompose to conjugated diene radicals, which react further to vinyl hydroperoxides that, after the homolytic cleavage of the hydroperoxide groups, undergo cyclization to form 2-pentylfuran. The second route from 9-hydroperoxide was more likely in this study because light was excluded during storage. The higher level of 2-pentylfuran may be due to lipoxygenase activity in the NHT flour. The lipoxygenase activity in the oats was shown to have a preference for the formation of 9-hydroperoxides over 13-hydroperoxides in the ratio of 88:12 (Heimann et al. 1973), whereas in autoxidation, the ratio of these hydroperoxides is equal. Therefore, the increased formation of 2-pentylfuran could be an indicator for enzymatic lipid oxidation in NHT oats.

The volatile profiles of the oat extrudates were dominated by hexanal and, in the case of the extrudate produced at 130 °C, also by hexanoic acid (Figure 8). Hexanal, the main volatile

product formed in the decomposition of 13-hydroperoxides of linoleyls (Ho and Chen 1994), is considered in low levels to contribute to the natural flavour of oats, and is greatly formed during lipid oxidation (Guth and Grosch 1993; Molteberg et al. 1996; Klensporf et al. 2008; Cognat et al. 2012). Hexanoic acid can be formed by further oxidation from hexanal and it is usually related to oxidation at high temperatures (Frankel 1998). The other selected key compounds, octane (from 10-OOH), 1-heptanol (from 11-OOH) and nonanal (from 9-OOH), are formed from oleys (Schaich et al. 2013.)

The volatile profiles showed that the extrudate produced at 70 °C was the most stable extrudate and comparable to the commercial HT oat flour, followed in stability by extrudates produced at 110 ºC, either at 100 rpm or 400 rpm. The extrudate produced at 130 °C had already started to oxidize during the extrusion process, and oxidation continued extensively during storage based on the high amounts of hexanoic acid. Previously, hexanoic acid was also considered to be an indicator of the intense oxidation of breakfast cereals (Paradiso et al. 2008). Based on these results, the increased extrusion temperature thus accelerated lipid oxidation, while the screw speed had only a minor influence on the oxidative stability. However, the extrudate produced at 100 rpm was slightly more prone to oxidation than the extrudate produced at 400 rpm. This may be related to differences in the binding of lipids. The binding of lipids was shown to improve their oxidative stability during the storage of maize extrudates with added lipids (Thachil et al. 2014).

A partial binding of lipids to the biopolymer matrix of oat extrudates was indicated by a decrease in extractability. Earlier, the binding of lipids to a gelatinized and denatured starch-protein matrix during extrusion was observed by Ho and Izzo (1992), Wicklund and Magnus (1997) and Thachil et al. (2014). The binding of lipids was the highest for the extrudates produced either at the highest temperature (130 °C) or the highest screw speed (400 rpm). Both the increasing temperature and increasing screw speed could increase the starch gelatinization and protein denaturation (Moraru and Kokini 2003). This may improve the binding of lipids to the polymer matrix of the extrudates. The lowest binding of lipids was obtained for the extrudate produced at the lowest screw speed (100 rpm). This could be explained with the results of the microstructure analysis, which displayed a more intact cell structure for the extrudate produced at 100 rpm compared to the other extrudates (Moisio et al. submitted). Therefore, it seems that the energy was too low at a screw speed of 100 rpm to degrade the cell structures. The SME of the extrudate produced at 100 rpm was 89 Wh/kg, which was the lowest value among all tested extrudates (Moisio et al. submitted).

Accelerated lipid oxidation by increasing the extrusion temperature above 120 ºC has been reported previously by Sjövall et al. (1997), Zadernowski et al. (1997), Gutkoski and El-Dash (1998) and Lehtinen et al. (2003). Factors which might increase the lipid oxidation at higher

extrusion temperatures include the increased degradation of endogenous antioxidants, for example, 40% of the total tocols were lost at an extrusion temperature of 120 °C, whereas 90% were lost at 200 °C in whole grain oat flour (Zieliński et al. 2001), and increased metal contamination from the extruder screw (Rao and Artz 1989). However, Parker et al. (2000) did not detect intensive formation of volatile lipid oxidation products in oat extrudates produced at higher temperatures (150 or 180 °C) than those used in this study. The better lipid stability in the study by Parker et al. (2000) may be caused by using a lower moisture content during extrusion (14.5% or 18%), which facilitated the formation of Maillard reaction products. Maillard reaction products have been shown to have radical scavenging activity, which could retard lipid oxidation (Elizalde et al. 1991). Nevertheless, the study from Parker et al. (2000) did not include a storage experiment, and mainly focused on flavour development. They also determined a rancid flavour for extrudates produced from debranned oat flour with a measurable lipase activity (the other oat flours in the study did not have any lipase activity left after commercial heat-treatment). They explained that the Maillard reaction was decreased in this extrudate by interactions between the Maillard reaction precursors and aldehydes from lipid oxidation.

To conclude, it was shown that 2-pentylfuran may be a useful indicator for lipoxygenase activity in oats, while hexanoic acid could be a good indicator for extensive lipid oxidation in oats. Further, as low extrusion temperature as 70 °C was shown to inactivate endogenous hydrolytic and oxidative enzymes in oats, and to improve lipid stability during storage. However, the study from Parker et al. (2000) indicated that not only the extrusion temperature, but also the water content during extrusion connected with the formation of the Maillard reaction products may play important roles in the lipid stability of oat extrudates. A similar effect was observed for the rye bran extrudates produced at different water contents (see 6.4). The effect of the Maillard reaction, and interactions of the Maillard reaction and lipid oxidation products, may be of interest for further study.

6.4 Lipid stability of rye bran extrudates

The ASE-extractabilities of lipids for the rye bran extrudates were similar at all tested extrusion parameters. Similar to what was observed for the oat extrudates, the extractability of lipids was lower for the rye bran extrudates than for the raw materials. Again, the decrease in extractability could be attributed to the binding of lipids. However, no formation of lipid-amylose complexes during extrusion could be detected in the differential scanning calorimetry (DSC) thermograms. Instead of lipid-amylose complexes, non-specific binding and interactions with fully gelatinized starch and denatured proteins (Ho and Izzo 1992) could have decreased the extractability of lipids.

