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Biochemistry and Health Benefits of Fatty Acids

Edited by Viduranga Waisundara

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Contributors
Augusta Caligiani, Veronica Lolli, Jesui Vergilio Visentainer, Luciana Pelissari, Lorena Visentainer, Vanessa Javera Neia, Caroline Zappielo, Oscar Santos, Liane Maldaner, Jessica Pizzo, Adriela Rydlewski Ito, Roberta Silveira, Marilia Galuch, Jeane Eliete Laguila Visentainer, Adela Mora-Gutierrez, Rahnat Attaie, Maryuri Nuñez De González, Maria Das Graças Tavares Do Carmo, Patricia De Velasco, Amanda Ferreira, Tarsis Marine Ferreira, Louise Crovesy De Oliveira, Raul Nunes Carvalho Jr, Ana Paula Souza Silva, Flávia Cristina Pires, Marielba De Los Angeles Salazar, Priscila Bezerra, Maria Caroline Ferreira, Eduardo Menezes, Glides Rafael Olivo Urbina, Jhonatas Rodrigues Barbosa, Wanessa Almeida Da Costa, Shahdat Hossain, Michio Hashimoto, Viduranga Waisundara

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Biochemistry

Volume 1

Dr. Viduranga Waisundara obtained her Ph.D from the Department of Chemistry, National University of Singapore in Food Science & Technology in 2010. She was a lecturer at Temasek Polytechnic, Singapore from July 2009 – March 2013. Following this, she relocated to her motherland of Sri Lanka and spearheaded the Functional Food Product Development Project at the National Institute of Fundamental Studies from April 2013 to October 2016. She was a Senior Lecturer on a temporary basis at the Department of Food Technology, Faculty of Technology, Rajarata University of Sri Lanka from January 2017 to July 2018. She presently serves as a Visiting Lecturer at the Australian College of Business and Technology – Kandy Campus, in Sri Lanka. Dr. Waisundara is a prolific writer with many research publications and articles in newspapers and magazines to her name. She is also the current Global Harmonization Initiative (GHI) Ambassador to Sri Lanka.

Book Series Editor and Editor of Volume 1: Viduranga Waisundara
Australian College of Business and Technology – Kandy Campus
Kandy, Sri Lanka

Scope of the Series

Biochemistry, the study of chemical transformations occurring within living organisms, impacts all of life sciences, from molecular crystallography and genetics, to ecology, medicine and population biology. Biochemistry studies macromolecules - proteins, nucleic acids, carbohydrates and lipids –their building blocks, structures, functions and interactions. Much of biochemistry is devoted to enzymes, proteins that catalyze chemical reactions, enzyme structures, mechanisms of action and their roles within cells. Biochemistry also studies small signaling molecules, co-enzymes, inhibitors, vitamins and hormones, which play roles in the life process. Biochemical experimentation, besides coopting the methods of classical chemistry, e.g., chromatography, adopted new techniques, e.g., X-ray diffraction, electron microscopy, NMR, radioisotopes, and developed sophisticated microbial genetic tools, e.g., auxotroph mutants and their revertants, fermentation etc. More recently, biochemistry embraced the 'big data' omics systems.

Initial biochemical studies have been exclusively analytic: dissecting, purifying and examining individual components of a biological system; in exemplary words of Efraim Racker, (1913 –1991) "Don't waste clean thinking on dirty enzymes." Today however, biochemistry is becoming more agglomerative and comprehensive, setting

out to integrate and describe fully a particular biological system. The 'big data' metabolomics can define the complement of small molecules, e.g., in a soil or biofilm sample; proteomics can distinguish all the proteins comprising e.g., serum; metagenomics can identify all the genes in a complex environment e.g., bovine rumen. This Biochemistry Series will address both the current research on biomolecules, and the emerging trends with great promise.

Contents

Preface

There are three major organic compounds that have rendered their assistance in putting together many natural and artificial substances of importance. They are carbohydrates, proteins, and lipids. Fatty acids come under lipids. This group of compounds have come into the attention of the scientific community because of its role in human and microbial health. Additionally, fatty acids have an industrial significance in terms of bio-fuel and alternative energy generation.

This book primarily focuses on three aspects: (1) Biochemical Aspects of Fatty Acids, (2) Industrial Applications of Fatty Acids, and (3) Fatty Acids in Health and Wellness. Although fatty acids are mostly seen as a molecule of dietary importance, they have various uses and applications, and it is hoped that readers of this book will see these multi-variate usages as well as be enlightened on other aspects in which they can be used.

I would like to extend my most sincere thanks to the authors who have generously consented to contribute chapters to this book. Without their helping hand, this project would not have been a success. Also, heartfelt thanks to IntechOpen with whom I have been working with for almost 2 years now, on quite a number of book projects. Last but not least, my appreciation goes to Mr. Nino Popović, the Author Service Manager assigned to this book, who has rendered his paramount support in obtaining contributors and putting the material together.

I hope that this book will serve as an update on fatty acid research as well as open new avenues for exploring investigations on this important biomolecule.

Dr. Viduranga Waisundara
Australian College of Business and Technology – Kandy Campus,
Kandy, Sri Lanka

Section 1

Biochemistry of Fatty Acids

Chapter 1

Introductory Chapter: Fatty Acids in Modern Times

Viduranga Y. Waisundara

1. Introduction to lipids and fatty acids

Before going into the chemical structure and properties of fatty acids, it is important to mention that they are merely one component of the major nutrient group commonly known as lipids. Lipids are biological compounds, which are soluble only in nonpolar solvents. They are typically known as fats and oils as well. However, fats and oil differ from each other based on their physical characteristics. The term "fats" is used to refer solid lipids at room temperature such as lard and butter, while "oils" are liquid lipids at room temperature such as sunflower oil, olive oil, etc. The classification of lipids is shown in **Figure 1**. Fatty acids appear under "triglycerides" since it is a component of this particular category of lipids.

To provide a brief introduction on fatty acids at a very basic level, they are the building blocks of the fat, which is physiologically present and obtained from the food we eat. During digestion, the body breaks down fats in the food products into fatty acids, which are subsequently absorbed into the blood. Upon absorption, fatty acid molecules are typically joined in groups of three, forming a molecule called a triglyceride. It has to be noted in this instance that triglycerides can even be made up from the carbohydrates in the food that we consume. There are several important functions of fatty acids in the body, including being a medium of storing energy and being involved in the cellular composition in the forms of phospholipids and

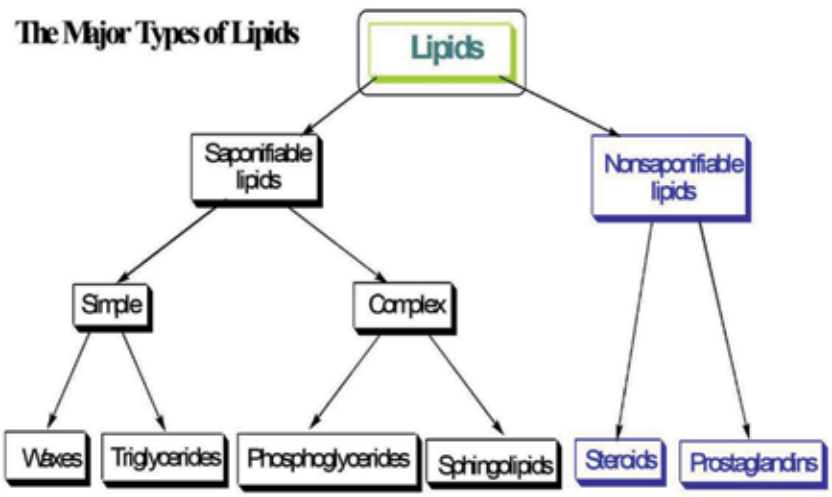

Figure 1.
Major types of lipids: fatty acids come under triglycerides according to this classification.

cholesterol esters. When glucose is unavailable for generation of energy in the cellular mechanism, the body uses fatty acids as fuel instead.

In terms of the chemical structure, a fatty acid is a carboxylic acid. It has an aliphatic chain, which is either saturated (having only single bonds) or unsaturated (having double bonds). Most of the naturally occurring fatty acids do not have branches in the aliphatic chain and contain an even number of carbon atoms (i.e., from 4 to 28). The long nonpolar tails of the fatty acids are responsible for the hydrophobic characteristics of fats and oils, while the carboxyl group of the fatty acids is polar or hydrophilic. Because of having both the hydrophobic and hydrophilic nature, when fatty acids are placed in an aqueous solution, they form spherical clusters or micelles. The micelles are arranged in such a way that the nonpolar straight chain is extended toward the interior of the structure and away from the water, while the polar carboxyl groups face outward in contact with the water.

There are two major types of classification of fatty acids, among many others. One classification is based on the length of the aliphatic chain, which is as follows:

- Short-chain fatty acids (SCFAs): less than five carbons in the aliphatic chain
- Medium-chain fatty acids (MCFA): aliphatic tails of 6–12 carbons, which form medium-chain triglycerides
- Long-chain fatty acids (LCFA): aliphatic tails of 13–21 carbons
- Very long-chain fatty acids (VLCFA): aliphatic tails of 22 carbons or more

Another classification is based on the level of saturation:

- Saturated fatty acids: no double bonds in the aliphatic tails. The general formula for saturated fatty acids is $C_nH_{2n+1}COOH$.
- Unsaturated fatty acids: one or more double bonds in the aliphatic tails.

Unsaturated fatty acids can be divided into *cis*- and *trans*-fatty acids. A *cis* configuration means that the two hydrogen atoms adjacent to the double bond appear on the same side of the aliphatic chain, whereas *trans* configuration means that the adjacent two hydrogen atoms lie on the opposite sides of the chain. Unsaturated fatty acids can also be divided based on the number of double bonds, where monounsaturated fatty acids would have only one double bond in the aliphatic tail and polyunsaturated fatty acids would have two or more double bonds. Polyunsaturated fatty acids may have the double bonds next to each other as conjugated double bonds or alternatively between single bonds as nonconjugated double bonds.

The *cis* configuration of unsaturated fatty acids will create a bend in the fatty acid chain that is not found in saturated fatty acids. These bends prevent unsaturated fatty acids from packing closely together. As a result, they form less London dispersion forces between the fatty acids. This leads to *cis*-fatty acids having lower melting points. *Trans*-fatty acids, on the other hand, are obtained via hydrogenation of polyunsaturated fatty acids, e.g., margarine. *Trans*-fatty acids will not pack as well as saturated fatty acids, but do not produce a bend as in *cis*-fatty acids. Therefore, the melting points of *trans*-fatty acids are between the melting points of saturated fatty acids and *cis*-fatty acids of the same carbon length.

2. Fatty acids and health

Fatty acids have gained much attention over the past few decades owing to its implications on human health. Much of this is due to the fact that the western diet has changed with the advent of "fast" and "convenient" foods [1]. These food products are energy dense, have a low dietary fiber content, and produce a comparatively lower satiety and satiation signals than low-energy-dense foods [2]. This newly introduced diet is markedly different to the historical diet of humans that the gut was adapted to over several millennia [3]. According to current evidence for most of the history on the human lineage, the diet consisted of more indigestible plant material, such as grasses, sedges, and tubers, than the present and is therefore likely to have contained a larger nondigestible component [4]. Recent systematic reviews of randomized trials [5, 6] and prospective cohort studies [7] have called for the re-evaluation of dietary guidelines based on these dietary transitions, primarily in terms of the intake, and a reappraisal of the effects of fatty acids on health. It is heartening to observe, nevertheless, that public health efforts to remove *trans* fats from the food supply in several countries have intensified [8].

Saturated fatty acids contribute to approximately 10% of energy to the North American diet [9, 10]. According to De Souza et al. [8], the main sources of fatty acids in the food supply of North Americans are animal-based food products, such as butter, cows' milk, meat, salmon, and egg yolks, and a few plant products such as chocolate and cocoa butter, coconut, and palm kernel oils. Despite attempts to completely remove *trans* fats from the diet, they evidently contribute to approximately 1–2% of energy in the North American diet [8, 11–13] and are primarily produced through industrial processing such as partial hydrogenation of liquid plant oils in the presence of a metal catalyst, vacuum, and high heat. Production of *trans* fats can also occur naturally in meat and dairy products, where ruminant animals biohydrogenate unsaturated fatty acids via bacterial enzymes [8]. According to De Souza et al. [8], the major industrially produced *trans*-fatty acids in the food supply are elaidic acid isomers, while the major ruminant-derived *trans*-fatty acid is vaccenic acid. Both these chemical compounds share the characteristic of having at least one double bond in the *trans*-configuration. Present dietary guidelines recommend that saturated fats should be limited to <10% (5–6% for those who would benefit from lowering of low-density lipoprotein, LDL cholesterol) and *trans* fats to <1% of energy or as low as possible in view of reducing the risk of ischemic heart disease and stroke [14–19].

3. Short-chain fatty acids (SCFAs)

There is increased attention on SCFAs given its importance in the human gut microbiota. The human microbiota is primarily the collection of microbes that live on and in our body, where the largest and most diverse cluster of microorganisms inhabits the gut [20]. Evidence has surfaced that the gut microbiota has coevolved with the host, which provides the microbes with a stable environment, while the microbes provide the host with a broad range of functions such as digestion of complex dietary macronutrients, production of nutrients and vitamins, defense against pathogens, and maintenance of the immune system [20]. Interactions between the microbiota and the distal gut are currently considered as fundamental determinants of human health.

As previously mentioned, SCFAs are a subset of saturated fatty acids containing six or less carbon molecules which include acetate, propionate, butyrate, pentanoic

(valeric) acid, and hexanoic (caproic) acid. SCFAs are the primary end products of fermentation of nondigestible carbohydrates that become available to the gut microbiota [21]. SCFAs represent the major flow of carbon from the diet, through the gut microbiome to the host. It was discovered recently that SCFA appears to be the natural ligands for free fatty acid receptors 2 and 3 (FFAR 2/3), found on a wide range of cell types, including enteroendocrine and immune cells [22–24]. This unearthing has led to renewed interest in the role of SCFA in human health.

SCFAs are mainly produced through saccharolytic fermentation of carbohydrates which are able to escape digestion and absorption in the small intestine [21, 25]. The pathways of SCFA production are well understood at present and have been recently described in detail recently by Flint et al. [26]. The major products formed as a result of the saccharolytic fermentation are formate, acetate, propionate, and butyrate. Lactate is also a major organic acid produced from the fermentation of selected often rapidly fermentable nondigestible carbohydrates [21]. Relatively minor amounts of branched-chain fatty acids are also produced in this biochemical pathway, mainly through the fermentation process of protein-derived branched-chain amino acids [27, 28].

Acetate is the most abundant SCFA in the colon and makes up more than half of the total SCFA detected in feces [29, 30]. The majority of acetate is produced by most of the enteric bacteria present in the gut as a result of carbohydrate fermentation [30]. In addition, approximately one-third of the colonic acetate has been detected to come from acetogenic bacteria, which are able to synthesize it from hydrogen and carbon dioxide or formic acid through the Wood-Ljungdahl pathway [25, 31].

It is evident from the insurmountable evidence on microbial interactions with dietary polysaccharides and the resulting SCFAs that these particular fatty acids are important energy and signaling molecules. It is becoming increasingly accepted that SCFA-producing bacteria have several beneficial effects on human health. However, it is still unclear whether beneficial effects are driven by the SCFAs per se or whether in combination with other metabolites produced from the gut bacteria [20]. It should be noted and understood in this instance that the gut microbiota produces many other classes of metabolites such as bile acids and amino acid derivatives, which may also have several essential signaling functions leading to health and wellness of the human physiological systems.

4. Conclusion(s)

Fatty acids remain an important component in human nutrition with growing amounts of scientific studies focusing on elucidating its roles in the biochemical pathways as well as its benefits upon consumption. Out of all fatty acids, the two most important types, which have garnered much attention, are *trans*-fatty acids, which are primarily not recommended and have a zero tolerance level of presence in food, and SCFAs owing to their vitality in maintaining the gut microbiota. The knowledge of the role of fatty acids in determining health and nutritional well-being among global consumers has expanded dramatically in the past few decades. The role of fatty acids in neonatal and infant growth and development, health maintenance, the prevention of cardiovascular disease, diabetes, cancers, and age-related functional decline is an aspect, which has been recognized by many experts; thus, despite several highlights of adverse effects of excessive fatty acid consumption, as a class of chemical components, fatty acids remain important to human health, and a complete elimination of fatty acids from the diet is definitely not considered as an intellectual recommendation. As briefly mentioned earlier, fatty

acids are the major components of the cell membrane structure, which participate in modulating gene transcription, function as cytokine precursors, and serve as energy sources in complex, interconnected systems. Therefore, it is indeed apparent that dietary fatty acids are vital for many of the physiological functions and, thereby, affect human health.

Conflict of interest

The author has no conflicts of interest to declare, financial or otherwise.

Author details

Viduranga Y. Waisundara
Australian College of Business and Technology, Kandy, Sri Lanka

*Address all correspondence to: viduranga@gmail.com

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Chapter 2

Fatty Acids: From Membrane Ingredients to Signaling Molecules

Michio Hashimoto and Shahdat Hossain

Abstract

Fatty acid constitutes the foundation cell membranes, provides metabolic energy, affects functions of membrane-bound enzymes/receptors, conducts signaling cascades, and helps in learning-related memory cognition in mammals, including humans. Structurally, the fatty acids are of two kinds: saturated and unsaturated; the latter are again of mono- and polyunsaturated types. From nutritional perspectives, they are of essential and nonessential types. Omega-6 linoleic acid (ω-6 LLA, C18:2) and ω-3 alpha linolenic acid (ω-3 αLLN, C18:3) and ω-6 arachidonic acid [(ω-6 AA, C20:4); it is conditional] are essential fatty acids (EFAs). In addition, mammalian brains cannot biosynthesize the ω-3 docosahexaenoic acid (ω-3 DHA, C22:6) in adequate amounts because of lack of necessary enzymes. Thus, DHA is essential for the growth and development of the brains. Deficiency of DHA produces visual- and learning-related memory impairments, and neurodegeneration in the aged brains and Alzheimer's disease brains. Finally, this chapter will highlight and broaden the awareness about the essentiality of different fatty acids with a special emphasis on DHA.

Keywords: docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, alpha-linolenic acid and linoleic acid, eicosanoids, docasonoids, brain cognition

1. Introduction

The concept of fatty acid was first introduced by the French chemist Michel Eugène Chevreul as *graisse acide* (acidic fat) [1]. Fatty acids are chemically defined as carboxylic acids with either saturated or unsaturated aliphatic chains and are derived after hydrolysis of fats or oils. A fatty acid has, therefore, an acid group at one end of its molecule and a methyl group at the other end [2, 3]. Fatty acids are essential structural components of the cell; they also play important roles in energy requirements and signaling cascades in the cell. Both plant and animal cells can synthesize fatty acids. Animal cells, however, cannot synthesize some of the fatty acids; they must take them from plant sources. These fatty acids are called essential fatty acids (EFAs) in the animal body. Some fatty acids are also synthesized by lower organisms such as phytoplanktons, which act as primary members of the food chain. On the basis of the location of the double bonds from the methyl terminal position of the unsaturated fatty acids (UFAs), they are named as ω-3 and ω-6 UFAs. Biologically, fatty acids are esterified with glycerol, phosphoglycerol, and cholesterol and are referred to as triacylglycerol, phospholipids, and cholesterol esters, respectively. Esterified fatty acids can constitute the structural components

or dietary fuels for cells and organisms; they can also form complex liposomal structures (including lipoproteins) for transporting lipid components from the hepatic tissues to extrahepatic tissues and vice versa.

1.1 Saturated versus unsaturated fatty acids

Fatty acids whose aliphatic carbon chains are fully saturated with hydrogen atoms or contain only C-C single bond and/or contain no C=C double bonds are simply referred to as saturated fatty acids (SFAs). Fatty acids containing C=C double bonds are referred to as unsaturated fatty acids (UFAs). UFAs are again classified as monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs): if they contain only one C=C double bond, they are MUFAs; if they contain more than one C=C double bond, they are then called PUFAs (see **Figure 1** for detail). Because of the presence of C-C single bonds or C=C double bonds, they have characteristic structural features and differences in physical as well as chemical properties and have significant roles in the constitution of cellular membranes.

1.2 Omega-3 (ω-3) versus omega-6 (ω-6) PUFAs

The Greek letter omega (ω) is used in the systemic nomenclature of the polyunsaturated fatty acids (PUFAs). The PUFAs that have a C=C double bond between the 6th and 7th carbon position counting from the terminal methyl end are called ω-6 and those with the double bond between the 3th and 4th carbon are called ω-3 PUFAs. The letter 'n' is also used to denote the position of the double bond. The locations of double bonds in the PUFAs confer huge differences in their physical, biochemical, and physiological properties. The essential fatty acid (EFA) linoleic acid (C18:2) is of ω-6 series, while the EFA α-linolenic acid is the member of ω-3 series. Some of the beneficial effects overlap between the ω-3 and ω-6, while many effects are antagonistic to each other. ω-6 PUFAs can be found in vegetable oils and seeds, whereas ω-3 PUFA is found more in fish/marine animals, walnuts, and canola oil.

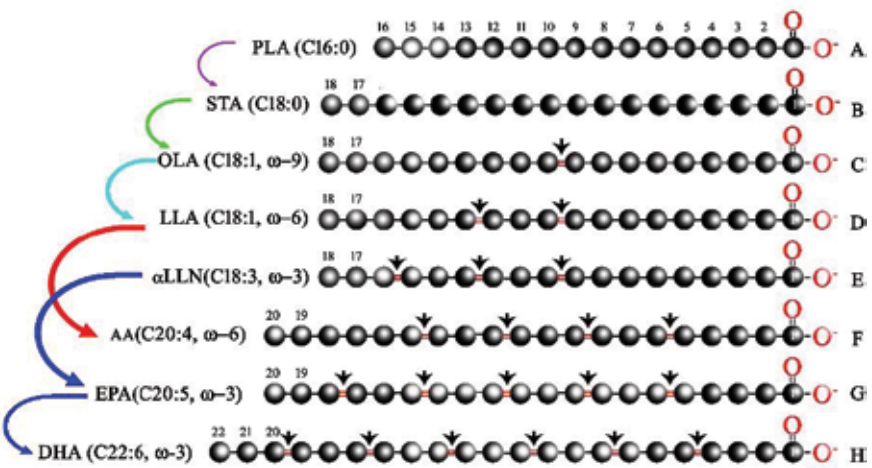

Figure 1.
The straight chain structural features of the most common fatty acids. PLA = palmitic acid, STA = stearic acid, OLA = oleic acid, LLA = linoleic acid, LLN = α-linolenic acid, AA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid. Omega (ω) is used to denote the position of double bonds from the methyl end of the fatty acid. Colored curved arrows = biological conversion is possible from the precursor by the actions of elongase/desaturase enzymes. Black arrow = indicates the position(s) of double bond.

