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Postprandial Lipid Metabolism Resulting from Heated Beef, Homogenized and Heat Treated Milk and Interesterified Palm Oil

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Food Chemistry and Food Development
Department of Biochemistry
University of Turku, Finland

Supervised by
Associate Professor Kaisa Linderborg, Ph.D.
Department of Biochemistry
University of Turku
Turku, Finland

Professor Emeritus Heikki Kallio, Ph.D.
Department of Biochemistry
University of Turku
Turku, Finland

Reviewed by
Associate Professor Dereck E.W. Chatterton, Ph.D.
Department of Food Science
University of Copenhagen
Copenhage, Denmark

Professor Ursula Schwab, Ph.D.
Institute of Public Health and Clinical Nutrition
School of Medicine
University of Eastern Finland
Kuopio, Finland

Opponent
Professor Ian Givens, Ph.D.
Institute of Food, Nutrition and Health
University of Reading
Reading, United Kingdom

Research director
Professor Baoru Yang, Ph.D.
Department of Biochemistry
University of Turku
Turku, Finland

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The journey, not the destination matters
T.S. Elliot

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ABSTRACT

Today, although our food is more processed than ever the effects of food processing on dietary lipids and their absorption and postprandial metabolism are not fully understood. The lipid composition of food impacts postprandial lipemia. The effects of different fats on the magnitude of postprandial lipemia depend on the fatty acid composition, mainly the chain length and degree of unsaturation of the fatty acids as well as the positioning of the fatty acids in the triacylglycerol backbone. Heightened or prolonged postprandial lipemia is a risk factor for atherosclerosis, obesity and type 2 diabetes. The technological properties of fat depend on its chemical and physical properties, thus, decreases in lipid droplet size or change of distribution of fatty acids in the triacylglycerol backbone during processing is often desired. Lipid oxidation is generally undesired and it deteriorates the quality of food. When absorbed, the products of lipid oxidation might participate in the development of atherosclerosis. The aim of the current work was to investigate the effects of homogenization of milk, heating of beef and interesterification of palm oil on postprandial lipid metabolism. Beef, cow´s milk and palm oil were selected as model foods since they are common commodities as such or as added incredients.

Beef steaks were investigated in relation to lipid oxidation caused by two differing cooking methods and in relation to the oxidized lipids found in plasma after consumption of these steaks. Lipid oxidation products increased with thermal processing and differences between pan-frying and sous-vide treatment were detected in the beef steaks as well as in the postprandial plasma.

Cow´s milk was subjected to heating and homogenization to compare the effects of different processing methods on gastrointestinal symptoms and postprandial lipemia in subjects who experience gastrointestinal symptoms related to cow´s milk consumption, even when neither lactose intolerance nor cow´s milk allergy had not been diagnosed. Raw milk, pasteurized milk, homogenized and pasteurized milk, and UHT treated homogenized milk were served to sensitive subjects with or without an ingestible pressure measuring capsule. Postprandial lipemia did not differ after consumption of the differently processed milk samples, but significant differences were found in the fatty acid composition of postprandial plasma. The amount, severity or duration of the reported symptoms or the intestinal pressure did not differ between the different milk samples.

Palm oil was interesterified to make the fat more solid and to increase the amount of palmitic acid in the *sn*-2 position and further to compare the effects of native palm oil and interesterified palm oil on postprandial lipemia in men with or without metabolic syndrome. In both volunteer groups, palm oil resulted in

greater, mainly prolonged, postprandial lipemia compared to interesterified palm oil. The total triacylglycerol content of chylomicrons or fatty acid concentrations did not differ significantly between the two volunteer groups.

In the research for this thesis, oxidized lipids were detected in the lipoproteins of postprandial plasma. High levels of lipid oxidation products were detected in the HDL fraction suggesting that the protective effect of HDL may extend to the reverse-transport of oxidized lipid species. Homogenization and heat treatment of milk did not seem to induce gastrointestinal symptoms in milk sensitive but lactose tolerant subjects nor did homogenization notably affect the intestinal absorption of triacylglycerols from milk fat globules. The different positioning of palmitic acid in a triacylglycerol had an effect on postprandial lipemia in healthy individuals and the effect was substantiated also in subjects with dyslipidemia. The results of this thesis shows that the effect of food processing on dietary lipids and postprandial lipemia depend on the processing method and the lipid matrix.

SUOMENKIELINEN ABSTRAKTI

Ruokamme on entistä prosessoidumpaa, mutta prosessoinnin vaikutuksia ravintorasvoihin, niiden imeytymiseen sekä aterianjälkeiseen aineenvaihduntaan ei tunneta vielä kovin hyvin. Ruuan sisältämä rasva vaikuttaa aterianjälkeiseen rasva-aineenvaihduntaamme. Se miten voimakas vaikutus on, riippuu rasvahappojen hiiliketjujen pituudesta ja kaksoissidosten määrästä sekä paikoista eli rasvahappokoostumuksesta sekä rasvahappojen paikasta triasyyliglyserolin rungossa. Aterianjälkeinen pitkittynyt veren runsas rasvapitoisuus on riskitekijä valtimonkovettumistaudille, lihavuudelle ja tyypin 2 diabetekselle. Ravintorasvojen tekniset ominaisuudet riippuvat rasvan kemiallisista ja fysikaalisista ominaisuuksista ja teollinen elintarvikkeiden prosessointi vaikuttaa usein rasvan koostumukseen. Esimerkiksi maidon homogenointi pienentää rasvapallosten kokoa ja interesteröinti muuttaa rasvahappojen paikkaa glyserolirungossa. Nämä muutokset ovat haluttuja, toisin kuin rasvojen hapettuminen. Hapettuminen on haitallinen ilmiö, joka heikentää elintarvikkeen laatua. Imeytyessään verenkiertoon hapettuneet rasvat voivat osaltaan lisätä valtimonkovettumistaudin riskiä. Tässä väitöskirjatyössä tutkittiin elintarvikkeiden kuumennuksen, homogenoinnin ja interesteröinnin vaikutuksia ravintorasvoihin. Väitöskirjan kokeellisen osan tarkoituksena oli myös selvittää, mikä vaikutus prosessoiduilla ravintorasvoilla on aterianjälkeiseen rasva-aineenvaihduntaan. Mallielintarvikkeina käytettiin naudanlihaa ja lehmänmaitoa sekä palmuöljyä, jota esiintyy yleisesti margariineissa, makeisissa ja leivonnaisissa.

Rasvojen hapettumista tutkittiin kypsentämällä naudanlihapihvejä kahdella eri tavalla, pannulla paistaen sekä vesihauteessa. Aterianjälkeisistä verinäytteistä selvitettiin, löytyykö rasvojen hapettumistuotteita verenkierrosta. Rasvojen hapettumista havaittiin molempien kypsennysmenetelmien yhteydessä. Myös aterian jälkeen otetuista verinäytteistä löydettiin rasvojen hapettumistuotteita.

Lehmänmaidon kuumennuksen ja homogenoinnin vaikutuksia vatsaoireisiin ja aterianjälkeiseen rasva-aineenvaihduntaan tutkittiin henkilöillä, jotka kokivat saavansa vatsaoireita prosessoidusta maidosta, vaikka heillä ei ollut todettu laktoosi-intoleranssia eikä lehmänmaitoallergiaa. Raakamaitoa, pastöroitua maitoa, pastöroitua ja homogenoitua maitoa sekä UHT-kuumennettua ja homogenoitua maitoa tarjottiin sensitiivisille tutkimushenkilöille yhdessä nieltävän paineenmittauskapselin kanssa ja ilman sitä. Aterianjälkeinen rasva-aineenvaihdunta ei eronnut eri maitojen välillä, mutta aterianjälkeisissä rasvahappokoostumuksissa havaittiin eroja maitojen välillä. Raportoitujen vatsaoireiden määrässä, kestossa sekä voimakkuudessa ei ollut merkitseviä eroja. Myöskään suoliston paine ei eronnut merkittävästä maitojen välillä.

Palmuöljyä interesteröitiin, jotta se olisi kiinteämpää huoneenlämmössä ja koska palmitiinihapon määrää haluttiin lisätä triasyyliglyserolin keskiasemassa. Interesteröidyn ja natiivin palmuöljyn vaikutusta aterianjälkeiseen rasva-aineenvaihduntaan tutkittiin metabolista oireyhtymää sairastavilla miehillä sekä terveillä verrokeilla. Kaikilla tutkimushenkilöillä aterianjälkeinen veren rasvapitoisuus nousi korkeammaksi natiivin palmuöljyn jälkeen verrattuna interesteröityyn palmuöljyyn. Prosessoimattoman palmuöljyn nauttimisen jälkeen kesti myös kauemmin, että veren rasvapitoisuus palasi takaisin lähtötasolle. Veren kylomikronien triasyyliglyserolien ja yksittäisten rasvahappojen pitoisuuksissa ei havaittu eroja vapaaehtoisryhmien välillä.

Rasvojen hapettumistuotteita havaittiin molempien naudanliha-aterioiden jälkeen kaikissa plasman lipoproteiiniluokissa. Lipidien hapettumistuotteita havaittiin runsaasti HDL-fraktiossa. Tämä saattaa tarkoittaa sitä, että HDL:n suojaava vaikutus ulottuu myös hapettuneiden rasvojen kuljettamiseen pois kudoksista. Lehmänmaidon homogenointi ja kuumennus eivät lisänneet vatsaoireita herkillä tutkimushenkilöillä. Maidon homogenointi ei myöskää vaikuttanut merkittävästi rasvan imeytymiseen rasvapalloista elimistöön. Palmitiinihapon paikka triasyyliglyserolin keskiasemassa interesteröidyssä palmuöljyssä vaikutti terveillä verrokeilla veren aterianjälkeistä rasvapitoisuutta pienentävästi, ja sama ilmiö oli nähtävissä myös metabolista oireyhtymää sairastavilla tutkimushenkilöillä. Väitöskirjan tulokset osoittavat, että elintarvikkeiden prosessoinnin aiheuttamat muutokset ravintorasvoissa ja aterianjälkeisessä rasva-aineenvaihdunnassa riippuvat käytetystä prosessointimenetelmästä sekä rasvamatriisista.

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
MAG	Monoacylglycerol
BMI	Body mass index
BT	Beef tallow
cDNA	Complementary deoxyribonucleic acid
CMRF	Chylomicron rich fraction
COP	Cholesterol oxidation product
CRP	C-reactive protein
CR1	Receptor for the complement fragment C3b
CR3	Receptor for the complement fragment C3bi
FA	Fatty acid
FGF21	Fibroblast growth factor 21
Flt3L	Fms-like tyrosine kinase 3 ligand
GI	Gastrointestinal
HDL	High-density lipoprotein
HOS	High oleic sunflower oil
HPM	Homogenized and pasteurized milk
iAUC	Incremental area under curve
INFγ	Interferon γ
INFλ	Interferon λ
IL	Interleukine
IPO	Interesterified palm olein
LDL	Low-density lipoprotein
MA	Moving average
MCFA	Medium chain fatty acid
MCP-1	Monocyte chemotactic protein-1
meq/kg	Milliequivalent per kilogram
MetS	Metabolic syndrome
MFG	Milk fat globule
MFGM	Milk fat globule membrane
MPa	Megapascal
MTP	Microsomal triglyceride transfer protein
PM	Pasteurized milk
PO	Palm olein
PUFA	Polyunsaturated fatty acid

PV	Peroxide value
RM	Raw milk
SFA	Saturated fatty acid
sn	Stereospecific numbering
TAG	Triacylglycerol
TBARS	Thiobarbituric reactive substances
TNFα	Tumor necrosis factor α
TRANCE	TNF-related activation-induced cytokine
UHT	Ultra high temperature
UHTM	Ultra high temperature treated homogenized milk
UPLC-ESI/MS	Ultra performance liquid chromatography electrospray ionosation mass spectrometry
VLDL	Very-low-density lipoprotein
w/w	Weight by weight

LIST OF ORIGINAL PUBLICATIONS

I. Nuora A.; Chiang V S-C.; Milan A.; Tarvainen M.; Pundir S.; Smith GS.; Markwort JF.; Ahotupa M.; Cameron-Smith D.; Linderborg KM. The impact of beef steak thermal processing methods on the lipid oxidation and human postprandial inflammation related responses. *Food Chemistry* **2015**, 184, 57-64.

II. Nuora A.; Tupasela T.; Tahvonen R.; Rokka S.; Marnila P.; Pohjankukka J.; Pahikkala T.; Viitanen M.; Mäkelä P.; Yang B.; Kallio H.; Linderborg K. Effect of milk processing on gastrointestinal symptoms, intestinal pressure and postprandial metabolism. *International Dairy Journal* **2018**, 79, 15-23.

III. Nuora A.; Tupasela T.; Jokioja J.; Tahvonen R.; Yang B.; Kallio H.; Viitanen M.; Linderborg KM. The effect of heat treatments and homogenization of cows' milk on gastrointestinal symptoms, inflammation markers and postprandial lipid metabolism. *International Dairy Journal* **2018**, 85, 184-190.

IV. Milan AM.; Nuora A.; Yang B.; Linderborg KM.; Markworth JF.; Poppitt P.; Fraser K.; Cameron-Smith D. Postprandial responses of overweight and hypertriglyceridemic men are affected by the positional distribution and fatty acid composition of palmitic acid rich oils in a randomized controlled trial. *Manuscript.*

1 INTRODUCTION

From the discussion in the media, it can be speculated that some consumers are concerned about processed food not being healthy, nutritious or natural. Farm sales are becoming more popular and so are foods that are organically produced or do not contain any food additives. Some consumers may believe that food processing affects the content, digestibility or availability of nutrients making processed food low in nutrients or less healthy than the unprocessed ones. The frequency of functional gastrointestinal disorders like irritable bowel syndrome has increased in western countries and patients may consider processed food as a trigger for the disease [1,2]. Food processing is, however, often necessary to ensure food safety and to provide a sufficiently long shelf-life. Processing can also be used to change the structure of food to be more digestible, to have desired sensory properties or to remove substances like lactose. Removing lactose from dairy produces products suitable for lactose intolerant individuals. Addition of nutritients that are not naturally present or are present in small quantities in foods, like vitamin D is also possible [3-5].

Fat is an essential part of our diet and according to the Nordic Nutrition Recommendations [6] 25 to 40 % of energy should come from fat. The effects of food processing on dietary lipids, their absorption and postprandial lipemia have not been intensively investigated. The postprandial state is especially important as the dietary lipids circulate in our blood for several hours after a meal and thus people in the western countries spend most of their lives in the postprandial state. From previous studies it is well known that prolonged clearance of triacylglycerols (TAGs) from blood to tissues is a risk factor for cardiovascular diseases and obesity, and it has been even linked to chronic inflammation [7,8]. Food processing, by altering the solid fat content, can affect the length and magnitude of postprandial lipemia even when the amount of dietary lipids in the food remains constant [9,10].

In food, fatty acids (FA) are mostly in TAGs esterified to a glycerol backbone. The positions of the FAs in the TAG molecule (*sn*-1, *sn*-2, *sn*-3; *sn*, stereospecific numbering) generate a distinctive stereospecificity which defines the physical properties of fats and influences their absorption, metabolism and uptake into tissues [11]. In processed foods, most of the fats and oils are present as oil-in-water emulsion. In the course of time, the emulsion starts to separate into an oil and aqueous layers via different mechanisms like creamin [12]. Milk is a classical example of an oil-in-water emulsion and homogenization of milk is performed for the purpose of physical stability to prevent creaming. Upon homogenization the size and structure of the milk fat globules is changed, resulting in smaller milk fat globules [13].

The positions of FAs in the TAG molecule affect the physical properties of the fat. Interesterification is a process which modifies the positions of FAs in the TAG molecules. The food industry uses interesterification to create fats with desirable melting characteristics for the use in spreads and bakery products without generation of *trans* FAs.

Processing of food may expose the lipids to oxygen, light and heat which can cause lipids to oxidize. In muscle foods, the temperature of cooking has a substantial effect on the production of free radicals that lead to the oxidation of lipids and could deteriorate sensorial and nutritive values as well as the color of the products [14]. The amount of lipid oxidation products in food is related to the amount and composition of lipids as well as to the storage, processing and preparation conditions [14,15]. Postprandially, inflammation arises from the interaction between the food components and the circulating immune cells. The most efficient triggers of postprandial inflammation response seems to be TAGs and saturated FAs [16]. Recent studies [17,18] have indicated that oxidized dietary lipids exist in human lipoproteins suggesting that a significant proportion of oxidative stress in the body may in fact be related to food. The pathophysiological significance on postprandial inflammation in the induction of insulin restistance and metabolic syndrome is one of the currently under research [16].

The research in this thesis concentrates on the effects of selected common food processing methods: heat treatment, homogenization and interesterification, and the influence of processed dietary lipids on postprandial lipid metabolism in humans. In the literature review, recent publications on the following are summarized: the effects of heat treatment on meat, homogenization of milk and interesterification of palm oil, and the current knowledge of acute effects of processed dietary lipids on postprandial lipid metabolism in humans is also discussed.