Although the extractability of fatty acid containing lipid classes decreased in extrusion, the amount of total tocols extracted from the coarse rye bran extrudates was increased. This indicated that the less-polar tocols were not bound as well as other lipids by the matrix, such as the phospholipids shown by Ho and Izzo (1992). Further, the smaller particle size of the milled extrudates may have enhanced the extractability of the tocols, compared to the non-milled coarse bran. In general, the differences in the extractability of the tocols from the extrudates and brans complicated the evaluation of their stability during the extrusion process. The results, however, indicate that no marked degradation occurred during extrusion. The degradation of the tocols was previously observed in the extrusion of whole grain oat flour (Zieliński et al. 2001). The lower tocol content of the fine rye bran extrudates was based on the losses of tocols during grinding. Thereby, the losses of the tocopherols were greater than those of the tocotrienols. Tocopherols are known to be concentrated in the germ, while tocotrienols are found mainly in the outer layers of the grains (Ko et al. 2003). Thus, the differences in the losses of tocopherols and tocotrienols, obtained for the fine rye bran, indicated the loss of germ fragments in grinding. In addition, tocols could also have been oxidized by frictional heat in the grinding process. However, the quantities of the main unsaturated fatty acids were not affected by the grinding process, which suggested that extensive oxidation did not occur during grinding.

Oxidation in the rye bran extrudates was determined by the formation of volatile secondary oxidation products and tocol losses. The extrudates produced at the same water content (22%) at different temperatures (80 to 140 °C) showed higher oxidation rates with increasing extrusion temperatures, as seen before for the oat extrudates. However, the effect was less pronounced than for the oat extrudates, and the rye bran extrudate produced at 140 °C was more stable than the one produced at 120 °C. The differences compared to the oat extrudates could be explained by the lower lipid content of rye bran extrudates (the rye bran extrudates contained only around one third of lipids as in the oat extrudates). The higher stability of the rye bran extrudate produced at 140 °C could be connected to Maillard reaction products found only in this extrudate in this extrusion series. As discussed earlier, the products of the Maillard reaction were observed to have radical scavenging activity (Elizalde et al. 1991).

Extrudates produced at low water content (13% and 16%) at 120 ºC, from either coarse or fine rye bran, contained even more volatile Maillard reaction products than the ones produced at 140 °C with a water content of 22%. These extrudates also showed the highest oxidative stabilities. The higher stability of the rye bran extrudates produced at a low water content could again be explained by the radical scavenging activity of the volatile Maillard reaction products (Elizalde et al. 1991). In addition, the low water content during extrusion has been suggested to minimize the loss of endogenous phenolics, resulting in the highest antiradical activity for extrudates produced at a low water content (Gumul et al. 2007). Fine bran extrudates were more stable than the coarse

extrudates produced at the same water content. However, the great loss of tocols by the grinding process reduced the nutritional value, and could decrease the lipid stability during longer storage.

The PCA analysis showed that the extrudate produced with the fine rye bran at 13% water was mainly associated with furfural, while the extrudate produced with the coarse bran at 13% water was mainly associated with 2,5-dimethylpyrazine. This showed a greater formation of furfural than of the 2,5-dimethylpyrazines or methylpyrazine in the fine rye bran extrudate. In all other extrudates containing volatile Maillard reaction products, the amount of both pyrazines was higher than the amount of furfural. A similar marked increase in furfural at a high temperature and low moisture was previously found for maize extrudates (Bredie et al. 1998). The shift from pyrazine towards furan formation in the Maillard reaction is dependent on pH (lower pH favours furan formation), energy (higher energy favours furan formation) and the substrate availability (Jousse et al. 2002). Although both rye brans were extruded at the same conditions (water content of 13% and temperature of 120 °C), the SME was still significantly higher during the extrusion of the fine rye bran (**VI**, Table 3). The higher energy during extrusion and the higher availability of reaction sites, indicated by the higher water solubility index (WSI) of the fine rye bran, might be responsible for the change in the Maillard product formation and the nearly burnt-like flavour mentioned earlier. Furans are known to cause sweet caramel-like flavour right up to burnt pungent flavours in foods (van Boekel 2006). The high formation of furfural could further indicate that other potentially harmful compounds, like acrylamide, have been formed during extrusion (Singh et al. 2007).

In the case of the extrudates produced at high water (22% and 30%), a stronger correlation with 2-pentylfuran than hexanal was observed for the fine rye bran extrudates. An unexpectedly high formation of 2-pentylfuran compared to hexanal was seen previously in the volatile profiles of the NHT oat flour during storage. However, the activity of the oxidative enzymes was not of concern in the rye bran extrudates as it was in oat extrudates (Meister et al. 1994), and singlet oxygen oxidation could again be excluded. In the case of the fine rye bran extrudates, increased binding of hexanal by the extrudate matrix rather than actual changes in the formation pathway of 2-pentylfuran was suspected. Grinding caused the degradation of the proteins and polysaccharides, which may have exposed new hexanal binding sites and, as seen and discussed in the volatile release study, hexanal is easily bound by a biopolymer matrix.

In summary, of the studied process parameters, the water content had a significant effect on lipid stability. Low water content (13% or 16%) in the extrusion of coarse or fine bran led to the best lipid stability during storage. The improved lipid stability for the extrudates produced at a low water content was mainly associated with the higher formation of Maillard reaction products, which could have functioned as antioxidants. The lipids in the fine rye bran extrudates showed a comparable stability to the lipids in the coarse rye bran extrudates, despite the loss of natural

antioxidants in the fine rye bran. However, a too-extensive Maillard reaction occurred in the fine rye bran extrudate at 13% water content, which caused an unpleasant burnt-like flavour. So far, this is the first study on the lipid stability in rye bran extrudates. Further studies, including other extrusion parameters, like different screw speeds not tested in this study, and studying other factors which may affect the oxidative stability, like phenolic compounds, would be of interest.

7 CONCLUSIONS

Lipid oxidation in solid food matrices with dispersed lipids was studied using spray-dried oil emulsion and extruded cereals as models. For spray-dried emulsions, the oxidation behaviour during storage at different RHs could be described by measuring the PVs, α-tocopherol losses and formation of hexanal. In the case of the oat and rye bran extrudates, volatile profiles measured by HS-SPME and neutral lipid profiles or tocol losses, respectively, were used to determine the lipid stability during storage. In all three storage experiments, the data obtained by the measured oxidation indicators correlated well with each other. Using several volatile secondary lipid oxidation products analysed by HS-SPME-GC-MS as oxidation indicators was shown to have an advantage over only using hexanal analysed by SHS-GC-FID as an indicator. In addition to volatile secondary lipid oxidation products, volatile Maillard reaction products were detected in the volatile profiles of rye bran extrudates produced at low moisture or high temperature. The development of these compounds was dependent on the extrusion condition used. Especially, low water content during extrusion facilitated the formation of volatile Maillard reaction products.