1.3 Essential versus nonessential fatty acids

The fatty acids, which mammals cannot synthesize in their body, are known as essential fatty acids (EFAs); they must be obtained by the mammals in a preformed condition, that is, from the exogenous dietary sources. EFAs were originally designated as vitamin F, until it was realized that they must be classified with fats [4]. Of all the 18-C UFAs, two unsaturated fatty acids are found to be essential fatty acids (EFAs): they are linoleic acid (**Figure 1D**) and α-linolenic acid (**Figure 1E**). Both of them can act as precursors of very long chain polyunsaturated fatty acids (LPUFAs), such as ω-6 linoleic acid acting as the precursor of arachidonic acid (C20:4, ω-6) and ω-3 α-linolenic acid acting as the precursor of eicosapentaenoic acid (EPA, C20:5, ω-3) and docosahexaenoic acid (DHA, C22:6, ω-3). The rest are nonessential. Some examples are (common names): stearic (C18:0), oleic (C18:1), palmitic (C16:0), myristic (C14:0), and lauric acid (C12:0). Being nonessential does not actually mean that they are not important. Our body does need them to function properly; it, however, can synthesize them without receiving them directly from food.

1.4 AA (C20:4, ω-6) versus DHA (C22:6, ω-3) or EPA (C20:5, ω-3)

AA is referred to as a conditionally essential fatty acid for animals [5–7], including humans, that experience persistent deficiencies of linoleic acid (LLA, C18:2, ω-6), or during prematurity and growth, or if there is a limited capacity to convert LLA to AA [5]. However, consumption of vegetable-based oils, with large amounts of LLA, and an adequate capacity to convert LLA to AA, can eliminate the need for exogenous supply of AA, excluding it thereby from the list of essential fatty acids.

1.5 EPA (C20:5, ω-3) and DHA (C22:6, ω-3)

Both EPA and DHA are the members of ω-3 PUFA family. Both can be biosynthesized from the precursor α-linolenic acid (C18:3, ω-3, LLN). However, they are believed to act differently in different organs. For example, the differential roles of EPA and DHA have been studied in lymphocytes [8], macrophages [9], vascular smooth muscle cells [10], and endothelial cells [11]. Their differential roles have also been seen in the brains. EPA constitutes a tiny part in the unsaturated fatty acid pool of the brain. DHA, however, constitutes >17% by weight of the total fatty acids in the brain of adult rats and >33% of the total fatty acids in the retina [12]. DHA is thus referred to as essential for the growth and development of the brains, and animals have to take it in preformed form. The brain has a limited capacity to convert αLNN to DHA because of the lack of synthesizing enzymes [13, 14]. DHA plays an important role in the learning-related memory of animals, including humans.

1.6 ω-7 and ω-9 Monounsaturated fatty acids (MUFAs)

Monounsaturated ω-7 and ω-9 fatty acids are also considered to be nonessential, as majority of them are obtained from dietary sources (**Figure 2**). They can also be biosynthesized in the body. The most common ω-7s are palmitoleic acid (PA) and *cis* and *trans*-vaccenic acid (VA) (11-cis-octadecenoic acid). The most common ω-9s include oleic acid (OA), erucic acid (EA) and mead acid (it is a triunsaturated fatty acid). Since the human body can create ω-9 unsaturated fatty acids, there is no need to include them in diet. Full-fat grass-fed dairy, wild-caught salmon, olives, sprouted nuts, etc. are the sources of ω-7 and ω-9 unsaturated fatty acids.

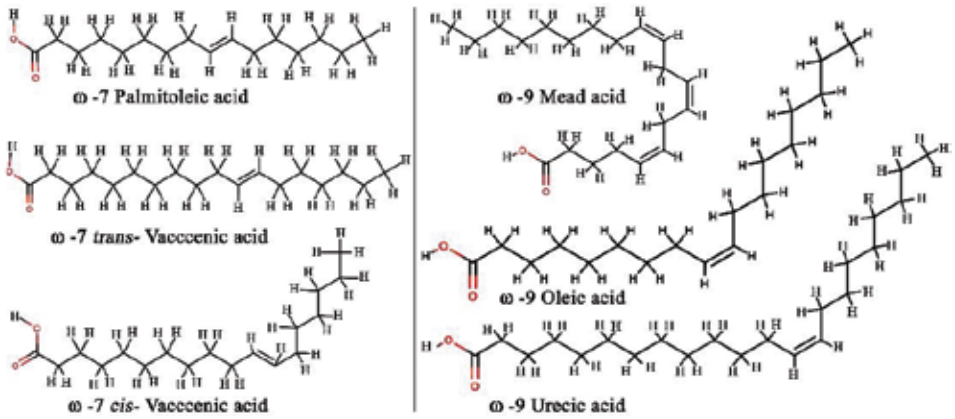

Figure 2.
The structural features of the most common ω-7 and ω-9 unsaturated fatty acids.

1.7 Cis-trans fatty acids

The naturally occurring unsaturated fatty acids have predominantly a *cis* carbon-carbon double bond (-C=C-). The C=C double bond typically lies on C-9 for the C18 unsaturated fatty acids. However, the artificial hydrogenation of C-18 unsaturated fatty acids such as linoleic acid (C18:2, ω-6) may produce *cis-trans* conjugated fatty acid (CLA), like isomers of *cis* Δ-9, *trans* Δ-11; *cis* Δ-9, *trans* Δ-12; and *trans* Δ-10, *cis* Δ-12. Hydrogenation may produce other forms of *trans* fatty acids (TFAs), such as *trans* Δ-8, *trans* Δ-9, and *trans* Δ-10 elaidic acid and *trans* Δ-11 vaccenic acid (**Figure 3**). *Trans* fatty acids (TFAs) are a kind of unsaturated fatty acids and also nonessential fatty acids. The primary TFAs are elaidic acid and vaccenic acid. The vaccenic acid is produced by bacteria in cattle rumen and thus may pass into humans via the milk of cows. The *trans* Δ-9 elaidic acid is the major industrial isomer of TFA [15].

The reports on the effect of CLAs on health and diseases are still scant. Raff et al. [16] reported that a 50:50 mixture of *cis* Δ-9, *trans* Δ-11 CLA and *trans* Δ-10, *cis* Δ-12 CLA caused a nonsignificant increase in SBP (by only 3 mmHg) without any effect on DBP in humans. Laso et al. [17] reported that CLA did not have any effect on blood pressure. Zock and Katan [18] reported that CLAs increase LDL-C and decrease HDL-C, thus indicating that CLA can act as a potential vascular risk factor. American Heart Association, the American Dietetic Association, the Institute of Medicine, US Dietary Guidelines, and the National Cholesterol

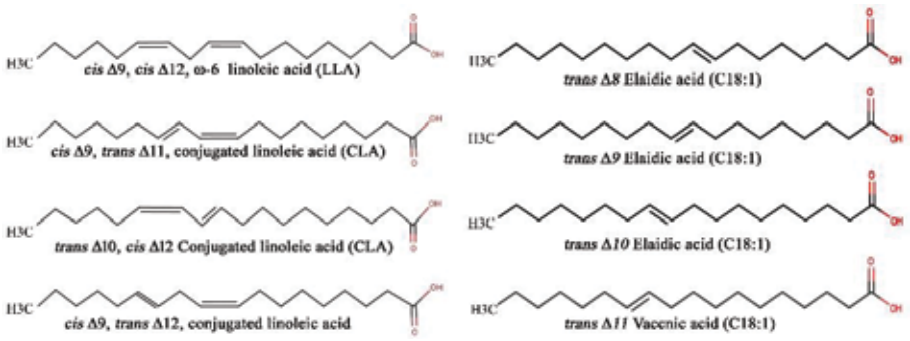

Figure 3.
The structural features of the most common cis-trans unsaturated fatty acids.

Education Program Adult Treatment Panel are claiming to limit *trans* fatty acids in the daily diet [19]. We have previously reported that *cis* Δ-9, *trans* Δ-11-conjugated linoleic acid promotes neuronal differentiation [20] in rats. These reports thus suggest that the effects of CLA remain to be resolved cautiously.

2. Physicochemical properties of fatty acids

Fatty acids are ubiquitous biological molecules. They are esterified to numerous complex lipid molecules, including triglycerides, phospholipids, and cholesterol esters. As being part of these molecules, fatty acids thus may govern some of their physical properties. The aliphatic chains and their lengths confer hydrophobicity to fatty acids. The hydrophobic nature of the fatty acids renders them insoluble in aqueous environments.

At very high pH, where the longer chained fatty acids are totally ionized, they form micelles, which are thermodynamically stable aggregates of molecules in aqueous solution [21]. This property confers the ionized fatty acids to detergent properties. However, to achieve a stable micelle formation, the fatty acids must be present in a solution at a pH greater than 9, which is generally unphysiologic. In fact, the most probable state of fatty acids at physiological temperature and pH is a membrane-like bilayer structure [22] (**Figure 4**, the middle one). The chain length of the fatty acid is interrelated with melting point; the higher the chain length, the lower the melting point. The double bonds in the (poly)unsaturated fatty acids further decrease their melting points [23]. This is very critical to the survival of mammals that live in extremely cold environments such as the polar areas of the earth. The presence of fatty acids in the bilayer membranes provides an excellent anisotropic solution for other membrane constituents. They confer fluidity to the membrane bilayer [24], wherein membrane-bound receptors, enzymes, and other proteins can diffuse laterally along the surface of the bilayer membrane. Phospholipids can also flip-flop between the bilayer leaflets and/or fatty acyl chains can have a vertical motion (translational motion). The word membrane fluidity can thus be referred to as the degree of stiffness or rigidity of the cellular bilayers. As saturated fatty acids are straight-chained, they can pack/stack easily with themselves and/or with the neighbor-cholesterol in the bilayer membrane. The (poly)unsaturated fatty acyl chains, on the other hand, retain bent(s) along the long axis of the chain at the position of double bonds; thus, they cannot align/stack tightly (**Figure 5**).

Consequently, they increase the degree of membrane fluidity. Therefore, the greater the degree of unsaturation of the fatty acids, the higher the fluidity of the membrane. We have previously reported the DHA, which has six double bonds, contributes to a greater extent in membrane fluidity than less-unsaturated

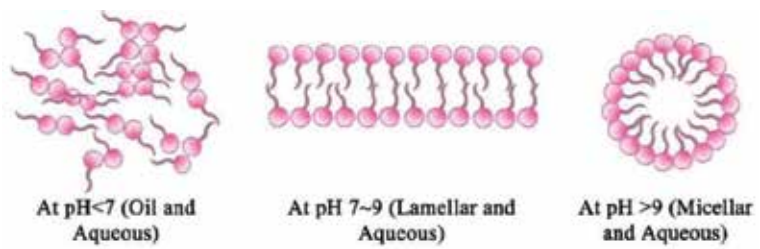

Figure 4.
The arrangements of fatty acids in aqueous environments at T > Tc. T = temperature. Tc = melting point of the fatty acid.

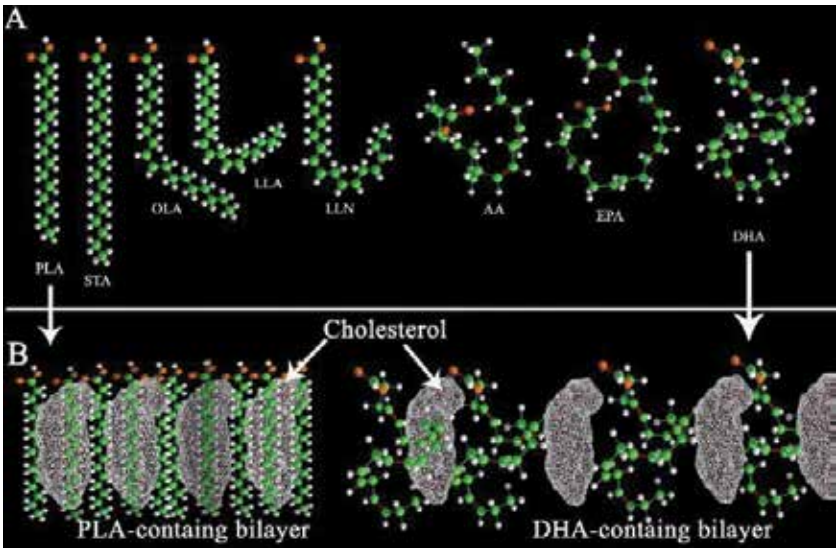

Figure 5.
The 3D structural features of the most common fatty acids (A). PLA = palmitic acid, STA = stearic acid, OLA = oleic acid, LLA = linoleic acid, LLN = α-linolenic acid, AA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid. Double bonds of the unsaturated fatty acids are denoted by red color (in A) (B). Because of the presence of double bond(s) along the long axis of (poly)unsaturated fatty acids, they occupy more space when they are esterified in the phospholipid bilayer and loosely align with 3D cholesterol, and increase the degree of disorder (membrane fluidity). However, when straight-chained saturated fatty acids like PLA highly align (stacks) with 3D cholesterol, the degree of packing in the bilayer increases (tightens); hence, the membrane bilayer becomes more rigid, that is, less fluid.

fattyvacids, such as EPA and/or saturated fatty acids [25, 26]. As a whole, the physicochemical properties of the fatty acids may affect the functions of these molecules [27], ultimately leading to altered functions of the cells and organisms.

3. Fatty acid oxidation

Fatty acids are usually oxidized by most of the cells of tissues in the body, except the RBCs. The cells of the central nervous system also do not use fatty acids for their energy requirements, using instead carbohydrates or ketone bodies. Heart cell fully depends on energy derived from fatty acid oxidation. Fatty acids constitute the principal source of energy for cells between meals, during hypoglycemia, and/or in diabetes. Beta-oxidation of fatty acids takes place in the mitochondria and, to some extent, in the peroxisomes, particularly the very long chain fatty acids [28]. Unlike in the mitochondria, beta-oxidation of fatty acid in the peroxisomes is not coupled to ATP; the high-potential electrons are rather transferred to O_2, yielding hydrogen peroxide (H_2O_2) and generating heat. The enzyme catalase, found exclusively in peroxisomes, converts H_2O_2 into water and oxygen. H_2O_2 is also used intracellularly to digest unwanted wastes like proteins and/or to defend against intracellular foreign particles including toxins or microorganisms. All fatty acids are not oxidized at the same rates, which implicates that the purposes of cellular accumulation of fatty acids are not the same for all cells. Some fatty acids might have been exploited for energy purposes, some of them might be exploited for the structural purposes, and some of them (or their derivatives) might help the cell for the signal transductions. For example, 30–40% of all esterified fatty acids in the neural plasma membrane phospholipids consist of DHA [29], while EPA constitutes only a tiny percent of the brain total fatty acid. Among the saturated fatty acids, lauric acid (12:0) is oxidized

at the fastest rate and is the most efficient energy substrate[30]. Oleic acid (18:1) is also oxidized at a remarkably faster rate, similar to that of lauric acid. Of the ω-6 essential fatty acids studied, linoleic acid (18:2, ω-6) is oxidized at a faster rate, with arachidonic acid (20:4, ω -6) being oxidized at the slowest rate. DHA and EPA possess different oxidizing properties [31, 32]. DHA is a poor substrate for both mitochondrial and peroxisomal beta-oxidation [33], while EPA can be oxidized and to a much greater extent than DHA [33, 34]. The mechanisms of these properties are not fully elucidated, although intensive investigations are continuously going on. Furthermore, ω-3 fatty acids are incorporated into cell membranes in a highly selective manner where they act as structural components influencing fluidity of the membrane [35]. The ω-3 fatty acids also compromise themselves for enzymatic biotransformations into eicosanoids/docosanoids that act as intracellular signaling molecules and, finally, they get involved in the activity of membrane-bound enzymes, ion channels, and receptors [36]. When EPA is administered to rats, both the EPA and DHA accumulate in different organs, including brain [37], indicating EPA is elongated to DHA. DHA administration also leads to an accumulation of EPA both in the plasma and brains, however, only a tiny percent [37]. As DHA seems difficult to metabolize, we thus speculate that DHA is retroconverted to EPA for further metabolism. Therefore, EPA and DHA imply different metabolic properties in the cells of the brains.

4. Roles of ω-6 and ω-3 PUFAs in physiology

4.1 Platelet physiology

Platelets are derived from megakaryocytes and cause aggregation and play important roles in physiological conditions and pathological conditions as well. Fatty acids are enriched in the plasma membranes of platelets and thus may contribute to the physiology and pathology of platelets. Oral administration of ω-3 PUFAs to rats decreases the degree of platelet aggregation both in rats and humans [38, 39]; hence, it is evident that fatty acids may affect the platelet physiology and atherosclerosis. The mechanisms through which PUFA affects the platelet aggregation is unclear; however, it is assumed that ω-3 PUFA deceases the levels of atherogenic ω-6 PUFA particularly platelet membrane-AA, which acts as a proaggregatory fatty acid. Therefore, ω-3 prevents platelet aggregation by inhibiting PLA2 and interrupting the prostaglandin/thromboxane pathways [40, 41]. In addition, ω-3 PUFAs modulate the platelet membrane fluidity [42], specific lipid domains that hold the receptors for a variety of aggregation factors, such as ADP, thrombin, fibrin, etc. [37], and doing so, they decrease platelet aggregation.

4.2 Effects of fatty acids on hypertension

The effect of fish oil on hypertension came into light when the Norwegian under Nazi invasion had to consume more fish rather than land-based food items during WWII [43]. The Norwegian had low blood pressure, low degree of platelet aggregation, and hypocholesterolemia as well. Afterwards, in studies on the Greenlandic Eskimos, Dyerberg and Bang [44] and Fischer et al. [45] reported that the Eskimos had also a low incidence hypertension and blood cholesterol levels. Then, oil components of marine animals and fish, in particular EPA and DHA, were attributed to lower incidence of cardiovascular risk factors, such as hypertension, hypercholesterolemia, and platelet hyperaggregation. We have previously reported that oral administration of EPA and DHA to rats

(hypercholesterolemic) decreased the hypertension [46] and hypercholesterolemia [47]. The results were consistent with many other published reports [48]. To understand the mechanism(s) of action of these PUFAs, we also pretreated the rat thoracic endothelial cells with these PUFAs and some interesting data emerged from our experiments. For example, the EPA and DHA increased the plasma levels of nucleotide products including ATP, ADP, AMP, and adenosine. The blood vessels of the PUFA-fed rats exhibited less sensitivity to noradrenaline and had caused an increased release of the total purines (ATP + ADP + AMP + Adenosine), concurrently with less contractility [47]. We hypothesized that these nucleotides and their derivatives decreased the noradrenaline sensitivity to purine-receptors of the blood vessels and decreased the blood pressure. The mechanism also might be related to the EPA/DHA-induced increase in the membrane fluidity of the endothelial cells (ECs). These hypotheses led us to preincubate the cultured ECs with EPA and DHA. As expected, the PUFAs increased the membrane fluidity of the ECS [49]. The inhibitory effects of fish oil ω-3 polyunsaturated fatty acids (PUFAs) have also been reported on the expression of endothelial cell adhesion molecules [50]. Hence, the ω-3 PUFAs might have played beneficial roles in reducing hypertension in the animal models as well as in human cases who consumed fish/marine animals' oils in their everyday life.

4.3 Effects of fatty acids on hepatic functions

Saturated and/or unsaturated fatty acids are indispensable for the functions of all tissues in the mammalian body. However, an adequate balance between saturated and (poly)unsaturated and between ω-6 and ω-3 PUFAs is essential to the proper functioning of the cells. Fatty acids after their absorption in the intestinal epithelial cells are first carried to the liver, which acts as a distribution center for the whole body. Therefore, fatty acids can affect the liver functions. Inadequate amounts of essential fatty acids may cause disorders of the liver, such as fatty liver, liver cirrhosis, metabolic syndromes, hyperlipidemia, hypercholesterolemia, and other liver problems [51, 52].

Oral administration of ω-3 DHA decreases the plasma as well as hepatic cholesterol and triacylglycerol levels [53]. The mechanism through which ω-3 PUFAs decrease the plasma cholesterol is not clear; however, it is attributed to the inhibition of hepatic HMG-CoA reductase by the PUFAs, including EPA and DHA. To prove the mechanism, we determined the levels of hepatic mRNA levels of HMG-CoA reductase (yet unpublished) of the DHA-fed rats. DHA decreased the expression of HMG-CoA reductase. Our results were also consistent with numerous other published reports [54–56]. The beneficial effects also emerged at lower levels of LDL-C and TG and high levels of HDL-C. The oral administration of DHA also increased the levels of ω-3 PUFAs and decreased the levels of ω-6 AA both in the plasma and liver tissues. It might be suggested that the oral administration of PUFAs like DHA increases the degrees of oxidative stress and mammalian tissues, including the liver. However, the levels of lipid peroxide (LPO) and reactive oxygen species (ROS) were not increased, thus demonstrating that the feeding of DHA does not pose an oxidative stress to the tissues. We suggest that the oral administration of DHA rather increases the levels of antioxidative enzymes, including glutathione peroxidase and catalase, and antioxidant substrate like GSH [53]. In a similar study, the levels of antioxidative enzymes and GSH increased in the brains of hypercholesterolemic aged rats after oral administration of DHA [57]. However, there are also contradictory results where consumption of PUFA was reported to promote oxidative stress [58]. Furthermore, we isolated and purified the canalicular plasma membranes of the hepatic cells of EPA/DHA-fed rats.

These membranes allow the transport and pump bile components in-and-out of the hepatic cells. The levels of PUFAs increased in the canalicular plasma membranes, concurrent with increases in the activities of membrane-bound enzymes such as Mg^{2-}-ATPase, 5′-nucleotidase. Membrane fluidity also increased in these membranes, thus suggesting that an increased fluidity might have helped in the pumping out the cholesterol via the bile (**Figure 6**). Otherwise, the levels of fecal cholesterol could have not been increased in the feces of the fish-oil-fed rats [53].