2 REVIEW OF THE LITERATURE

2.1 Effects of selected food processing methods on dietary lipids

2.1.1 Lipid oxidation in red meat during cooking

Lipid oxidation in muscle foods is a major cause of quality deterioration as it adversely affects the color, flavor, shelf-life and nutritional value [19]. However, cooking of meat causes several positive effects, such as taste and flavor enhancement, destruction of micro-organisms and improved digestibility [20,21]. Moreover, the cooking method as well as the time and temperature combinations can affect the lipid oxidation and thus the resulting oxidation products [15]. Roasting, stewing, grilling and pan-frying are probably the most common methods used in the cooking of meat. They all use high temperatures in the presence of oxygen leading not only to lipid oxidation but also to the formation of chemical carcinogens, especially hetero- and polycyclic amines [22,23].

Cooking significantly increases the concentration of primary lipid oxidation products measured as thiobarbituric acid reactive substances (TBARS) and many studies have reported higher levels of TBARS in different kinds of cooked meat products in comparison to the raw meat [24-27]. In a Study by Ferioli et al. [28] lipid oxidation was investigated from fresh minced meat and from pan-fried meat patties made from fresh minced meat. In fresh meat the levels of cholesterol oxidation products (COPs), peroxide value (PV) and TBARS were low. Pan frying of the meat patties for 15 min did not influence the concentration of COPs nor affected the levels of TBARS but the PV increased significantly [28]. In contrast, Broncano et al. [15] discovered that grilling, frying, roasting and microwaving of ham from Iberian pigs significantly increased the lipid oxidation (TBARS and hexanal values as well as and the concentration of COPs) compared with fresh ham. The meat from Iberian pigs contains more polyunsaturated FAs compared other pig meats due to the special diet of Iberian pigs which may possibly make it more sensitive to oxidation. The TBARS value was most affected by roasting and frying followed by microwave and grilling. The levels of hexanal were highest after frying and microwave cooking. Cooking also increased the COPs values of the ham samples and three different COPs (7a-hydroxycholesterol, 7b-hydroxycholesterol and 25-hydroxycholesterol) were found in all the samples analyzed. No significant differences in the levels of COPs were detected between the cooking methods [15]. Saboloá et al. [29] found significantly higher levels of 7-ketocholesterol in meatloaf baked at 250 °C compared to 180 °C, especially in the crust. Frozen storage of the cooked

meatloaves did not hinder the formation of COPs, but on the contrary increased it, when the content of COPs in the freshly cooked and frozen and thawed meatloafs were compared.

Thurner et al. [30] reported only modest increases in COP concentrations after the pan-frying of beef and pork meat compared to raw meat. The concentration of COPs was higher after pan frying in pork meat compared to beef. The authors suggested that the short heating time and relatively low heating temperature (180 °C) kept the concentration of COPs low.

In a Study by Roldan et al., [22] sous-vide cooking with different lengths of time (6, 12 and 24 h) and temperature (60, 70, 80 °C) combinations induced lipid oxidation in lamb loins. Higher cooking temperature and time combinations increased the concentrations of conjugated dienes and decreased the TBARS values and hexanal concentrations. The levels of conjugated dienes remained relatively constant throughout the cooking time at temperatures of 60 and 70 °C but were significantly increased with time when the lamb loins were cooked at 80 °C. For TBARS, the values increased up to 6 hours in all cooking temperatures and then started to decrease after 24 hours of cooking [22]. Sanchez del Pulgar et al. [31] measured the highest TBARS values in pork cheeks sous-vide cooked at 60 °C for 12 h compared to those cooked for 5 hours or cooked at 80 °C for 5 or 12 hours. The measured levels were significantly high, but in accordance with previous reports, possibly due to the long cooking time [32].

Roldan et al. [33] also investigated the volatile compound profile of the lamb loins and how they were affected by a cooking time (6 and 24 h) and temperature (60 and 80 °C) combination. Aliphatic hydrocarbons and aldehydes were the most abundant chemical groups in the cooked samples. In samples cooked in low or moderate conditions (60 °C for 6 and 24 h, 80 °C for 6 h), the value of the volatile compounds linked to lipid oxidation were high, and a more severe time-temperature combination (80 °C and 24 h) resulted in higher concentrations of volatile compounds due to Streckers degradation of amino acids. A Study by Soladoye et al. [34] showed that pan-frying of bacon resulted in higher lipid oxidation (measured as TBARS) compared to microwave cooked bacon. Contrary to Soladoye, Domínguez et al. [35] reported higher TBARS levels in microwave cooked foal meat compared to pan-frying. In the same Study roasting of foal meat resulted in the highest lipid oxidation concentration followed by microwaving, pan-frying and grilling.

Higher cooking temperatures and longer cooking times seem to induce more lipid oxidation. In addition, the lipid oxidation reactions take place at a higher rate producing further reactions with other compounds present in the meat giving rise to a variety of different compounds compared with lower cooking temperatures and shorter cooking times [22,33,36,37]. The studies investigating

the effect of different cooking methods of red meat on lipid oxidation are summarized in **Table 1**.

Table 1. Effect of different cooking methods of red meat on lipid oxidation.

HEATING METHOD	COMPOUND CLASS	REFERENCE
Pan frying minced meat	Lipid peroxides, oxidized cholesterol	Ferioli et al., 2010
Grilling, frying, roasting, microwaving iberian ham	TBARS, hexanal, oxidized cholesterol	Broncano et al., 2009
Foal meat	TBARS	Domínguez et al., 2014
Oven baking meatloaf	Oxidized cholesterol	Saboloá et al., 2017
Sous-vide cooking of lamb loins	Conjugated dienes, TBARS	Roldan et al., 2014
Sous-vide cooking of pork cheeks	TBARS	Sanchez del Pulgar et al., 2012
Sous-vide cooking of lamb loins	Aldehydes	Roldan et al., 2015
Pan-fried and microvawed bacon	TBARS	Soladoye et al., 2017

2.1.1.1 Mechanisms of lipid oxidation

Lipid oxidation is a complex process that involves the loss of essential FAs and generation of toxic compounds like malondialdehyde and COPs [31]. In addition to the degree of unsaturation of the FAs, lipid oxidation depends on various factors including: light, temperature, concentration of oxygen in the surroundings, the amount and composition of phospholipids, the presence of antioxidants, metal ions, haem pigments, use of mechanical processing, and the processing conditions [19]. The free radical chain mechanism is the main pathway for lipid autoxidation. This pathway proceeds through initiation, propagation and termination, leading to series of complex chemical changes [38]. TAGs with unsaturated FAs are especially vulnerable to oxidation, **Figure 1** shows the autoxidation of linoleic acid. In the presence of initiators like heat or light, unsaturated FAs easily lose a hydrogen atom producing free radicals, which will react with oxygen to form peroxyl radicals [39]. The peroxyl radicals further attack new TAGs creating a reaction cycle which can be repeated several thousand times during propagation until no hydrogen source is available [38]. This makes lipid oxidation a self-propagating process. Lipid hydroperoxides are formed as primary oxidation products. However, they could further react and

form secondary oxidation products like TBARS, aldehydes and ketones, which could contribute to off-aromas and flavors [38,40]. At low temperatures, as in cold storage, the breakdown of hydroperoxides is accelerated by metal ions and in the case of muscle foods, haem provides the metal ion for this reaction [39]. In the termination stage of oxidation, radicals neutralize each other through radical-radical coupling or radical-radical disproportionation where two radicals react to form two stable, non-radical products [38,39].

Hydroperoxides are the first compounds formed during the oxidation process and they are often used to determine the lipid oxidation status [41]. The extent to which hydroperoxides occur in fat is often described with PV, which represent the quantity of active oxygen (in meq) in 1 kg of lipid [42]. A PV of 5 meq/kg is considered to be the maximum acceptable level for muscle foods [19,43]. The TBARS value determines the malondialdehyde (**Figure 2**) content and is a common method of measuring lipid oxidation in meat products [44,45]. The acceptable level of TBARS in the cooked meat products during storage is 0.50 – 1.0 mg/kg [46]. A rancid flavor can be detected when the TBARS value exeeds 1 mg of malondialdehyde / kg meat product [19]. Hexanal is one of the major lipid oxidation products and because of this it is used to follow lipid oxidation and volatile compound formation during the cooking of meat products (**Figure 2**) [47].

Dietary cholesterol is oxidized via a free radical mechanism resulting in the formation of oxidation products such as 7α and 7β-hydroxy-cholesterol, 7-ketocholesterol and 5α, 6α epoxy-cholesterol (**Figure 2)** [48]. The most common method for determing the concentration of COPs in a food, is to use cold saponification and solid phase extraction for the purification and GC or HPLC for the determination [49-52]. COPs have been demonstrated to have cytotoxic, mutagenic, carcinogenic and atherogenic effects in mammalian cells [53,54]. A positive correlation seems to exist between TBARS and COPs, indicating that COPs formed in meat during cooking can be predicted from the TBARS values [15,24,55,56]. Conjugated dienes are formed when the radical state of hydroperoxides, formed from polyunsaturated FAs, is stabilised via a double-bond rearrengement (**Figure 2**) [41]. Conjugated dienes are relatively stable and they absorb light in the UV range, which can be measured by spectrophotometric methods to asses the level of lipid oxidation [41,57]. Measurement of conjugated dienes is often used to monitor LDL oxidation [58].

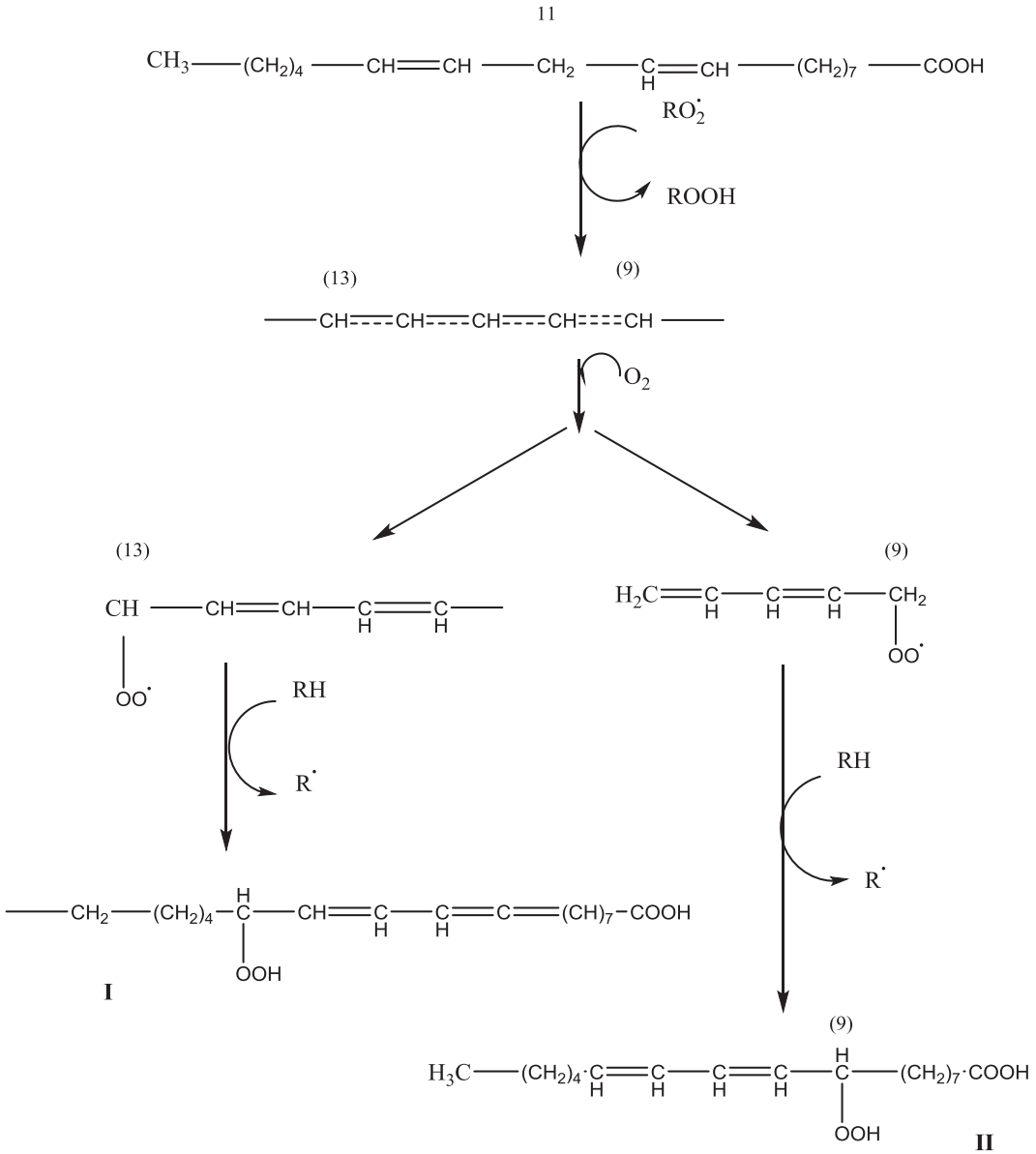

Figure 1. Autoxidation of linoleic acid. Primary reaction products: I 13-Hydroperoxyoctadeca-9,11-dienoic acid; II 9-Hydroperoxyoctadeca-10.12-dienoic acid.

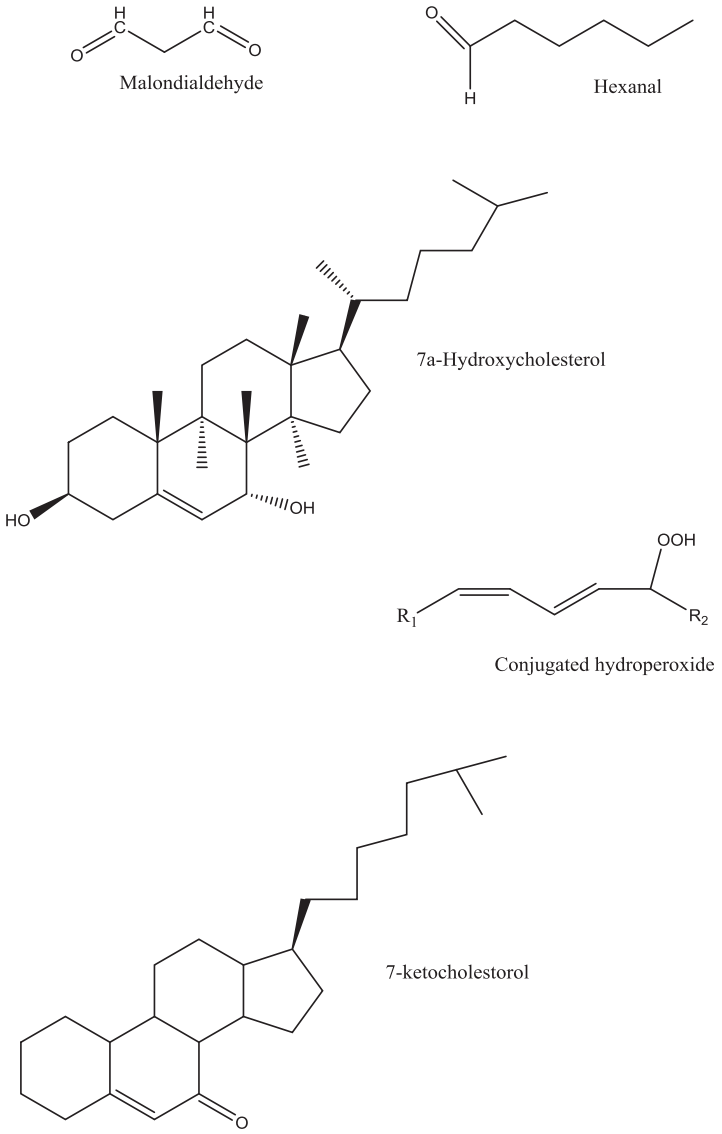

Figure 2. Examples of oxidation products of lipids.

2.1.2 Physico-chemical changes in milk lipids during pasteurization, UHT treatment and homogenization

2.1.2.1 Pasteurization

Cow's milk is pasteurized for two distict reasons (i) to make milk safe for human consumption by reducing the number of viable pathogenic bacteria and (ii) to

extend shelf-life by reducing the number of spoilage bacteria [59]. Pasteurization of milk is defined as heating of every particle of milk to a standardized temperature for a defined period of time without allowing recontamination of the milk during the process [60].

The commercial heating of milk does not affect the lipid content of milk and very low numbers of lipid oxidation products are found in pasteurized milk [61]. The fat globules in pasteurized, non-homogenized milk are on average 3 to 5 μm in size and are mainly covered by the milk fat globule membrane (MFGM) which constituets predominantly of phospholipids and MFGM proteins. During digestion, components of the MFGM inhibit the effect of pancreatic lipase, slowing down the digestion of pasteurized milk [62,63]. Due to large amount of phospholipids on the MFGM, it takes longer time for the pancreatic lipase to penetrate trough the membrane and reach the core full of TAGs [62].