The profiles of the volatile oxidation products from the spray-dried emulsions analysed by HS-SPME were influenced by the RH. The effect of the RH was linked to the water-induced changes in hydrophilicity, structure and the binding ability of the Na-caseinate-maltodextrin matrix, and to the partitioning and solubility of the volatiles in the matrix. At water contents of 3.1% and 5.2% (RH 11% and 33%, respectively) the highest overall amount of released volatiles was obtained. An increase above these water contents altered the volatile profile towards lower molecular weight compounds. At the driest condition (RH ~0%), the water content was too low to facilitate the release of hydrophobic volatile compounds. Also, the cross-linking of the Na-caseinate and HS-SPME extraction conditions (temperature and agitation) were shown to have an effect on the overall amount of volatiles released. However, the effects were smaller and always dependent on the RH. Based on the results, both matrix-related factors (RH and cross-linking) and the extraction conditions should be considered in the interpretation of the HS-SPME results. However, the results further indicated that the HS-SPME may be a useful method for studying matrix-related changes in solid foods.

Both the total and surface lipid fractions of the spray-dried emulsions with sunflower oil in a Na-caseinate-maltodextrin matrix showed improved oxidative stability with increasing RH during storage. The higher oxidative stability at higher RHs was related to the water-induced changes that caused the matrix to collapse, which resulted in caking of the powder. This limited oxygen availability as a rapid gas-phase diffusion in the loose packed matrix was replaced by slower modes in the collapsed matrix. Further, the improved oxidative stability may also have been associated with water-induced differences in the reactivity (e.g., antioxidant activity of protein) of

the Na-caseinate-maltodextrin matrix. The modification of the protein layer surrounding the oil droplets through cross-linking had a minor influence on the lipid stability. At RH 54%, the highest improvement in the stability of the total and surface lipids was seen. The results thus indicated that to use cross-linked protein as part of the wall material could be suitable to improve the oxidative stability of the encapsulated oils, but further research is needed; it would be of interest to determine the effect of different extents of cross-linking.

Even at the lowest extrusion temperature (70 °C), extrusion was shown to inactivate lipases and possibly other lipid degrading enzymes in oats as efficiently as the traditional heat-treatment of oat grains. The necessity of some kind of heat-treatment was seen in the storage stability of the non-heat-treated oat flour, in which high amounts of FFAs and by lipoxygenase activity catalysed oxidation were found. Based on the volatile profiles of the non-heat-treated oat flour, 2-pentylfuran may be a useful indicator for lipoxygenase activity in oats. A high extrusion temperature (130 °C) promoted extensive lipid oxidation and degradation of the TAGs in the extruded oats during storage. Hexanoic acid was found to be an indicator of extensive oxidation in oat extrudates. The formation of hexanoic acid caused the levelling and decreasing of hexanal, another commonly used indicator of lipid oxidation. Therefore, lipid oxidation could be underestimated if only hexanal is considered as an oxidation indicator. Compared to the extrusion temperature, the influence of the screw speed on the oxidative stability during storage was small. However, some binding of lipids at the highest screw speed was observed, which improved the oxidative stability slightly.

In the case of rye bran extrudates, low water content during the extrusion of both coarse and fine rye bran led to the best storage stability of lipids. The better oxidative stability at low water was associated with the increased formation of Maillard reaction products, which can have radical scavenging activity, and possibly a better retention of phenolic compounds, which too have antioxidant activity. The small particle size of the rye bran also improved the formation of Maillard reaction products, and decreased the quantities of the lipid-derived volatiles and losses of tocols. However, grinding prior to extrusion caused high losses of tocols, which decreased the nutritional value of the final product, and could decrease the stability during longer storage. In addition, the high formation of furfural, which could indicate the formation of other potentially harmful compounds, was observed in the fine rye bran extrudate produced at 13% water.

Therefore, low temperature and low water content in extrusion were shown to be beneficial for the lipid stability of oat and rye bran extrudates, respectively. Also, an increased screw speed and particle size reduction showed some potential for improving the oxidative stability of the cereal extrudates. However, further studies may concentrate on the interactions of the Maillard reaction and lipid oxidation products, and on other compounds which could affect lipid stability, such as

phenolic compounds, in cereal extrudates, and how these are affected by different extrusion conditions.

The knowledge gained in this thesis will benefit future studies on lipid oxidation in solid foods by showing possibilities, but also limitation of using HS-SPME-GC-MS to study lipid oxidation. Further, this thesis demonstrated the usefulness of applying different commonly used analytical methods together to gain a more complete picture of oxidation behaviour. Future studies on extrusion of cereals, especially of cereal brans, will profit from the presented effects of extrusion parameters on the oxidative stability of products. Further, the shown formation of Maillard reaction products during extrusion of rye bran gives the possibility in future to modify the flavour of cereal brans, which may expands the use of bran material in food products.

8 REFERENCES

Aberkane L, Roudaut G, Saurel R. 2014. Encapsulation and Oxidative Stability of PUFA-Rich Oil Microencapsulated by Spray Drying Using Pea Protein and Pectin. Food Bioprocess Technol 7(5):1505-1517.

Alam SA, Järvinen J, Kirjoranta S, Jouppila K, Poutanen K, Sozer N. 2013. Influence of particle size reduction on structural and mechanical properties of extruded rye bran. Food Bioprocess Technol 7(7): 2121-2133.

Anderson JW, Baird P, Davis Jr RH, Ferreri S, Knudtson M, Koraym A, Waters V, Williams CL. 2009.Health benefits of dietary fiber. Nutr Rev 67(4):188-205.

[AOAC] Association of Official Analytical Chemists. 2001. Official Method 996.06 Fat (Total, Saturated, and Unsaturated) in Foods. In: Official Methods of Analysis of AOAC International, 17th ed. Gaithersburg, MD, USA: AOAC Int.

Artz WE, Rao SK. 1994. Lipid oxidation in extruded products. In: Parliament TH, Morello MJ, McGorrin RJ, eds. Thermally generated flavors: Maillard, microwave and extrusion. Washington, DC, USA: American Chemical Society. pp. 296-314.