4.4 Anti-inflammatory responses

ω-6 PUFA like arachidonic acid (AA, C20:4, ω-6) generates 2-series prostanoids, namely prostaglandins PGE_2, PGI_2, PGD_2, and $PGF_2\alpha$ (largely produced by monocytes and macrophages) and thromboxanes TXA_2 and TXB_2 by COX-1/COX-2 enzymes. Prostaglandin PGI_2/PGE_2 has proinflammatory effects. AA by the action of LOX also produces leukotrienes such as 5-HETE and 5-HPETE, LTE_4, LTB_4, LTC_4, and LTD_4. They are strong proinflammatory agents and have vasoconstriction effects and platelet- and/or neutrophil- and macrophage-activating effects [59–61]. Interestingly, the eicosanoids derived from the action COX and/or LOX on EPA and DHA produces 3-series prostaglandins and thromboxanes and 5-series leukotrienes, and they are less inflammatory and even have anti-inflammatory effects, as compared to the eicosanoids derived from AA. These lipid mediators antagonize the effects of those derived from AA, thus conferring beneficial effects on inflammatory responses [62].

4.5 Effects on skeletal muscles

Skeletal muscle is the largest organ in the human body, comprising approximately 40% of total body weight [63]. This muscle has a plastic-like property and has adapting capability to physical activity. Strenuous muscle exercise increases muscle fatigue and decreases muscle strength, leading to an increase in muscle oxidative stress. It is believed that the response of skeletal muscle to exercise can be modified by the nutritional status of the muscles. There

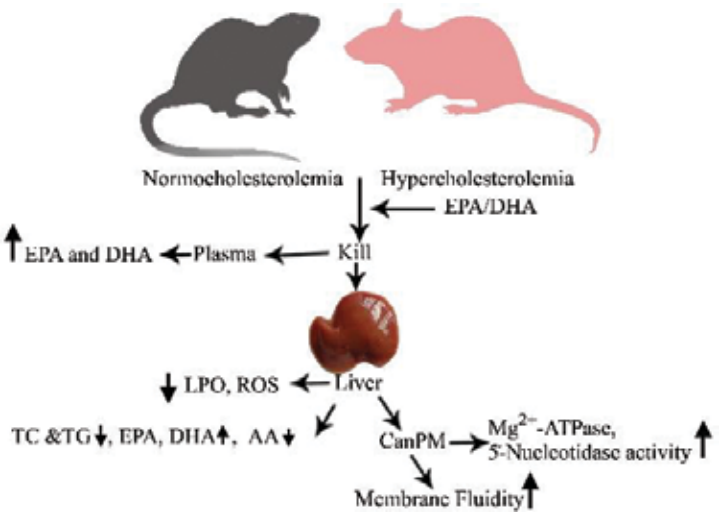

Figure 6.
Effects of oral administration of EPA and DHA on the plasma and hepatic lipid profile (TC = total cholesterol, TG = triacylglycerol), LPO = lipid peroxide, ROS = reactive oxygen species, CanPM = canalicular plasma membrane of hepatic cells).

are numerous reports on the beneficial effects of EPA and DHA on muscle. Therefore, the effects of these PUFAs on muscle strength have been investigated with increasing interest. Hess et al. [64] reported that dietary algae and marine fish increase the levels of EPA and DHA in the equine skeletal muscles. Guen et al. [65] reported that DHA-enriched supplementation improves endurance exercise capacity and skeletal muscle mitochondrial function in murine skeletal muscle. Stebbins et al. [66] reported that DHA + EPA enhances skeletal-muscle blood and vascular conductance in active skeletal muscle (especially type I and IIa fibers) and that the increase in muscle blood is due to an increase in cardiac output secondary to increases in vascular conductance [66]. However, we believe that there are differential effects of PUFAs on the muscle [67]. AA deposition in the fast-twitch muscle of aging rats reduced cell volume with an increase in oxidative stress [68].

5. Effect of ω-3 DHA/EPA on brain cognition

As neurons are the structural and functional units of brain, electrochemical properties of the neurons allow them to transmit signals over long distances and send information to each other. Neurons form the basis of the brain activity and brain cognition and dictate the whole body when and how to work and maintain the behavior of the animals, including humans. Numerous reports have been published stating that the PUFAs have colossal roles in brain growth and development, learning, and memory. At the same time, deficiency of PUFAs such as DHA has been reported to cause neurodegeneration leading to impairments of memory and brain cognition.

Henriksen et al., reported that the level of DHA was low in the preterm infants (born at <33 weeks gestation, body weight < 1.5 Kg). Concurrently, the preterm infants had learning disabilities, reduced IQ, and weak visuospatial relations. However, when these infants were supplemented with DHA, they exhibited normal growth and development in terms of body weight, height, head circumference, visual acuity, and mental development [69]. The study thus suggests that DHA is important before birth. Infants (9-month-old, growth spurt period) fed with DHA-supplemented traditional formula showed higher problem-solving activities, when compared with those fed with traditional formula-only, suggesting thus that DHA also plays an important role during growth spurts and development [70]. Infant's gray matter autopsy (of human/nonhuman primate) study showed that brain DHA levels have also 40% higher in the DHA-supplemented formula-fed infants than those in the formula-fed only infant brains [29, 71]. In addition, DHA declines in aging and age-related neurodegenerative diseases such as Alzheimer's disease [72–74]. All these investigations thus suggest that DHA is important for brain cognitions, such as learning and memory, thought processes, tracing of new information, and comprehension, and that brain DHA deficiency can be recovered by the dietary DHA supplementation. Though cerebral endothelial cells and brain astroglial cells can synthesize DHA and/or α-LLN, EPA from the diet can act as precursors for the DHA; however, the endogenous synthesis or conversion of DHA is extremely low [75] . Thus, dietary DHA is the ultimate source for the DHA in the brain.

We have previously reported that oral administration of DHA for 12 weeks significantly increased the learning-related memory, as evaluated by the 8-armed-radial maze task in DHA-deficient young and old rats [76, 77]. Not only DHA increases the memory of DHA-deficient young and old rats, DHA

also had an extraordinary ability to increase the learning-related memory of Alzheimer's disease model rats [78, 79] (**Figure** 7). EPA also increased the learning-related memory, however, only after their conversion into DHA [80]. The roles of ω-6 AA on the brain cognitions have also recently been investigated; however, the results are controversial. Memory-enhancing effect of AA has been reported previously [81]. In our investigation, the ω-6 AA failed to increase memory of rats (yet unpublished). DHA always exhibited a positive effect on memory. However, the mechanisms by which DHA increases the memory remain to be clarified. Numerous mechanisms of action of DHA on memory have been proposed. DHA-induced increases in synaptic plasma membrane fluidity [26]; antioxidative effects [76–79]; anti-apoptotic effect [78]; increased expressions memory-related proteins, including postsynaptic PSD-95, presynaptic synaptophysin, NMDA-receptor unit NR2A [75], and c-fos [82]; and reductions of brain Aβ-burdens [83] have been attributed to the beneficial effects of DHA in the normal and AD rats, respectively. To examine the mechanism(s) of the reduction of amyloid burden, we tested whether DHA affects the *in vitro* Aβ peptide ($A\beta_{25–35}$, $A\beta_{1–40}$, and $A\beta_{1–42}$ are the most toxic amyloids) fibrillation, a process that assumes to increase the Aβ deposition in the brains. We found that DHA inhibits *in vitro* Aβ fibrillation both at the initial stage of Aβ-seed formation and oligomerization and also causes dissolution of mature Aβ peptide fibers [84–86] (**Figure 8A**). It is thus conceivable that DHA, by decreasing the amyloid fibrillation, decreases the brain Aβ-burden and hence contributes to the amelioration of memory of AD model rats. DHA also caused a clearance of circulating Aβs by increased lipid raft-dependent degradation pathways [87].

We later tested whether DHA affects neurogenesis, which is of great interest in the modulation of memory both in the aging and neurodegenerative Alzheimer's disease. As expected, DHA accelerated both *in vitro* and

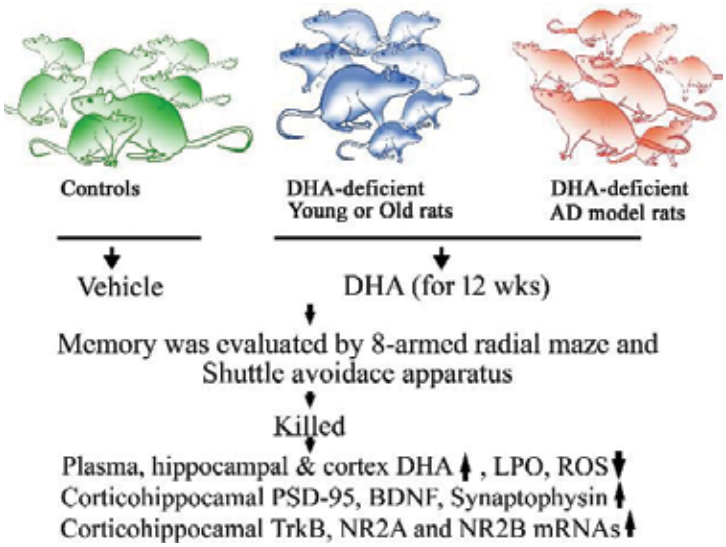

Figure 7.
Effect of oral administration of DHA on the learning-related memory of DHA-deficient young/old and Alzheimer's disease model rats. Protein levels of postsynaptic density protein (PSD-95), brain-derived neurotropic factor (BDNF), and presynaptic synaptophysin were measured. Also, the mRNA levels of BDNF-receptor tyrosine Kinase B (TrkB) and NMDA receptor units NR2A and NR2B were determined by RT-PCR to examine whether they were affected by the oral administration of exogenous DHA. All these parameters were ameliorated by the oral administration of DHA.

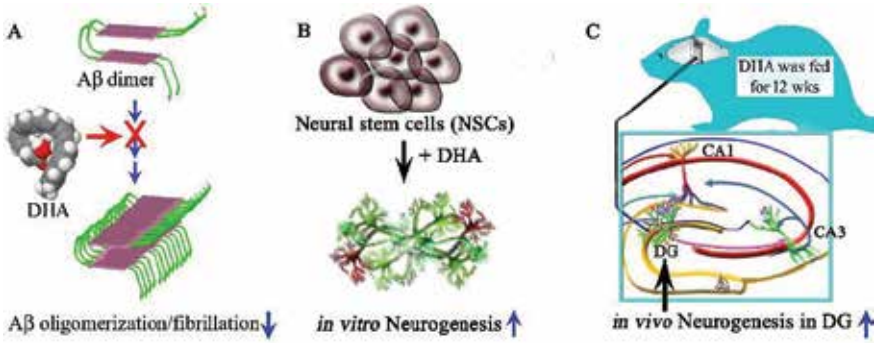

Figure 8.
Effect of incubation of DHA on in vitro amyloid beta (Aβ) peptide fibrillation (A) and in vitro neurogenesis in NSCs culture (B) and, effect of oral administration of in vivo neurogenesis (C). Neurogenesis occurred primarily in the dentate gyrus (DG) region.

in vivo neurogenesis [88] (**Figure 8B, C**), which is conducive to inhibition of the impairments of memory in aging and/or AD model rats. DHA stimulated the differentiation of neural stem cells into mature neurons by triggering the activating-type bHLH transcription factors, including neurogenin, Mash1, and NeuroD and inhibiting the repressor-type transcription factor Mes1 [89]. We also reported that DHA-derived docosanoids, such as neuroprotectin D1, help increase the memory of rats [90]. Consistent with our results, Bazan et al. [91] also reported that endogenous signaling by DHA-derived mediators sustains neuronal function and protects synapses and circuits, thus demonstrating that DHA and/or its docosanoid products might act as signaling molecules during memory processing. Finally, DHA is essential for the growth and development of brain and might play crucial roles in the formation of learning-related brain cognition.

6. Conclusion

For the last several decades, fatty acid nutrition, in terms of quality, has been dramatically changed [92]. Consumption of saturated fatty acids, ω-6 PUFAs, and *trans* fatty acid intake has been increased [93]. Optimal dietary ω-6:ω-3 ratio should be around 1–4:1; however, this ratio has now increased to 10:1 to 20:1 in the Western diet [92]. Concurrently, the incidence of diseases involving inflammatory diseases, cardiovascular disease, obesity, rheumatoid arthritis, cancer, neurodegenerative, and psychiatric illnesses, such as AD and depression, are increasing with an ever-increasing rate [94]. The results of our investigations and those of the others, finally, suggest that DHA is accumulated in the synaptic plasma membranes, represses oxidative stress by increasing the antioxidative defense, decreases cholesterol in the detergent-insoluble membrane fraction (DIMF) of the brain tissues, increases synaptic plasma membrane fluidity, inhibits amyloid fibrillation and decreases amyloid toxicity and burden in the brain tissues, improves the neuronal morphology, increases memory-related protein substrates, and hence ameliorates the memory-related brain cognition (**Figure 9**). In conclusion, a balanced intake of ω-3 and ω-6 PUFAs is a must, as well as an increased intake of DHA, which might act as a signaling molecule to protect the brains from preterm-, postnatal-, and other age-related neurological diseases, such as Alzheimer's disease.

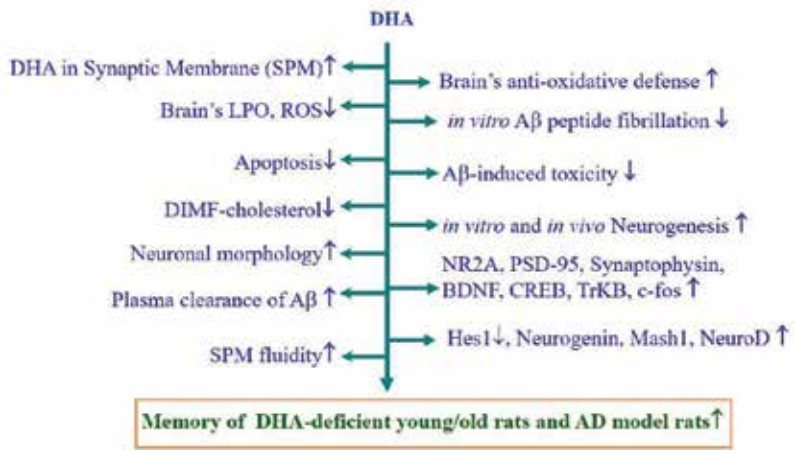

Figure 9.
Outlines of the effect of DHA on learning-related memory of rats. SPM = synaptic plasma membrane. DIMF = detergent-insoluble membrane fraction. All other abbreviations are same as for other figures.

Author details

Michio Hashimoto[1*] and Shahdat Hossain[2]

1 Department of Environmental Physiology, Shimane University Faculty of Medicine, Japan

2 Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka, Bangladesh

*Address all correspondence to: michio1@med.shimane-u.ac.jp

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Chapter 3

Cyclic Fatty Acids in Food: An Under-Investigated Class of Fatty Acids

Augusta Caligiani and Veronica Lolli

Abstract

Cyclic fatty acids are an unusual class of minor fatty acids generally produced by bacteria and less frequently by plants. Among plants, the most known cyclic fatty acid is sterculic acid (9, 10-methyleneoctadecenoic acid) produced by *Sterculia foetida*. Bacteria (e.g., lactic acid bacteria) synthetize cyclopropane fatty acids, such as dihydrosterculic acid (9, 10-methylene octadecanoic acid) and lactobacillic acid (11, 12 methylene octadecanoic acid), to strength their membrane, improving their resistance to environmental stress. Another class of cyclic fatty acids is omega-cyclohexyl fatty acids, present in milk and probably produced by rumen bacteria. Cyclopropane and omega-cyclohexyl fatty acids have been recently found in bovine meat and dairy products, representing important foodstuffs in human diet. In this chapter, a review of literature data concerning the presence of cyclic fatty acids in foods, their metabolism in humans, and their potential bioactivity will be provided. The role of some cyclic fatty acids as molecular markers for food authenticity will also be highlighted.

Keywords: cyclopropane fatty acids, cyclohexyl fatty acids, food authentication, metabolism, bioactivity

1. Introduction

Lipids are water insoluble organic biomolecules that have several important biological functions within the cell, providing energy storage, participating in the formation of cell membranes, and exerting regulatory functions in transduction and signaling processes in multiple metabolic pathways [1]. Through these actions, dietary lipids can affect health, well-being, and the risk of developing disease, such as cardiovascular, inflammatory, and cognitive disorders, among many others [2].

The term lipid is known to describe fatty acids, their esters, and different lipophilic structures. Most dietary lipids consist of triglycerides, but there is also little amount of other lipid classes, such as phospholipids, present in the cell membranes of all food that we eat [3].

Fatty acids (FA) are carboxylic acids with an aliphatic chain of varying lengths: short chain (C < 6), medium chain (6C-12C), long chain (13C-22C), and very long chain (C > 22). The most common chain length range for fatty acids is between C12 and C22 and they can be characterized by saturated and unsaturated (mono or poly) chains [4]. Most of the FA existing in nature have an even number of carbon

atoms and linear hydrocarbon chains, although some of them, found primarily in bacteria, may contain branched or cyclic structures [5–7]. Fatty acids containing a carbocyclic unit naturally occur in specific genera of bacteria and in plants.

In some cases, alicyclic fatty acids, such as cyclopropane (CPFA) and omega-cyclohexyl fatty acids (CHFA), are essential for cell survival, as they could affect the membrane fluidity that enables certain microorganisms to survive under extreme environmental conditions [8]. In plants, CPFA are usually minor components, where cyclopropene fatty acids are the most abundant. *Sterculia foetida* seed oil contains 65–78% of cyclopropene fatty acids (principally sterculic acid), suggested to have antifungal and enzyme inhibitor activities [9].

CPFA, especially dihydrosterculic (9,10-methylene-octadecanoic acid) and lactobacillic (11,12-methylene-octadecanoic acid) acids, have been identified as minor component of lipid profile in a wide range of milk and dairy products [10, 11] and, more recently, in meat and fish [12] representing important foodstuffs in human diet.

CPFA concentration ranges from 200 to 1000 mg/kg fat in dairy products and bovine meat [13]; therefore, their dietary intake may not be negligible, and their potential role in human health should not be underestimated.

However, due to their recent identification, so far CPFA have not been yet considered for their occurrence in humans, and several aspects related to their bioavailability and putative bioactivity as well as the bacterial strains producing CPFA in feeds and in which conditions still must be explored.

ω-Cyclohexyl fatty acids (CHFAs), mainly cyclohexyl-undecanoic and tridecanoic acids, occur in several acido-thermophilic bacteria such as *Alicyclobacillus acidocaldarius* and can be biosynthesized by these bacteria species, even by adding cyclohexyl acid to the bacteria culture [11]. 11-cyclohexyl undecanoic acid was first isolated as a minor component of butter fat, then in sheep fat but it is almost certainly produced by bacteria in the rumen. 13-cyclohexyltridecanoic acid has been considered as a potential marker of ruminal acidosis in cow [11]. Recently, both ω-cyclohexyl fatty acids, 11-cyclohexylundecanoic acid and 13-cyclohexyltridecanoic acid, were detected in meat fat, especially in bovine meat but not in pork and horse meat. Therefore, the presence of ω-cyclohexyl fatty acids in foods was related to a ruminal origin and, combined with other fatty acids as branched chain fatty acids, could be proposed as marker of species [14].

This chapter reviews the literature data about the origin and natural occurrence of cyclic fatty acids, their presence in foods, especially in meat and dairy products, and their potential bioavailability and bioactivity in mammals. Finally, the application of some cyclic fatty acids as molecular markers for food authenticity will be provided.

2. Cyclic fatty acids: natural occurrence and biosynthesis

2.1 Cyclopropane and cyclopropene fatty acids

Cyclopropane fatty acids (CPFA), containing three carbon rings located at different sites of the fatty acid chain (**Figure 1**), occur widespread in several microorganisms as major lipid component [8] and in certain eukaryotes, including protozoa, fungi, and plants [9, 15]. Many cyclopropane-containing natural compounds have shown biological activity, and their presence in the cellular membrane seems to be related to its physicochemical properties [16]. However, the real significance of these compounds in their natural context is often less well known as well as their occurrence in higher animals. The major investigations which have been

n,m	Common name
9,5	lactobacillic acid
6,7	dihydromalvalic acid
7,7	dihydrosterculic acid

Figure 1.
Most commonly found cyclopropane fatty acids in bacteria.

published about their occurrence, biosynthesis, and their physiological role in the cellular membrane are described in more detail in the following paragraphs.

2.1.1 Distribution

A study of the fatty acid composition of *Lactobacillus arabinosus* first reported the isolation of lactobacillic acid (cis-11,12-methylene octadecanoic acid), a 19-carbon cyclopropane analogue of cis-vaccenic acid, the major unsaturated fatty acid in *L. arabinosus* membrane [17].

Subsequently, lactobacillic acid and other cyclopropane fatty acids have been identified in a variety of microorganisms of both Gram-negative and Gram-positive bacteria such as Lactobacilli, Streptococci, Enterobacteria, Clostridia, and Brucellaceae [18]. Some microorganisms contain cis-9,10-methylene octadecanoic acid (dihydrosterculic acid), derived from oleic acid, together with other isomers (C16 or C20 in chain length, as cis-9,10-methylene hexadecenoic acid).

CPFA are suggested to be associated with the occurrence of unsaturated fatty acids (UFA) in the bacterial membrane, generally palmitoleic (cis-9-hexadecenoic acid), cis-vaccenic (cis-11-octadecenoic acid), and oleic (cis-9-octadecenoic acid) acids [5]. Furthermore, it seems that they predominate at the end of the growth cycle of bacteria, when the majority of UFA are converted to cyclopropane fatty acids by the cyclopropane synthase [8].

Cyclopropane and the structurally related cyclopropene fatty acids have also been found in certain eukaryotes, including trypanosomatid protozoa and plants [19, 20].

In plants, cyclopropene fatty acids, such as sterculic acid (cis-9,10-methylene-9-octadecenoic acid) and malvalic acid (cis-8,9-methylene-heptadecenoic acid), are distributed across several families, mainly in Sterculiaceae, Malvaceae, Bombacaceae, Tiliaceae, and Sapindaceae. It has been reported that cyclopropene fatty acids are often accompanied by smaller proportion of cyclopropanic fatty acids, such as dihydrosterculic and dihydromalvalic acids, which are the dihydro analogues of cyclopropene fatty acids [21].

Sterculia foetida is a tropical tree belonging to the Sterculiaceae family of order Malvales. Its seeds are rich in oil (55% dry weight) and contain up to 78% of cyclopropenoid fatty acids (especially sterculic and malvalic acids), representing one of the highest source of carbocyclic fatty acids reported in nature [19].

CPFA were the major lipid component (42%) in the seed oil of *Litchi chinensis*, belonging to Sapindaceae family. The CPFA fraction in *Litchi chinensis* seed oil mainly contains dihydrosterculic acid, and cis-7,8-methylenehexadecanoic acid, cis-5,6-methylene-tetradecanoic acid, and cis-3,4-methylenedodecanoic acid in smaller amounts [22].