The digestibility of milk proteins is not affected by pasteurization to any significant extent [64]. The whey proteins are more sensitive to heat compared to caseins. However, during standard pasteurization at 72 °C for 15 seconds the bioactive whey proteins retain most of their activity [65]. Immunoglobulins are the most heat sensitive whey proteins. They will start to denaturate already at temperatures around 60 °C [66]. β-lactoglobulins and bovine serum albumin begin to denaturate at a temperature around 70 °C, whilst α-lactalbumin is the most heat stable of the whey proteins and denaturates at temperatures over 70 °C [67]. The denatureated whey proteins form aggregates with the caseins, especially with κ-casein [68]. Mainly the functional properties of the milk proteins are modified (emulsifying and water binding ability, solubility) during pasteurization with very little effect on their nutritional value [69].

Pasteurization does not significantly affect the contents of lactose, hormones, growth factors or proteins that bind to the minerals in milk [70]. Enzymes, like lipoprotein lipase, alkaline phosphatase and catalase, are inactivated during pasteurization but proteinase and lactoperoxidase stay active [71]. Around 10 % of B group vitamins (thiamine, folate acid, pyridoxine and cobalamin) and about 25 % of vitamin C are destroyed during pasteurization (**Table 2**) [71]. However, the small losses of vitamins do not affect the nutritional value of milk and the levels of fat-soluable vitamins in milk are not affected by pasteurization.

2.1.2.2 Ultra high temperature treatment

Ultra High Temperature (UHT) treatment of milk combined with aseptic packaging produces sterile milk with advantageous shelf-life from 6 to 9 months at room temperature [72]. In the standard UHT treatment, milk is heated at 130-150 °C for 2-6 seconds [64]. There are two main types of UHT heating: direct and indirect. In the direct heating, super-heated steam is mixed with milk, while

in the indirect heating, a heat exchanger tranfers heat across a partition between milk and the heating medium, which is either pressurized hot water or steam [73,74]. In the direct heating systems either steam injection or steam infusion is used. In the steam injection system, superheated steam is injected into the milk stream, whereas in the steam infusion system milk is sprayed into a superheated steam. With both processes the milk temperature is immediately raised [73]. A major requirement for the direct UHT plant is a high-quality, culinary steam to ensure that there is no flavor carryover from the steam.

During the UHT treatement, 60 % of whey proteins, mainly α-lactalbumin and β-lactoglobulin, denaturate [75]. Lactulose is formed in the UHT treatment by isomerization of lactose, the reaction is catalyzed by free amino groups of casein [76]. UHT treatment also causes nonenzymatic modications in the milk proteins, which may have negative influence on the nutritional properties. The Maillard reaction modifies the side chains of α-lactalbumin, β-lactoglobulin and caseins. Especially the ε-amino groups of a lysine residues represent a primary target for lactose to form lactulosyllysine [64]. The lactulosyllysine may then undergo series of advanced reactions resulting in a variety of modified structures [77]. The Maillard reaction between lactose and lysine continues during storage, leading continuous production of the lactulosyllysine [73]. Blockage of the ε-amino groups of lysine residues hinders the action of the digestive enzymes and reduces protein digestibility [78]. In addition, UHT treatment can cause interchange of disulfide bonds between proteins [64]. A non-native protein complexes are formed which are resistant to digestion [79].

UHT treatment does not affect the levels of fat-soluable vitamins in milk. However, during UHT treatment 20 % of B group vitamins and 30 % of vitamin C is destroyed (**Table 2**). Practically all enzymes are inactivated during UHT treatment. The shelf-life of UHT treated milk is limited by age-gelation, a phenomenon where the viscosity of milk is increased during storage. Eventually, when a gel is formed, milk loses its fluidity [80]. The gel is composed of cross-linked chains between the whey proteins and casein. UHT milk heated by the direct method tends to gel sooner during storage than milk heated with the indirect method [73]. The indirect heating method leads to production of more stable casein micelles compared to the direct heating method. The indirect heating method also increases the level of whey protein denaturation. The denaturation of whey proteins slows down the formation of complexes between the whey proteins and the caseins and delayes the onset of gelation of milk [73,81].

UHT treatment induces flavors in milk which are described as being “cooked”, “stale” and “flat”. These off-flavor compounds, which are low in concentration in pasteurized and raw milk, are often the reason why UHT milk is poorly accepted by consumers [82]. The compounds in UHT milk are due to thermal

denaturation of proteins, lipid oxidation or non-enzymatic reactions [72]. The amount of dissolved oxygen in milk is an important factor in the flavor change of UHT milk as many of the off-flavors are produced by oxidation of milk components during storage [73]. The "cooked" flavor is mainly due to sulphur-containing aroma compounds, raising from denaturation of the milk serum proteins during UHT treatment [83]. A number of different ketones contribute to the overall off-flavor of UHT milk and especially the methyl ketones contribute to the "stale" flavor appearing during storage [72,84]. Aldehydes, although low in concentration, may strongly contribute to the "flat" flavor in UHT milk [85].

Table 2. Major changes in milk induced by homogenization and heating. (Adabted from Michalski and Januel, 2006).

PROCESS	RELATED REACTION	CONSEQUENCES
Homogenization	Fat globule disruption	Smaller fat globules with new interface
	Dispersion of casein miselles	Formation of fat-protein complexes
	Activation of some enzymes	Oxidized taste, rancid taste
Heat treatment	Destruction of micro-organisms	Increased microbiological quality and shelf-life
	Whey protein denaturation	Formation of casein-whey complexes
	Lactone formation	Enhanced flavor and taste
	Enzyme inactivation	Increased quality and shelf-life
Pasteurization	Destruction of water-soluble vitamins	<10% vitamin B; <25% vitamin C
UHT	Destruction of water-soluble vitamins	<20% vitamin B; <30% vitamin C
Storage of packed:	Reactivation of enzymes	Organoleptic deects (proteolysis, lipolysis)
Pasteurized milk	Growth of psychotropic bacteria	Bitter taste due to proteolysis
UHT milk	Destruction of water-soluble vitamins	<30% of vitamins B and C
	Age gelation	Formation of protein-mineral complexes
	Destruction of water-soluble vitamins	<50% of vitamin B; >90% vitamin C

2.1.2.3 Homogenization

Milk lipids are packed in milk fat globules (MFGs) where non-polar lipids, mainly TAGs, are surrounded by a primary membrane and a lipid bilayer composed of phospholipids, proteins, enzymes, cholesterol and glycoproteins (**Figure 3**) [86]. Milk is homogenized to prevent the separation of the MFGs on the surface of the milk, i.e. cream separation. Homogenization of whole milk causes major physical changes in the structure and size of MFG [13]. Natural MFGs are decreased in size during homogenization from an average of 3 – 5 μm to less than 1 μm and at the same time the total surface area of the fat globules is increased more than 10-fold [13]; in addition, caseins and some whey proteins are adsorped at the droplet interface. **Figure 3** shows the structure of native MFGs and the homogenized ones.

The efficiency of the homogenization increases at temperatures between 42 °C to 72 °C and stabilizes at around 72 – 77 °C, though the temperature and pressure vary with the apparatus and valve type [66,87]. Pressurized milk (8 – 20 MPa) is forced between a valve needle and a seat, resulting in significant reduction of the size of the MFGs due to shear stress, inertial force and cavitation [66]. A two-stage homogenizer is usually used. Aggregates formed during the first homogenization stage are separated during the second stage which is operated at a lower pressure than the first one. There is a density difference between the milk fat and aqueous phase and according to Stokes law, the cream separation rate is dramatically decreased due to the smaller milk fat globule sizes resulting after homogenization. Homogenization also prevents coalescence of MFG particles to some extent, making the milk emulsion more stable [66]. In addition to regular homogenizators, also microfluidization is used as a homogenization technique. In microfluidization, milk is forced under high pressure in a reaction chamber and is divided into two jets colliding at 180 degrees at high speed [88]. Microfluidization produces a smaller mean diameter and a narrower MFG size distribution compared to regular homogenization, which improves long-term emulsion stability [88]. High-pressure homogenization is conducted at a higher pressure (> 50-200 MPa) than those used during regular homogenization. The size of the fat droplets decreases when the pressure is increased and under certain conditions of temperature and pressure the fat droplets produced are significantly smaller than the regularly homogenized ones [89]. High pressure homogenization reduces bacterial microflora [90], inactivates plasmin [91] and reduces activities of alkaline phosphatase and lactoperoxidase [90]. The disadvantages of high pressure homogenization are the off-flavors possibly due to oxidation. Regular homogenization does not typically cause these off-flavors [66].

The composition of the MFGM is altered by homogenization (**Table 2**). Due to the rupture of fat globules and an increase in the total surface area, the MFGM cannot cover the entire newly formed fat surface area, leading to other surface active components being adsorbed and forming a new membrane around the fat droplets [13,92]. A fourfold increase in the total proteins in the newly formed fat droplet interface occurs during homogenization compared to native MFGM [93]. Casein micelles are fragmented during homogenization and caseins are the major protein fraction which is adsorbed on the newly formed fat globule surface [66]. Structural measurements of fat dropleds indicates that three types of particles can be found in homogenized milk (i) regular homogenized MFGs with a fraction of surface covered with caseins and the rest being covered by MFGM; (ii) small (< 500 nm) lipid-protein complexes which have new membranes composed of caseins; (iii) miniature native MFGs (~100 nm) originally present in milk which have not been affected by homogenization [94]. Dietary lipids are mainly organized as droplets and the size of the droplets varies between 0.3 and 20 μm depending on the source [95]. The lipid droplet size of milk is a key physico-chemical factor affecting the digestive properties of milk FAs in humans. The droplet size has a function in the activity of gastric lipase in humans [96]. In fact, with the same amount of lipids, smaller lipid droplets will result in a larger lipid/water interface area and faster lipolysis by gastric lipase compared to larger droplets. The larger interface area of lipid droplets allows more lipase molecules to bind to the substrate. In addition, the larger interface area will delay the inhibition of gastric lipolysis by long chain FAs.

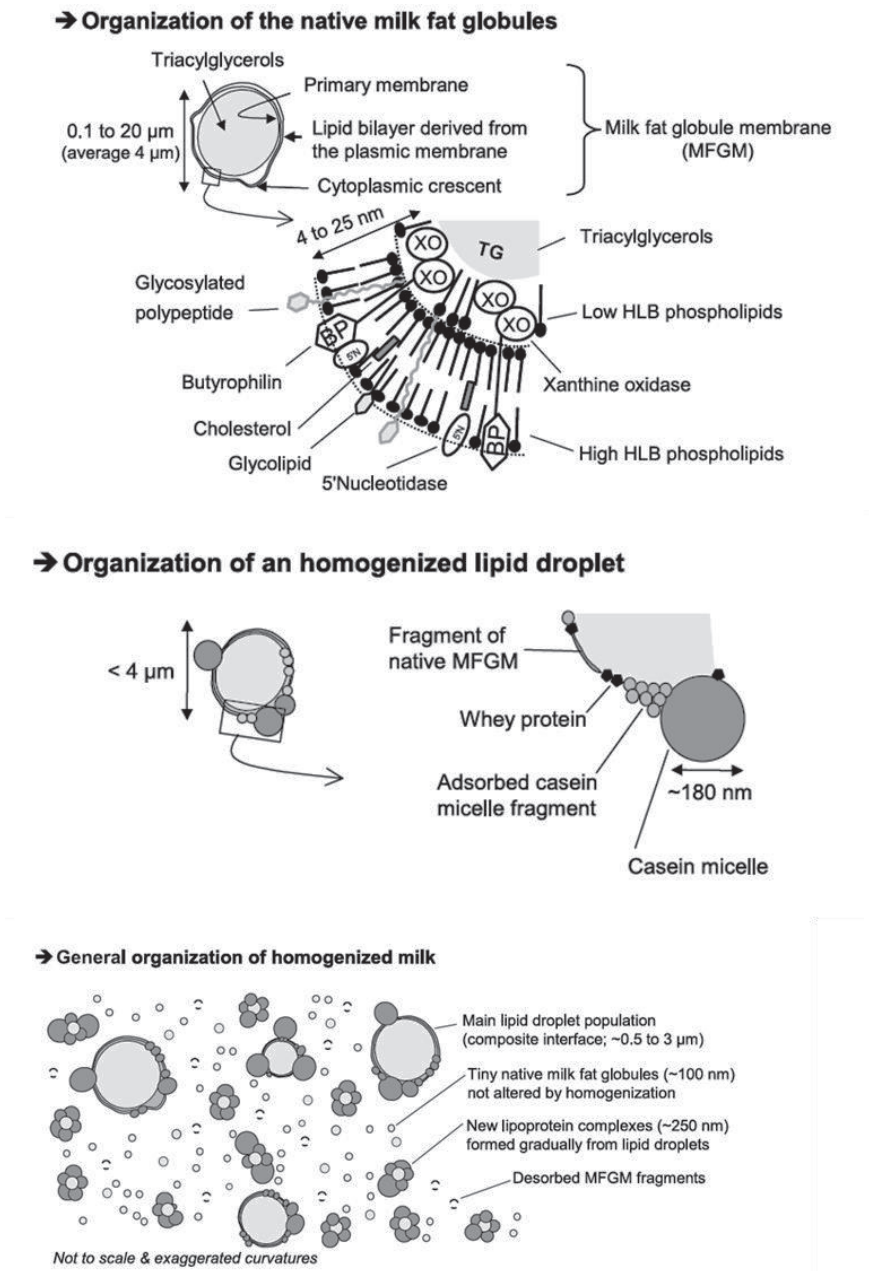

Figure 3. Structure of native milk fat globule membrane (MFGM) compared to homogenized fat droplet, and a suggested organization of the lipid particles in homogenized milk. HLB phospholipid, hydrophilic-lipophilic balance phospholipid. Reprinted from *Trends in Food Science & Technology*, 17, Michalski et Januel, Does homogenization affect the human health properties of cow's milk? 423-437, Copyright (2006), with the permission of Elsevier Ltd.

2.1.3 Interesterification of vegetable oils

The overall FA composition and regiospecific structure, meaning the positional distribution of FAs along the TAG backbone, differs between plant oils and animal fats. Generally, most vegetable oils contain unsaturated FA in the *sn*-2 position and saturated FA in the *sn*-1 and/or *sn*-3 position [11]. The three hydroxyl groups in the glycerol backbone are numbered by the stereospecific numbering (*sn*) system, designating the FA to be either internal *sn*-2 position or external *sn*-1 and *sn*-3 position [97]. In cocoa butter, palm and soybean oils, palmitic and stearic acids are mainly located in the *sn*-1 and *sn*- 3 positions while the most common FAs in the *sn*-2 position are oleic and linoleic acids [97]. However, in olive oil, unsaturated FAs are equally distriputed among the three regiospecific position and in coconut oil the saturated fatty acids are widespread between the positions [11]. Unlike vegetable oils, some animal fats have saturated FA largely in the *sn*-2 position and unsaturated FA in the *sn*-1 and *sn*-3 positions. In lard as well as in bovine and human milk, palmitic acid is mainly located in the *sn*-2 position, while oleic acid distriputed in the *sn*-1 and/or *sn*-3 positions in these fats [98,99]. In contrast, beef tallow palmitic acid is distributed more evenly throughout the different positions [11].

Interesterification reaction redistributes FAs within and between TAGs until thermodynamic equilibrium is reached [86]. The resulting products maintain the FA composition and degree of saturation of the original TAGs but present different regiochemistry [87-89]. Chemical and enzymatic interesterification are used to modify the structure and function of TAGs. With chemical interesterification the reaction is faster and a random FA distribution is reached, while enzymatic interesterification gives stereospecificity, which is dependent on the properties of the enzyme [100]. Currently, interesterification is used in many applications by the fats and oils industry, especially in the manufacturing of spreads.

The solid fat content may determine the suitability of fats and oils for specific industrial applications as the solid fat is responsible for some sensory properties like thickness and flavor release as well as spreadability, stability, and ease of packing [101]. Chemical and enzymatic interesterifications often increase the desired spreadability and plasticity without production of *trans* FAs. One strategy for this is to use fully hydrogenated oils, free from *trans* FAs and rich in stearic or palmitic acid and to blend them with a vegetable oil followed by interesterification (chemical or enzymatic) [102]. Ahmadi et al. [102] blended high oleic sunflower oil (HOS) and fully hydrogenated canola oil and interesterified them both chemically and enzymatically. The interesterification of different blends led to a broader melting transition and lower melting point for the interesterified blends. The plasticity range was wider in the interesterified

blend compared to the original one. However, in the case of sunflower oil and canola oil, the crystallization kinetic parameters were not affected by interesterification when compared as a function of the solid fat content [102]. In a Study by Soares et al., [103] chemical interesterification of palm stearin, coconut oil and canola oil blends led to a reduced softening and melting point of the blends.