Baik M-Y, Suhendro EL, Nawar WW, McClements DJ, Decker EA, Chinachoti P. 2004. Effects of antioxidants and humidity on the oxidative stability of microencapsulated fish oil. J Am Oil Chem Soc 81(4): 355-360.

Balasubramanian S, Panigrahi S. 2011. Solid-phase microextraction (SPME) techniques for quality characterization of food products: A review. Food Bioprocess Technol 4(1):1-26.

Bao S-S, Hu X-C, Zhang K, Xu X-K, Zhang H-M, Huang H. 2011. Characterization of spray-dried microalgal oil encapsulated in cross-linked sodium caseinate matrix induced by microbial transglutaminase. J Food Sci 76(1):E112-E118.

Barriuso B, Astiasarán I, Ansorena, D. 2013. A review of analytical methods measuring lipid oxidation status in foods: A challenging task. Eur Food Res Technol 236(1):1-15.

Bartosz G and Kołakowska A. 2011. Lipid Oxidation in Food Systems. In: Sikorski ZE, Kołakowska A, Eds. Chemical, Biological, and Functional Aspects of Food Lipids. Second edition. Boca Raton, FL, USA: Taylor and Francis Group. pp. 163-181.

Berton-Carabin CC, Ropers M-H, Genot C. 2014. Lipid Oxidation in Oil-in-Water Emulsions: Involvement of the Interfacial Layer. Comp Rev Food Sci Food 13(5):945-977.

Biermann U, Wittmann A, Grosch W. 1980. Occurrence of bitter hydroxy fatty acids in oat and wheat [Vorkommen bitterer Hydroxyfettsäuren in Hafer und Weizen]. Fett Wiss Technol 82(6): 236-240.

Black PD, Lewis D. 2009. Dietary fiber: consumer attitudes and preferences. Cereal Foods World 54(5): 213-215.

Bowen SE, Gray DA, Giraud C, Majzoobi M, Testa CEM, Pérez LAB, Hill SE. 2006.Lipid oxidation and amylopectin molecular weight changes occurring during storage of extruded starch samples. J Cereal Sci 43(3):275-283.

Bredie WLP, Mottram DS, Guy RCE. 1998. Aroma volatiles generated during extrusion cooking of maize flour. J Agri Food Chem 46(4):1479-1487.

Brennan MA, Derbyshire E, Tiwari BK, Brennan CS. 2013. Ready-to-eat snack products: the role of extrusion technology in developing consumer acceptable and nutritious snacks. Int J Food Sci Technol 48(5):893-902.

Bushuk W. 2001. Rye production and uses worldwide. Cereal Foods World 46(2):70-73.

Camire ME, Dougherty MP. 1998. Added phenolic compounds enhance lipid stability in extruded corn. J Food Sci 63(3):516-518.

Camire ME, Dougherty MP, Briggs JL. 2007. Functionality of fruit powders in extruded corn breakfast cereals. Food Chem 101(2):765-770.

Choe E, Min DB. 2006. Mechanisms and Factors for Edible Oil Oxidation. Comp Rev Food Sci Food Safety 5(4):169-86.

Coleman III WM. 1996. A study of the behavior of Maillard reaction products analyzed by solid-phase microextraction-gas chromatography-mass selective detection. J Chromatogr Sci 34(5)213-218.

Coleman III WM. 1997. A study of the behavior of polar and nonpolar solid-phase microextraction fibers for the extraction of Maillard reaction products. J Chromatogr Sci 35(6):245-258.

Cognat C, Shepherd T, Verrall SR, Stewart D. 2012. Comparison of two headspace sampling techniques for the analysis of off-flavour volatiles from oat based products. Food Chem 134(3):1592-1600.

Delcour JA, Hoseney RC. 2010. Principles of cereal science and technology, 3rd ed. St. Paul, MN, USA: American Association of Cereal Chemists International. Inc. 270p.

Ding QB, Ainsworth P, Plunkett A, Tucker G, Marson H. 2006. The effect of extrusion conditions on the functional and physical properties of wheat-based expanded snacks. J Food Eng 73:142–148.

Dobarganes MC, Velasco J. 2002. Analysis of lipid hydroperoxides. Eur J Lipid Sci and Technol 104 (7):420-428.

Doehlert DC, Angelikousis S, Vick B. 2010. Accumulation of oxygenated fatty acids in oat lipids during storage. Cereal Chem 87:532-537.

Drusch S, Serfert Y, Schwarz K. 2006. Microencapsulation of fish oil with n-octenylsuccinate-derivatised starch: Flow properties and oxidative stability. Eur J Lipid Sci Tech 108(6):501-512.

Ekstrand B, Gangby I, Åkesson G. 1992. Lipase activity in oats – Distribution, pH dependence, and heat inactivation. Cereal Chem 69(4):379-381.

Elizalde BE, Rosa MD, Lerici CR. 1991 Effect of maillard reaction volatile products on lipid oxidation. J Am Oil Chem Soc 68(10):758-762.

Frankel EN. 1980. Lipid oxidation. Prog Lipid Res 19(1-2):1-22.

Frankel EN. 1998. Lipid Oxidation. Dundee, Scotland, UK: The Oily Press Ltd. Inc. 303p.

Gharsallaoui A, Roudaut G, Chambin O, Voilley A, Saurel R. 2007. Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Res Int 40: 1107-1121.

González RJ, Torres RL, Mario De Greef D, Guadalupe BA. 2006. Effects of extrusion conditions and structural characteristics on melt viscosity of starchy materials. J Food Eng 74(1):96-107.

Grattard N, Salaün F, Champion D, Roudaut G, Le Meste M. 2002. Influence of physical state and molecular mobility of freeze-dried maltodextrin matrices on the oxidation rate of encapsulated lipids. J Food Sci 67(8):3002-3010.

Gray DA, Bowen SE, Farhat I, Hill SE. 2008. Lipid oxidation in glassy and rubbery-state starch extrudates. Food Chem 106(1):227-234.

Guichard E. 2002. Interactions between flavor compounds and food ingredients and their influence on flavor perception. Food Rev Int 18(19):49-70.

Gumul D, Korus J, Achremowicz B. 2007 The influence of extrusion on the content of polyphenols and antioxidant/antiradical activity of rye grains (secale cereale l.). ACTA Sci Pol Technol 6(4):103-111.