Malvalic, sterculic, and dihydrosterculic acids have also been detected in Baobab seeds oil from plant belonging to Adansonia species (Bombacaceae family) of Madagascar. Seed lipids containing CPFA are extensively consumed by humans, especially in those tropical areas [19, 23].

However, carbocyclic fatty acids seemed not to be confined to seeds. Long-chain cyclopropane fatty acids have been described in various polar lipid classes of leaves of early spring plants, whereas both cyclopropane and cyclopropene fatty acids were found in root, leaf, stem, and callus tissue in plants of the Malvaceae [9].

The presence of CPFA has also been documented in some aquatic invertebrates, marine isolates [24, 25] and in the lipid composition of mushrooms, mainly belonging to the family *Boletaceae* [15]. Overall, the natural distribution of CPFA among eukaryotes appears much less common than among bacteria.

2.1.2 Biosynthesis and physiological aspects

CPFA synthesis involves the transfer of a methylene group from S-adenosyl methionine by the CPFA synthase to the cis double bond of the precursor unsaturated fatty acids, already integrated into phospholipids of cellular membrane [5]. A proposed pathway for the biosynthesis of dihydrosterculic acid from oleic acid is shown in **Figure 2**.

The reaction is a post synthetic modification and has been widely studied in microorganisms such as *E. coli* [26–29], Pseudomonas, Mycobacterium, *Lactobacillus* spp. and *Leishmania* spp. [20, 30, 31].

Cyclopropane fatty acids are not essential fatty acids, but the bacterial production of cyclopropane ring seems to be related to changes in the membrane fatty acid composition of that microorganisms. In fact, the presence of these specific fatty acids seems to favor the stress tolerance of several bacteria strains to adverse environments (including ethanol, osmotic and oxidative stress, hot temperature, and low pH) and likely plays a role in the pathogenesis of bacterial infections [32].

The acid tolerance of individual strains of *E. coli* appears to be correlated with membrane cyclopropane fatty acid content and may enhance the survival of microbial cells exposed to low pH [29]. During acid habituation, monounsaturated fatty acids (cis-16:1 and cis-18:1) are either converted to their cyclopropane derivatives or replaced by saturated fatty acids. On the contrary, *E. coli* mutants deficient in CPFA seemed to be more sensitive to adverse conditions such as repeated freeze-drying and pressure [33].

Natural CPFA occur widespread with a cis configuration about cyclopropane moiety [5]; however, trans cyclopropane fatty acids are common in the cell

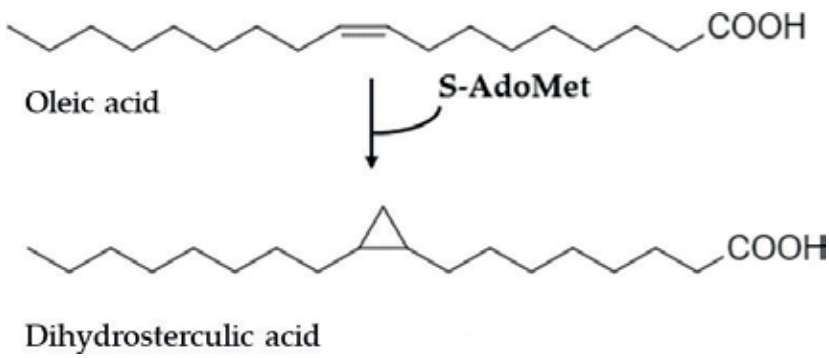

Figure 2.
Biosynthesis of dihydrosterculic acid from oleic acid by CPFA synthase.

envelope of *Mycobacterium tuberculosis* and play a role in regulating virulence. Cyclopropanation of mycolic acids has been suggested to be correlated with the persistence of the pathogen and modulates the innate immune response of the host [34, 35].

Cyclopropane fatty acids tend to promote the fluidity of lipid bilayers by interfering with lipid packing, improving the formation of gauche defects originating partly from the steric restraints caused by the methylene moieties and increasing lipid diffusion [31]. This could explain how cyclopropane fatty acids can improve the stability of the membrane against adverse conditions and, at the same time, reduce its permeability against toxic compounds.

CPFA are cellular components of lactic acid bacteria (LAB), such as *Lactobacillus bulgaricus*, *L. helveticus*, *L. sanfranciscensis*, and *L. acidophilus*, and are synthetized to strength their membrane, improving their resistance to unfavorable conditions to which LAB are exposed during their proliferation and lactic fermentation in foods as well as the response to osmotic and ethanol stresses [30].

Recently, we reported the presence of CPFA in ensiled feeds (as maize silage) and in milk and cheeses from cow fed with silages [10, 11]. Some LAB strains, both homofermentative such as *Lactobacillus plantarum* and heterofermentative (i.e., *Lactobacillus buchneri* and *Lactobacillus brevis*), are known to represent major constituents of the microbial ecosystem in silages [36]. Crop ensiling technology is based on the natural fermentation of plant tissue juice mediated by the lactic acid bacteria naturally present in the plant leaves. LAB convert soluble carbohydrates to organic acids, mainly lactic acid, under anaerobic conditions, resulting in a pH drop from 6.0–6.5 to 5.0–3.7 [36]. Therefore, the presence of CPFA in milk was related to their presence in ensiled products, where they are released by bacteria during silage fermentation conditions. Further studies on dairy products [10] demonstrated that LAB, ubiquitous in fermented milk and cheeses, were not able to release significant amount of CPFA in the medium during milk fermentation, and their presence in fermented milk products derives only from their starting content in milk.

2.2 Omega-cyclohexyl fatty acids

Omega-cyclohexyl fatty acids (CHFAs), as 11-cyclohexyl undecanoic and 13-tridecanoic acids (**Figure 3**), occur in several acido-thermophilic bacteria, mainly in *Alicyclobacillus acidocaldarius* [8, 37, 38].

Cyclohexanecarboxylic acid starter unit in omega-cyclohexyl fatty acid synthesis is derived from shikimic acid, and it is probably related to glucose metabolism [37].

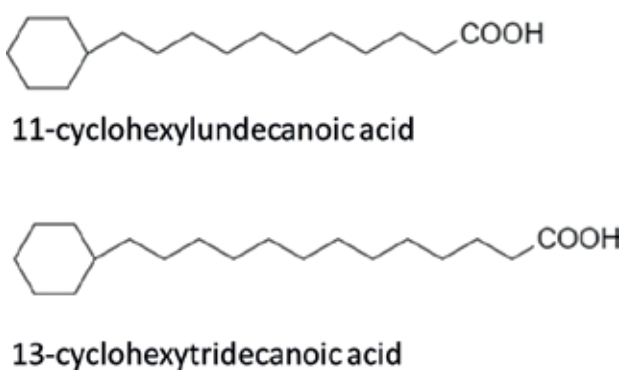

Figure 3.
Chemical structure of 11-cyclohexylundecanoic and 13-cyclohexytridecanoic acids.

In the following paragraph, information about the distribution of omega-cyclohexyl fatty acids, their biosynthesis, and their role on the cellular membrane will be provided.

2.2.1 Distribution and structure

Omega-cyclohexyl fatty acids are the principal lipid component of saponifiable fraction of *Alicyclobacillus acidocaldarius*. They also occur in thermoacidophile strains, such as *A. acidoterrestis* and *A. cycloheptanicus*, and in the mesophile *Curtobacterium pusillum*, where the percentage concentration of these fatty acids in the cellular membrane increases at pH 3–4 as well as at elevated temperatures [8, 37]. In fact, omega-cyclic fatty acids are suggested to have special physiological importance for the cells both at hot temperature and acid pH. Model membranes, consisting of lipids containing omega-cyclohexyl fatty acids, are relatively dense and closely packed even at the phase transition temperature [8].

The occurrence of a fully saturated and monosubstituted cyclohexane ring is rare but derivatives of cyclohexyl acid, precursor of omega-cyclohexyl fatty acid biosynthesis, have been isolated from the extract soil and shoots of *Achyranthes aspera* and from several *Streptomyces* antibiotics, including ansatrienin A synthetized by *S. collinus* [8]. However, in this case, omega-cyclohexyl fatty acids do not seem to play a similar membrane stabilizing role as in *A. acidocaldarius*.

In eukaryotes, the identification of 11-cyclohexylundecanoic fatty acid has been documented as a minor component of butter fat [40], then in sheep fat (0.05% of the total weight of fatty acids) [39] and more recently in cow milk [11]. In this previous work [11], they focused on the identification and characterization of cyclic fatty acids in cow milk to study the effect of diverse types of dairy diet on milk fat composition. 13-cyclohexyl tridecanoic acid methyl ester was well detectable in all milk samples by GC-MS analysis of fatty acid methyl esters (FAME), and its presence was confirmed by mass spectra and the synthetized standard.

The presence of omega-cyclic fatty acids in milk could be related to acidic ruminal fermentation patterns. An increase in starch as well as a decrease of less digestible fiber content favors the growth of amylolytic bacteria. This leads to an increased concentration of rumen volatile fatty acids such as butyric and propionic acids, a decrease of rumen odd and branched fatty acids [41] and lactate accumulation, resulting in a pH drop, which favors not only the development of the subacute ruminal acidosis (SARA) [42] but also the growth of thermo-acidophilus bacteria, which produce omega-cyclohexyl fatty acids. In this context, the presence of omega-cyclohexyl fatty acids in milk, especially 11-cyclohexylundecanoic and 13-cyclohexyltridecanoic acids, could be represented as a parameter to detect SARA, as proposed by other authors for odd- and branched-chain fatty acids [41]. However, this hypothesis has never been confirmed by experimental data.

2.3 Cyclic fatty acids in human nutrition

Cyclic fatty acids are generally secondary compounds in fatty acid profiles of food; however, due to the recent discovery, some gaps of knowledge must be fulfilled. In some cases, especially cyclopropane fatty acids, they could reach the g/kg of total fat content in meat and dairy products [12, 43] and their dietary intake may be not negligible.

Therefore, it would be interesting to investigate on their metabolism in humans and eventual physiological effects, considering that bacteria produce cyclic fatty acids to enforce their membranes. The aim of these studies is to achieve a first never reported picture of the occurrence of CPFA in humans and their possible health effects.

In the following paragraphs, we focused on the investigation of CPFA content in foods to estimate their dietary intake and on their potential bioaccessibility in humans. Finally, a review of literature data about their potential biological effects on mammals will be provided.

2.3.1 Cyclopropane fatty acids presence in food

Data reported on CPFA, mainly in dairy products, meat, and fish, were obtained in previous publications [10–12]. The content of cyclopropane fatty acids has also been evaluated in other food categories such as probiotic food supplements, vegetable edible oils (e.g., extra virgin olive, corn, soy, and peanuts oils) and cocoa butter, soy-derived products, and mushrooms (data not published). CPFA content in food categories, resulted positive in previous analysis, is shown in **Table 1**.

Results showed that among all the analyzed food categories, the most important CPFA food source is Grana Padano cheese, reaching concentration levels of 1 g/kg total fat (**Table 1**). CPFA were detected not only in commercial bovine meat (200–400 mg/kg total fat) but also in some species of fish (eels and mullets) with concentrations between 400 and 800 mg/kg total fat [12], probiotics, and in mushrooms (data not published). On the contrary, poultry, pork meat, vegetable oils commonly consumed (e.g., extra virgin olive, corn, soy, and peanuts oils), and cocoa butter were all negative to CPFA (data not shown), indicating that CPFA presence in foodstuffs of animal origin is correlated with the use of silages in the animal feedings, whereas plant organisms generally do not produce CPFA. As a whole, our results demonstrate the bacterial and fungal origin of CPFA in foods [16, 44]. Finally, the estimated daily, weekly and monthly CPFA dietary intake in the total Italian population (all sex and ages) [45] resulted in the milligrams order, so not negligible in view of a possible physiological action by CPFA on humans. Furthermore, food processing, manufacturing, seasoning steps, and fermentation [10] seemed not to affect CPFA content in the analyzed food matrices. Certainly,

Food	No. of positive samples to CPFA[2]/tot	Mean ± SD (mg/kg total fat)	Range (mg/kg total fat)
Dairy products[1]			
Cow milk	49/50	310 ± 240	70–830
Grana Padano (Lombardy, Italy)	72/72	540 ± 110	300–1000
Other cow cheeses	30/79	360 ± 180	180–1000
Commercial butter	6/10	200 ± 100	90–335
Yoghurt/fermented cow milk	4/4	200 ± 20	170–240
Meat[1]			
Commercial beef meat	5/5	200 ± 100	200–400
Fish[1]			
Eel	2/2	500 ± 250	400–590
Mullet	1/1	700 ± 100	600–800

[1]*Results obtained combining previous analysis [10–12, 43].*

[2]*CPFA = cyclopropane fatty acids as the sum of total isomers (dihydrosterculic and lactobacillic acids) as reported by Caligiani et al. [43].*
SD = standard deviation.

Table 1.
CPFA food sources.

CPFA can be considered unknown components of the human diet, and additional information about their possible impact on humans is useful to provide a further understanding on the link between diet and human health.

2.3.2 CPFA digestibility and potential bioaccessibility

Triglycerides (TG) are the major components of dietary fats, and once ingested, they are submitted to a hydrolytic process catalyzed by lipases present in gastric and especially in duodenal digestive juices [46]. Nowadays, the evolution of the triglycerides during digestion is a subject of great interest in lipid research, as much as the development of methodologies able to evaluate both qualitatively and quantitatively all the products generated from this process [13].

As reported in the previous paragraphs, no information is present in literature about the fate of CPFA within the human body, and a thorough investigation of how CPFA accumulate and are metabolized in humans is needed. In [13], the rate of CPFA digestibility has been assessed through their lipolysis and resistance to *in vitro* simulated human gastrointestinal (GI) digestion in Grana Padano cheese, one of the most relevant sources of CPFA [43]. Results showed a high percentage of digestibility of the lipid fraction (more than 90% of free fatty acids and 1-monoglycerides were obtained after digestion). Furthermore, CPFA were all released from TG and the cyclopropane ring was not degraded, proving its resistance to GI digestion, mainly due to the acid pH of the gastric environment. Results of CPFA concentration in fat before and after *in vitro* digestion are reported in **Table 2**.

These observations are encouraging, since CPFA seemed to be potentially efficiently absorbable and, ideally, bioavailable. Certainly, additional research is needed to evaluate the diffusion of these unusual fatty acids through the membrane of the small intestine epithelial cells as well as their presence in human plasma. For this purpose, an *in vivo* study needs to be conducted to determine the eventual CPFA presence in human plasma after a CPFA-rich diet.

2.3.3 Potential biological effects of cyclic fatty acids

Cyclopropane and omega-cyclohexyl fatty acids play a significant role in increasing the chemical and physical stability of bacterial membranes to adverse conditions [8]. To the best of our knowledge, little information is reported in literature about the effect of cyclic fatty acids in higher animals. However, some papers concern about biological activity of cyclopropene fatty acids, mainly sterculic acid, in mammals [9, 47–49]. On the contrary, omega-cyclic fatty acids remain an under investigated lipid class from a nutritional and physiological significance.

Many seed lipids containing cyclopropene fatty acids are extensively consumed by humans, especially in tropical areas [9]. It has been documented that their dietary leads to the accumulation of hard fats and other physiological disorders in animals [9].

Some studies suggested the effect of sterculic acid on lipid metabolism in mammals, especially in dairy sheep [47] and in human Caco2 cells [48], as inhibitor of Δ9-desaturase, which has a key role in the endogenous synthesis of cis-9, trans-11 conjugated linoleic acid (rumenic acid), known to have interesting properties in improving human health.

It is also known that sterculic acid is a potent inhibitor of stearoyl-CoA desaturase (SCD) involved in the biosynthesis of monounsaturated fatty acids (MUFA). SCD catalyzes the NADH- and O_2-dependent desaturation of palmitate (16:0) and

Lipid classes	CPFA before digestion	CPFA after digestion
TG	540 ± 20 mg/kg	≤LOD (60 mg/kg)
FFA and MG	≤LOD (60 mg/kg)	520 ± 10 mg/kg

Results are expressed in mg/kg of total extracted fat as mean ± standard deviation. FFA, free fatty acids; MG, monoglycerides; TG, triglycerides. Cyclopropane fatty acids (CPFA) refer to the sum of dihydrosterculic and lactobacillic acids [13].

Table 2.
Free (as FFA and MG) and bound (as TG) CPFA in Grana Padano cheese before and after in vitro simulated human GI digestion.

stearate (18:0) at carbon 9 to produce palmitoleate (cis-9, 16:1) and oleate (cis-9, 18:1), respectively, and has a crucial role in regulation of adipocyte proliferation/differentiation of adipocytes, mainly in ruminant species [49, 50]. Due to the structural analogies between cyclopropene and cyclopropane fatty acids, it is possible to hypothesize a possible physiological role for CPFA in lipid metabolism as well.

Fatty acids (FA) with a cyclopropane in the structure, especially cyclopropaneoctanoic acid 2-hexyl (CPA2H), have also been recently identified in human serum and adipose tissue of obese patients [32, 51] suggesting that they are absorbed as the other fatty acids and can be selected markers of metabolic disorders such as dyslipidemia, inflammation, and increased cardiovascular risk. These studies reported that both obese patients with hypertriglyceridemia and non-obese patients with chronic kidney disease (CKD) presented elevated serum levels of CPA2H, suggesting a positive correlation between high serum levels of CPA2H and high serum TG and cholesterol concentrations rather than to body mass or body mass index (BMI). These results show that CPA2H negatively affect the cellular lipid metabolism; however, the relevance of altered serum concentrations of this fatty acid remains still unclear.

Previously, it has been reported that cyclopropane fatty acids can influence the pathogenicity of *Mycobacterium tuberculosis*, demonstrating they could modulate the host immune response [34, 35].

TNF is an inflammatory cytokine produced by activated macrophages and plays a key role as a mediator of intestinal inflammation [52]. In [52], they studied select strains of human-derived *Lactobacillus reuteri,* which are involved in human TNF immunomodulatory activity in gut. This work showed that the bacterial enzyme cyclopropane fatty acid synthase is involved in the anti-inflammatory effect of select strains of *L. reuteri*. Indeed, only the strains contained a cyclopropane fatty acid, lactobacillic acid, were able to inhibit TNF in activated macrophages, whereas cyclopropane fatty acid synthase mutants (lacking cyclopropane fatty acid synthase activity) do not suppress the production of the proinflammatory cytokine. However, lactobacillic acid seemed not to be responsible for mediating the repression of human TNF production, indicating that lactobacillic acid indirectly contributed to *L. reuteri* immunomodulatory activity, probably altering the composition and permeability of bacterial membrane, resulting in a decrease of the membrane fluidity or in an altered expression of immunomodulins.

Since significative amount of dihydrosterculic acid had been found in foods, mainly in dairy products, this fatty acid can be considered a new as well as unknown component of human diet. However, no specific works both *in vitro* and *in vivo* about the effect of dihydrosterculic acid are available in the literature, for example on enzyme activity, cellular membranes, and metabolism in mammals.

Future studies should elucidate how and whether this uncommon FA may have a biological role and clarify its healthy or unhealthy effects in humans.

2.4 Cyclic fatty acids in food authentication

In the last decades, food frauds have been on the rise [53]. For this reason, food authentication represents an important strategic issue for food industry because consumers are becoming increasingly interested in the quality and origin of foods. This is especially true when consumers purchase expensive certified and high added-value products, such as protected denomination of origin (PDO) or protected geographical indication (PGI) products [54].

Assuring food authenticity is not only an economical issue for food industries but it also concerns consumer safety, due to the substitution of food grade materials by cheaper non-food grade materials or to the presence of undeclared ingredients. The broad objective in food authentication is to identify unique or groups of markers to characterize the authenticity of food or their potential adulterants/contaminants and use them to resolve authenticity problem [55, 56].

As previously reported, cyclopropane and ω-cyclohexyl fatty acids, isolated respectively in lactic and rumen bacteria, have been identified in milk and dairy products as well as in meat. The occurrence and the content of cyclopropyl fatty acids in dairy products and meat were mainly correlated with the presence in forage of maize silage, whereas omega-cyclohexyl fatty acids have been proposed as marker of species, especially for ruminant species. In the following paragraphs, we will focus on the role of cyclic fatty acids as quality markers in food authentication mainly in dairy products and meat.

2.4.1 Cyclopropane fatty acids as quality markers in dairy products

In dairy sector, the most critical issue in authentication is related to PDO cheeses, which are high commercial value products confined according to legislative and proper labeling rules. The higher prices of PDO products encourage more frequent food fraud [57]. Their authenticity is associated with several factors, such as the geographical area of production, materials, and technology used. In fact, cheese production can differ according to the feeding system of the animals providing all the ingredients as milk, the starters used, and the presence or lack of preservatives as well as other parameters (i.e., the heating temperature, the salting, the ripening time). All of these generate defined characteristics, which in turn can be detected by several analytical techniques, and provide a trace of the cheese origin [58, 59].

Among PDO cheeses, Parmigiano-Reggiano is probably the most worldwide appreciated Italian PDO cheese. It represents a fully natural high-quality cheese only made in a very small and specific region of Italy, without the use of additives and from milk of local cows fed with hay and not silage as fodder commonly used worldwide in livestock feeding. It is made according to the Production Specification Rules laid down by Parmigiano-Reggiano Cheese Consortium (CFPR) according to EU regulations (EU 510/2006 and following 1151/12). Due to the high quality of the raw material, the long ripening, and the strict rules of production, it is an expensive cheese, but despite this, a constant growing of the national and international market is registered [60]. Consequently, the problem of fraud is a critical issue. In fact, several grated varieties branded as Parmesan and with "Italian sound" elements on the pack were found to be inauthentic, some of them containing non-declared additives. Therefore, mislabeling is a severe problem concerning unfair competition and deception to the detriment of consumers.

As previously reported, data collected by the authors [10, 11, 43] on hundreds of milk and dairy samples confirm the strict correlation between the use of silages in the feeding and the presence of CPFA in milk fat. Cyclopropane fatty acids (CPFA) were present only in dairy products from cows fed with silages, and their

determination has been demonstrated to be molecular markers of quality for PDO cheeses, as Parmigiano-Reggiano, where the use of silage is forbidden [10]. In this context, an example of successful case was the innovative method (UNI11650) that has been developed for Parmigiano-Reggiano cheese authentication based on the presence or absence of CPFA [43]. As results, Grana Padano (GP) samples were always positive to CPFA, reflecting that silages are not forbidden for their production (**Figure 4**). The amount of CPFA found in Grana Padano is variable, and it ranges from 300 to 830 mg/kg of fat, with a global mean value of 540 ± 110 mg/kg [43].