Blends rich in palmitic and lauric acids, like palm stearin and palm kernel oil or coconut oil are the most commonly used interesterified fats by the European food industry. Typically, a hard stock is created from palm stearin and kernel oils and blended with vegetable oils [25].

2.1.3.1 Chemical interesterification

Two types of chemical interesterification methods are commonly used: random and directed. Reactions carried out at temperatures above the melting point of the highest-melting TAG in the mixture, results a complete randomization of FAs among all TAGs [104]. If the interesterification reaction is perfomed at temperatures below the highest-melting TAG, which is usually a trisaturated TAG, the reaction will result in a mixture enriched with this component. When the trisaturated TAG is produced, it will crystallize and precipitate from the solution which will push the equilibrium of the interesterification reaction towards an increased production of trisaturated TAGs [105].

Metal alcoholate catalysis is usually applied in chemical interesterification. Sodium methoxide is the most commonly used catalyst, as sodium alkylates are easy to use, inexpensive, only required in small amounts and active at relatively low temperatures (50 – 90 °C) [106]. However, they are extremely sensitive to moisture as water reacts with alkylate and produces the corresponding alcohol and completely inactivates the catalyst. The other disadvantages are that a pre-activation period is needed to form the active catalyst and the loss of oil due to saponification [107]. With dry oil without impurities the amount of catalyst needed is only 0.2 % (w/w) [108]. The chemical catalyst is added to the heated oil or fat in such a form that it disperses rapidly and completely [108]. The reaction is allowed to continue for a fixed time period before it is terminated by the use of water and sometimes acid [104].

2.1.3.2 Enzymatic interesterification

In enzymatic interesterification the modifications are carried out using random, regiospecific or fatty acid -specific lipases as catalyst [104]. These commercial lipases are from plant, animal or microbial origin [100]. Usually, lipases are classified into random (no regiospecifisity) and specific (1,3-regiospecific)

categories. Random lipases include *Candida rugosa* lipase, *Geotrichum candidum* lipase and *Staphylococcus aureus* lipase. Specific lipases include pancreatic lipase, *Mucor miehei* lipase, *Aspergillus niger* lipase, *Pseudomonas fluorescens* lipase and *Rhizopus arrhizus* lipase [104]. Enzymatic interesterification gives an increased amount of control over the reaction and the final product as a consequence of the specificity of the lipases compared to chemical interesterification.

Enzymatic interesterification offers milder reaction conditions compared to the chemical interesterification, resulting in lower degradation of polyunsaturated FAs [109] and fewer by-products [110-112]. The oil-water interface is the place where the lipase can acces the substrate and catalyze the interesterification reaction. The activity of the enzyme is increased by increasing the interface area, allowing more enzyme molecules to adsorb onto the oil-water interface [113]. When adsorbed, the enzyme is activated and the substrate can bind to the active site leading to catalysis of the interesterification reaction [113]. Because the lipases used in the enzymatic interesterification act at the oil-water interface, they must undergo a conformational change. During the conformational change, the active site of the enzyme is exposed for the substrate to bind [114]. The active site consists of a catalytic triad of asparagine/glutamine-histidine-serine, of which the serine is the reactive nucleophile and the other two amino acids improve the catalysis [114].

2.2 Effects of selected food processing methods on postprandial lipid metabolism

2.2.1 General overview of digestion and absorption of triacylglycerols

The orosensory detection of fat in the mouth already provokes signals that stimulate the digestion and absorption of lipids [115,116]. The lingual serous glands in mouth, secrete the lingual lipase into the oral cavity, starting the hydrolysis of TAGs during oral processing [117]. Digestion of dietary lipids continues in the stomach. As an acidic enzyme, the optimal pH for lingual lipase is around 4, but it is active also at a pH of 6 – 6.5 and therefore also works in the stomach until the pH in the stomach decreases [118]. When the dietary lipids arrive in stomach they are emulsified, which creates a larger lipid-water interface making it possible for the lipases to interact with the water-insoluble lipid droplets [95,96]. Protonated long chain FAs accumulate at the surface of lipid droplets and entrap gastric lipase, stopping its activity when the FA concentration reaches 121-172 μmoles/m2 interface area [119].

Gastric lipase is secreted by the chief cells of the fundic mucosa of the stomach. In healthy humans, gastric lipolysis leads to hydrolysis of 5-40 % of ingested TAGs, generating mainly free FAs and diacylglycerols [96,120,121]. Gastric lipase also ensures the emulsification of lipids and stimulates cholecystokinine secretion by releasing long-chain free FAs [122]. This enhances the lipid digestion in the duodenum by pancreatic lipase. Gastric lipase is active at pH range 3.0 – 6.0 which allows the enzyme to also function in duodenum [123]. TAG hydrolysis continues in the duodenum with the synergistic effects of gastric lipase and pancreatic lipases at a pH of around 6.0, leading to the formation of 2-monoacylglycerols (2-MAGs) and free FAs [121]. The effective work of pancreatic lipase in the presence of bile salts is dependent on co-lipase [122]. Co-lipase binds to the lipid droplets covered with bile salts making it possible for pancreatic lipase to bind to the lipid/water interface [124]. In the duodenum, pancreatic lipase hydrolyzes 40-70 % of TAGs and gastric lipase accounts for additional an additional 7.5 % of the lipolysis [96,125].

The digested lipids are solubilized in the lumen of the intestine by bile salt micelles and unilamellar vesicles composed mainly of phospholipids [126]. Uptake into enterocytes occurs either by passive diffusion or active transport [127,128]. Free FAs and 2-MAGs are transported through the cell cytoplasm with the aid of cytosolic proteins, like intestine-type fatty acid-binding protein and liver-type fatty acid-binding protein, and are re-esterified to form TAGs mainly through the MAG pathway [128-130]. However, if the supply of 2-MAGs is insufficient the glycerol-3-phosphate pathway becomes the major pathway for the formation of TAGs [95]. The assembly of synthesized TAGs takes place in the endoplasmic reticulum of the enterocyte leading to formation of lipoproteins, mainly chylomicrons, after a meal [95]. Chylomicrons are formed with TAG, cholesteryl esters and free cholesterol in the lipid core and phospholipids and apolipoproteins B-48, A-I and A-IV on the surface. Chylomicrons are released from the enterocytes to the lymph glands, from where they reach the blood which transports the TAGs to the adipose tissue, skeletal muscle and myocardium [131]. A summary of TAG digestion and absorption is shown in **Figure 4**.

The absorption of medium chain FAs (MCFA), with 8 – 10 carbons, differs from that of the long chain FAs. MCFAs are absorbed in the intestine and transported directly to the liver via the portal vein. In the liver, the MCFAs are rapidly metabolized by β-oxidation [132,133]. The postprandial TAG response is reduced since the MCFAs are not absorbed and released as a component of chylomicrons into the circulation [132].

Lingual lipase prefers TAGs with short or medium chain FAs over long chain FAs [134]. Gastric lipase has a higher affinity for FAs in the *sn*-3 position compared to FAs in *sn*-1 position and it prefers medium chain FAs [135]. Pancreatic lipase prefers FAs in the *sn*- 1 position, but also hydrolyzes FAs from

the *sn*-3 position and favours diacylglycerols over TAGs [135,136]. The structure of TAGs highly affects the activity of lingual, gastric and pancreatic lipases as the enzymes release short and medium chain FAs quicker than long chain FAs located in the *sn*-1 and *sn*-3 positions [137]. Absorption of FAs is also affected by the structure of the TAG as FA esterified in the *sn*-2 position are effectively absorbed as 2-MAGs. Long chain saturated FAs especially, are poorly absorbed when located at the *sn*-1 or *sn*-3 position and when realeased from the glycerol backbone, they tend to form insoluable soaps with calcium and magnesium cations in the small intestine [138,139]. Of the FAs in the *sn*-2 position, approximately 75 % are retained from the dietary TAGs during the re-esterification process in the enterocyte, whereas the FAs in the *sn*-1 and *sn*-3 positions are randomized and, also partly come from the endogenous FA pool [140-142]. In addition, the structure of the TAG affects the lipid droplet size and emulsification properties. In fact, the emulsification properties of TAGs increase with the number of carbons and unsaturation level of FAs attached to the glycerol backbone [95].

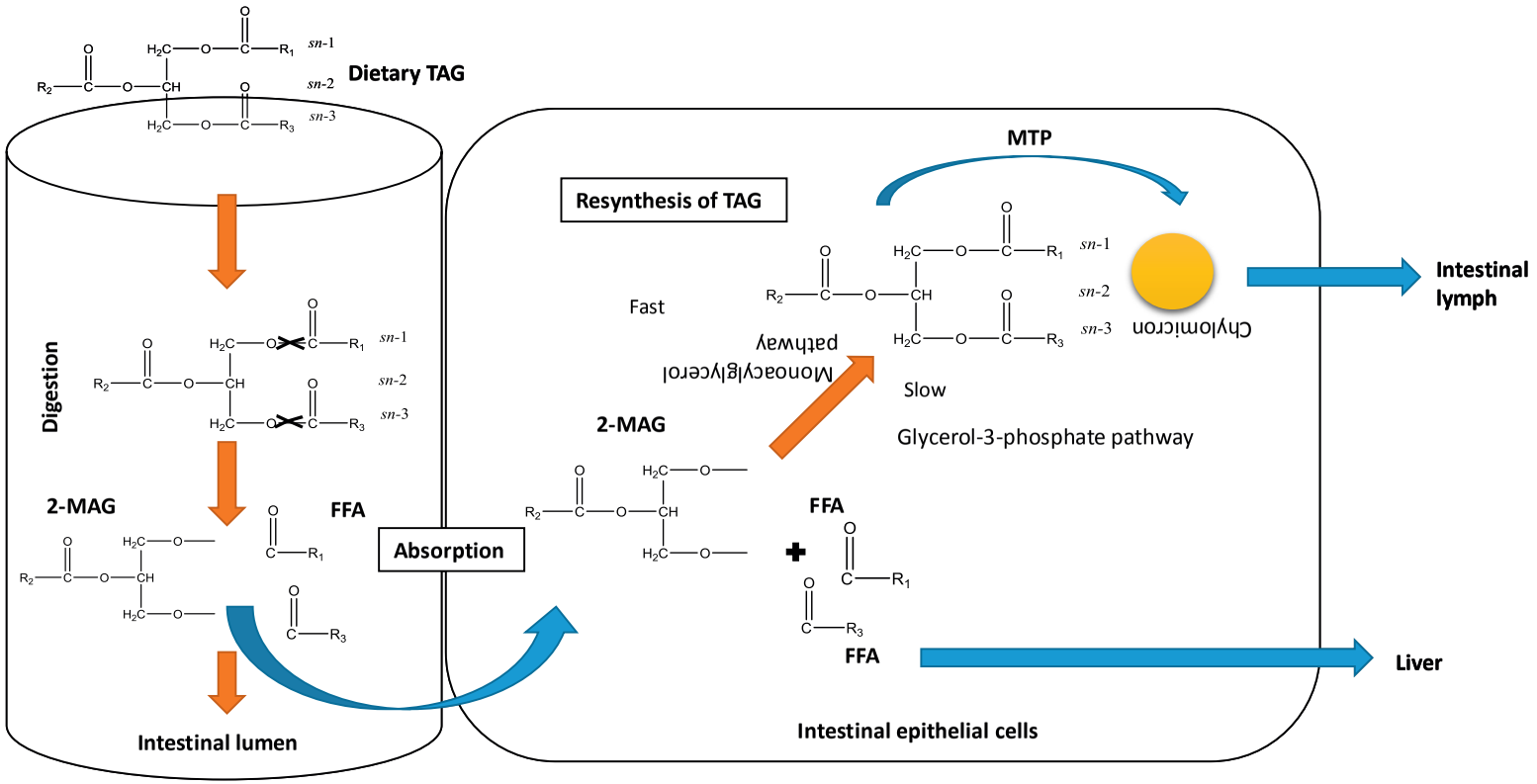

Figure 4. Digestion and absorbtion of dietary TAG. Dietary TAGs are disgested as FFAs and 2-MAGs in the intestinal lumen. Lipases hydrolysate FAs from the *sn*-1 and *sn*-3 positions and the FFAs and 2-MAGs are then absorbed by intestinal epithelial cells. Here, 2-MAGs are rapidly resynthesize to TAGs which are then packed into chylomicrons by MTP and transported in the intestinal lymph. FFAs, via the glycerol-3-phosphate pathway, could also be transported by the portal system to the liver for oxidation. TAG, triacylglycerol; 2-MAG, 2-monoacylglycerol; FFA, free fatty acid; MTP, microsomal triacylglycerol transfer protein. Adabted from Alfieri et al., 2008.

2.2.2 Effect of lipid oxidation products on postprandial lipid metabolism

There is a possibility that the Western diet contains large quantities of oxidized fatty acids, oxidized cholesterol and their breakdown products because a large proportion of food in the diet is consumend in a heated, and long-stored form [48]. **Table 3** summarize the health effects of oxidized lipids. During food processing and storage reactions leading to lipid oxidation occur and the gastrointestinal (GI) tract and especially the stomach is affected by the oxidized food compounds. Additionally, it has been hypothesized that the stomach and its gastric fluids could be a medium for further lipid oxidation or antioxidation [143,144]. Knowledge of the transport of lipid oxidation products from the food into the circulation is restricted. A limited number of studies have documented lipid peroxides in the chylomicrons [145,146] and plasma [147,148] of humans after a diet rich in lipid peroxides. Ursini et al. [147] discovered a marked increase in plasma lipid hydroperoxides in humans during the postprandial period after a high fat meal. Moreover, ingestion of lipid peroxides from cooked meat during a 7-day trial, has been shown to create increased amounts of malondialdehyde in the urine [149]. Wilson et al. [148] investigated the absorption of [U-^{13}C]-labeled hydroxy and dihydroxy TAGs, markers of lipid peroxidation, in healthy women. The labeled hydroxy TAGs were absorbed and found in the postprandial plasma, with monohydroxy at higher concentrations than dihydroxy, indicating better absorption of the former. A Study by Staprans et al. [146] demonstrated that in humans dietary oxidized lipids are absorbed in the small intestine, incorporated into chylomicrons and appear in the bloodstream where they contribute to the total body pool of oxidized lipids. The amount of oxidized FAs in the test meal, in the form of heated corn oil, was proportional to the quantity of oxidized FAs found in the chylomicrons, measured as conjugated dienes [146]. The same group demonstrated in that the oxidized dietary FAs significantly increased the levels of oxidized FAs in chylomicrons of diabetic patiens with poor glycemic control, compared to levels in healthy controls or in diabetic patiens with good glycaemic control [145].

The presence of oxidized lipids in the chylomicrons may have adverse consequenses, as oxidized lipids are metabolized in a similar manner to non-oxidized lipids and thus with a substantial portion of oxidized lipids reaching the liver. Oxidized lipids derived from chylomicrons have been shown to be incorporated into very low-density lipoproteins (VLDL) [150] and oxidized lipoproteins are important in the development of atherosclerosis [18,151,152]. Ahotupa et al. [17] demonstrated that food derived lipid peroxides are incorporated into triacylglycerol-rich lipoproteins and low-density lipoproteins (LDL), directing the flow of lipid peroxides towards peripheral tissues. They also

speculated that high-density lipoprotein (HDL) tends to have the opposite function by transporting lipid peroxides in the reverse direction and thus having a protective role as regards oxidative stress [17]. This transport of atherogenic lipid peroxides by serum lipoproteins may partly explain their specific role in atherosclerosis.

Oxidized cholesterol has been found to be one type of oxidized lipid in the lipoproteins in human serum [153]. Staprans et al. [153] found serum levels of α-epoxy cholesterol to correlate with the α-epoxy levels in the diet. Additionally, no α-epoxy structures were found in the serum samples of subjects on non-oxidized cholesterol diet. α-Epoxy cholesterol was found in the chylomicrons and chylomicron remnants as well as in LDL, VLDL and HDL particles. After administration of the test meal, epoxy cholesterol was present in all lipoprotein fractions over a 10 h period and LDL exhibited the highest levels. Oxidized cholesterol levels in the serum remained elevated for 72 h mainly in other lipoprotein fractions than chylomicrons. It seems that α-epoxy cholesterol from the diet is first incorporated into chylomicrons or chylomicron remnants and then with the help of a cholesteryl ester transfer protein transferred to LDL and HDL contributing to lipoprotein oxidation [153]. Wilson et al. [154] also investigated the absorption of [U-^{13}C]-labeled epoxy and diepoxy FAs. Monoepoxy FAs were better absorped compared to diepoxy FAs and their absorption was simultaneous with normal FAs. The difference in the absorption of mono- and diepoxies is important when concidering the relative toxicity of epoxidized material in the food chain.

It seems that lipid oxidation products are digested and absorbed from the diet and quite easily incorporate into the lipoproteins. Circulating lipid oxidation products could promote atherosclerosis and increase the oxidative stress in the body.

Table 3. Effect of lipid oxidation products on human health.