Guth H, Grosch W. 1993. Odorants of extrusion products of oat meal-changes during storage [Geruchsstoffe von extrudiertem Hafermehl, Veränderungen bei der Lagerung]. Z Lebensm Unters For 196(1):22-28.

Gutkoski LC, El-Dash AA. 1998. Effects of moisture and extrusion temperatures on the oxidative stability of milling oat products with granularity below 532 μm. Braz Arch Biol Technol 41(4):401-408.

Hamberg M, Hamberg G. 1996. Peroxygenase catalyzed fatty acid epoxidation in cereal seeds. Plant Physiol 110:807-815.

Hardas N, Danviriyakul S, Foley, JL, Nawar WW, Chinachoti P. 2002. Effect of relative humidity on the oxidative and physical stability of encapsulated milk fat. J Am Oil Chem Soc 79(2): 151-158.

He J, Vazquez-Landaverde P, Qian MC, Eskin NAM. 2013. Chapter 12 - Off-Flavors in Milk. In: Eskin, NAM, Shahidi F, eds. Biochemistry of Foods, 3rd ed. San Diego, CA, USA: Academic Press. pp. 419-478.

Heimann W, Dresen P, Klaiber V. 1973. Formation and decomposition of linoleic acid hydroperoxides in cereals quantitative determination of the reaction products [Über die Bildung und den Abbau von Linolsäurehydroperoxiden in Cerealien Quantitative Bestimmung der Reaktionsprodukte]. Z Lebensm Unters For 153(1):1-5.

Heiniö RL, Katina K, Wilhelmson A, Myllymäki O, Rajamäki T, Latva-Kala K, Liukkonen KH, & Poutanen K. 2003a. Relationship between sensory perception and flavour-active volatile compounds of germinated, sourdough fermented and native rye following the extrusion process. LWT-Food Sci Technol 36(5):533–545.

Heiniö RL, Liukkonen KH, Katina K, Myllymäki O, Poutanen, K. 2003b. Milling fractionation of rye produces different sensory profiles of both flour and bread. LWT-Food Sci Technol 36(6):577-583.

Heiniö RL, Liukkonen KH, Myllymäki O, Pihlava JM, Adlercreutz H, Heinonen SM, Poutanen K. 2008. Quantities of phenolic compounds and their impacts on the perceived flavour attributes of rye grain. J Cereal Sci 47(3):566-575.

Ho CT, Chen Q. 1994. Lipids in Food Flavors. In: Ho CT, Hartman TG, eds. Lipids in food flavor. Washington, DC, US: American Chemical Society. pp. 2-14.

Ho CT, Izzo MT. 1992. Lipid-protein and lipid-carbohydrate interactions during extrusion. In: Kokini JL, Ho CT, Karwe MV, eds. Food Extrusion Science and Technology. New York, NY, USA: Marcel Dekker. pp. 415-425.

Ishida T, Haruta M. 2007. Gold catalysts: Towards sustainable chemistry. Angew Chem Int Edit 46(38):7154-7156.

Jackson LS, Lee K. 1991. Microencapsulation and the food industry. LWT-Food Sci Technol 240(4):289-297.

Jeleń HH, Majcher M, Dziadas M. 2012. Microextraction techniques in the analysis of food flavor compounds: A review. Anal Chimica Acta 738:13-26.

Jeleń HH, Obuchowska M, Zawirska-Wojtasiak R, Wąsowicz E. 2000. Headspace solid-phase microextraction use for the characterization of volatile compounds in vegetable oils of different sensory quality. J Agri Food Chem 48(6):2360-2367.

Jeleń HH, Wąsowicz E. 2012. Lipid-Derived Flavor Compounds. In: Jeleń HH, ed. Food Flavors: Chemical, Sensory and Technological Properties. Boca Raton, FL, USA: CRC Press/Taylor & Francis Group. pp. 65-93.

Jimenez M, García HS, Beristain CI. 2004. Spray-drying microencapsulation and oxidative stability of conjugated linoleic acid. Eur Food Res Technol 219(6):588-592.

Jouquand C, Ducruet V, Le Bail P. 2006. Formation of amylose complexes with C6-aroma compounds in starch dispersions and its impact on retention. Food Chem 96(3):461-70.

Jousse F, Jongen T, Agterof W, Russell S, Braat P. 2002. Simplified kinetic scheme of flavor formation by the Maillard reaction. J Food Sci 67(7):2534-2542.

Kamal-Eldin A, Lærke H, Bach Knudsen K, Lampi AM, Piironen V, Adlercreutz H, Katina K, Poutanen K, Åman P. 2009. Physical, microscopic and chemical characterisation of industrial rye and wheat brans from the Nordic countries. Food Nutr Res doi:10.3402/fnr.v53i0.1912(0).

Kasprzak M, Rzedzicki Z, Wirkijowska A, Zarzycki P, Sobota A, Sykut-Domańska E, Błaszczak W. 2013. Effect of fibre-protein additions and process parameters on microstructure of corn extrudates. Cereal Sci 58(3):488-494.

Klensporf D, Jeleń HH. 2005. Analysis of volatile aldehydes in oat flakes by SPME-GC/MS. Polish J Food Nutr Sci 14/55(4):389-395.

Klensporf D, Jeleń HH. 2008. Influence of the addition of raspberry seed extract on changes in the volatile pattern of stored model breakfast cereal. J Agri Food Chem 56(9):3268-3272.

Kiokias S, Varzakas TH, Arvanitoyannis IS, Labropoulos AE. 2009. Lipid Oxidation and Control of Oxidation. F. Yildiz, ed. Advances in Food Biochemistry. Boca Raton, FL, USA: CRC Press/Taylor & Francis Group. pp. 383-408.

Klinkesorn U, Sophanodora P, Chinachoti P, McClements D J, Decker EA. 2005. Stability of spray-dried tuna oil emulsions encapsulated with two-layered interfacial membranes. J Agri Food Chem 53(21):8365-8371.

Ko S-N, Kim C-J, Kim H, Kim C-T, Chung S-H, Tae B-S, Kim I-H. 2003. Tocol levels in milling fractions of some cereal grains and soybean. J Am Oil Chem Soc 80:585-589.

Kruse A, Dahmen N, Dinjus E, Ederer H. 2006. Oxidation of hexanal to hexanoic acid in supercritical carbon dioxide. 1. experiments in a tubular reactor and modeling. J Supercrit Fluid 39(2)211-219.