Because grated parmesan is particularly vulnerable to being adulterated with other cheeses, mix of Parmigiano-Reggiano with a cheese produced with milk from ensiled-fed cows have been analyzed to establish the minimum level of adulteration. The GC-MS method [43] was able to detect frauds that included 10–20% of cheaper cheeses. Adulteration behind this value has scarce commercial significance. Furthermore, the optimized GC-MS method was subjected to validation in terms of precision, accuracy, linearity, detection, and quantitation limits following the recommendations of the International Conference on Harmonization [61]. Therefore, due to the innovative and encouraging results, an application for an official standardization of the method (UNI method 11650) has been validated and included in Parmigiano-Reggiano Product Specification Rules among the official controls, enforcing the current analytical methods adopted for the quality controls.

In conclusion, the presence of CPFA in milk and dairy products probably derives from their presence in ensiled feeds, where CPFA can be released by bacteria during fermentation. The environmental conditions developed in silos seem to be essential for the production and release of CPFA from bacteria, whereas shelf-life, manufacturing, seasoning steps, and lactic fermentation did not affect CPFA content [11]. As a whole, CPFA demonstrated to be interesting molecular markers, able to distinguish cheeses obtained from cows fed with or without silages. Moreover, the quantitative

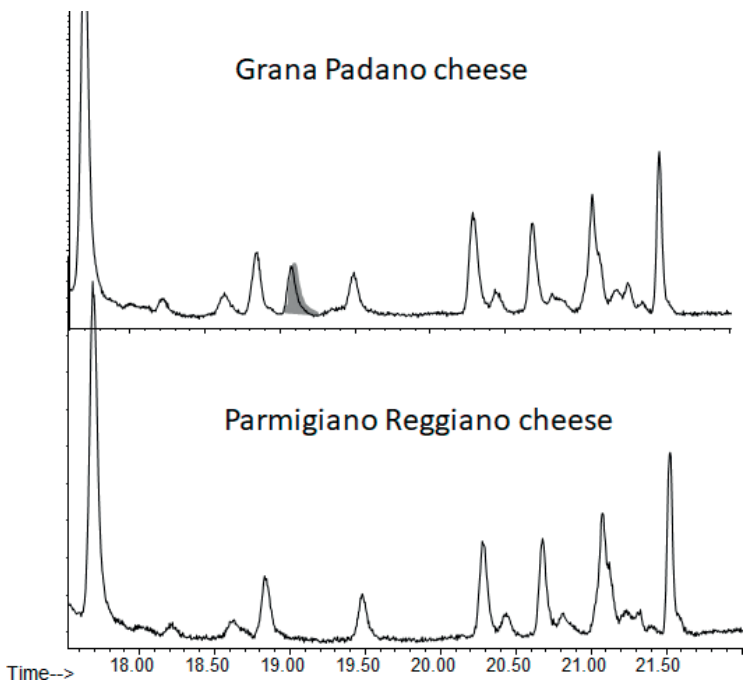

Figure 4.
Enlarged view of CPFA peak elution zone and comparison between a sample of Grana Padano cheese positive to CPFA and a sample of Parmigiano-Reggiano cheese negative to CPFA [43].

GC-MS method developed is relatively simple, assures a quick sample preparation, and relies on available instrumentation, thus making it suitable for the screening of many samples with a good cost-per-analysis ratio.

2.4.2 Cyclic fatty acids as quality markers in meat

An increasing critical issue is the substitution of higher commercial valued meats by low-priced ones and the fraudulent labeling of meat species [62]. In this context, there is the need for new, fast, and reliable analytical methodologies and easily quantifiable markers to be used for meat authentication and to protect both consumers and producers from illegal substitutions. Current methods to identify the origin of species present in commercial meat are based on DNA and ELISA, but also UPLC, Raman spectroscopy, low-field NMR, and mass spectrometry have been considered [63].

Fatty acids in food authentication were mainly used for the determination of the cow feeding system, which affect the milk and meat fat composition [64, 65]. Previously, many data have been collected confirming the association between the use of ensiled feeds and the presence of CPFA in the fatty profile of dairy products.

As suggested by Lolli et al. [12], cyclopropane fatty acids (mainly dihydrosterculic acid) have also been detected in animal fat, especially in bovine meat fat. As results, CPFA were detected in the fatty profiles of commercial bovine meat samples but they were absent in the samples of certified meat from cows not fed with fermented forages, reflecting the same correlation observed in dairy products [10, 11, 43]. In the case of meat of other animal species (pork, pork cured meat, and chicken), results did not show the presence of cyclopropane fatty acids as shown in **Table 3**. These preliminary results suggested that CPFA might be proposed as markers of silage feedings and for the authentication of high quality costly meat whose producers declare the absence of silages in the feeding as in dairy products. Certainly, it will require the construction of a robust database of certificated meat for the feeding system. Moreover, CPFA (mainly lactobacillic acid) were recently found [12] in farmed fish. Therefore, this approach could also be extended to fish to eventually distinguish farmed from wild fish.

Regarding omega-cyclohexyl fatty acids, they have been detected [11] (mainly 11-cyclohexyl undecanoic and 13-cyclohexyl tridecanoic fatty acids) in the GC-MS fatty profile of cow milk, suggesting their presence in milk fat could represent a reliable method to evidence rumen acidosis in cows. However, as mentioned above, this hypothesis has never been confirmed by experimental data.

Recently, they have been also identified in animal fat, mainly in meat of ruminants, especially bovine and ovine meat [14]. Preliminary data (not published) showed that omega-cyclohexyl fatty acids, combined with other fatty acids as branched chain fatty acids [41], permitted to discriminate beef from pork meat.

Sample of meat	N° sample	Range CPFA (mg/kg total fat)
Commercial bovine meat	5	200–400
Bovine meat of certified origin (not fed with silages)	2	Negative[a]
Other meats (pork and chicken)	4	Negative[a]
Pork cured meat	3	Negative[a]

[a] *<LOD (60 mg/kg total fat) [12].*

Table 3.
Presence of CPFA in meat samples.

As suggested by Marseglia et al. [11], 11-cyclohexyl undecanoic fatty acid methyl ester from milk and meat fat eluted in the chromatographic region of isomers of the oleic acid, so it was detected by the characteristic molecular ion 282 m/z in the mass spectrum.

13-cyclohexyl tridecanoic fatty acid methyl ester was detectable in the GC-MS profile as it elutes just before the eicosanoic acid (arachidic acid) and after eicosenoic acid. However, due to the presence of interfering signals, the identification of 13-cyclohexyl tridecanoic was confirmed by the mass spectrum with the characteristic molecular ion 310 m/z and the previous biosynthesized compound [11]. The characteristic mass spectra of 13-cyclohexyl tridecanoic fatty acid detected by GC-MS analysis is shown in **Figure 5**.

The results from the GC-MS analysis showed that omega-cyclohexyl fatty acids, both 11-cyclohexylundecanoic acid and 13-cyclohexyltridecanoic acid, were present only in bovine and ovine meat samples with values between 90–230 and 20–200 mg/kg of the total meat fat, respectively [14]. On the contrary, they were absent in pork, horse, chicken, and rabbit, reflecting the ruminal origin and a possible application for the detection of bovine/pork ratio in commercial minced meat [14].

As mentioned above, current analytical methods in meat authentication are mainly based on protein or DNA measurement, which are not directly comparable to labeled meat expressed as percentage (w/w) [66]. Furthermore, analytical procedures based on protein analysis are sensitive to heat treatment. Therefore, they could not be applied to cooked products for the quantitative analysis. In this context, a quantitative GC-MS method is going to be developed on mixtures of beef and pork meat, both raw and cooked (ragout), based on the method previously applied to determine CPFA [43] and combining other fatty acids, as iso-branched chain fatty acids, of ruminal origins [41].

Preliminary results [14] showed that omega-cyclohexyl and iso-branched chain fatty acids content decreased in minced meat, both raw and cooked, as function of bovine meat percentage in the sample, as shown in **Table 4**.

Therefore, the analysis of omega-cyclohexyl fatty acids combined with that of specific iso-branched chain fatty acids was able to detect until 20% of pork meat in beef, representing potential markers for ruminant meat, also detectable in complex matrix and after thermal treatment in ragout samples.

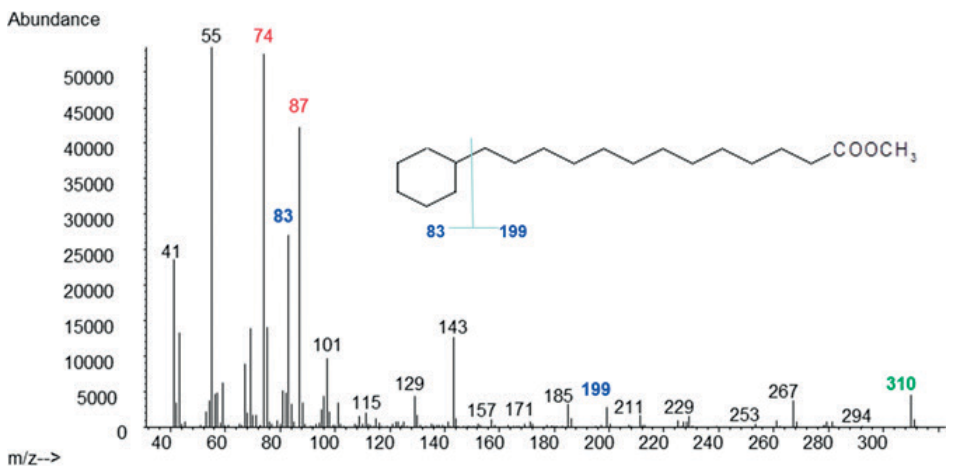

Figure 5.
Mass spectra of 13-cyclohexyl tridecanoic fatty acid methyl ester [11].

Percentage of bovine/ pork in fresh meat	11-cyclohexylun decanoic acid (mg/kg)	13-cyclohexyltri decanoic acid (mg/kg)	Iso methyl C16:0 (mg/kg)	Iso methyl C17:0 (mg/kg)
100 bovine	175 ± 7	55 ± 7	1500 ± 240	2240 ± 721
80 bov/20 pork	120 ± 28	35 ± 7	1005 ± 49	1595 ± 219
60 bov/40 pork	40 ± 1	20 ± 14	650 ± 14	1055 ± 148
40 bov/60 pork	20 ± 1	15 ± 7	690 ± 42	860 ± 283
20 bov/40 pork	15 ± 7	5 ± 7	200 ± 100	385 ± 21
100 pork	nd	nd	90 ± 1	145 ± 21
Percentage of bovine/ pork in ragout	**11-cyclohexylun decanoic acid (mg/kg)**	**13-cyclohexyltri decanoic acid (mg/kg)**	**Iso methyl C16:0 (mg/kg)**	**Iso methyl C17:0 (mg/kg)**
100 bovine	100.0 ± 10.5	32.5 ± 1.9	821.7 ± 79.0	1166.0 ± 17.0
80 bov/20 pork	80.0 ± 5.7	26.5 ± 0.1	690.8 ± 61.9	943.4 ± 125.4
60 bov/40 pork	40.0 ± 1.7	11.1 ± 1.9	381.8 ± 33.3	539.7 ± 39.1
40 bov/60 pork	6.8 ± 5.7	nd	238.9 ± 2.0	296.6 ± 20.2
20 bov/40 pork	5.5 ± 1.3	nd	202.8 ± 35.2	233.0 ± 32.0
100 pork	nd	nd	29.3 ± 1.9	56.2 ± 18.9

Results are expressed as mean ± SD of two replicates; nd = not detectable.

Table 4.
Concentration (mg/kg total fat) of omega-cyclohexyl and iso-branched chain fatty acids found in minced meat, both raw and cooked (ragout), as function of bovine meat percentage [14].

In conclusion, omega-cyclohexyl fatty acids can be proposed as markers of ruminant meat, especially of beef meat, which could enforce current analytical methods applied for labeling regulations.

3. Conclusions

Cyclopropane and omega-cyclohexyl fatty acids are carboalicyclic fatty acids widely distributed among microorganisms, enhancing the chemical and physical stability of bacterial membranes. Significant variations in the membrane content of cyclic fatty acids have been identified in a multitude of physiological situations. Recently, they have been detected in food of animal origins, so representing new components in human diet. In some cases, these cyclic fatty acids can act as markers of quality and their detection could enforce current analytical methods adopted in food authentication.

However, little is known regarding the actual role that these fatty acids play, their release, and the chemical basis of their effects on the cellular membrane, especially in higher animals.

Conflict of interest

We declare that we have no conflict of interest.

Author details

Augusta Caligiani* and Veronica Lolli
Department of Food and Drug, University of Parma, Parma, Italy

*Address all correspondence to: augusta.caligiani@unipr.it

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Chapter 4

Fatty Acids, Gut Microbiota, and the Genesis of Obesity

Patricia de Velasco, Amanda Ferreira, Louise Crovesy, Tarsis Marine and Maria das Graças Tavares do Carmo

Abstract

Obesity is a major public health problem, which is growing around the world. It is a multifactorial disease and a risk factor for other noncommunicable diseases (e.g., cardiovascular diseases, type 2 diabetes mellitus, and hepatic steatosis). Among the etiological factors, gut microbiota and diet, especially lipids, have been highlighted, which seem to have an important potential as a modulator of its composition, being the key factor in the link between microbiota and obesity. Gut microbiota interacts with the host metabolism in the development of this disease through dietary fatty acids or when produced by intestinal bacteria. Short-chain, saturated, and polyunsaturated fatty acids have an impact with respect to gut microbiota and health, presenting central and systemic effects associated with the genesis of obesity. Finally, gut microbiota seems to play a significant role in controlling the endocannabinoid system, and imbalance in this system can be associated with obesity.

Keywords: gut microbiota, obesity, saturated fatty acids, short-chain fatty acids, polyunsaturated fatty acids, endocannabinoid system

1. Introduction

Obesity currently affects around 600 million adults worldwide (13%), being more prevalent among women (15%) than men (11%) [1]. It is described as an increase in the adipose tissue that releases a wide variety of proinflammatory cytokines and chemokines, called adipokines, promoting inflammation, recruitment of macrophages, and insulin resistance [2]. This inflammatory process is manifested systemically and is characterized by a chronic low-intensity reaction [3], which is linked to the pathophysiology of several chronic diseases such as type 2 diabetes mellitus (DM2), cardiovascular diseases, and nonalcoholic fatty liver disease, among others [1, 4]. Although the etiology of obesity is multifactorial, there is often an energy imbalance with an increase in intake and/or absorption of calories and a decrease in energy expenditure or metabolic efficiency, which can be caused by several factors, such as genetics, environment, psychological factors, endocrine disorders, and some drugs. Recently, gut microbiota has been receiving attention [1, 4, 5].

The differences in gut microbiota between lean and obese animals or human subjects suggest a link between gut microbiota and energy homeostasis [7]. In fact, modifications in gut microbiota composition are associated with greater energy breakdown and absorption from food, greater efficiency in storing fat in adipose

tissue, as well as the stimulation of metabolic endotoxemia, low-grade inflammation, and dysbiosis that favors obesity [5, 6]. Dysbiosis leads to increased permeability of the intestinal barrier, allowing for the translocation of lipopolysaccharides (LPSs), toxins that are responsible for the initiation of the inflammatory cascade, affecting mainly adipose tissue, liver, muscle, and brain, which reduce insulin sensitivity in these organs. In addition, LPS also disrupts the endocannabinoid system, further increasing intestinal permeability and allowing greater LPS translocation. The increase in circulating LPS suppresses the fasting-induced adipose factor, thus modulating lipoprotein lipase (LPL) activity, which starts to exacerbate functions in adipose tissue and muscles, favoring the accumulation of triglycerides in these organs [7].

In cell membranes, fatty acids act as phospholipids constituents and may also be released as signaling molecules, regulating energy production and inflammation [8]. Fatty acids are also precursors of endocannabinoids and their structural congeners. The endocannabinoid (eCB) system plays a pivotal role in the regulation of eating behavior and modulation of the immune and inflammatory response [9]. Fatty acids contribute between 94 and 96% of the total weight of different fats and oils and can be classified as saturated fatty acids (SFAs), which contain no double bonds; monounsaturated fatty acids (MUFAs), which feature one double bond; and polyunsaturated fatty acids (PUFAs), which contain multiple double bonds [10]. There are two families of polyunsaturated fatty acids (PUFAs): n-3 (or omega-3 or n-3) and n-6 (omega-6 or n-6). An adequate balance in the dietary intake of n-6/n-3 PUFA is a determining factor in the maintenance of gut microbiota balance, considering the role of this n-6/n-3 ratio in inflammatory response. Over the last decades, there has been a significant modification in the dietary pattern of the western diet, with the increased consumption of industrialized products, generating an increase in the dietary saturated fat and n-6/n-3 ratio [2]. This dietary pattern may increase gut permeability, which results in a greater translocation of LPS from the intestinal lumen to the bloodstream [11]. On the other hand, n-3 fatty acids, e.g., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have an anti-inflammatory effect, which attenuate the endocannabinoid receptor system tone and may contribute to microbiota balance [12]. Therefore, the quality of fatty acids in diet can modulate gut microbiota composition, which in turn may interfere in host metabolic health [10].

Considering that all factors mentioned above are relevant in the context of obesity, the aim of this chapter is to describe the different mechanisms that link the consumption of SFA or PUFA to the composition of gut microbiota and the impact on the regulation of the target inflammation and, consequently, on the insulin resistance and possible risks of type 2 diabetes and cardiovascular disease, among obese individuals.

2. Gut microbiota composition

Microbiota is the set of microorganisms that inhabit the human body in a symbiotic relationship, which can intervene in the digestion process, metabolism, or in the regulation of fatty acid tissue composition. In the gastrointestinal tract of humans, there are approximately 100 trillion bacteria that make up the microbiota in this system. These bacteria are divided into phyla in which the main gut microbiotas are *Firmicutes* (60–65%) and *Bacteroidetes* (20–25%), followed in smaller quantities by *Proteobacteria* (5–10%), *Actinobacteria* (3%), and *Verrucomicrobia* (<1%) [13–15].

The colonization of the human body begins in uterine life, through contact with the bacteria belonging to the maternal microbiota, and is an important health factor for the mother, including nutritional, metabolic, and immunological status.

Both endogenous and exogenous effects can modify the composition of gut microbiota. Other elements that influence the formation of gut microbiota include host genetics, type of birth delivery (vaginal or cesarean section), diet (breastfeeding or infant formula, and dietary introduction), and use of medications (antibiotic, probiotic, prebiotic, and symbiotic) [16].

Gut microbiota composition seems to have different specificities between lean and obese individuals. Regarding the bacterial species, obese individuals present a greater amount of *Lactobacillus reuteri* and smaller concentrations of *Bifidobacterium animalis*, *Lactobacillus casei*, *Methanobrevibacter smithii,* and *Escherichia coli*, with the demonstrated association with adequate body weight only for *L. casei* and, with obesity, the combination of a decrease in *B. animalis* and an increase in *L. reuteri* [17]. Gut microbiota impacts body fat accumulation and insulin resistance in an experimental study with germ-free mice that received transplanted gut microbiota from wild-type mice. In 2005, the first study that identified the difference between the composition of the microbiota of obese and nonobese animals was published [18]. Obese mice (ob/ob) had a 50% decrease in *Bacteroidetes* and an increase, of equal percentage, in *Firmicutes*, when compared with the wild types [19]. Studies have also shown that this change in microbiota acts at the hypothalamic level, promoting a decrease in the secretion of anorectic hormones [peptide YY (PYY) and glucagon-like peptide-1 (GLP-1)], which, associated with low-insulin sensitivity in the hypothalamus, reduce satiety by increasing food intake [7].

Although studies with animal models have defined that with obesity there is an increase in the *Firmicutes/Bacteroidetes* ratio [18–20], in humans, this hypothesis has not yet been verified, as seen in the conflicting results specifically concerning this ratio [21]. A systematic review found 11 interesting studies on humans that connect gut microbiota composition and body mass index (BMI). Among the main findings, a study reported high concentrations of *B. fragilis* and *Lactobacillus* sp. among obese and overweight children when compared with the lean children. In addition, a negative correlation was found between BMI and *Bifidobacterium* spp. The results suggest the differences between the microbial diversity in lean and obese individuals and thus influence the important metabolic pathways [22]. However, studies of differences in gut microbiota between obese and lean people have not always provided consistent results, which is one of the issues requiring further studies.

3. Fatty acids and gut microbiota

3.1 Short-chain fatty acids and gut microbiota

As lipid factors that influence the intestinal balance and the development of obesity, in this chapter, we will first highlight the short-chain fatty acids (SCFAs). SCFAs are metabolites produced by bacterial fermentation in the gut from dietary fermentable carbohydrates. The main SCFAs are acetate, butyrate, and propionate, which play important roles as nutrients for colon epithelium, modulating gut lumen, intracellular pH, cellular volume, and functions associated with ion transport, proliferation, differentiation, gene expression, reduction of oxidative stress, inflammation recovery, and satiety [23].

Propionate has a role as a substrate for hepatic gluconeogenesis, in addition to inhibiting the synthesis of hepatic cholesterol. Acetate is the most abundant SCFA, being absorbed and metabolized rapidly by the liver, where it serves as a substrate for cholesterol synthesis and lipogenesis [24]. It is also involved in the satiety process by inhibiting pro-opiomelanocortin (POMC) in the hypothalamus [25]. As acetate and propionate exert opposite functions in lipid metabolism in the

liver, the proportion of these two SCFAs is important to maintain lipogenic balance and cholesterol synthesis [26]. Butyrate is found in a lower quantity than the other SCFA in the blood circulation. It is the main source of energy for enterocytes. It acts mainly in cell regulation and proliferation, especially in colon intestinal cells, and plays a role in anomalous cell apoptosis. In addition, it has a function in the maintenance of tight junctions and is responsible for preserving the integrity of the intestinal barrier [27].

SCFA can also modulate the endocrine, immune, and nervous system responses. Propionate interacts mainly with G protein-coupled receptors (GPCR) 41 and GPCR43 in enteroendocrine cells, stimulating the secretion of PYY and GLP-1 hormones, respectively, which has anorexigenic effects, thus contributing to the reduction of food intake. Both hormones promote satiety by acting on the hypothalamic arcuate nucleus, suppressing the neuropeptide Y (NPY), which has an orexigenic effect, and stimulating POMC, which is anorexigenic, in addition to delaying gastric emptying [28]. Binding of SCFA to GPCR43 is also related to increased insulin secretion, improving insulin sensitivity and gut immune response. SCFA interacting with GPCR41 and GPCR43 in adipose tissue also stimulates the secretion of leptin, which suppresses adipogenesis. The interaction with GPCR41 can enhance energy expenditure by increasing the activity of the sympathetic nervous system [29].