COMPOUND CLASS	CONSEQUENSES ON HUMAN HEALTH	REFERENCE
Oxidized lipids	Promotes the risk of atherosclerosis	Staprans et al., 1994, 1999
Lipid hydroperoxides	Proinflammatory, oxidize lipoproteins, promote the risk of atherosclerosis, and increase foam cell formation	Urisini et al., 1998; Wilson et al., 2002; Ahotupa et al., 2010; Kanner 2007
Oxidized cholesterol	Oxidize lipoproteins, promote the risk of atherosclerosis	Staprans et al., 2003; Wilson et al.,2002b; Birukov et al., 2006; Vasankari et al., 2001

2.2.3 Effect of the lipids in processed milk on postprandial lipid metabolism

Knowledge about the impact of milk fat globule structures on digestive lipolysis has recently increased and interest has grown concerning its effects on lipid bioaccessibility and intestinal lipid absorption. *In vitro* studies have revealed that human gastric and pancreatic lipases hydrolyse TAGs from small droplets faster than from large droplets (0.5 vs. 3 μm) [155,156]. Smaller droplets result in greater lipolysis due to their larger surface area [95,96] and homogenization results in a smaller lipid dropled size compared to raw milk, which may favor the lipolysis of homogenized milk. During digestion of homogenized milk in the stomach, coagulation of lipid droplets and casein proteins occurs [157]. The coagulation of homogenized milk differs from that of raw milk in the stomach as the much finer and casein-coated lipid droplets of homogenized milk are likely to interact with the casein matrix of the gastric clot [158]. *In vitro* studies using simulated human gastro-duodenal digestion [159], recombinant human pancreatic lipase and pancreatic lipase proteins 1 and 2 [63] and pancreatic-lipase-catalyzed digestion [62] have revealed that the small native MFGs present in both raw and homogenized milk as well as MFGs in homogenized milk are more rapidly hydrolyzed by the gastric and pancreatic lipases compared to large native MFGs; this is due to the increased surface area for lipase adsorption. According to Berton et al. [63] there is a lag phase in the hydrolysis of native MFGs due to the phospholipid layer of MFGM, which prevents the access of the pancreatic lipase to the interface. With the homogenized milk, there is no lag phase and the interface is directly accessible to the pancreatic lipase. Armand et al. [96] demonstrated in healthy subjects that smaller lipid droplets (0.7 μm) are indeed lipolyzed more efficiently compared to larger ones (10 μm), however, the overall chylomicron response did not differ between the two emulsions with different droplet sizes.

Differently structured dairy products seem to have different effects on the profiles of postprandial lipemia. Vors et al. [160] hypothesized that postprandial lipemia is modulated by the physicochemical structure (emulsified or non-emulsified) of fat. They served emulsified and non-emulsified milk fat to healthy subjects and revealed that the emulsified milk fat resulted in an earlier and sharper increase in plasma chylomicron rich fraction TAGs compared to the non-emulsified milk fat. Clemente et al. [161] investigated the effects of three fat-rich meals with similar composition but different physical structure on postprandial lipemia. They showed that, in type 2 diabetic patients, the area under the postprandial plasma TAG concentration curve did not differ after milk, mozzarella cheese and butter but the timing of the TAG peak did. The peak time after butter and mozzarella cheese was significantly delayed compared to milk.

Sanggaard et al. [162] investigated the effect of whole milk and fermented milk (both homogenized) on postprandial lipid metabolism in men. After the fermented milk ingestion, the gastric emptying rate was slower than after whole milk. However, whole milk showed a slower, lower and longer rise in postprandial TAG compared to fermented milk, which resulted in a higher increase in TAG in the lipoprotein fraction. The impact of the structure of dairy products on lipemia remains controversial and the presently published results warrant further research in this field.

2.2.4 Effect of interesterified vegetable oils on postprandial lipid metabolism

FAs may have different absorption patterns based on chain length and saturation as well as on the regiospecific position on the TAG molecule [11]. The melting temperature of FAs depends on their chain length and unsaturation. Saturated FAs with a long chain length have the highest melting point, above 40 °C [97]. Palmitic and stearic acids have lower coefficients of absorption than unsaturated FAs, because their melting points are above the body temperature [11] and they can form insoluble soaps with calcium and magnesium ions when cleaved from the *sn*-1 and *sn*-3 positions. Thus, they could be theoretically better absorped from the *sn*-2 position compared to *sn*-1/3. In addition, the position of the FA on the TAG molecule affects the melting temperature of TAG. Unlike pure compounds, natural fats and oils are mixtures of different TAGs and they do not have a single melting point but a melting and crystallization range [97]. The presence of a solid fat phase affects digestion, absorption and metabolism of dietary lipids by limiting the enzymatic hydrolysis of the TAG and then their absorption [163,164]. Hence, mixtures of TAG with the same FA composition but a different regiodistribution with different melting and crystallization properties and percentage of lipids at the solid state at body tempertature may present different rate of digestion leading to different FA absorption kinetics and bioavailability [97,163,164]. For instance, after consumption of sunflower oil, which has 0 % of solid lipids at body temperature, the kinetics of postprandial plasma TAG is higher compared to shea butter, which has 22 % of solid lipids at body temperature due to the high concentration of stearic acid [163,165].

Many studies have compared the effect of interesterified fat to a native fat on postprandial plasma lipid profiles in humans, as shown in **Table 4**. Yli-Jokipii et al. [10] investigated the effects of palm oil and interesterified palm oil on postprandial lipid response in healthy women. They revealed that the positional distribution of FAs in chylomicrons TAGs was reflective of the dietary fats and

that native palm oil resulted in larger incremental area of total TAGs in postprandial plasma compared to the interesterified palm oil.

In a Study by Sanders et al., [9] 50 healthy subjects were served palm olein and interesterified palm olein (39.1 % 16:0 at *sn*-2), lard (70.5 % 16:0 at *sn*-2) and high oleic sunflower oil (HOS) on four separate occasion to investigate whether lard and interesterified palm olein, rich in palmitic acid in the *sn*-2 position, decreases postprandial lipemia compared to native palm olein and HOS. The iAUC of plasma TAGs was significantly lower after lard compared to palm olein and HOS, but it did not differ between interesterified palm olein, palm olein and HOS. Furthermore, the plasma TAG increase was slower after lard and interesterified palm olein compared to the other two fats. Hall et al. [166] discovered that in older men (age 40 – 70) with a fasting TAG concentration higher than optimal, the interesterified palm oil decreased the plasma TAG concentration during the first 4 h of the postprandial period compared to native palm oil. During the 4 – 6 h postprandial period the chylomicron concentration was slightly reduced after interesterified palm oil compared to native palm oil. However, no significant differences were found in the plasma intermediate-density lipoprotein fraction TAGs, cholesterol or apolipoprotein B-48 between the meals over the total 6 h postprandial period [166].

In another Study of Hall et al., [167] a mixture of palm stearin and palm kernel oil (80:20), rich in palmitic and lauric acids, was interesterified and fed to male subjects (age 18 – 45). A non-interesterified mixture of the same oils was used as a control. Contrary to their earlier Study, the increase in the postprandial serum TAG concentration was higher after the interesterified blend versus non-interesterified blend and the iAUC for the plasma TAG was 51 % lower after non-interesterified blend compared to the interesterified blend [166]. Moreover, following the ingestion of the interesterified blend the postprandial plasma TAG concentrations peaked 3 hours after the meal and started to decline after that. With the non-interesterified blend, the rise of the postprandial TAG concentration was slower and it did not reach peak concentration during the 4 hour postprandial period [167]. The solid fat content of the non-interesterified blend was higher (24 %) than that for the interesterified blend (21 %), and the percentage of palmitic acid at the *sn*-2 position was 54 % for the non-interesterified blend and 36 % for the interesterified blend. The higher solid fat content and more palmitic acid at outer positions might explain the lower postprandial lipemia after non-interesterified blend.

Robinson et al. [168] compared the effects of chemical and enzymatic interesterification on acute postprandial lipid responses in male subjects (age 40 – 70) which were either obese with a high risk of type 2 diabetes and cardiovascular diseases or non-obese controls. A stearic acid-rich fat blend of HOS and canola stearin (70:30) was used as test fat and as interesterified with

two different methods – chemically and enzymatically with non-specific *Candida Antarctica* lipase. In the non-obese controls the interesterification did not affect the postprandial TAG concentration compared to the non-interesterified blend. The obese subjects had an 85 % higher postprandial plasma TAG concentration after the chemically interesterified blend compared to the non-interesterified blend. No differences were seen in the obese group between enzymatically interesterified and non-interesterified blend or between enzymatically and chemically interesterified blends. Serum oleic acid concentrations were significantly increased after the non-interesterified blend in both groups, whereas, serum oleic and stearic acid concentrations were increased after both interesterification treatments in both groups [168].

High proportions of palmitic acid in a *sn*-2 position seem to decrease postprandial lipemia in healthy subjects. However, there might be differences in the responses to interesterification between healthy individuals and obese individuals who are already at risk for type 2 diabetes and cardiovascular diseases. In addition, the solid fat content of the fat at 37 °C appears to be a major determinant of the postprandial response of the interesterified fat.

Table 4. Effect of interesterification of vegetable oils on human health.

FAT SOURCE	SUBJECT GROUP	CONSEQUENCES ON HUMAN HEALTH	REFERENCE
Interesterified palm oil	Young, healthy men; Older men with increased fasting TAG; Healthy, premenopausal women	Reduced postprandial lipemia compared to native palm oil	Sanders et al. 2011; Hall et al., 2014;
Interesterified palm stearin and palm kernel oil 80:20	Healthy men	Increased postprandial plasma TAG concentration	Hall et al., 2017
Interesterified blend of high-oleic sunflower oil and canola kernel 70:30	Men; obese with risk of T2D or CVD or non-obese control	Non-obese: no effect on posptrandial TAG concentration Obese: chemical interesterification increased postprandial TAG concentration comparered to blend of native oils	Robinson et al., 2009

2.3 Gastrointestinal symptoms related to milk consumption

GI symptoms triggered by food have increased in frequency in the western countries [1,2]. Milk and dairy products are often reported as a source of GI discomfort. Lactose intolerance, where bacteria in the colon ferment undigested lactose into short chain FAs, hydrogen, methane and carbon dioxide resulting in symptoms such as abdominal pain, bloating and diarrhea is an extremely common condition worldwide [169]. The activity of the lactose hydrolyzing enzyme lactase drops in the intestine leading to lactose malabsorption and to symptoms of lactose intolerance [170]. Lactose intolerance can be tested by lactose challenge [171] and/or hydrogen breath test [172].

An increasing population of people claim to experience GI symptoms related to milk consumption even when neither lactose intolerance nor cow´s milk allergy can be diagnosed [173-175]. Processing of milk has been suggested to be the trigger of the GI symptoms in milkhypersensitive individuals [173]. Homogenization of milk not only decreases the size of MFGs, but also introduces caseins and whey proteins at the interface [13,93,176,177]. The denatured casein and whey proteins at the surface of MFGs could be more allergenic than intact proteins in raw milk [175]. One theory is that in milkhypersensitive individuals processed milk could increase the levels of circulating inflammation markers and thus induce chronic inflammation in the GI tract leading to discomfort. Yet, the scientific evidence on the matter is conflicting [175,178,179]. The known risks of raw milk consumption are associated with foodborne pathogens [180]. The consumption of raw milk has been justified with reductions of GI symptoms, however, the scientific evidence is lacking [172].

Several research groups [172,174,175,179,181] have investigated the GI symptoms and inflammation marker levels as response to raw milk and differently processed milk samples. Raw milk in relation to pasteurized milk and pasteurized and homogenized milk did not reduce the GI symptoms in lactose intolerant subjects [172,181]. When raw milk and homogenized and pasteurized milk was served to milkhypersensitive but not lactose intolerant subjects no significant differences were seen in the symptoms between the milk samples. Approximately half of the 44 subjects tolerated the processed milk better and the other half tolerated the raw milk better [174].

Conflicting results have been reported in relation to inflammation related compounds and milk hypersensitivity. Increased phagocyte activity and complement receptor expression of phagocytes was detected in milkhypersensitive subjects [179]. However, complement receptor expression (CR1 and CR3, indicators of milk induced immunological changes) failed to differ between homogenized milk, non-homogenized milk and milk-free control

drink in any of the three subject groups (lactose intolerant, milkhypersensitive and control) studied [175]. Lack of significant results from the studies could be due to relatively small group sizes.

2.4 Postprandial inflammation response

Food and food components initiate an acute innate immune response that only lasts for few hours but is repeated several times during a day following eating [16]. The probable importance of postprandial inflammation response in the induction of insulin resistance, metabolic syndrome and atherosclerosis has only recently been discovered [182-184]. The most efficient triggers of postprandial inflammation seem to be TAGs, saturated FAs, oxysterols and glucose [185-188]. Meals may contain oxidative components which upon absorption could initiate oxidative stress/inflammatory responses [16]. Several endo- and exogenous modifiers influence the levels of inflammation markers and cause significant between-individual variations. These modifiers include: age, body fatness, physical activity or inactivity, sex, genetics, smoking, diet, the composition of the gut microbiota and certain medications [156].

Common blood cellular markers of inflammation include various subclasses of leucocytes. During inflammation response increased numbers of leucocytes are found in the blood stream as well as increased concentrations of inflammatory cytokines (TNF, IL-1β, IL-6 and IFN-γ) and chemokines (IL-8, MCP-1) [189]. **Table 5** summarises the biological functions of 14 common markers of inflammation.

Table 5. Common blood markers of inflammation and their biological functions[1].

INFLAMMATION MARKER	BIOLOGICAL FUNCTION(S)
TNFα	Cytokine; important role in systemic inflammation and acute phase reaction; regulate inflammation cells
MCP-1	Chemokine secreted by monocytes, macrophages and dendritic cells; involved in several inflammation related diseases; Leucocyte recruitment
IL-1α	Central role in the regulation of immune responses, in infection responsible for initiation of inflammation and promotion of fever
IL-1β	Promotion of fever; neutrophil attraction and activation; B and T lymphocyte activation; acute-phase protein synthesis by liver
IL-12	Pro-T helper cell 1 (Th1) and anti-Th2 function; Th1 and natural killer cell (NK) proliferation; stimulation of T and NK cell cytotoxic activity
IL-17A	Potentiates chemokine and pro-inflammatory cytokine release
IL-18	Induction of IL-1β, INF-γ and TNF production, in combination with IL-12 suppresses Th2-mediated B-cell responses
IL-6	Promotion of fever; neutrophil and lymphocyte activation; inhibition of TNF and IL-1 and activation of IL-10 secretion
IL-10	Decreases pro-inflammatory cytokine expression; improves B-cell survival and antibody production
IL-4	Cytokine; induce differentiation of Th0 cells to Th2; stimulate activated B cells and T cells proliferation
IL-5	Stimulates B cell growth; increases immunoglobulin secretion
INF-λ	Antiviral activity
INF-γ	Natural killer cell and macrophage activation; promotes T helper 1 cell (Th1) differentiation and suppresses Th2 differentiation; viral replication inhibition
CRP	Host defence by clearance of pathogens and dead cells by opsonisation and complement activation

[1]Collected from references [16,189,190].

3 AIMS OF THE STUDY

The hypothesis of the study was that processed dietary lipids have an effect on the postprandial lipid metabolism. The primary aim of the study was to investigate the effects of the heating of beef, the homogenization, pasteurization and UHT treatment of milk and the interesterification of palm oil on postprandial lipid metabolism. The second aim was to study whether these processing methods contribute to gastrointestinal symptoms and inflammation. Cow's milk and beef steak were chosen as model foods as they are commonly consumed in the Nordic diet and palm oil because it is globally used in the food industry.

The objectives of the individual studies were to:

I. Investigate the impact of different methods of thermally processing beef steak on lipid oxidation and human postprandial inflammation-related responses.

II. Investigate the effect of homogenized and pasteurized cow's milk versus raw cow's milk on gastrointestinal symptoms, intestinal pressure, postprandial lipemia and inflammation markers in sensitized subjects.

III. Investigate the effect of different thermal treatments and homogenization of cow's milk on gastrointestinal symptoms, postprandial lipemia and inflammation markers in sensitized subjects.

IV. Investigate the effect of interesterification of palm olein on postprandial lipid metabolism in healthy individuals and in individuals with metabolic syndrome.

4 MATERIALS AND METHODS

4.1 Study design and ethical considerations

A randomized cross-over design was used in all the studies. Subjects consumed the Study meals after an overnight fast on two (I, II), three (III) or four (IV) occasions separated by one (I, IV) or two (II, III) weeks. The Study plans were approved by the Ethics Committee, Hospital District of South-West Finland, Finland (II, III) and University of Auckland Human Participants and Ethics Committee, New Zealand (I) and the Southern Health and Disability Ethics Committee, New Zealand (IV). Each subject gave their written informed consent for the Study, and each subject was free to discontinue their participation at any point in the Study without explanation.