Kühn J, Considine T, Singh H. 2006. Interactions of milk proteins and volatile flavor compounds: Implications in the development of protein foods. J Food Sci 71(5):R72-R82.

Labuza, TP. 1971. Kinetics of lipid oxidation in foods. CRC Crit. Rev. Food Sci. Nutr. 2:355-405.

Labuza, TP. 1980. The effect of water activity on reaction-kinetics of food deterioration. Food Technol 34(4):36-41, 59.

Lampi A-M, Piironen. 2009. Chapter 3: Tocopherols and tocotrienols. In: Shewry PR, Ward JL, eds. Analysis of bioactive componts in small grain cereals. St. Paul, MN, USA: AACC International Inc. pp. 15- 24.

Lehtinen P, Kaukovirta-Norja A. 2011. Oat lipids, Enzymes, and Quality. In: Webster FH, Wood PJ, Editors. Oats; Chemistry and Technology, 2nd ed. St. Paul, MN, USA: AACC International Inc. pp. 143-156.

Lehtinen P, Kiiliäinen K, Lethtomäki I, Laakso S. 2003. Effect of heat treatment on lipid stability in processed oats. J Cereal Sci 37:215-221.

Lehtonen M, Lampi A-M, Ollilainen V, Struijs K, Piironen V. 2011. The role of acyl moiety in the formation and reactions of steryl ester hydroperoxides. Eur Food Res Technol 233(1):51-61.

Le Meste M, Champion D, Roudaut G, Blond G, Simatos D. 2002. Glass transition and food technology: A critical appraisal. J Food Sci 67(7):2444-2458.

Lojzova L, Riddellova K, Hajslova J, Zrostlikova J, Schurek J, Cajka T. 2009. Alternative GC-MS approaches in the analysis of substituted pyrazines and other volatile aromatic compounds formed during Maillard reaction in potato chips. Anal Chim Acta 641(1-2)101-109.

Le Thanh M, Thibeaudeau P, Thibaut MA, Voilley A. 1992. Interactions between volatile and non-volatile compounds in the presence of water. Food Chem 43(2):129-135.

Madene A, Jacquot M, Scher J, Desobry S. 2006 Flavour encapsulation and controlled release - A review. Int J Food Sci Technol 41(1):1-21.

Márquez-Ruiz G, Velasco J, Dobarganes C. 2003. Oxidation in dried microencapsulated oils. In: Kamal-Eldin A, ed. Lipid Oxidation Pathways. Champaign, IL, USA: AOCS Press. pp. 245-264.

Meister U, Schramm G, Symmank H. 1994. Determination of activities of lipase, lipoxygenase and peroxidase in native and extruded cereal brans [Bestimmung der Aktivitäten von Lipase, Lipoxygenase und Peroxidase in nativen und extrudierten Getreidekleien]. Z Lebensm Unters For 199:275-280.

Meynier A, Rampon V, Dalgalarrondo M, Genot C. 2004. Hexanal and t-2-hexenal form covalent bonds with whey proteins and sodium caseinate in aqueous solution. Int Dairy J 14(8):681-690.

Mildner-Szkudlarz S, Jeleń HH, Zawirska-Wojtasiak R, Wąsowicz E. 2003. Application of headspace - solid phase microextraction and multivariate analysis for plant oils differentiation. Food Chem 83(4):515-522.

Min DB, Boff JM. 2002. Chemistry and reaction of singlet oxygen in foods. Comp Rev Food Sci Food Safety 1(2):58-72.

Moisio T, Damerau A, Lampi A-M, Piironen V, Forssell P, Partanen R. 2014. Interfacial protein engineering for spray–dried emulsions – Part I: Effects on protein distribution and physical properties. Food Chem 144:50-56.

Moisio T, Forssell P, Partanen R, Damerau A, Hill SE. Reorganisation of starch, proteins and lipids in extrusion of oats. Submitted to J Cereal Sci.

Molteberg EL, Magnus EM, Bjørge JM., Nilsson A. 1996. Sensory and chemical studies of lipid oxidation in raw and heat-treated oat flours. Cereal Chem 73:579-587.

Mora-Gutierrez A, Attaie R, Kirven JM, Farrell HM. 2014. Cross-linking of bovine and caprine caseins by microbial transglutaminase and their use as microencapsulating agents for n-3 fatty acids. Int J Food Sci Tech 49:1530-1543.

Morales A, Dobarganes C, Márquez-Ruiz G, Velasco J. 2010 Quantitation of hydroperoxy-, keto-and hydroxydienes during oxidation of FAMEs from high-linoleic and high-oleic sunflower oils. J Am Oil Chem Soc 87(11):1271-1279.

Moraru CI, Kokini JL. 2003. Nucleation and expansion during extrusion and microwave heating of cereal foods. Comp Rev Food Sci Food Safety 2:147-165.

Nelson K, Labuza T. 1992. Relationship between water and lipid oxidation rates. Water activity and glass transition theory. In: St. Angelo AJ, ed. Lipid Oxidation in Foods. ACS Symposium Series 500. Washington, DC, USA: American Chemical Society. pp. 93-103.

Nordlund E, Heiniö RL, Viljanen K, Pihlava JM, Lehtinen P, & Poutanen K. 2013. Flavour and stability of rye grain fractions in relation to their chemical composition. Food Res Int 54(1): 48-56.

Paradiso VM, Summo C, Pasqualone A, Caponio F. 2009. Evaluation of different natural antioxidants as affecting volatile lipid oxidation products related to off-flavours in corn flakes. Food Chem 113(2):543-549.

Paradiso VM, Summo C, Trani A, Caponio F. 2008. An effort to improve the shelf life of breakfast cereals using natural mixed tocopherols. J Cereal Sci 47(2):322-330.

Park EY, Murakami H, Mori T, Matsumura Y. 2005. Effects of protein and peptide addition on lipid oxidation in powder model system. J Agri Food Chem 53(1):137-144.

Parker JK, Hassell GME, Mottram DS, Guy RCE. 2000. Sensory and instrumental analyses of volatiles generated during the extrusion cooking of oat flours. J Agri Food Chem 48(8):3497-3506.

Partanen R, Forssell P, Mackie A, Blomberg E. 2013. Interfacial cross-linking of β-casein changes the structure of the adsorbed layer. Food Hydrocolloids 32(2):271-277.