Propionate and butyrate induce gluconeogenesis in gut cells, thereby regulating food intake and increasing insulin sensitivity. The interaction of butyrate with GPCR109A expressed in the gut reduces the inflammation mediated by interleukin (IL) 8 and IL-10 and promotes lipolysis in adipose tissue. Acetate and propionate can regulate systemic blood pressure by binding to olfactory receptors located in blood and kidney vessels [29].

Acetate plays a role in appetite directly in the hypothalamus. In the brain, acetate is converted into acetyl coenzyme A (CoA) that participates in the cycle of citric acid leading to the accumulation of adenosine triphosphate, which leads to the reduction of the adenosine monophosphate–activated protein kinase (AMPK) and consequent inhibition of acetyl-CoA carboxylase, favoring the accumulation of malonyl-CoA. This, in turn, has been related to the activation of POMC and the suppression of NPY and peptide related to agouti in the hypothalamus, which leads to a suppression of appetite and, consequently, a reduction in food intake [25].

The SCFAs appear to be involved in lipid oxidation and energy expenditure, increasing both and contributing to weight loss [30]. The mechanism by which SCFAs increase fat oxidation was studied in an animal model. Acetate activates AMPK in adipose tissue and skeletal muscles and regulates peroxisome proliferator gene (PPAR), stimulating lipid oxidation in these organs [31]. In addition, SCFAs are responsible for the increase in PYY and mitigation of lipolysis [32].

Acetate and propionate also appear to inhibit intracellular lipolysis. Propionate increases lipidic buffering in adipose tissue through an increase in the uptake of triglycerides by LPL in adipocytes, favoring the adipogenesis in adipose tissue and reducing the circulation of FFA. These effects improve the insulin sensitivity and reduce the accumulation of ectopic fat, especially in muscles and liver [33, 34]. The mechanisms involved in the prevention of fat stores in the liver occur through the inhibition of fatty acid synthesis by propionate and the increase in AMPK activity and PPAR genes by acetate and butyrate, which raise glycogenesis and FA oxidation in the mitochondria, thus reducing fat stores in hepatocytes [35].

In this context, it appears that these metabolites may be the key between the microbiota and the development of obesity, playing an important role in body weight control and insulin sensitivity. Studies *in vitro* and *in vivo* demonstrate that SCFAs regulate energy homeostasis, glucose, and lipid metabolism and have effects on adipose tissue [36, 37]. As discussed above, they act on appetite through

endocrine regulation, stimulating the secretion of leptin by adipocytes, and on neuronal receptors, modulating neural activity and visceral reflexes [26, 36].

Thus, it is possible to assert that SCFAs, produced by gut bacteria, have an impact with respect to gut microbiota and health, presenting central and systemic effects that may contribute to the maintenance of adequate metabolism, in addition to regulating mechanisms that can prevent the development of obesity and its morbidities [36].

3.2 Long- and medium-chain saturated fatty acids and gut microbiota

Based on their structure, saturated fats can be subclassified into short-chain, medium-chain, and long-chain fatty acids. Medium-chain fatty acids have 8–10 carbon atoms, and long-chain fatty acids have, in general, 12 or more carbon atoms [37]. Animal sources are the main sources of saturated fatty acids in the diet: dairy products, meat, butter, margarine, and hydrogenated vegetable oils. These fatty acids are quite heterogeneous in nature, and their potential effects may also vary in relation to their health effects.

A high-fat diet (HFD), predominantly SFA, has been associated with changes in gut microbiota composition and a reduction in diversity [38, 39], independently of the host genotype [40]. A study conducted with subjects at risk of metabolic syndrome showed that SFA intake increased the composition of pathogenic bacteria [41]. A decrease in *Bacteroidetes* and an increase in *Firmicutes* and *Proteobacteria* after the consumption of HFD have also been reported [42]. A HFD significantly reduced levels of *Bacteroidetes* spp., *Eubacterium rectale*/*Clostridium coccoides,* and *Bifidobacterium* spp. These results reflected in insulin resistance, low-grade systemic inflammation, and higher concentrations of endotoxins in plasma [43]. The levels of *Bilophila wadsworthia*, associated with increased gut inflammation, were elevated when mice were fed with a milk fat–enriched diet [44]. Likewise, higher levels of *B. wadsworthia* were observed in mice that were fed a lard diet than fish oil as a lipid source [39].

As previously reported, an imbalance in bacterial population may lead to an increase in intestinal permeability, with a high translocation of bacteria into systemic circulation, inducing an elevation in circulating LPS and metabolic endotoxemia [45]. HFDs are also associated with a reduction in proteins of the narrow junction [45], whose function is to block the intercellular space, preventing the passage of substances through the intestinal epithelium. Additionally, it is relevant to consider that SFAs represent an essential component of the lipid portion of LPS derived from pathogenic bacteria [46].

Studies also demonstrate that SFA can bind to Toll-like receptor 4 (TLR4) and activate inflammatory signaling pathways [47, 48]. The signaling pathway of the TLR4 is recognized as one of the main triggers of the inflammatory response induced by obesity [2]. Subsequent *in vitro* and *in vivo* studies suggest that fatty acids are able to activate proinflammatory signaling pathways mediated by TLR4 and TLR2, leading to insulin resistance [3]. In addition, a normal weight individual who consumed high-calorie meals (910 Kcal), with high lipid (51 g) and carbohydrate content (88 g), demonstrated distinguishable changes in the TLR postprandial stage, implying a greater activation of TLR2 and TLR4 on blood mononuclear cells. A high-fat meal also resulted in a greater activation of NF-κβ in the postprandial stage and an increase in leukocytes' activation, evaluated by surface expression of CD11a, CD11b, and CD62L [49].

The palmitic acid binding to TLR4 also activates the c-jun N-terminal kinase (JNK) and the inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-β) proteins and increases the expression and secretion of pro-inflammatory cytokines [50], also causing damage in insulin signaling [51]. It also impairs insulin signaling pathways, inducing the phosphorylation of IRS-1 at the position of the serine residue

307 [52]. This process reduces the interaction with the insulin receptor and, consequently, decreases its action. In addition, SFAs induce insulin resistance due to the antagonistic action of peroxisome proliferator-activated receptor-1 alpha coactivator, promoting the expression of mitochondrial genes that are involved in oxidative phosphorylation and glucose uptake, which is mediated by insulin [51, 53, 54].

Lauric and palmitic FA activates an inflammatory response through TLR4 signaling pathways [55]. It was reported that the lauric, palmitic, and stearic FA could induce inflammation from cyclooxygenase-2 expression by means of a dependent mechanism of NF-κβ in a macrophage cell line, through a TLR4 connection [56]. It was observed that TLR4 was the main signaling pathway to stress the endoplasmic reticulum caused by a high-fat diet among obese mice, increasing inflammation of the adipose tissue even more [57]. NF-κβ can also be activated by the binding of lauric acid with TLR2, as other studies have demonstrated [47, 56]. Mice fed with a high-fat diet (palmitic) demonstrated a greater expression of TLR2 and inflammatory reduction when TLR2 was inhibited [58]. When palmitic acid binds with TLR4, it also activates JNK and IKK-β kinase protein and also increases the expression of NF-κβ [50], impairing insulin signaling [59].

From another perspective, a recent study argues that long-chain saturated fatty acids (lcSFAs) are not direct agonists of TLR4. The authors suggest that the activation of the inflammatory cascade by saturated fats depends on an initial sensitization of the TLR4-dependent macrophages, which can be generated, for example, by a metabolic endotoxemia resulting from LPS infiltration. The authors justify that lcSFAs take several hours to initiate inflammatory signaling, while LPS activates it in minutes. Macrophages of an insulin-sensitive healthy adipose tissue, in the absence of a priming or a sensitizing signal, do not respond to the inflammatory effects of lcSFA. These data demonstrate that further studies are still needed to elucidate, in detail, the signaling pathways involved in this inflammatory process but highlight the interesting interaction of the intestine-inflammation-obesity axis [60].

Medium-chain saturated fatty acids (mcSFAs) are present in coconut oil and breast milk and are known for their antimicrobial properties, preventing LPS-mediated endotoxemia [61]. Rats fed with mcSFA for 1 week prior to an acute intravenous dose of LPS presented a significant improvement of intestinal permeability compared to rats fed with corn oil, which showed increased gut permeability [62]. Medium-chain fatty acids have also been shown to have a direct impact on the gut bacteria of Gram-positive (low LPS) and Gram-negative (high LPS) subdivisions. A change was observed in the population distribution of *Bacteroidetes/Porphyromonas/Prevotella* phyla and in the genus *Clostridia/Streptococcus* with mcSFA consumption [68]. McSFAs also specifically modulated bacterial populations in specific regions, such as jejunum and colon, promoting the growth of *Escherichia/Hafnia/Shigella* bacteria and the genus *Clostridia* [63].

Given this, it is understood that saturated fats play an important role in the development of obesity, either from their influence on the profile of gut microbiota or from their performance in inflammatory processes. The understanding that the immune system and the different metabolic pathways are closely related and functionally dependent is essential for studies that focus on obesity and the possible metabolic repercussions (**Table 1**).

3.3 Polyunsaturated fatty acids and gut microbiota

Linoleic acid (C18:2 n-6; LA) and α-linolenic acid (C18:3 n-3; ALA) are the parent compounds of the n-6 and n-3 polyunsaturated fatty acid families, respectively. Humans do not have enzymes to insert a double bond in the n-6 or n-3 position, which makes n-3 and n-6 fatty acids essential [64]. Many vegetable oils, such as

FA	Sources	Effects on microbiota and Ref.	Systemic effects and Ref.
Saturated fatty acids			
Short chain (<8 carbon atoms)	Bacterial fermentation (fermentable carbohydrates)	Integrity of the intestinal barrier [27]	Regulate energy homeostasis, glucose, lipid metabolism, and appetite [26, 36, 37]
Medium chain (8–10 carbon atoms)	Coconut oil and milk	Preventing LPS-mediated endotoxemia [61, 62]	Regulate energy homeostasis and satiety [64]
Long chain (>12 carbon atoms)	Meat	Increased pathogenic bacteria [39, 41, 44]	Increased inflammation and insulin resistance [43, 47–49]
Polyunsaturated fatty acids			
n-6 Polyunsaturated	Vegetable oils (e.g., corn, sunflower, and soybean)	Increased endotoxemia [69]	Inflammatory precursors [69]
n-3 Polyunsaturated	Fish oil	Regulate the tight junction functioning, intestinal balance [69, 70, 73]	Anti-inflammatory precursors [70]

FA, fatty acids; LPS, lipopolysaccharide; Ref., references.

Table 1.
Studies in vivo and in vitro about the effects of saturated and polyunsaturated fatty acids in gut microbiota.

corn, sunflower, and soybean oils, are rich in n-6 fatty acids, mainly as LA, but linseed is also a rich source of ALA. In humans, dietary LA can be metabolized to arachidonic acid (lcPUFA—AA, 20:4, n-6), and dietary ALA can be metabolized to long-chain n-3 as eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), mainly in the liver [65]. LcPUFAs are synthesized from dietary LA and ALA by microsomal desaturase and elongation enzymes that metabolize both the n-6 and n-3 families of PUFA, and the delta-6 (delta-6) desaturase is the rate-limiting enzyme in this process [66]. Binding affinity for delta-6 desaturase is highest for ALA, high for LA, and lowest for oleic acid [64], and, for this reason, desaturation and elongation of n-9 PUFA are only observed when combined n-3 and n-6 EFA deficiency occurs. This conversion of the dietary ALA to lcPUFA is limited, and, in humans, it is estimated that only 8% of ALA is converted to EPA and even less to DHA (less than 4%) [67].

Dietary sources of preformed n-3 lcPUFA can provide large amounts of these fatty acids and are primarily derived from certain species of fish (and fish oils or marine lipids). These lcPUFAs are part of the structural components of cell membranes and so affect many aspects of membrane functions such as membrane permeability, receptor functions, and membrane-associated enzyme activities [67].

Dietary intake of n-3 PUFA and its role in inflammatory responses is discussed extensively in the literature and is usually associated with fewer inflammatory effects, while PUFA n-6 effects are more inflammatory. PUFAs have the ability to change the composition of gut microbiota [68]. Intestinal dysbiosis has been implicated in obesity pathogenesis, and the supplementation with n-3 led to the increase in *Lactobacillus* species and decrease in *Bacteroidaceae* family bacteria by improving dysbiosis. On the other hand, supplementation with n-6 had a negative association with the abundance of *Akkermansia muciniphila* [69].

Thus, gut integrity appears to be an important factor for n-3 PUFA action because increased permeability is associated with several disorders (e.g., obesity, DM2, and inflammatory bowel disease). n-3 PUFAs are capable of maintaining the integrity of the intestinal epithelium and thus influence inflammatory bowel status. They serve as precursors to anti-inflammatory substances or even regulate the functioning of tight junctions [70]. In a HFD model (high in saturated fat), an increase was observed in the number of *Bacteroides,* which could lead to decreased gut permeability [71]. Using the same diet protocol, a study reported an increase in *Enterobacteria* that elevate gut permeability, and this phenomenon would lead to a systemic elevation of LPS and endotoxemia and also dysbiosis [72]. Furthermore, an imbalance in the gut microbiota environment (dysbiosis) could lead to a low-grade systemic inflammation by increasing gut permeability to LPS and endocannabinoid system activity [6].

Animal studies have demonstrated that n-3 PUFA protects against dysbiosis, reversing bacterial overgrowth induced by n-6 PUFA dietary intake [73]. On the other hand, among healthy humans, the effects of PUFA, especially n-3, on microbiota are less established, and the literature is limited. However, in a study among patients with inflammatory bowel disease, the supplementation with n-3 was able to revert the microbiota to a healthier composition, with a decrease in the genus *Faecalibacterium* and an increase in the *Lachnospiraceae* family, and the *Roseburia* and *Bacteroidetes* genus. In addition, n-3 PUFAs increase the production of anti-inflammatory compounds like SCFA acids that could be associated with disease reversal [74]. Based on the well-established anti-inflammatory effects of n-3 PUFAs (as EPA and DHA) that are related to a reduction in metabolic endotoxemia and positive changes in gut microbiota, further studies are needed to better understand the role of n-3 PUFA dietary intake in preventing diseases associated with dysbiosis, such as obesity (**Table 1**).

4. Endocannabinoid system

The endocannabinoids (eCBs) are a family of biologically active lipids—derivatives of arachidonic acid, an omega-6 PUFA—that bind to and activate CB1- and CB2-cannabinoid receptors, widely distributed throughout the brain areas, in the central nervous system and in peripheral tissues such as liver, adipose tissue, pancreas, and gut. The main types of eCB are anandamide [N-arachidonoyl ethanolamine (AEA) and 2-arachidonoyl glycerol (2-AG)]. This eCB system is a signaling system with a variety of physiological functions, such as the modulation of the immune and inflammatory response, synaptic plasticity and learning, and regulation of metabolism and energy homeostasis [75]. There is growing evidence that the eCB system, high-fat diets, inflammation, and obesity are interconnected [76, 77].

The CB1 appears to be the key factor in this eCB system for the genesis of obesity. Stimulation of CB1 increases the food intake, enhances the reward aspects of eating, and promotes the energy conservation [78]. In fact, obesity is characterized by increased endocannabinoid system tone and the altered expression of CB1 mRNA, accompanied by increased eCB levels in adipose tissues [79]. On the other hand, evidence also suggests that a high-fat diet increases hepatic AEA levels and CB1 density, which leads to increased fatty acid synthesis and contributes to diet-induced obesity [80]. AEA and 2-AG levels are also increased in adipose tissue and pancreas in diet-induced obese mice [76]. At the same time, studies on animals lacking CB1 receptors (CB1−/− mouse model) demonstrate that they are hypophagic, leaner, and lighter in relation to wild types. Another study showed that when CB1 receptor activity is blocked in obese mice induced by diet, an improvement in the

gut barrier and metabolic endotoxemia was observed, including an alteration in gut microbiota composition, a reduction in body fat, and an improvement in obesity-associated parameters [81].

Studies have emphasized that gut microbiota modulates the intestinal eCB system tone, which, in turn, regulates gut permeability and plasma LPS, and is able to stimulate peripheral endocannabinoids in the gut and adipose tissue [76]. This hyperactivity of the CB1 receptor increases the permeability of the gut barrier, favoring the translocation of more LPS into the bloodstream, which will further stimulate the eCB system, generating a cycle in which both remain altered. In adipose tissue, eCB disturbance leads to adipogenesis, contributing to the accumulation of body fat and, consequently, obesity [82]. LPS and eCB regulate, in different ways, the apelinergic system in adipose tissue, reducing the secretion of apelin and the expression of its AP1 receptor. The apelinergic system plays a role in energy and glycemic homeostasis [83]. Thus, gut microbiota seems to play a significant role in controlling the endocannabinoid system and, consequently, as modulators of obesity and energy homeostasis.

A sham-feeding protocol in rats used to identify fatty-acid profile of dietary fat components that could trigger small-intestinal endocannabinoid signaling. Results have shown that sham-feeding emulsions containing oleic acid (18:1) or linoleic acid (18:2) induce a nearly twofold accumulation of jejunal endocannabinoids, whereas emulsions containing stearic acid (18:0) or linolenic acid (18:3) had no such effect [79]. In a mice model, it was observed that increasing the percentage of linoleic acid (18:2 n-6) in the diet led to increased levels of 2-AG and AEA, which are derived from arachidonic acid (20:4 n-6), which, in turn, is formed from linoleic acid in the body. Interestingly, this was associated with a higher body weight but not with an increased food intake [76]. Studies with rodents and human subjects have also shown that increasing the relative proportion of n-3 long-chain PUFA in the diet can lead to a decrease in the formation of the n-6 (arachidonic acid)-derived endocannabinoids AEA and 2-AG [79, 80]. Thus, dietary lipids can modulate eCB system tone.

5. Modulation of gut microbiota and inflammation by fatty acids: the role of diet

As previously discussed, there are several actions of different types of FA in inflammatory pathways and in gut microbiota. Changes in the dietary quality of lipids can improve inflammatory markers and provide an intestinal balance, preventing the development of diseases [84]. In general, therapeutic protocols suggest that a lower intake of foods rich in saturated fats, trans fats, and sugars and an increase in dietary sources of bioactive fiber and lipids are considered beneficial [92]. Also, the intake of bioactive lipids, which include monounsaturated and PUFA, phytosterols, and medium-chain lipids is beneficial [85].

Regarding fat consumption, it has been reported that postprandial endotoxemia is influenced by the fatty acid composition of the diet and not by the fat content as a whole. Subjects consuming meals rich in omega-3 decreased serum levels of endotoxemia, unlike those who consumed omega-6-rich foods. The omega-6 fatty acids can increase serum triglyceride levels and also cause gut hyperpermeability, favoring the accumulation of fat in adipose tissue and stimulating the inflammatory processes [86].

The consumption of sardines and fish enriched in omega-3 caused a decrease in the *Firmicutes* filo and in the *Firmicutes*/*Bacteroidetes* ratio in patients with no DM2 treatment [87]. The adoption of a Mediterranean diet or low-fat and carbohydrate diet by obese individuals resulted in different changes in gut microbiota, with high

levels of the genus *Roseburia* and *F. prausnitzii*, respectively, demonstrating the protective effects of both diets on the development of DM2 [88]. The consumption of extra virgin olive oil enriched with phenolic compounds has been shown to have a cardio-protective effect, which could be mediated by the increase in the population of bifidobacteria, together with the increase in microbial metabolites and phenolic compounds, which has antioxidant activities [89].

Saturated fats, such as palmitic, lauric, and stearic acids, may be related to the activation of inflammatory processes. A study with healthy young people showed that a reduction in the palmitic acid/oleic acid ratio in the diet resulted in lower secretion of IL-1β, IL-18, IL-10, and tumor necrosis factor alpha stimulated by LPS [90]. A study conducted with subjects at risk of metabolic syndrome showed that the consumption of saturated fat increased the composition of pathogenic bacteria [41].

In infants, the percentage of fat in the complementary diet has been reported to be negatively correlated to the diversity of gut microbiota [91]. In infant formulas, lipids are typically added as vegetable oils, with long-chain fatty acids predominating [92]. However, human milk has a low content of n-6 fatty acids and a high content of medium-chain fatty acids, acting as a prebiotic [93]. Sources of SCFA may be useful for remodeling gut microbiota and reducing obesity.

The beneficial role of the fibers in a high-fat (HF) diet on inflammatory markers and gut microbiota was also verified [94]. A decrease in adipocyte size and IL-6 levels was observed when the fibers were administered as part of a HF diet over a period of 6 weeks. These fibers were able to alter gut microbiota and increase fermentation rates, mainly by stimulating the production of SCFA, demonstrating its role in the prevention of intestinal inflammation, and may increase the beneficial forms of microbiota diversity.

6. Conclusion and recommendations

Determination of the fatty acid composition in the diet is essential for two reasons: first, to understand the pathogenesis and responsiveness of some diseases, such as obesity, and second, to address potential therapeutic interventions. In this chapter, we have demonstrated that there is a direct relationship between excessive fat consumption and microbiota composition. Obesity is closely associated to microbiota composition as well. In other words, an overconsumption of fat induces changes in microbiota composition, which, by its turn, becomes influential to obesity. These connections have been demonstrated both in obese people and in rodent models, that consume an excess of fat, have shown alteration in their microbiota composition. Increased intake of saturated fatty acids and LPS is negatively associated with this process, while a higher intake of food sources of short- and medium-chain fatty acids and n-3 PUFA has shown positive effects (**Figure 1**). It is evidently observed the relevant roles of these lipids in inflammatory processes, in the endocannabinoid system, brain functioning, and behavior, influencing the composition of gut microbiota and, therefore, the functioning of the gut-brain axis. All of these factors have significant implications for the reduction of meta-inflammation and, consequently, insulin resistance and the risk of DM2 and cardiovascular diseases in obese individuals.