Blood samples were drawn from an antecubital vein at 0, 60, 120, 180, 240 (I) or 0, 20, 40, 60, 90, 120, 180, 240 (II) or 0, 20, 40, 60, 90, 120, 180, 240, 300 (III) or 0, 30, 60, 90, 120, 180, 240, 300 (IV) minutes postprandially.

4.2 Model foods

Sous-vide beef rump steak (I), pan-fried beef rump steak (I), raw milk (RM) (II), pasteurized milk (PM) (III), homogenized and pasteurized milk (HPM) (II, III), UHT treated and homogenized milk (UHTM) (III), palm olein (PO) (IV) and interesterified palm olein (IPO) (IV) as well as beef tallow (BT) (IV) and high-oleic sunflower oil (HOS) (IV) were studied. RM, PM, HPM and UHTM samples originated and were processed at the Natural Resources Institute Finland. Sous-vide and pan-fried beef rump steaks were purchased from the Waikato region, New Zealand and were processed at the University of Auckland, New Zealand. PO and IPO were sustainably produced and originated from Malaysia were the chemical interesterification was also conducted. HOS originated from Malaysia and beef tallow was of New Zealand origin.

4.3 Subjects

Volunteers were recruited from the urban area of Auckland, New Zealand (I, IV) or from the district of Turku, Finland (II, III).

For Study I the volunteers (twelve male) were healthy, aged between 18-25 years and with a BMI between 18.5-24.9 kg/m^2.

For Study II the volunteers (five males and six females) self-reported to be sensitized to cow's milk but otherwise healthy, were aged between 24-68 years,

with a BMI between 20.2-30.9 kg/m^2 and met the following criteria: normal liver, kidney and thyroid function, no history of cardiovascular diseases, diabetes or any GI conditions including history of gastric bezoar, suspected strictures, fistulas, GI obstruction, GI surgery within the past three months, no dysphagia, no Crohn's disease or diverticulitis, no implanted or portable electro-mechanical medical devices, no regular medication, no trouble with swallowing, no coeliac disease, no lactose intolerance diagnosed by a medical doctor, not a regular smoker and not having participated in another intervention two months prior to the present Study. Four out of the 11 subjects were carriers of the C/C genotype which is associated with low lactase enzyme activity. Four of the 11 were carriers of the C/T genotype and three subjects had the T/T genotype. C/T and T/T genotypes are linked with lactase persistency [191].

For Study III, the characteristics of the volunteers (six males and eight females) self-reported to be sensitized to milk but otherwise healthy, between 20-45 years of age, with a BMI between 19.3-28.5 kg/m^2, met the following criteria: normal liver, kidney and thyroid function, no history of cardiovascular diseases, diabetes or any GI conditions or GI surgery within the past three months, no dysphagia or Crohn's disease or diverticulitis, no implanted or portable electro-mechanical medical devices, no regular medication, no coeliac disease, no lactose intolerance diagnosed by medical doctor, not a regular smoker and not having participated in another intervention two months prior to the present Study. Three out of the 14 subjects were carriers of the C/C genotype. Nine were carriers of the C/T genotype and two had the T/T genotype.

For Study IV the volunteers (42 males) consisted of subjects with metabolic syndrome and healthy controls. The characteristics of the healthy controls (n = 19) were: healthy, 45-60 years of age, BMI 18-25 kg/m^2, waist circumference < 90 cm, fasting triacylglyserols < 1.66 mmol/L, HDL > 1.03 mmol/L, systolic blood pressure < 130 mmHg and diastolic blood pressure < 85 mmHg, fasting plasma glucose < 5.6 mmol/L, normal liver and kidney functions. The characteristics of the subjects with metabolic syndrome (MetS, n = 19) were: 45-60 years of age, BMI > 25 kg/m^2, waist circumference ≥ 90 cm, fasting triacylglyserols ≥ 1.66 mmol/L, HDL < 1.03 mmol/L, systolic blood pressure ≥ 130 mmHg and diastolic blood pressure ≥ 85 mmHg, fasting plasma glucose ≥ 5.6 mmol/L, normal liver and kidney functions.

The subjects in all four studies were asked to fast overnight (10 hours) and advised to restrain from vigorous exercise the day prior to the intervention day. The subjects were advised to eat and drink normally and except for Studies II and III the subjects were on a non-dairy diet five days prior to intervention and one to three days after the intervention day. In Study IV, the subjects were given a standardized low fat meal of pasta Bolognese (640 kcal, 32 g protein, 10 g fat and 105 g carbohydrate) on the night before their intervention.

4.4 Test meals

In Study I, 270 ± 20 grams of pan-fried or sous-vide cooked beef rump steak containing 11.5 ± 0.9 grams of fat was served together with two slices of commercial white bread (50 g; 1.0 g fat) and commercial tomato sauce (20 g). The meal was consumed within 15 minutes. For pan-frying, a non-stick frying pan was pre-heated to 240 °C and the steak was grilled for 3 minutes on one side and 2 min on the other side. For sous-vide, the water bath was pre-heated to 80 °C and the vacuum packed steak was immersed and maintained under water for six hours. The steaks were immediately served to the subjects. The moisture, ash, protein and lipid content of the steaks were measured according to the Association of Official Analytical Chemist [192].

In Study II, 4 dl of RM or HPM was served together with a cereal bar (72 g: 2 g of fat, 50 g of carbohydrate and 2 g of fiber) and an ingestible pressure and pH measuring probe, the SmartPill® capsule. The meal was consumed within 10 minutes. Both milk samples were served cold from paper cups covered with a lid and aluminum foil. Milk samples were well shaken before serving. A straw was used to drink the milk to make the mouth feel as similar as possible for both samples. The milk was first homogenized with a two-stage homogenizer and then pasteurized. The homogenization pressure was 160 bars and the pasteurization conditions were 72 – 73 °C for 15 seconds. The raw milk was chilled and packed unprocessed.

In Study III, 4 dl of PM or HPM or UHTM was served together with 24 g rice cakes, 85 g turkey cold cuts and 50 g cucumber. The meal was consumed within 15 minutes. All three milk samples were served cold from paper cups covered with a lid and aluminum foil. Milk samples were shaken before serving. A straw was used to drink the milk to make the mouth feel as similar as possible for all three samples. Pasteurization conditions were 72 – 73 °C for 15 seconds and UHT treatment was done at a temperature of 135 °C for 3 seconds. Homogenization was done prior to pasteurization and UHT treatment at a pressure of 160 bars. Packing of the milk samples was done aseptically.

In Study IV, four muffins containing 50 g of either PO, IPO, BT or HOS together with a milkshake of 220 ml skimmed milk and 15 g Nesquik milkshake mix (Nestlé New Zealand, Auckland New Zealand) was served providing 846 kcal; 14 g protein, 85 g carbohydrate, 50 g fat. The meal was consumed within 10 minutes. The muffins were baked at the Human Nutrition Unit, University of Auckland, New Zealand and they were frozen prior to use and thawed before each intervention day. The milkshake was mixed from skimmed milk and milkshake mix in the morning of each intervention.

4.5 Methods

4.5.1 Clinical analysis

For Studies II and III, plasma insulin, glucose and TAGs were analyzed with a Cobas 8000 (Roche Diagnostics, Basel, Switzerland) at Turku University Hospital Laboratory, TYKS lab, Finland. Insulin was analyzed with electro-chemiluminescence immunoassay. Plasma glucose was analyzed enzymatically with a hexokinase assay. Plasma TAGs were analyzed enzymatically with a colorimetric method. Incremental areas under the response curves were calculated using trapezoidal rule. For Study IV, plasma TAGs and chylomicron rich fraction (CMRF) TAGs were measured at the Liggins Institute, University of Auckland, New Zealand, using a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan) by an enzymatic colorimetric assay (Roche, Mannheim, Germany).

Indicators of low grade inflammation including a tumor necrosis factor alpha (TNFa), a monocyte chemotactic protein-1 (MCP-1) and interleukins 1α, 1β, 4, 5, 6 and 10 and interferon λ (INFλ) were analyzed using a flow cytometric multiplex array (Milliplex® MAP Kit Human Metabolic Hormone Magnetic Bead Panel Assay; Millipore, Billerica, MA) for Study I or by using a Q-Plex™ High Sensitivity Human Cytokine Array (Quansys Biosciences, West Logan, Utah) for Study II, as instructed by the kit provider. High-sensitivity C-reactive protein (CRP) was analyzed by immunoturbidimetry (Roche Diagnostics, Mannheim, Germany) in Study I and by using high sensitive ELISA-kit (IBL International EU59151, Hamburg, Germany) in Study II. For Study III, 92 biomarkers of inflammation were analyzed using cDNA-based multiplex immunoassay (Olink Proteomics, Uppsala, Sweden). For the same Study, leukocytes were counted with an automatic cell count (TYKS lab, Turku, Finland).

In Studies II and III the activity of the lactase enzyme was investigated. The ability to digest lactose is associated with SNP rs4988235 located in the MCM6 gene in European Caucasian populations [193]. Sanger sequencing was used to genotype this SNP from the blood samples in order to investigate adult-type hypolactasia in the subjects. For genotyping, Qiagen's blood and tissue kit was used to extract DNA from the blood samples. Altogether 400bp around rs4988235 was amplified using the primer pair 5'-ACCCCCTTTTCAAAGACGAC and 5'-TGCTCATACGACCATGGAAT. An amplified DNA fragment was sequenced and individual genotypes were determined from the chromatograms.

In Study I the diene conjugation, resembling the amount of lipid peroxidation, was measured separately from the HDL fraction and the non-HDL fraction of plasma as explained later in section 4.5.2.

4.5.2 Lipidomic analysis

The meat samples from Study I were homogenized with Ultra Turrax (IKA) and the muffins and milkshake from Study IV were blended with a domestic blender before lipid extraction. CMRF (IV) containing chylomicrons and their large remnants, were separated from the plasma by their density [194] in 4.7 ml OptiSeal tubes (Beckman Coulter, CA) using an Optima MAX-XP ultracentrifuge with a BTA-110 rotor.

Lipids were extracted with chloroform/methanol (2:1 v/v) (I, II, III, IV) [195]. TAGs were separeated from the lipid mixture using silica columns and solvent mixtures with different polarities [196] (I, II, III, IV).

Fatty acid methyl esters of TAGs were prepared using sodium methoxide catalyzed transesterification [197] (I, II, III, IV) and analysed by gas chromatography (column DB-23, 60 m, i.d. 0.25 mm, liquid film 0.25 µm, Agilent technologies, J.W. Scientific, Santa Clara, CA). A Shimadzu GC-2010 gas chromatograph equipped with a flame ionisation detector (Shimadzu Corporation, Japan) was used to analyze the FA composition.

Diene conjugation (I) was measured by dissolving the extracted lipids in 2,2,4-trimethylpentane (meat) or cyclohexane (HDL and non-HDL fractions). The absorbance was measured spectrophotometrically at 234 nm for meat and 300-220 nm for HDL and non-HDL fractions. The HDL fraction was isolated and precipitated from the plasma by phosphowolframate/$MgCl_2$ method [198].

Secondary lipid oxidation was determined using the 2-thiobarbituric acid reactive species (TBARS) method [199].

For UPLC-ESI/MS analyses of oxidized lipids (I) a Waters Acquity UPLC was used with Waters Quattro Premier triple quadrupole mass spectrometer equipped with electrospray ionisation (ESI) probe (Waters co., Milford, MA). Full scans of mass range of m/z 190 to 1100 were acquired in positive ionisation mode. Amounts of oxidised lipids in the samples were evaluated by extracting selected ion chromatograms from the total ion current mass chromatograms and comparing the integrated areas of the peaks. Oxidised lipids were identified by retention times and mass spectra. Synthesised, oxidised triacylglycerol standards were used as references. Minimal handling of samples was needed as no derivatization was done before the UPLC-ESI/MS analysis minimazing the potential for unwanted oxidation during sample handling. The UPLC increased the speed of the analysis and enabled screening of larger sample amounts of

biological origin compared to the conventional HPLC. However, the high back pressure of UPLC analysis was a disadvantage. Formed by high flow rates and increased chromatographic resolution together with high-viscose solvents like isopropanol and water, the operating pressure increased near to the upper limit of the equipment.

4.5.3 Intestinal pressure, pH and temperature

The SmartPill GI Monitoring System® (Given Imagine, Yoqneam, Israel) was used to monitor the changes in pH, pressure and temperature in the GI tract (II). The system consists of an ingestible but non-digestible capsule (26mm x 13 mm), a receiver for acquiring and storing the signals from the capsule and the MotiliGI software for displaying the data on a computer. The SmartPill's pH sensor has an operating range of 1.0-9.0 ± 0.5 units, the pressure sensor has a range of 0-350 ± 5 mmHg and the temperature sensor has a range of 20-42 ± 1 °C. The software uses the change from the acidic gastric pH to the alkaline duodenal pH to determine the gastric emptying time. SmartPill technology is able to assess the transit times of the small bowel, the colon, and the whole gut. The aim (II) was to investigate whether there is a correlation between the severity of GI symptoms and pressure measured in the gut.

The SmartPill software generates reports of continuos measures of temperature, pH and pressure in the GI tract (**Figure 5**). The pressure in the GI tract was calculated as 45 minutes moving average (MA) pressure and was expressed as AUC in the stomach, small intestine, large intestine and whole gut.

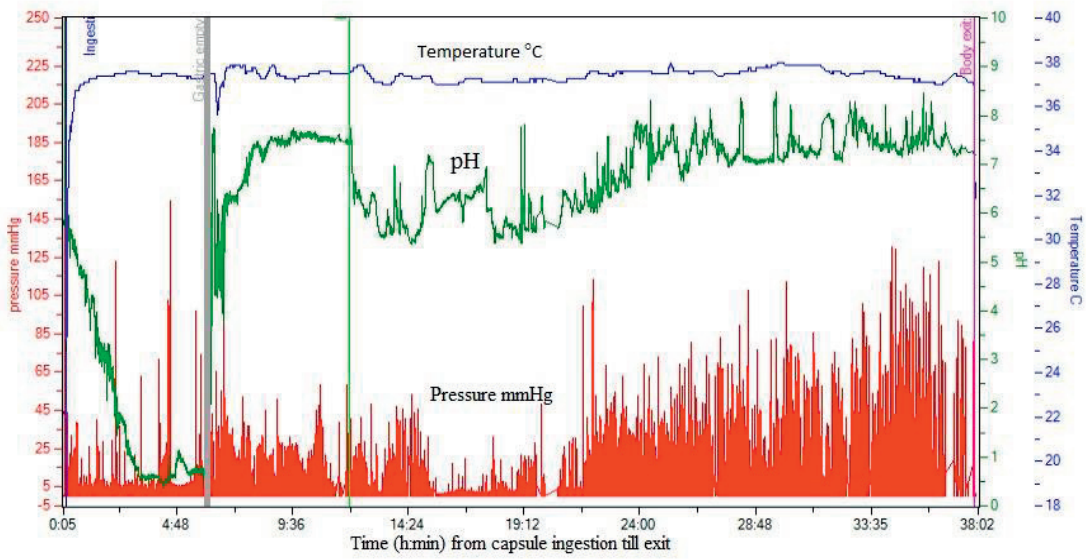

Figure 5. A graph created by the SmartPill software from the data recorded by the SmartPill capsule as it travels trough the GI tract. The pressure is marked as red bars, pH as a green line and the temperature as a blue line. A grey vertical line marks the gatric emptying, a light green vertical line mark the entry from small bowel to large bowel and a purple vertical line the body exit of the capsule. Reprinted from the original publication II (Nuora, Tupasela, Tahvonen, Rokka, Marnila, Pohjankukka, Pahikkala, Viitanen, Mäkelä, Yang, Kallio, Linderborg, 2018), with the permission of Elsevier Ltd.

4.5.4 Diary of symptoms

Subjects were required to keep a diary of symptoms (II, III) during their Study visit and the following day. They were asked to write down the type of symptom: abdominal pain/cramping, bloating, bowel movement, diarrhea, gas or nausea; the severity of the symptom in a scale of 1 to 3, one meaning mild pain, two being moderate pain and three being intense pain and the duration of the symptom. The diary was divided into time slots, shorter slots during the Study visit and longer slots on the following day. In each time slot, the subjects indicated whether they had symptoms or not and the type of the symptom.

4.6 Sample size calculations

Power calculation analyses for required sample size for Study II were carried out in G*power software (version 3.1.9) using a power curve, with alpha=0.05, power of 0.8. It is estimated that 15 subjects per group (RM and HPM) would be needed to detect a 1.05 SD difference between the two milk samples.

For Study III power calculations were not done due to similar setting with Study II. Three different milk samples and 20 subjects per group (PM, HPM and UHTM) would be enough to detect a 1.05 SD difference between those three groups.

Power calculations analyses for required samples size for Study IV were carried out in Minitab (v.16, Pennsylvania State University, USA) using a power curve, with alpha=0.05, power of 0.8 and SD=0.41. It is estimated that 20 subjects per group (lean versus MetS) would be needed to detect a 1.0 SD difference between these two groups. Allowing for withdrawals (n=8) a total of 48 subjects will be recruited.