Partanen R, Hakala P, Sjövall O, Kallio H, Forssell P. 2005. Effect of relative humidity on the oxidative stability of microencapsulated sea buckthorn seed oil. J Food Sci 70(1): E37-E43.

Partanen R, Raula J, Seppänen R, Buchert J, Kauppinen E, Forssell P. 2008. Effect of relative humidity on oxidation of flaxseed oil in spray dried whey protein emulsions. J Agri Food Chem 56(14): 5717-5722.

Partanen R. Yoshii H. Kallio H. Yang B. Forssell P. 2002. Encapsulation of sea buckthorn kernel oil in modified starches. J Am Oil Chem Soc 79(3):219-223.

Ponginebbi L. Nawar WW. Chinachoti P. 2000. Effect of relative humidity on lipid oxidation in freeze-dried emulsions. Grasas Aceites 51(5):348-354.

Prättälä R, Elasoja V, Mykkänen H. 2001.The consumption of rye bread and white bread as dimensions of health lifestyles in Finland. Public Health Nutrition 4(3):813-818.

Pulkkinen M. 2012. Volatile compounds as indicators of lipid oxidation in oats [Haihtuvat yhdisteet kauran lipidien muutosten indikaattoreina]. EKT-sarja 1586, Helsinki, Finland: University of Helsinki. Inc. 97p.

Ralet MC, Thibault JF, Della Valle G. 1990. Influence of extrusion-cooking on the physico-chemical properties of wheat bran. J Cereal Sci 11(3):249-259.

Ramos Diaz JM, Kirjoranta S, Tenitz S, Penttilä PA, Serimaa R, Lampi A-M, Jouppila K. 2013. Use of amaranth, quinoa and kañiwa in extruded corn-based snacks. J Cereal Sci 58(1):59-67.

Rao SK, Artz WE. 1989. Effect of extrusion on lipid oxidation. J Food Sci 54(6):1580-1583.

Ré M. 1998. Microencapsulation by spray drying. Drying Technol 16(6): 1195-1236

Rey AI, Hopia A, Kivikari R, Kähkönen M. 2005. Use of natural food/plant extracts: Cloudberry (rubus chamaemorus), beetroot (beta vulgaris "vulgaris") or willow herb (epilobium angustifolium) to reduce lipid oxidation of cooked pork patties. LWT-Food Sci Technol 38(4):363-370.

Robin F, Schuchmann HP, Palzer S. 2012. Dietary fiber in extruded cereals: Limitation and opportunities. Trends Food Sci Tech 28:23-32.

Roos YH. Karel M. 1991. Phase-transition of mixtures of amorphous polysaccharides and sugar. Biotechnol Progr 7:49-53.

Santala O, Kiran A, Soze, N, Poutanen K, Nordlund E. 2014. Enzymatic modification and particle size reduction of wheat bran improves the mechanical properties and structure of bran-enriched expanded extrudates. Cereal Sci, Article in Press.

Schaich KM, Shahidi F, Zhong Y, Eskin NAM. 2013. Chapter 11 - Lipid Oxidation. In: Eskin NAM, Shahidi F, eds. Biochemistry of Foods, 3rd ed. San Diego, CA, USA: Academic Press. pp. 419-478.

Schwartz H, Ollilainen V, Piironen V, Lampi A-M. 2008. Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats. J Food Comp Anal 21(2):152-161.

Sharma S, Kaur S, Dar BN, Singh B. 2014. Storage stability and quality assessment of processed cereal brans. J Food Sci Technol 51(3):pp. 583-588.

Singh S, Gamlath S, Wakeling L. 2007. Nutritional aspects of food extrusion: a review. Int J Food Sci Technol 42: 916-929.

Sjövall O, Lapveteläinen A, Johansson A, Kallio H. 1997 Analysis of volatiles formed during oxidation of extruded oats. J Agri Food Chem 45(11):4452-4455.

Soupas L, Huikko L, Lampi AM, Piironen V. 2005. Esterification affects phytosterol oxidation. Eur J Lipid Sci Technol 107(2):107-118.

Thachil MT, Chouksey MK, Gudipati V. 2014. Amylose-lipid complex formation during extrusion cooking: Effect of added lipid type and amylose level on corn-based puffed snacks. Int J Food Sci Technol 49(2):309-316.

Van Boekel MAJS. 2006 Formation of flavour compounds in the Maillard reaction. Biotechnol Adv 24:230-233.

Vasanthan T, Gaosong J, Li J. 2002. Dietary fiber profile of barley flour as affected by extrusion cooking. Food Chem 77(1):35-40.

Vega C, Roos Y. 2006. Invited review: Spray-dried dairy and dairy-like emulsions - compositional considerations. J Dairy Sci 89(2): 383-401.

Velasco J, Dobarganes C, Holgado F, Márquez-Ruiz G. 2009a. A follow-up oxidation study in dried microencapsulated oils under the accelerated conditions of the Rancimat test. Food Res Int 42(1):56-62.

Velasco J, Dobarganes C, Márquez-Ruiz G. 2003. Variables affecting lipid oxidation in dried microencapsulated oils. Grasas Aceites 54(3):304-314.

Velasco J, Holgado F, Dobarganes C, Márquez-Ruiz G. 2009b. Influence of relative humidity on oxidation of the free and encapsulated oil fractions in freeze-dried microencapsulated oils. Food Res Int 42(10):1492-1500.

Velasco J, Marmesat S, Dobarganes C, Márquez-Ruiz G. 2006. Heterogeneous aspects of lipid oxidation in dried microencapsulated oils. J Agri Food Chem 54(5):1722-1729.

Villière A, Rousseau F, Brossard C, Genot C. 2007. Sensory evaluation of the odour of a sunflower oil emulsion throughout oxidation. Eur J Lipid Sci Technol 109(1):38-48.

Viscidi KA, Dougherty MP, Briggs J, Camire ME. 2004. Complex phenolic compounds reduce lipid oxidation in extruded oat cereals. LWT-Food Sci Technol 37(7):789-796.

Voilley A, Souchon I. 2006. Flavour retention and release from the food matrix: an overview. In: Voilley A, Etiévant P, eds. Flavour in food. Sawston, Cambridge, UK: Woodhead Publishing Limited. pp. 117-132.

Waraho T, McClements DJ, Decker EA. 2011. Mechanisms of lipid oxidation in food dispersions. Trends Food Sci Technol 22:3-13.