As most studies have been conducted on animals and cell culture, we strongly recommend well-controlled, long-term clinical studies in humans, so that we can better understand this complex interaction among dietary fatty acids, gut microbiota, and obesity. Due to the strong anti-inflammatory activities of n-3 PUFA, as well as dietary fibers stimulating the production of SCFA and, therefore,

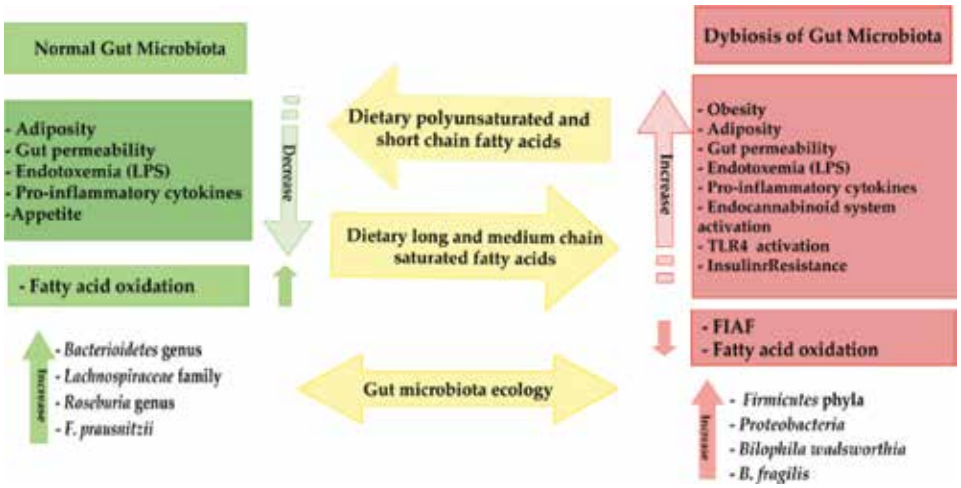

Figure 1.
Impact of dietary fatty acids in the gut microbiota composition and health. A healthy microbiota is associated with the balance between pathogenic and nonpathogenic bacteria that may reflect on systemic consequences. Dietary lipid profile can modulate its composition (yellow arrows). Excessive consumption of SFA (right yellow arrow) will cause negative outcomes to the metabolism and microbiota content (red boxes and arrows). On the other hand, SCFA and PUFA (left yellow arrow) reveal positive effects in inflammation, fat deposition, and microbiota (green boxes and arrows). The two-way arrow (in yellow) represents changes in gut microbiota ecology, depending on what type of FA has been consumed. The equilibrium among these factors seems to be another way to explain the obesity development. FIAF, fasting-induced adipose factor; LPS, lipopolysaccharides; TLR4, Toll-like receptor 4.

influencing the composition of gut microbiota and the host health, we also recommend that dietary fiber and n-3 PUFA should be part of the diet of obese individuals and that dietary SFA and n-6 PUFA should be under control.

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Conflict of interest

The authors declare no conflicts of interest.

Abbreviations

2-AG	2-arachidonoylglycerol
AEA	anandamide
AMPK	adenosine monophosphate-activated protein kinase
BMI	body mass index
CB1	type 1 cannabinoid receptor
CB2	type 2 cannabinoid receptor
CoA	coenzyme A
DM2	type 2 diabetes mellitus

eCBs	endocannabinoid
FA	fatty acid
GLP-1	glucagon like peptide 1
GPCR	G protein-coupled receptor
HFD	high-fat diet
IL-1β	interleukin 1β
IL-6	interleukin 6
IL-8	interleukin 8
IL-10	interleukin 10
IL-18	interleukin 18
JNK	c-jun N-terminal kinase
lcSFA	long-chain saturated fatty acid
LPL	lipoprotein lipase
LPS	lipopolysaccharide
mcSFA	medium-chain saturated fatty acids
NF-κβ	nuclear factor-kappa beta
NPY	neuropeptide Y
POMC	proopiomelanocortin
PPAR	peroxisome proliferator-activated receptor
PUFA	polyunsaturated fatty acid
PYY	peptide YY
SCFA	short-chain fatty acid
SFA	saturated fatty acid
TLR-2	Toll-like receptor 2
TLR-4	Toll-like receptor 4

Author details

Patricia de Velasco, Amanda Ferreira, Louise Crovesy, Tarsis Marine and Maria das Graças Tavares do Carmo*
Institute of Nutrition Josué de Castro, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

*Address all correspondence to: tcarmo@nutricao.ufrj.br

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Section 2

Benefits of Fatty Acids

Chapter 5

Commercial and Therapeutic Potential of Plant-Based Fatty Acids

Ana Paula de Souza e Silva, Wanessa Almeida da Costa, Marielba de Los Angeles Rodriguez Salazar, Priscila do Nascimento Bezerra, Flávia Cristina Seabra Pires, Maria Caroline Rodrigues Ferreira, Eduardo Gama Ortiz Menezes, Glides Rafael Olivo Urbina, Jhonatas Rodrigues Barbosa and Raul Nunes de Carvalho

Abstract

This chapter reviews plant-based fatty acids as well as their methods of production, applications in the industry, and benefits in treatments of cardiovascular and cerebral diseases, besides being a source of food. The fatty acids obtained from vegetable matrices have been acting as alternatives to the use of lipids of animal origin, due to their limitation in relation to the increase in demand. Thus, plants have been investigated in order to act as sources of fatty acids and assist in the supply of such demands. Vegetable oils represent not only an economical alternative but also a beneficial source of human health.

Keywords: fatty acids, plants, nutraceuticals, cardiovascular diseases, brain functions

1. Introduction

Lipid components, especially fatty acids, are present in the most diverse forms of life, playing important roles in the structure of cell membranes and metabolic processes. In humans, omega-series fatty acids are required to maintain cell membranes, brain functions, and the transmission of nerve impulses under normal conditions. These fatty acids also play a key role in the processes of transfer of atmospheric oxygen to blood plasma, hemoglobin synthesis, and cell division. They are called essential because the human body does not synthesize them [1].

Fatty acids are classified according to the presence of double bonds between the carbon chains. They are called saturated fatty acids (SFA) if there are no double bonds; monounsaturated fatty acids (MUFA) if there is one double bond; and polyunsaturated fatty acids (PUFA) if two or more double bonds are present. Regarding the size of the carbon chain, PUFAs have number of carbons ≥ 16 and are also called long-chain polyunsaturated fatty acids, whereas those with number of carbons ≥ 20 are referred

to as very long-chain polyunsaturated fatty acids. The PUFAs omega-3 and omega-6 are distinguished by their beneficial effects on human health, including their role in the synthesis of tissues [2].

Among the organisms that produce fatty acids, fish is the most consumed worldwide. However, its production has not been sufficient to supply the demand of the world market. Due to this fact, sources from agriculture have been replaced by fish oil. Moreover, nowadays, nutritionists also recommend the ingestion of vegetable oils as an important part of a healthy diet [3, 4].

The production of vegetable oils has advantages over the production of fish oil, since the methods of obtaining and purification of vegetable oils are simpler, resulting in cheaper processes [5].

2. Plants as sources of fatty acids

Fatty acids are present in both animal and plant species. Among the animal species, fish are the main sources of fatty acids, but there are a number of limitations regarding the use of fish oil as a supply of fatty acids. Among them, problems caused by harmful contaminants such as carcinogenic, teratogenic, mutagenic, and noncarcinogenic agents (antibiotics and heavy metals, for example) are highlighted. Other limitations on the production and commercialization of fish oil are also widely discussed, such as oil stability problems and unpleasant taste and odor, which result in higher production costs and create difficulties in the oil purification [6–10].

Species	Fatty acid composition (%)				Reference
	C16:0	C18:1	C18:2	C18:3	
Nuts and seeds					
Arachis hypogaea L.	16.02 ± 0.01	53.80 ± 0.01	25.10 ± 0.10	—	[13]
Bertholletia excelsa H.B.K.	14.04 ± 0.75	34.55 ± 1.85	40.15 ± 2.13	0.09 ± 0.05	[14]
Camellia L.	14.76 ± 0.01	22.71 ± 0.03	56.27 ± 0.03	0.33 ± 0.01	[15]
Chenopodium quinoa Wild.	9.58 ± 0.02	25.84 ± 0.06	49.55 ± 0.07	8.51 ± 0.02	[16]
Cucurbita maxima	12.05 ± 0.73	23.90 ± 1.01	57.33 ± 0.41	0.32 ± 0.02	[17]
Dipteryx alata vogel	5.71 ± 0.01	53.35 ± 0.01	24.59 ± 0.01	4.12 ± 0.01	[18]
Helianthus annuus L.	6.70 ± 0.30	25.60 ± 3.0	65.80 ± 2.90	0.07 ± 0.02	[19]
Heliophila africana	7.60 ± 1.00	23.4 ± 2.20	22.00 ± 4.3	4.2 ± 0.60	[20]
Lupinus albus	6.30 ± 0.30	56.10 ± 0.30	18.40 ± 0.40	7.80 ± 0.10	[21]
Myristica fragrans	17.53 ± 0.01	59.44 ± 0.03	13.83 ± 0.02	1.94 ± 0.01	[22]
Moringa oleifera Lam.	23.65 ± 0.68	5.92 ± 0.02	6.84 ± 0.05	—	[23]
Vaccinium myrtillus L.	6.10 ± 0.10	23.30 ± 0.00	33.70 ± 0.20	35.50 ± 0.00	[24]
Zea mays L.	12.57 ± 0.01	29.70 ± 0.11	52.68 ± 1.44	1.12 ± 0.01	[25]

Species	Fatty acid composition (%)				Reference
	C16:0	C18:1	C18:2	C18:3	
Leaves, stems, roots and palms					
Cassia tora L.	18.6 ± 0.08	11.2 ± 0.04	13.2 ± 0.06	16.1 ± 0.02	[26]
Elaeis guineensis Jacq.	36.30 ± 3.40	47.40 ± 2.50	9.40 ± 2.10	0.50 ± 0.30	[27]
Morus alba	26.38 ± 0.01	2.86 ± 0.01	14.76 ± 0.91	34.97 ± 1.84	[28]
Olea europaea L.	20.30 ± 0.80	29.20 ± 1.70	5.80 ± 0.30	32.30 ± 0.50	[29]
Panax ginseng Meyer	2.22 ± 0.01	1.0 ± 0.01	6.58 ± 0.01	0.39 ± 0.01	[30]
Stevia rebaudiana (Bertoni)	12.57 ± 0.01	—	33.14 ± 0.01	—	[31]
Fruits					
Caryocar brasiliense	31.90 ± 0.10	61.40 ± 0.30	2.30 ± 0.10	0.40 ± 0.00	[32]
Euterpe oleracea	23.47 ± 0.01	57.73 ± 0.01	15.54 ± 0.01	—	[33]
Morus nigra L.	11.93 ± 1.30	6.0 ± 0.14	75.85 ± 1.82	1.51 ± 0.27	[34]

Table 1.
Content of the main plant-based fatty acids (%).

Fish oil is a limited resource. In 2017, there was an increase in its world production compared to 2016, but it did not reach the market expectation for 2017, resulting in an increasing trend in the global price [4].

One way to minimize the problems caused by the limitations in fish oil production is to use alternative sources such as vegetable oils. Lipids found in plants present, in their composition, polyunsaturated fatty acids (PUFA), mainly omega-6 and omega-3, which are derived from linoleic acid and α-linolenic acids, respectively. Both are synthesized by plants and not by animal tissues and are classified as essential for good health and disease prevention [11].

Over the years, lipids derived from plants have been gaining prominence in the biotechnology area, mainly for the development of products with pharmacological potential [12].

When evaluating a potential source of fatty acids, its sustainability and ability to meet any demand must be considered. The possibility of a scalable production based on agriculture, coupled with low costs and ease of production, highlights the potential of plants as sources of fatty acids for human diet. **Table 1** presents some plant matrices evaluated as sources of fatty acids.

3. Obtaining and commercial applications of fatty acids

Fatty acids present many applications, due to their physical, biological, and alimentary properties. Regarding their obtaining, there is a great diversity of conventional and modern techniques [35]. According to [36], one of the most traditional methodologies for obtaining oils rich in fatty acids, which involve the use of organic solvents, is Soxhlet [36, 37]. Another technique used is cold pressing, even though it is a millenarian technique [38]. More sophisticated procedures, such as ultrasound-assisted extraction, supercritical fluids extraction, and enzyme extraction are also used to obtain such oils [39].

The extraction of oils can be carried out by Soxhlet using different parameters and organic solvents [40]. The most used solvent in this methodology is petroleum ether, due to its high solvation capacity, inertness, and good stability with oils. Time, temperature, and number of cycles are parameters that directly influence the oil yield obtained in the process [41]. In the methodology of [42], the classic extraction protocol based on chloroform and methanol is used, and it is performed in two different steps: the first one with homogenization and filtration and the second one with washing of the filtrate obtained in the first step, in order to collect the clean oil [42].

The methodology of [37] is an adaptation of that performed by [43], developed due to the need of a faster and simpler process, maintaining its efficiency and reproducibility. According to the needs faced by researchers, several adaptations based on these methodologies have already been developed, most of them with the purpose of obtaining oil of different matrices, with use of less aggressive solvents [40, 43].

A technique of low environmental impact is the extraction by cold pressing, which in addition to being faster, when compared with other techniques, and of low cost, no solvent is used. However, it does not have the ability to completely remove the lipid fraction of the matrix, and due to the absence of selectivity, an extract with low degree of purity is obtained; that is, it contains a greater variety of compounds besides the fatty acids. This type of oil is widely used in food industries [44].

Among the most modern techniques, the extraction by supercritical fluid outstands, which does not use toxic organic solvents, is efficient in obtaining oils rich in fatty acids and is a process with high selectivity [44]. The most commonly used solvent is carbon dioxide, because it is nontoxic, inert, and has low critical properties. In this process, the solvent is pressurized until it becomes a fluid in the supercritical state, with characteristics of liquid and gas, and presents high solubilization and diffusion power, which is able to extract the lipid fraction [45]. The ultrasonic scanning, on the other hand, occurs through ultrasonic waves that create bubbles in the solvent used; these bubbles rupture near the cell walls causing their rupture, and consequently leading to the release of the lipid fraction [46].

In addition to these techniques, there is the microwave-assisted enzymatic extraction, in which some enzymes (cellulase, pectinase, and proteinase), due to their potential of oil release from within membranes, come into contact with the aqueous matrix and react under agitation and microwave resonance. It is a clean methodology because it does not use organic solvents, and it is possible to obtain results similar to the traditional techniques regarding the content of fatty acids [47].

With a wide range of methodologies for obtaining oils rich in fatty acids, which go beyond those mentioned in this chapter, it is possible to obtain oils of the most varied qualities for the most varied applications. The fatty acids are high-value compounds used in the food and pharmaceutical areas. They are marketed predominantly in the form of edible oils, supplements in the form of gelatinous tablets, intravenous emulsions, and in oil-based products of topical use.

Edible vegetable oils rich in fatty acids are products widely consumed worldwide, and also the main sources of omega-9 (oleic acid). Omega-9 is the most consumed fatty acid in America through its main marketed sources, such as olive oil, oleaginous fruits (almonds and nuts), grape seeds, canola, sesame, sunflower, soybean, coconut, and palm oils, among others. Most of these products are linked to a healthy lifestyle of their consumers [48].

However, the increased incidence of diseases related to the lack of a balanced diet and the presence of sedentary lifestyles is one of the main factors driving the global market for fatty acid supplements, which directly increases the demand for isolated omega supplements (3, 6, and omega-9), or their main plant sources. Such products are indicated as a source of lipids to meet the energetic needs of patients who require

parenteral nutrition when oral or enteral feeding is impossible, insufficient, or contraindicated [49]. It is also indicated in the treatment of rheumatoid arthritis [50].

Omega-3 supplements are generally consumed in the form of gelatinous tablets due to their residual taste, caused by their high instability to oxidation, which causes the product to exhibit odor and taste of fish. One of its main plant sources is linseed oil. These products are indicated for the prevention/treatment of cardiovascular diseases; for the reduction of triglycerides rates, total cholesterol and arterial pressure, and also in neurological treatments, improving concentration, memory, motivation, and motor abilities, besides neutralizing the stress and preventing degenerative brain diseases [51, 52]. They are also indicated during pregnancy, reducing the risk of postpartum depression and mood swings, as well as improving health after child's birth [53].

Omega-6 supplements are usually marketed as evening primrose oil (EPO), which is its most popular form, being indicated in the prevention/treatment of problems related to premenstrual syndrome, diabetes, cardiovascular diseases, inflammation, skin problems, and cancer, as well as assisting the attention deficit/hyperactivity disorder, reducing arterial hypertension and osteoporosis [54]. On the other hand, omega-9 supplements are the most commercialized in the form of intravenous emulsion. They are mostly made from refined olive and soybean oils, and found in the market in tablet form. Omega-9 supplementation is indicated for the reduction of waist circumference, combating total and bad cholesterol (LDL), and increasing the good one (HDL). Also, it presents anti-inflammatory activity and is involved in the prevention of coronary diseases, cancer, and aging [55].

Another way of commercializing fatty acids is in topical products. These products are aimed at the dermoprotection, being found in the market in liquid form, and in bandages soaked in oil. Some are indicated for nail strengthening due to their antifungal character (for example, oils derived from melaleuca, clove, thyme, and rosehip), whereas others are indicated for the treatment of all types of skin lesions, such as pressure sores, and venous stasis ulcer. Because of their emollient and healing properties, they improve the skin barrier function and reduce the symptoms of inflammation in atopic dermatitis and psoriasis, diminishing the transepidermal water loss [56, 57].

These fatty acid-based products may contain one or both fatty acids plus other substances, such as vitamin A, E, and soy lecithin. They can also integrate medium-chain triglyceride formulations, linoleic and linolenic acids, mainly responsible for this therapeutic effect, since they are the main constituents of the epidermal water barrier layer. The medium-chain triglycerides present in such formulations contain predominantly caprylic, capric, caproic, and lauric acids, which can be used as a nutritional source, solvents, and product stabilizers. When present in topical products, these acids have the function of lubricating the skin and hair, making the skin more resistant to infections, protecting it from chemical and enzymatic agents, preventing dryness, and accelerating the cicatricial processes. In addition, they are free of side effects [58–60].

Therefore, the application of fatty acids in food and pharmaceutical areas is of fundamental importance, in order to promote the development of new natural products that offer numerous benefits to their consumers.

4. Nutraceutical functions

A new paradigm of food health is evolving, in which the positive aspects of diet are more emphasized. Therefore, consumers are looking for beneficial, complementary or alternative products, and the nutraceutical ones particularly stand out. Nutraceutical

comes from the combination of the words "nutrition" and "pharmaceutical," which was coined in 1989 by Dr. Stephen Defelice. It is considered food or part of food, or any substance of both plant and animal origin, which has positive effects on the health, playing an important role in maintaining the normal physiological function that keeps humans healthy, including the prevention and/or treatment of diseases [61–64].

Some of the most common ways of classifying nutraceuticals can be based on food sources, mechanisms of action, chemical nature, etc. Food sources used as nutraceuticals can be categorized as dietary fiber, prebiotics, probiotics, PUFAs, antioxidant vitamins, polyphenols, and other different types of herbal foods. These nutraceutical products help fight diseases such as obesity, cardiovascular diseases, cancer, osteoporosis, arthritis, diabetes, cholesterol, among others [53, 61, 65].

Long-chain PUFAs are also called essential fatty acids because they are necessary for vital functions in humans, as well as being an important source of energy for most tissues. In this sense, PUFAs can be divided into two groups: omega-3 and omega-6. The major omega-3 fatty acids are α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA is the precursor of EPA and DHA, and its main sources are linseed, soybeans, and canola. The omega-6 fatty acids consist mainly of linoleic acid (LA), γ-linolenic acid (GLA), and arachidonic acid (ARA). LA occurs mainly in corn, soybean, and sunflower oils. Essential fatty acids should be consumed through diet, since humans do not possess the enzymes to produce them [64–66].

Recent studies show the importance of PUFAs as essential fatty acids, and their nutritional value in human health and disease prevention. Intake of PUFAs has been associated primarily with decreased risk of cardiovascular diseases and normal brain development [66–68]. They also present anti-inflammatory effects and contribute to the good functioning of vision. In the study carried out by [69], it was shown that among PUFAs, ALA can serve as a great anti-inflammatory agent of the ocular surface. Its anti-inflammatory effects are comparable to those of corticosteroids. ALA's inhibition of pro-inflammatory cytokines was associated with a significant reduction of I-κBα. According to [70], dietary supplementation with PUFAs is a promising therapeutic option for patients with rheumatoid arthritis, considered as a chronic disease, in which many inflammatory pathways contribute to structural damage, joint swelling, and systemic inflammation.

More recently, studies have shown the benefits of PUFAs in cancer prevention. According to [71], PUFAs exert inhibitory effects on the growth of colon cancer cells. Metabolites of PUFAs such as prostaglandins and leukotrienes play an important role in colon cancer. Also, they are reported in the treatment of diabetes mellitus [72], based on clinical intervention studies performed in diabetic patients. It corroborates that dietary supplementation with 0.42–5.2 g of PUFAs per day for 8 weeks might become an alternative treatment against type 2 diabetes mellitus.

PUFAs have also been attributed to anticoagulant, vasodilator, and antiaggregant activities [66, 67]. Specifically, the biological activities of essential fatty acids (ALA and LA) influence on the functions and responsiveness of cell membranes, tissue metabolism, hormone signals, and others. The beneficial effects of PUFAs can be mediated by different mechanisms, including altering or regulating cell membrane structures, regulating intracellular signaling pathways, modulating gene transcription, and regulating the production of bioactive lipid mediators or production of eicosanoids (prostaglandins, leukotrienes, and thromboxanes) [68, 73].

5. Studies on cardiovascular diseases

Essential fatty acids are considered nutraceutical or functional foods, exhibiting cardioprotective effect, due to their anti-inflammatory, hypolipidemic,

antiatherogenic, antiarrhythmic, and antithrombotic properties, being thus used as risk reducers of cardiovascular diseases [74, 75].

Cardiovascular diseases have been reported as the leading cause of death in the western countries. According to data obtained in 2017 by the World Health Organization, cardiovascular diseases cause approximately 31% of deaths worldwide, making it clear that prevention is important for reducing these numbers [76].

Currently, the number of deaths due to cardiovascular diseases is increasing, and this is an important reason to carry out studies in order to obtain products that can contribute to a healthier diet through the intake of substances beneficial to heart health, such as unsaturated fatty acids (omega series), as well as reduce the intake of saturated fatty acids [76–82].

Researchers have investigated the reduction of the risk of cardiovascular diseases by implementing a diet rich in PUFAs. These studies confirm the efficiency of the intake of these acids, demonstrating a positive effect on lipid metabolism, and also emphasizing that diets rich in SFAs promote cardiovascular damage, as well as an increase in hypercholesterolemia. Nutritionists recommend that intakes of SFAs are maintained by up to 10% relative to total energy, based on dietary guidelines for prevention of cardiovascular diseases [80, 82–84].