4.7 Statistical analyses

Normal distripution of the data was tested with the Shapiro Wilk´s test (I, II, III, IV) and the logarithmic transformation for non-normally distriputed data were performed where applicable (II, III). Depending on the normality of the data a paired samples t-test or Wilcoxon matched pairs signed ranks test was used to compare the measured responses (I, II, III). Where appropriate, a two-way repeated measures ANOVA was performed with a Bonferroni multiple comparisons test (I) or a three-factor repeated measures ANOVA followed by Sidak adjusted post hoc test (IV). Where the Mauchly's sphericity test failed, the Huynh-Feldt correction was applied (IV). Statistical significance was indicated as $P < 0.05$ (I, II, III, IV) and for Studies III and IV the statistical significance was also indicated as $P < 0.001$. Statistical analyses were conducted with SPSS 23.0 software (IBM Inc., Armonk, NY) (I, II, III, IV) and GraphPad Prism (v6.0, La Jolla, CA) (I).

5 RESULTS AND DISCUSSION

5.1 Composition of test meals

In Study I, the raw beef steaks chosen for thermal treatment contained 4.6 ± 0.5 % fat, 21.8 ± 0.3 % proteins, 0.9 ± 0.0 % ash and 72.8 ± 1.7 % moisture (n = 6). The moisture content was reduced to 55.0 ± 2.0 % after sous-vide cooking ($P < 0.0001$) and to 60.4 ± 1.1 % after pan frying ($P < 0.001$). During sous-vide cooking the protein content of the steak increased to 35.1 ± 1.8 % and during pan frying the protein conted increased to 27.8 ± 2.4 %. The increase in protein content is most propably due to moisture loss during cooking. No differences were found between sous-vide cooking and pan frying in the resultant ash content. The fat content of sous-vide cooked beef did not differ from that of raw beed steak but the fat content of pan-fried steak was higher (12.8 ± 1.1 %) than that of raw ($P < 0.0001$) and sous-vide cooked steaks (6.9 ± 0.4 %, $P < 0.001$). The most abundant FAs in the beef steaks were oleic acid (18:1), palmitic acid (16:0), stearic acid (18:0), linoleic acid (18:2), palmitoleic acid (16:1) and α-linolenic acid (18:3). No differences were seen between sous-vide cooking and pan frying in the fatty acid composition. This study aimed to replicate real cooking conditions which gave tender and medium rare beef steaks. On the contrary, previous studies [146,147,200] investigating the effects of lipid oxidation in meals and the acute effects of those products when ingested, have used extreme levels of lipid oxidation not indicative to habitual cooking conditions.

In Study II no significant differences were observed in the lactose and protein content or in the fatty acid composition between the two milk samples. The major fatty acids in milk were: palmitic acid, oleic acid, stearic acid and myristic acid (14:0). The protein content of RM was 34.8 ± 1.7 g/kg and that of HPM was 34.6 ± 0.6 g/kg, respectively. The lactose content of RM was 45.8 ± 1.7 g/kg and of HPM milk 44.8 ± 0.5 g/kg. Lactose and protein content were in the levels normal to cow's milk. The cereal bar served together with the milk sample was provide by the SmartPill capsule manufacturer. The cereal bar was encouraged to use with the capsule to ensure the peristaltics.

In Study III the composition of the milk was not investigated as Study II showed that processing has no effect on the protein and lactose content or fatty acid composition. The test meal composition other than milk differed from Study II as the cereal bar contained allergens which excluded few volunteers. Also, the blood samples were drawn for 5 hours during which time the subjects restrained from eating, the test meal was then designed to contain more calories. The milk dose in both Studies II and III (4 dl per milk type) was in accordance with

previous studies were similar kind of amounts of milk were served to subjects [179,181,201].

In Study IV the fatty acid composition of the test meals was investigated. The most abundant fatty acids were palmitic, stearic, oleic and linoleic acids, respectively. Several previous studies have used a similar kind of test meal with muffins and a milk shake when investigating the effects of interesterification or solid fat content on postprandial lipemia [9,164,166,167,202].

5.2 Postprandial responses

5.2.1 Glucose, insulin and inflammation markers

In Study I, plasma insulin and glucose concentrations were not measured. However, plasma levels of inflammation markes were measured in response to two differently cooked beef steaks. No differences in the plasma levels of interleukin-6 and CRP were observed between pan-fried and sous-vide cooked meals. The levels of TNFα and MCP-1 remained at baseline after the pan-fried meal, but decreased after the sous-vide cooked meal from 3 h to 4 h postrandially. For TNFα and MCP-1, the difference between pan-fried and sous-vide cooked meals reached statistical significance at 4 h ($P < 0.001$ for both). Previous studies have found increased levels of CRP after high read meat intake in Teherani [203], European [204] and American women [205]. One study also found increased levels of interleukin-6, TNFα and CRP after ingestion of Wagu beef in healthy subjects [206]. In contrast to those studies, our Study failed to observe increases in the inflammation markers, possibly due to use of a different type of beef as feeding patterns influence beef macronutrients and the specific lipid composition [207]; this could then affect the postprandial responses to the beef [208]. The results did demonstrate differences in the postprandial response of MCP-1 and TNFα between the thermal processing methods used, and the inflammatory response was comparatively lower after the sous-vide than the pan-fried meal.

In Study II, the postprandial plasma insulin and glucose concentrations did not differ between the raw milk samples and the pasteurized and homogenized milk samples when fed to milk sensitive subjects. No statistically significant differences were found in the effects of treatments on plasma inflammatory markers. The CRP levels remained at baseline for the whole 4 h follow up period. All measured inflammatory markers remained within the normal recommended ranges for healthy individuals [209,210].

In Study III, no significant differences were found in the postprandial insulin and glucose concentrations between PM, HPM and UHTM. Postprandial plasma leucosyte levels did not change significantly between the milk types at baseline and 3 and 5 h postprandially and remained at levels comparable to the reference

value for healthy adults. The 92 inflammation markers measured from postprandial plasma did not show any significant differences between the three milk types at baseline and 5 h postprandially. However, the levels of a few biomarkers changed between baseline and 5 h after UHTM, HPM and PM. The levels of interleukin-6 increased significantly after UHTM and HPM. The concentrations of fibroblast growth factor 21 (FGF21), interleukin-8, and Fms related tyrosine kinase 3 ligand (Flt3L) as well as TNF-related activation-induced cytokine (TRANCE) decreased significantly. Dencker et al. [211,212] investigated the effect of food intake on 92 biomarkers of cardiovascular disease and 92 neurological biomarkers in 22 healthy subjects. Blood samples were taken at baseline and 30 and 120 minutes postprandially. As in our Study, the results showed the meals had a very modest effect on those biomarkers.

Sampling of blood samples differed between Studies I, II, III and IV. In Study I the levels of glucose and insulin were not investigated and the first postprandial blood sample was drawn 60 min after the test meal. In contrast, in Studies II, III and IV also the glycemic responses was of interest and first postprandial blood samples were drawn during the first 60 minutes after the ingestion of the test meals. As the peak time for glycemia is always an estimation different laboratories use slightly different time points. For Studies II and III blood samples were drawn 20 and 40 min postprandially and as for Study IV the first postprandial blood sample was drawn at 30 min time point.

5.2.2 Lipidomics

5.2.2.1 Lipid analysis

In Study II, the posprandial TAG concentration did not differ significantly between RM and HPM at any time point, despite the differing fat droplet sizes (**Figure 6 A**). No significant differences were seen in the iAUC of postprandial lipemia between the two milk types, suggesting that the difference in lipid droplet size does not influence the intestinal absorption of TAGs from the lipid droplets.

In Study III, the posprandial TAG concentration was significantly higher 90 minutes after ingestion of UHTM compared to HPM ($P = 0.04$)). No such difference was found between UHTM and PM or HPM and PM (**Figure 6 B**). The postprandial lipemia, measured as iAUC, did not differ between the three milk types.

In Study IV, the postprandial TAG concenteration did not differ between MetS and healthy controls, but differed between the four different meals (interaction time × fat $P < 0.05$). After the HOS meal the plasma TAG increased rapidly at 1 h while, with all other meals, the plasma TAG did not increase untill after 2 h.

IPO and PO, delayed the postprandial elevation of TAGs in MetS and healthy subjects. This supports previous findings in healthy young and older individuals [9,166] and strongly indicates that interesterification of palmitic acid to *sn*-2 position suppresses the postprandial TAG concentration regardless of the fasting lipemia. However, the decrease in TAG concentration in MetS subjects did not normalize the hypertriacylglyceridaemia down to that of the healty subjects. Nevertheless, the reduction of hypertriacyglyceridaemia after IPO meal in MetS subjects was significant compared to the PO and BT meals. This achievement in lipaemic reduction is of substantial importance as the risk of cardiovascular diseases is increased in MetS. The chylomicron rich fraction (CMRF) TAG response did not differ between the MetS group and the Healthy group, which was unexpected. The increase in the CMRF TAGs was delayed after the BT and IPO meals compared to the PO and HOS meals (time × fat interaction, $P < 0.05$). The HOS and PO meals increased the CMRF TAG concentrations after ingestion until the end of the 5 h experimental period ($P < 0.05$ each from baseline).

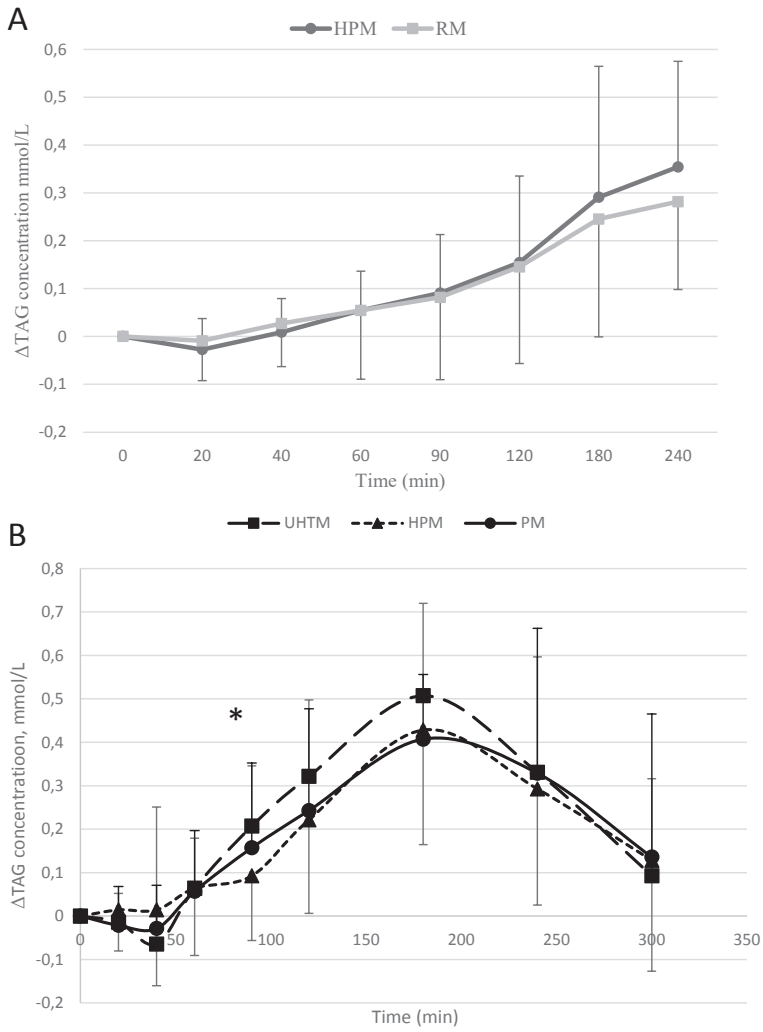

Figure 6. Plasma postprandial triacylglycerol (TAG) (Δ = deviation from baseline) concentration. **A)** In Study II, after homogenized and pasteurized milk (HPM, dark grey line with dot) and raw milk (RM, light grey line with square), n = 11, values are mean ± SD. No significant differences were observed between the milk samples. **B)** In Study III, after UHT homogenized milk (UHTM, black longer dashes), homogenized, pasteurized milk (HPM, black short dashes) and pasteurized milk (PM, black line), the TAG concentrations of UHTM were significantly higher at the 90 min time point compared HPM (P = 0.04), marked with *, n = 14, values are mean with SD. Reprinted from the original publication II (Nuora, Tupasela, Tahvonen, Rokka, Marnila, Pohjankukka, Pahikkala, Viitanen, Mäkelä, Yang B, Kallio, Linderborg, 2018) and III (Nuora, Tupasela, Jokioja, Tahvonen, Kallio, Yang, Viitanen, Linderborg, 2018), with the permission of Elsevier Ltd.

5.2.2.2 Fatty acid composition

The FA composition of postprandial plasma lipids was investigated in Studies II and III and the FA composition of CMRF was determined in Study IV.

In Study II, the most abundant fatty acids in the postprandial plasma were oleic, palmitic, linoleic, stearic, myristic and α-linolenic acids after ingestion of RM and HPM (**Figure 7 A**). Four hours after ingestion of HPM, significantly more myristic ($P = 0.021$), palmitic ($P = 0.047$) and stearic ($P = 0.028$) acids were observed compared to RM. The linoleic acid concentration tended to be higher ($P = 0.07$) after HPM compared to RM.

In Study III, myristic, palmitic, stearic, oleic and linoleic acids were the most abundant fatty acids after UHTM, PM and HPM meals (**Figure 7 B**). Two hours after ingestion of UHTM meal there were significantly more myristic ($P = 0.016$), palmitic ($P = 0.001$), oleic ($P = 0.012$) and linoleic ($P = 0.019$) acids compared to HPM. In addition, 2 hours after ingestion of PM significantly more myristic ($P = 0.048$), palmitic ($P = 0.008$), oleic ($P = 0.018$) and linoleic ($P = 0.03$) acids were detected compared to HPM. No significant differences were observed between UHTM and PM at the 2 hour time point. It is not clear why the fatty acid compositon of UHTM and PM differed from HPM at this one time point only. On the other hand, at the 4 hour time point linoleic acid differed significantly between UHTM and HPM ($P = 0.001$), and between UHTM and PM ($P = 0.001$) as well as between HPM and PM ($P = 0.035$). FA composition analyses showed significant differences mainly in saturated fatty acids.

In Study IV, the most abundant CMRF FAs were palmitic, stearic, oleic and linoleic acids after HOS, PO, IPO and BT meals in boht Healthy and MetS groups. At the 4 h time point the MetS subjects had higher concentrations of palmitic and oleic acids compared to the Healthy subjects, regardless the fat ingested (group x time interaction $P = 0.006$ and $P = 0.005$). In both study groups, palmitic acid concentrations did not differ between the PO and IPO, but were lower after the BT at 4 h postprandially (fat x time interaction $P = 0.001$; $P < 0.05$ vs BT each). Stearic and oleic acid concentrations did not differ between the PO and IPO. After the PO the linoleic acid concentrations were higher compared to the IPO at both 2 h and 4 h postprandially (fat x time interactions $P = 0.003$, $P = 0.045$ PO vs IPO).