Wardencki W, Michulec M, Curyło J. 2004. A review of theoretical and practical aspects of solid-phase microextraction in food analysis. Int J Food Sci Technol 39(7):703-717.

Whitfield FB. 1992. Volatiles from interactions of Maillard reactions and lipids. Critical Rev Food Sci Nutr 31(1/2):1-58.

Wicklund T, Magnus EM. 1997. Effect of extrusion cooking on extractable lipids and fatty acid composition in sifted oat flour. Cereal Chem 74:326-329.

Wood PJ. 2007. Cereal β-glucans in diet and health. J Cereal Sci 46:230-238.

Xu R. 2012. Oat fibre: overview on main biological properties. Eur Food Res Technol 234:563-569.

Zadernowski R, Nowak-Polakowska H, Rashed AA. 1999. The influence of heat treatment on the activity of lipo-and hydrophilic components of oat grain. J Food Process Preserv 23(3):177-191.

Zadernowski R, Nowak-Polakowska H, Wicklund T, Fornal Ł. 1997. Changes in oat lipids affected by extrusion. Nahrung-Food 41(4):224-227.

Zhou K, Slavin M, Lutterodt H, Whent M, Eskin NAM, Liangli Y. 2013. Chapter 1 - Cereals and Legumes. In: Eskin NAM, Shahidi F, eds. Biochemistry of Foods, 3rd ed. San Diego, CA, USA: Academic Press. pp. 419-478.

Zhu K, Huang S, Peng W, Qian H, Zhou H. 2010. Effect of ultrafine grinding on hydration and antioxidant properties of wheat bran dietary fiber. Food Res Int 43(4):943-948.

Zieliński H, Kozłowska H, Lewczuk B. 2001. Bioactive compounds in the cereal grains before and after hydrothermal processing. Innov Food Sci Emerg 2(3):159-169.

APPENDIX 1

Volatile compounds identified in oxidized spray-dried emulsions and stored cereal extrudates by HS-SPME-GC-MS (**II-VI**).

Compounds	Spray-dried emulsions (**II**)	Oat extrudates (**III**)	Rye bran extrudates (**IV**)
Hydrocarbons			
hexane		x	x
1-hexene		x	
1-heptene		x	
octane†	x	x	x
nonane		x	
1-nonene		x	
decane†		x	
1-decene		x	
4-decene (E)		x	
5-undecene (E)		x	
Alcohols			
1-pentanol*	x	x	x
1-penten-3-ol	x	x	x
1-hexanol	x	x	x
1-heptanol†	x	x	
6-methyl-1-heptanol			x
1-octanol	x	x	x
1-octen-3-ol*†	x	x	x
3,5-octadien-2-ol (E,E)		x	
Aldehydes			
propanal	x	x	x
butanal	x	x	x
2-butenal (E)	x		
2-metylbutanal			x
3-methylbutanal			x
pentanal *	x	x	x
2-pentenal (E)	x		
2-methyl-2-pentenal (E)	x		x
hexanal*†‡	x	x	x
2-hexenal (E)			x
heptanal*	x	x	x
2-heptenal (E)*	x	x	x
2,4-heptadienal (E,E)			x
octanal*†	x	x	x
2-octenal (E)*	x	x	x

Compounds	Spray-dried emulsions (**II**)	Oat extrudates (**III**)	Rye bran extrudates (**IV**)
2,4-octadienal (E,E)	x		
2-butyl-2-octenal (E)		x	
nonanal*†	x	x	x
2-nonenal (E)	x	x	x
2,4-nonadienal (E,E)	x		
decanal	x	x	x
2-decanal (E)*	x	x	
2,4-decadienal (E,E)	x	x	
undecanal	x		
benzaldehyde			x
Ketones			
2-butanone		x	x
1-penten-3-one			x
2-hexanone		x	x
2-heptanone†		x	x
6-methyl-5-hepten-2-one (E)	x		x
2-octanone	x	x	x
1-octen-3-one	x	x	
3-octen-2-one (E)*	x	x	x
3,5-octadien-2-one (E,E)	x		x
3-nonen-2-one (E)†	x	x	x
2-decanone	x	x	
6-undecanone		x	
6-dodecanone	x		
Acids			
butanoic acid		x	
pentanoic acid	x	x	
hexanoic acid*†	x	x	x
heptanoic acid		x	
octanoic acid*	x	x	x
2-octenoic acid (E)		x	
nonaoic acid		x	
2-furancarboylic acid		x	
Esters			
1-butylformate		x	
1-pentylformate		x	
1-hexylformate		x	
methyl propenoate			x
2-ethoxyethyl acetate			x
1-heptyloctanoate		x	

Compounds	Spray-dried emulsions (**II**)	Oat extrudates (**III**)	Rye bran extrudates (**IV**)
Lactones			
γ-hexalactone	x	x	
δ-octalactone	x		
γ-nonalactone		x	
Furans			
2-methylfuran		x	x
2-ethylfuran		x	x
2-butylfuran†	x	x	x
2-pentylfuran*†‡	x	x	x
2-heptylfuran		x	
2,5-dimethylfuran			x
2-furanmethanol (2-furylmethanol)	x		x
furfural (2-furaldehyde)‡			x
5-methyl-furfural (5-methyl-2-furaldehyde)			x
2-acetylfuran (1-(2-furyl)ethanone)			x
2-propionylfuran (1-(2-furyl)-1-propanone)		x	
5-methyl-2(5H)-furanone			x
5-butyl-2(5H)-furanone	x	x	
5-pentyl 2(3H)-furanone	x		
5-pentyl 2(5H)-furanone*	x	x	x
Pyridines			
pyridine			x
N,N-dimethyl-3-pyridinamine			x
Pyrazines			
pyrazine			x
2-methylpyrazine‡			x
2-ethylpyrazine			x
2-propylpyrazine			x
2,3-dimethylpyrazine			x
2,5-dimethylpyrazine‡			x
2-ethyl-3-methylpyrazine			x
2-ethyl-6-methylpyrazine			x
2-ethenyl-6-methylpyrazine			x
2-ethyl-3,5-dimethylpyrazine			x
2-acetylpyrazine (1-(2-pyrazinyl)ethanone)			x
sulfur-containing compounds			
1,3-thiazole			x

* selected indicator compounds in oxidized spray-dried emulsion (**II**)

† selected indicator compounds in stored oat extrudates (**III**)

‡ selected indicator compounds in stored rye bran extrudates (**IV**)