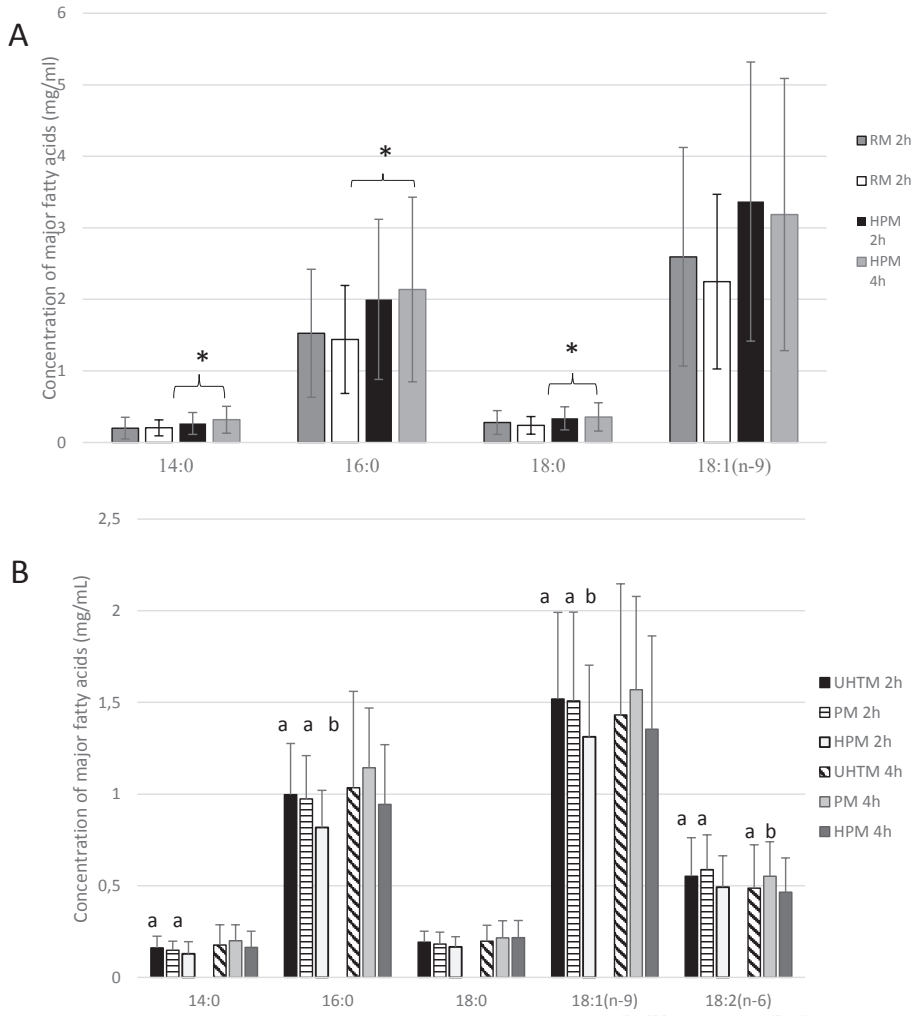

Figure 7. Concentration of major fatty acids in postprandial plasma at 2 and 4 h hour time points. **A)** Study II, raw milk (RM, black and light grey bars) and homogenized and pasteurized milk (HPM, striped and dark grey bars). n =11, values are mean with SD. Myristic (14:0), palmitic (16:0) and stearic (18:0) acids differed significantly between RM and HPM at 4 hour time point. Significant differences ($P < 0.05$) are marked with an asterisk. **B)** In Study III, UHT homogenized milk (UHTM, black and diagonally striped bars), pasteurized milk (PM, horizontally striped and grey bars) and homogenized, pasteurized milk (HPM, light grey and dark grey bars). n = 14, values are mean with SD. Significant differences ($P < 0.05$) are marked with different letters with in each fatty acid. Reprinted from the original publication II (Nuora, Tupasela, Tahvonen, Rokka, Marnila, Pohjankukka, Pahikkala, Viitanen, Mäkelä, Yang B, Kallio, Linderborg, 2018) and III (Nuora, Tupasela, Jokioja, Tahvonen, Kallio, Yang, Viitanen, Linderborg, 2018), with the permission of Elsevier Ltd.

5.2.2.3 Lipid oxidation

In Study I, from the sous-vide and pan-fried beef steaks, 23 possible oxidation products were detected, with more from the pan-fried steak compared to the sous-vide cooked steak (0.42 vs 0.36 % of all TAGs). Out of those 23 possible oxidation products, 15 were also found in the postprandial plasma samples. The number of different oxidized lipid species found in the plasma was from 0 to 6 and varied between the individuals and the thermal processing method. No statistically significant difference was found in the number of different oxidation products between thermal treatments ($P > 0.05$). It cannot be concluded that all 15 lipid oxidation products found in the plasma arose from the cooked meat, as further modifications of oxidized lipid species can occur in the digestive tract [213]. Previous studies in pigs [214], rats [215,216] and humans [146] have shown that dietary oxidized lipids or their degradation products [217,218], including hydroperoxides [147,200], are present in plasma lipoproteins. In these studies the level of lipid oxidation in the meals was extreme and not indicative of habitual meals. In the present Study, the aims was to replicate real cooking conditions, which resulted in a tender or medium rare steak.

Secondary oxidation products, measured as malondialdehyde present in the samples, were found in both raw and thermally treated beef steaks (I). Malondialdehyde is one of the degradation products of lipid hydroperoxides and it is widely used as an indicator of the degree of lipid oxidation [219]. As anticipated, with pan frying the levels of secondary oxidation products increased by 200 % in relation to sous-vide cooking and raw steak ($P < 0.05$; $P < 0.01$).

In pan-fried steak the conjugated diene concentration was 43 ± 2.7 µmol/g and in sous-vide cooked steak 37 ± 4.5 µmol/g (I). Despite limited sample size ($n = 3$ replicates), this difference was statistically significant ($P < 0.05$). In the plasma HDL fraction the diene conjugation concentration was reduced after ingestion of pan-fried steak at the 2 h time point ($P < 0.05$), this was not observed after ingestion of sous-vide cooked steak. In the non-HDL fraction the conjugated diene concentration was generally lower than in the HDL fraction but there were no statistical differences at the 2 h time point or between thermal treatments. Lipoproteins like chylomicrons and LDL transport oxidation products to the circulation and might exert oxidative damage in the peripheral tissues. However, a key role of HDL is reverse-transport, trafficking lipids, including cholesterol, from the periphery to the liver. Lipid oxidation products transported by HDL are speculated to have protective and antioxidative effect [220].

Glutathione, as free radical scavenger, plays an important role in protecting cells against oxidative damage [221]. The plasma glutathione levels (I) differed significantly from the baseline to the 2 h time point between the pan-fried and

sous-vide cooked meals (Δ(0–120 min), $P < 0.05$). This change in glutathione from the baseline between the two meat thermal treatments may indicate greater oxidative stress following the pan-fried meal. However, the measured glutathione levels were very low and it is known that glutathione can be unstable in plasma [222]. No significant between time point differences were observed in the plasma glutathione concentrations (sous- vide 0 vs. 2 h, $P = 0.055$; pan frying 0 vs. 2 h, $P = 0.062$). Nor were there any significant between meal differences between the fasting samples and the 2 h time point samples ($P > 0.05$).

Suprinsingly, there was little difference between the two thermal processing methods as regards the the quantity and quality of oxidized lipids that was either detected with mass spectrometry or measured as TBARS values, diene conjugation or glutathione levels. Previously, higher cooking temperature and longer cooking times have been reported to significantly increase diene conjugation and volatile lipid oxidation products in lamb meat [22]. In addition, the amount of lipid oxidation products in the postprandial plasma varied between the subjects, suggesting that there might be differences between men in their susceptibility to lipid oxidation.

5.3 Intestinal pressure, pH and temperature

In Study II, the 45 min moving average (MA) pressure did not differ between the two milk samples in the stomach ($P = 0.963$), small intestine ($P = 0.643$) and whole gut ($P = 0.53$). The area under the curve of the moving average pressure in the large intestine tended to be larger after HPM compared to RM ($P = 0.068$) but did not reach a significant difference. The pressure during reported GI symptoms in the stomach, small intestine and large intestine could not be differentiated from the corresponding 45 min MA pressure in the stomach, small intestine, large intestine or whole gut (stomach $P = 0.428$; small intestine $P = 0.723$; large intestine $P = 0.292$; whole $P = 0.158$). The gastric emptying time was delayed in 27 % of the cases, suggesting that in these cases the capsule necessarily did not travel with the test meal and in that case did not record the "required" pressure in the GI tract. This could be one reason why the pressure during the reported GI symptoms could not be differentiated from the MA pressure. Previous studies by Timm et al. [223] and Willis et al. [224] faced similar problems with delayed gastric emptying. The large size of the SmartPill capsule has been suggested to be the reason why the capsule becomes to "suspended" in the stomach and skews the gastric emptying time [225]. The intestinal transit times did not differ between the HPM and RM. The emptying or transit times for RM and HPM were: 4.6 ± 2 versus 5.4 ± 5 h for gastric, 4.7 ± 1 versus 4.1 ± 1 h for small intestine and 21.0 ± 10 versus 26.4 ± 12 h for large

intestine. Previous studies [223,226,227] have also reported intestinal transit times in healthy subjects in ranges similar to these.

5.4 Diary of symptoms

In Studies II and III the subjects were required to keep a diary of their symptoms during their intervention day and the following day. Numerically more GI symptoms were reported after HPM (108) compared to RM (55) in Study II, but the difference was not statistically significant (P = 0.103). In Study III events of GI symptoms were reported 100 times after UHTM, 97 times after PM and 83 times after HPM. There were no significant differences between the milk samples.

The most commonly reported symptoms in both Studies II and III were flatulence, bloating and abdominal pain or cramping. Previously, other studies have reported similar kind of symptoms [172,174,175]. None of the subjects reported diarrhea. These symptoms are also observed in lactose intolerance. None of the subjects had previously diagnosed lactose intolerance (II, III), however, the role of lactose in the induction of GI symptoms cannot be entirely ruled out. In Study II, six subjects reported the highest number GI symptoms after HPM, three subjects after RM and two subject had no difference in the number of reported symptoms between the two milk samples. In Study III, five subjects reported the highest number of GI symptoms after UHTM, three subjects after HPM and four after PM.

No significant difference was found in the duration of the GI symptoms between the milk samples (III). Several previous studies have investigated GI symptoms related to milk consumption, similar to this Study, have found hardly any differences between the responses to raw milk and processed milk [172,174,175]. Mummah et al. [172] even investigated whether raw milk could reduce the symptoms of lactose intolerance compared to PM but found no difference in the symptoms severities.

6 SUMMARY AND CONCLUSION

The experimental part of the thesis concentrated on the acute postprandial effects of differently processed dietary lipids. The hypothesis was that processing of dietary lipids would affect their postprandial lipid metabolism. Lipid oxidation and the absorption of oxidized lipids were investigated using beef steak as a model food in a setting using two different thermal processing methods. The role of milk pasteurization, homogenization and UHT treatment on the severity of gastrointestinal symptoms as well as TAG absorption from MFGs was researched. In addition, the influence of the regioisomerism of TAGs on postprandial lipemia, especially the position of palmitic acid on the glycerol backbone, was investigated in healthy and unhealthy subjects.

Thermal processing of beef steak influenced the lipid oxidation in the steaks, despite the low level of unsaturation in fatty acids and typical cooking conditions. Plasma glutathione and certain inflammatory markers indicated that consumption of sous-vide cooked meat caused lower oxidative stress and inflammation than pan-fried meat. High levels of conjugated dienes were detected in the HDL fraction of the postprandial plasma indicating that the protective effect of HDL may not only be the result of the transport of cholesterol from the peripheral tissues but also the transport of oxidized lipid species to the liver. Large individual variation was detected in the levels of oxidized lipid species in the postprandial plasma despite the use of a defined cohort of young healthy males, which warrants further studies with multiple meals, and isolated plasma lipoprotein fractions.

We hypothesized, based on the self-reported symptoms of our volunteers, that homogenized and UHT treated milk would create more symptoms than pasteurized milk or raw milk in sensitive subjects. Homogenization and pasteurization of milk did not seem to influence the intestinal absorption of TAGs from the milk fat globules, but it influenced intestinal absorption and/or clearance rates of different FAs within the measured time-frame. However, this effect was not constant in the two studies, and the detected significant difference in the postprandial plasma TAG concentration was found at one time point only. In the context of a mixed meal, such differences in plasma FAs are unlikely to be of importance. These findings further support earlier studies in which no significant difference was found in the number of gastrointestinal symptoms in sensitive individuals between raw and processed milk or between differently processed milk samples. However, the results obtained with the SmartPill capsule on pressure in the large intestine (large intestine pressure $P = 0.068$) suggest that, although no significant differences were found between raw and

processed milk samples, further studies in this area are needed with a larger subject group and with longer exposure times, such as to several consecutive meals, and to the differently processed milk types.

In healthy subjects interesterified palm olein has resulted in a delayed lipemic response compared to native palm olein. Our study demonstrated that also in individuals with increased cardiometabolic risk, the lipemic response is similarly delayed after ingestion of interesterified palm olein. These individuals, did not have a markedly different lipemic response to interesterified palm olein relative to native palm olein compared with healthy volunteers. These findings demonstrate that interesterified palmitic acid rich fats have no increased lipemic potential for individuals with MetS, however additional work is required to determine the long-term impacts of these lipids in this at risk population.

The studies included in this thesis demonstrated that the processing of dietary lipids affects their digestion and absorption in the gastrointestinal tract, thus proving the hypothesis. The effect could either be beneficial, such as enhancing lipolysis of TAGs and improving absorption of certain FAs, or negative, by inducing lipid oxidation and increasing the oxidative stress load of the body. The studies did not substantiate the effect of food processing on the adverse gastrointestinal symptoms experienced by sensitive individuals.

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DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

1. **REINO R. LINKO (1967)** Fatty acids and other components of Baltic herring flesh lipids. (Organic chemistry).
2. **HEIKKI KALLIO (1975)** Identification of volatile aroma compounds in arctic bramble, *Rubus arcticus* L. and their development during ripening of the berry, with special reference to *Rubus stellatus* SM.
3. **JUKKA KAITARANTA (1981)** Fish roe lipids and lipid hydrolysis in processed roe of certain *Salmonidae* fish as studied by novel chromatographic techniques.
4. **TIMO HIRVI (1983)** Aromas of some strawberry and blueberry species and varieties studied by gas liquid chromatographic and selected ion monitoring techniques.
5. **RAINER HUOPALAHTI (1985)** Composition and content of aroma compounds in the dill herb, *Anethum graveolens* L., affected by different factors.
6. **MARKKU HONKAVAARA (1989)** Effect of porcine stress on the development of PSE meat, its characteristics and influence on the economics of meat products manufacture.
7. **PÄIVI LAAKSO (1992)** Triacylglycerols – approaching the molecular composition of natural mixtures.
8. **MERJA LEINO (1993)** Application of the headspace gas chromatography complemented with sensory evaluation to analysis of various foods.
9. **KAISLI KERROLA (1994)** Essential oils from herbs and spices: isolation by carbon dioxide extraction and characterization by gas chromatography and sensory evaluation.
10. **ANJA LAPVETELÄINEN (1994)** Barley and oat protein products from wet processes: food use potential.
11. **RAIJA TAHVONEN (1995)** Contents of lead and cadmium in foods in Finland.
12. **MAIJA SAXELIN (1995)** Development of dietary probiotics: estimation of optimal *Lactobacillus* GG concentrations.
13. **PIRJO-LIISA PENTTILÄ (1995)** Estimation of food additive and pesticide intakes by means of a stepwise method.
14. **SIRKKA PLAAMI (1996)** Contents of dietary fiber and inositol phosphates in some foods consumed in Finland.
15. **SUSANNA EEROLA (1997)** Biologically active amines: analytics, occurrence and formation in dry sausages.
16. **PEKKA MANNINEN (1997)** Utilization of supercritical carbon dioxide in the analysis of triacylglycerols and isolation of berry oils.
17. **TUULA VESA (1997)** Symptoms of lactose intolerance: influence of milk composition, gastric emptying, and irritable bowel syndrome.
18. **EILA JÄRVENPÄÄ (1998)** Strategies for supercritical fluid extraction of analytes in trace amounts from food matrices.
19. **ELINA TUOMOLA (1999)** *In vitro* adhesion of probiotic lactic acid bacteria.
20. **ANU JOHANSSON (1999)** Availability of seed oils from Finnish berries with special reference to compositional, geographical and nutritional aspects.
21. **ANNE PIHLANTO-LEPPÄLÄ (1999)** Isolation and characteristics of milk-derived bioactive peptides.
22. **MIKA TUOMOLA (2000)** New methods for the measurement of androstenone and skatole – compounds associated with boar taint problem. (Biotechnology).
23. **LEEA PELTO (2000)** Milk hypersensitivity in adults: studies on diagnosis, prevalence and nutritional management.
24. **ANNE NYKÄNEN (2001)** Use of nisin and lactic acid/lactate to improve the microbial and sensory quality of rainbow trout products.
25. **BAORU YANG (2001)** Lipophilic components of sea buckthorn (*Hippophaë rhamnoides*) seeds and berries and physiological effects of sea buckthorn oils.
26. **MINNA KAHALA (2001)** Lactobacillar S-layers: Use of *Lactobacillus brevis* S-layer signals for heterologous protein production.
27. **OLLI SJÖVALL (2002)** Chromatographic and mass spectrometric analysis of non-volatile oxidation products of triacylglycerols with emphasis on core aldehydes.
28. **JUHA-PEKKA KURVINEN (2002)** Automatic data processing as an aid to mass spectrometry of dietary triacylglycerols and tissue glycerophospholipids.
29. **MARI HAKALA (2002)** Factors affecting the internal quality of strawberry (*Fragaria x ananassa* Duch.) fruit.
30. **PIRKKA KIRJAVAINEN (2003)** The intestinal microbiota – a target for treatment in infant atopic eczema?
31. **TARJA ARO (2003)** Chemical composition of Baltic herring: effects of processing and storage on fatty acids, mineral elements and volatile compounds.
32. **SAMI NIKOSKELAINEN (2003)** Innate immunity of rainbow trout: effects of opsonins, temperature and probiotics on phagocytic and complement activity as well as on disease resistance.
33. **KAISA YLI-JOKIPII (2004)** Effect of triacylglycerol fatty acid positional distribution on postprandial lipid metabolism.
34. **MARIKA JESTOI (2005)** Emerging *Fusarium*-mycotoxins in Finland.
35. **KATJA TIITINEN (2006)** Factors contributing to sea buckthorn (*Hippophaë rhamnoides* L.) flavour.
36. **SATU VESTERLUND (2006)** Methods to determine the safety and influence of probiotics on the adherence and viability of pathogens.
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