Limiting the intake of SFAs becomes an important measure for the prevention of cardiac ischemia, since healthy eating habits can affect the development of diseases and reduce the risk of their occurrence in the myocardium. Thus, daily intake of PUFAs such as omega-3 and omega-6 is important. Experimental tests were carried out with people with ischemic heart failure, and the beneficial effect of PUFAs consumption was verified by the reduction of cardiovascular risk factors such as reduction of the inflammatory process in myocardial tissue, and acceleration of the healing process of the myocardium fibrous tissue [78, 83–89].

Coronary heart disease relates to the development of atherosclerosis, characterized by chronic inflammation of the tunica intima of large and medium-sized arteries. Such inflammation is caused by the interaction between the smooth muscle of the arterial walls and the plasma lipids, or platelets, lipoproteins, endothelium and monocytes, causing narrowing of the coronary arteries. Thus, the maintenance of a diet rich in dietary lipids acts as a therapeutic alternative in the prevention and treatment of various cardiovascular diseases such as strokes and thrombosis [90–92].

6. Effects on brain functions

The importance of fatty acids of vegetable origin in neural development, aging, and neurodegeneration has been addressed in several studies. The brain is an organ rich in phospholipids, which make up about 25% of its dry weight [93]. Some of these fatty acids participate in the structure, biochemistry, physiology, and consequently of the cerebral function, being necessary to maintain, under normal conditions, the cellular membranes, increasing their fluidity and functionality. They also aid in the nerve impulses transmission, reinforcing the importance of the adequate consumption of these lipids to benefit patients with neurological diseases [94, 95].

The consumption of PFAs is related to the reduction, prevention, and non-pharmacological treatment of some neurological diseases [96, 97]. Some studies show how eating habits can affect the brain development by making a comparison between the "Mediterranean diet" and the "western diet," for example. Whereas the Mediterranean diet is rich in long-chain PFAs derived from the combination of fruits, vegetables, cereals, olive oil, and other foods, the western diet is characterized by an increase in the consumption of SFAs and transfats due to the introduction of highly processed foods [98, 99]. Experimental data relate these dietary components

to neurological, neurodegenerative, and psychiatric disorders, since diets with high cholesterol rates increase the risk of developing such diseases, whereas diets with low saturated-fat intake reduce the risk of dementia [100–102], confirming the important role of diet in pathological mechanisms related to the brain.

The hypothesis that diet-induced changes affect brain circulation may be linked to changes in brain structure [103, 104]. The fatty acid content may affect the production and function of dopamine and serotonin [105], since omega-series fatty acids are fundamental for the maintenance of dopaminergic function in the brain, whereas irregularities of these fatty acids can interfere in the function of the dopaminergic receptors [104]. Healthy aging of humans on a regular diet was associated with neuroprotective properties, such as increased volume of the cortex's gray matter, higher total brain volume, and less white matter hyperintensities (lesions) [106], whereas high-energy transfat diets are associated with increased brain atrophy, and reduced total brain volume and numbers of neurons [107].

Adequate dietary intake of fatty acids or their precursors is also important during the perinatal period (before and after the baby's birth), since to ensure the normal development of the brain, newborns need more lipids than adults do. They are essential for fetal growth and development, and for neurological, behavioral, and learning functions [108–110]. Therefore, insufficient supplementation during early life may also aid in the development of diseases related to poor brain development, such as coordination disorder, dyspraxia (neurological motor dysfunction), and attention-deficit/hyperactivity disorder [109]. And the ingestion of fatty acids, mainly of the ω-3 type, positively affects the functioning and development throughout life, increasing cognitive functionality, such as learning, memory, and attention [110, 111].

7. Conclusion

Vegetable oils rich in essential fatty acids have been consumed instead of animal oils due to their high potential of production scalability, and because the fish oil market has not been able to satisfactorily meet the current consumers' demands. Several commercial applications have been developed with wide acceptability, and studies aimed at treating cardiovascular diseases and improving brain functions have reached promising results. In this sense, the use of plants as sources of fatty acids presents itself as a potential alternative not only in the economic scope, but also for human health.

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Author details

Ana Paula de Souza e Silva, Wanessa Almeida da Costa,
Marielba de Los Angeles Rodriguez Salazar, Priscila do Nascimento Bezerra,
Flávia Cristina Seabra Pires, Maria Caroline Rodrigues Ferreira,
Eduardo Gama Ortiz Menezes, Glides Rafael Olivo Urbina,
Jhonatas Rodrigues Barbosa and Raul Nunes de Carvalho*
Program of Post-Graduation Food Science and Technology (LABEX)/FEA (Faculty of Food Engineering), Federal University of Para (UFPA), Belém, Pará, Brazil

*Address all correspondence to: raulncj@ufpa.br

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Chapter 6

Lipids and Fatty Acids in Human Milk: Benefits and Analysis

Jesui Vergilio Visentainer, Oscar Oliveira Santos, Liane Maldaner, Caroline Zappielo, Vanessa Neia, Lorena Visentainer, Luciana Pelissari, Jessica Pizzo, Adriela Rydlewski, Roberta Silveira, Marilia Galuch and Jeane Laguila Visentainer

Abstract

Human milk is related to the physiological and nutritional welfare of newborns, providing the necessary dietary energy, physiologically active compounds and essential nutrients for breastfed babies. Human milk fat has an important position as energy source, structural and regulatory functions, being one of the most important components of breast milk. It provides approximately 50–60% of the energy of the human milk, and its composition in fatty acids defines its nutritional and physico-chemical properties. Furthermore, human milk contains the long-chain polyunsaturated essential fatty acids (LCPUFA) eicosapentaenoic acid (EPA), arachidonic acid (AA) and docosahexaenoic acid (DHA), which is important for appropriate development of baby's organs, tissues and nervous system. This chapter will address the benefits associated with the consumption of human milk (health, nutritional, immunological and developmental benefits) as well as the analysis applied to determine the lipid quality of this powerful food.

Keywords: lipids, fatty acids, human milk, omega-3, children's health

1. Introduction

Human milk is especially complete and suitable to provide the essential to the infants due to its composition including a variety of nutrients, bioactive compounds and immunological factors, which are indispensable for the newborn growth and optimal development [1]. Moreover, studies have demonstrated the benefits of human milk for newborns concerning gastrointestinal problems, growth, neurological development and immune system [2]. Breastfeeding is recognized as the gold standard for feeding infants and should be the exclusive feed source during the primary 6 months of life, without the demand to supplement with additional food or liquid. Besides, the breastfeeding should be prolonged even after food introduction during the primary 2 years of life [3].

Lipids are allocated in groups according to its solubility in apolar and organic solvents insoluble in water, being classified as neutral lipids: triglycerides (TAG), diglycerides (DAG) and monoglycerides (MAG), polar lipids: phospholipids and glycolipids, and miscellaneous lipids: sterols, carotenoids and vitamins [4].

In human milk, the lipids are present as fat globules form, mainly constituted of TAG surrounded by a structural membrane composed of phospholipids, cholesterol, proteins and glycoproteins [5]. The fat from human milk is its main energy source, consisting 98% (m/m) of neutral lipids (TAG, DAG and MAG) [6]. Hence, the fatty acid composition of these constituents defines the nutritional and physicochemical properties of human milk fat [7].

TAG are molecules of glycerol esterified to three fatty acids (FA), which may be located at the TAG sn-1, sn-2 and sn-3 positions. However, the FA position in TAG is also related to the human milk quality. In TAG from human milk, for example, palmitic acid is positioned normally on sn-2 (the central carbon atom) [8], which facilitates the action of pancreatic lipase. Besides, it leads to improved absorption of fat and calcium by newborns due to the subsequent metabolism of these TAG in the infant's body [9].

Therefore, numerous analytical techniques are employed to attempt the verification of the FA composition as well as TAG structure present in human milk fat. This chapter will address the benefits associated with the consumption of human milk, as well as analytical techniques employed to assess its lipid quality.

2. Benefits and analysis of human milk

2.1 Importance of human milk in newborn health

Breastfeeding provides numerous health benefits, both short and long-term for breastfed newborn [10]. The short-term benefits include immune system development, reduction of gastrointestinal diseases (diarrhea), respiratory diseases (pneumonia), skin diseases (atopic dermatitis), allergies, leukemia, sudden death syndrome, diabetes and ear inflammation during childhood [10, 11]. Long-term evidence has shown various benefits to public health problems such as improved cognitive development [12] and reduction of chronic diseases, for example diabetes (type 1 and 2), obesity, hypertension, cardiovascular diseases, hyperlipidemia and selected categories of cancer in adult life [13].

2.1.1 Importance of different lipid classes of human milk

Milk TAG are formed in the endoplasmic reticulum from circulating FA or newly synthesized in the mammary epithelial cells of glucose. The initial step in the FA synthesis is the conversion of acetyl-CoA to malonyl-CoA, afterwards, the synthase enzyme catalyzes the sequence of fatty acid reactions, then each sequence adds two-carbon unit to the growing chain, resulting in the de novo synthesis of medium-chain and intermediate chain FA as well as explaining the elevated content of these FA in milk [14].

Long-chain TAG are digested by a lingual lipase, while the medium and short chain TAG undergoes the action of a stomach lipase and are absorbed in the stomach as FA and glycerol. In the intestine, TAG non-hydrolyzed, especially long chain triglycerides, undergo the action of bile salts and pancreatic enzyme, being reduced to MAGs, FA and glycerol, which are absorbed, distributed and utilized by the tissues [15].

Phospholipids contribute to 1–2% of the total lipids of human milk [16]. The major phospholipids of milk fat globule membrane are phosphatidylcholines, phosphatidylethanolamines and sphingomyelins, and each of it contributes to 20–40% of the total phospholipids [17]. The nutritional importance of these lipids is based on the variety of specific lipids provided, plus it also has particular bioactivities in the gastrointestinal.

The sphingomyelin demonstrates robust anti-tumor activity, may influence the cholesterol metabolism, and exhibits anti-infective activity [4]. The phosphatidylcholine and sphingomyelin contribute to approximately 10% of the total choline intake of infants [18]. Thus, in quantitative terms, water-soluble choline in milk is more significant, although there are good indications that the metabolization of free and esterified dietetic choline is distinctive and it may have specific effects on plasma cholesterol levels and even in the brain development of the baby [19].

Cholesterol content in human milk is low (0.5%), serving as structural component of the milk fat globule membrane, this characteristic is related to the provision of sufficient stabilization and fluidity, it is also essential in lipid metabolism [5]. Breastfed babies present higher plasma cholesterol levels in comparison to babies receiving infant formulas; however, early exposure may favor the metabolic regulation of cholesterol homeostasis in adult life [20].

2.1.2 Importance of fatty acids in human milk

Human milk fat accomplish an important position as energy source, structural and regulatory functions, [21] in which FA are essential for the development of the central nervous system [22] antiprotozoal activity (free fatty acid (FFA) produced during gastric and intestinal digestion of milk fat), increased immune response, anticarcinogenic agents and antidiabetic effects [23].

The principal saturated fatty acid (SFA) in human milk is the palmitic acid (16:0) [24]. It is located in the TAG sn-2 region, simplifying the pancreatic lipase action that specifically hydrolyzes the FA at the sn-1 and sn-3 positions converting the palmitic acid to sn-2 MAG, which is generally well absorbed resulting in improvement of intestinal discomfort, decreasing colic and crying of the newborn. [25] In addition, the palmitic acid position influences the n-acylentanolamides, including levels of anandamide, which presents analgesic effects contributing to the enlightenment of the association between the palmitic acid position and the baby cry behavior [14].

It is also noteworthy that the butyrate SFA (4:0) present functions as modulation of the gene expression regulation and reduction of inflammation processes in the intestine. The SFAs caproic (6:0), caprylic (8:0), capric (10:0) and lauric (12:0) acids are linked to antimicrobial biological activities [26].

In particular, the most significant FA in human milk are the long-chain polyunsaturated fatty acids (LCPUFA) [22]. The homologs of linoleic acid (18:2n-6; LA) from n-6 series are precursors of arachidonic acid (20:4n-6; AA), while homologs of α-linolenic acid (18:3n-3; ALA) from n-3 series are precursors of eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). Therefore, breast milk contains the indispensable FA precursors (LA and ALA) to produce AA and DHA, which present crucial function in visual, immune, cognitive and motor development in newborns. Besides, it present important function in allergy protection, asthma, improvement of lung function and reduction of childhood inflammation and obesity rates, plus an additional advantage is the increase of 4.5 IQ points in infants breastfed in comparison to infants that did not received it [24].

2.2 Nomenclature and terminology of main fatty acids in human milk

The IUPAC nomenclature system is technically clear. The fatty acid names are excessively long, principally the long chain acids, therefore, for convenience, common or trivial name and abbreviated notations are often employed in scientific texts. Researchers working in different study areas on fatty acid composition are familiar with the chemical structure and commonly use of the notation C:D to

represent the FA, being C the number of carbon atoms and D the number of double bonds in the carbon chain. Some researches frequently employ the "omega" system (n minus system), Shorthand Designation, as a notation to define the different series, such as n-3, n-6, n-9, n-12. This system is applicable to unsaturated fatty acids from natural sources (cis configuration). Unsaturated fatty acids have double bond in the carbon chain and, commonly, in the PUFA, the double bonds of carbon chain is interrupted by a methylene group (cis, cis-1,4-pentadiene group).

Thus, the term n minus refers to the position of the double bond closest to the methyl end carbon chain. However, in this system, the position of another double bond in PUFA acid carbon chain is not denoted and the configuration (cis or trans) is not specified. So, this system is not employed for FA with trans configuration and PUFA group, although it is widely used by researchers. Therefore, the IUPAC-IUB Commission does not recommend the 'omega' system [27].

2.2.1 Main fatty acids in human milk

Cis fatty acid composition is one of the major components of woman's breast milk, and it is influenced by different factors, that can be grouped as follows: (i) variable: method of feeding, genetic factors, dietary habits, maternal diet composition, hormones, gestational age at birth, parity, seasonality, between lactation daily, caloric content of food and mutual proportions of particular dietary components (carbohydrate and fat contents), (ii) positive modulation: duration of the lactation period, adiposity, stage of lactation and maternal age, (iii) negative modulation: maternal malnutrition, infectious (mastitis), metabolic disorders (diabetes) and medications [28, 29].

In particular, trans fatty acids (TFA) in human milk have raised concerns because of the possible adverse effects on infant growth and development. The TFA have been associated with adverse effects on LCPUFA and essential fatty acids (LA, 18:2n-6 and ALA, 18:3n-3) metabolism, oxidative stress and low density lipoprotein cholesterol levels. These negative effects of TFA are predominantly associated with different isomers from hydrogenated vegetable shortening, such as 6/7/8/9/10 (trans) 18:1, and barely with TFA of natural sources such as ruminants fats as 11 (trans) 18:1 [30]. Composition of the TFA in human milk from Canadian and American woman has reduced since the mandatory TFA labeling was introduced in those countries [30, 31].

In this chapter, the group prepared a review concerning the composition of FA commonly encountered in studies of human milk from different countries, including five regions of China, Canada, Spain, Brazil, Poland, Germany, Hungary, Finland, Sweden Slovakia, United Kingdom, Denmark, Egypt, Uganda, and Tanzania. A list of FA is elaborated on **Table 1**, including: saturated FA, monounsaturated FA, polyunsaturated FA, branched chain FA, trans FA and conjugated linoleic [14, 26, 31–34].

2.3 Contribution of omega-3 fatty acids from human milk on immunity

Human milk contains numerous growth and antimicrobial factors, as well as cells and antibodies from mother, which are responsible by innate and acquired immune responses in the newborn [35]. The breastfeeding permits these components to cover the neonatal gastrointestinal tract, the main access of microorganisms in early life, and influences the maturation of immune system. It is assumed that human milk can perform in the induction of specific immune responses in the intestine, favoring a microbiota that competes with pathogenic bacteria [36].

Trivial name	Shorthand designation	Trivial name	Shorthand designation
SFA		**MUFA**	
Butiric	4:0	Lauroleic	12:1n-3
Caproic	6:0	Tsuzuic	14:1n-10
Enantic	7:0	Physeteric	14:1n-9
Caprylic	8:0	None	14:1n-7
Pelargonic	9:0	Myristoleic	14:1n-5
Capric	10:0	None	15:1n-5
Undecylic	11:0	Sapienic	16:1n-10
Lauric	12:0	None	16:1n-9
Tridecylic	13:0	Palmitoleic	16:1n-7
Myristic	14:0	None	17:1n-8
Pentadecylic	15:0	None	17:1n-7
Palmitic	16:0	Petroselinic	18:1n-12
Margaric	17:0	None	18:1n-10
Stearic	18:0	Oleic	18:1n-9
Arachidic	20:0	Vaccenic	18:1n-7
Heneicosylic	21:0	None	18:1n-6
Behenic	22:0	None	18:1n-5
Tricosylic	23:0	None	18:1n-4
Lignoceric	24:0	None	18:1n-3
BCFA		None	18:1n-2
Isotridecylic	13:0 iso	Gadoleic	20:1n-11
Anteisotridecylic	13:0 anteiso	Gondoic	20:1n-9
Isomyristic	14:0 iso	Paullinic	20:1n-7
Isopentadecylic	15:0 iso	Cetoleic	22:1n-11
Anteisopentadecylic	15:0 anteiso	Erucic	22:1n-9
Isopalmitic	16:0 iso	Nervonic	24:1n-9
Isomargaric	17:0 iso		
Anteisomargaric	17:0 anteiso		
PUFA		**TFA**	
Linoleic	18:2n-6 (LA)	Myristelaidic	t-14:1n-5
Gamma-linolenic	18:3n-6	None	t-15:1n-5
Alpha-linolenic	18:3n-3 (ALA)	Isomers	3/4/5/6/7/8/9/10/11/12/13 (trans) 16:1
None	20:2n-6	Isomers	6/7/8/9/10/11/12/13/14 (trans) 18:1
Stearidonic	18:4n-3	Linolelaidic	trans,trans 18:2n-6
Meadacid	20:3n-9	None	cis-9, trans-12 18:2
Dihomo-γ-linolenic	20:3n-6	None	trans-9,cis-12 18:2
Dihomo-ALA	20:3n-3	None	trans-11,cis-15 18:2

Trivial name	Shorthand designation	Trivial name	Shorthand designation
None	20:4n-3		
Aracdonic	20:4n-6 (AA)	**CLA**	
Timnodonic	20:5n-3 (EPA)	Rumenic	cis-9, trans 11 18:2
Adrenic	22:4n-6	None	trans-9, cis-11 18:2
Osbond	22:5n-6	None	trans-11, cis-13 18:2
Clupadonic	22:5n-3	None	trans-11,trans-13 18:2
Cervonic	22:6n-3 (DHA)		

Fatty acids abbreviations: SFA—saturated fatty acid, MUFA—monounsaturated fatty acid, PUFA—polyunsaturated fatty acid, BCFA—branched chain fatty acid, TFA—trans fatty acid, CLA—conjugated linoleic acid.

Table 1.
Fatty acids commonly encountered in human milk.

Other components constantly present in human milk are the LCPUFA DHA and AA [37], essentials as cell membranes components and also as immunomodulators, by production and regulation of inflammatory cytokines, leukotrienes, prostaglandins, and thromboxanes, recognized as eicosanoids [38].

LCPUFA in human milk can modulate immunological responses, affecting the balance between T-helper cell type-1 (Th1) and Th2 [39], and regulatory T and T helper 17 cells from the acquired immune response [40].

These subsets of CD4+ T cells, Th1, Th2 [41], Th17 [42], and regulatory T (Treg) cells [43] participle producing cytokines with the most diverse functions. Interferon-γ (IFN-γ), tumor necrosis factor-alpha (TNF-α) and interleukin-2 (IL-2) are the products of Th1, and IL-4, IL-5, IL-9, IL-10, IL-13, and IL-25 are of Th2 cells. Th17 cells produce IL-17A, IL-17F, IL-21 and IL-22; while Treg cells produce IL-10 and TGF-β1.

Th1 cells present important functions in cellular immunity against intracellular bacteria and protozoa, while Th2 cells mediate the response against extracellular parasites, as helminths, and participate in allergies [44]. Th17 cells apparently perform against different classes of pathogens and autoimmune conditions [45], and Treg cells perform regulating the inflammation, autoimmunity, allergy, infection, and tumors.

In general, preterm infants have an immature immunoregulatory system, with potential for chronic inflammation [46], but an increase in Tregs and their function in early neonates has been observed [47], suggesting a transient increase of activated Treg in mature and full-term infants.

Although, generally, AA is considered proinflammatory and DHA immunoregulatory, the addition of it to infant formula has been indicated to increase the immunoregulatory system and to reduce inflammatory cytokines in infants, indicating an effect of LCPUFA on immune maturation [48].

However, according to [49], diets rich in DHA can reduce suppressive and migratory functions of regulatory T-cells [48]. Thereby, we must carefully examine the influence of FA during breastfeeding, since the knowledge from the DHA data on immune response in preterm infants, and the generation and maintenance of Tregs are still not well comprehended. Finally, the addition of AA and DHA in infant formulas should consider balancing its amounts, as DHA in excess may suppress the benefits provided by AA [50].

2.4 Atopic disease and polyunsaturated fatty acids (PUFA)

Atopic disease is defined as a set of disease such as atopic dermatitis, asthma and rhinitis, but it may differ according to the authors. The prevalence of childhood atopic

disease has been increasing and studies suggested the possibility that breastfeeding may reduce allergic manifestations in high-risk individuals [51, 52]. This association is possible linked with the breast milk PUFA, which are essential for adequate growth and development. It is also recognized that an early ingestion of it may affect the growth as well as the neurological and immune functions in later life [53].

Part of the PUFA breast milk composition depends on the mother dietary, and it has provided support to the hypothesis that omega-3 (n-3) PUFA in breast milk possibly protect against atopic diseases. The ratio between n-3 and omega-6 (n-6) PUFA levels seems to influence the development of atopic disorders [54, 55].

The n-3 FA supplementation, during pregnancy, and lactation have been extensively studied. Pregnant mothers consuming n-3 FA may enhance levels of IgA (adaptative immune response) and soluble CD14 (innate immune response) in breast milk. This theory reinforces the importance of the PUFA immune modulation and the idea that it can improve the immune system directly and indirectly [56].

The composition of breast milk has been shown to reflect in the infant's serum, by increase of immunomodulatory cytokines, such as TGF-1 and TGF-β2, associated to the protection against atopic diseases [57]. Moreover, EPA and DHA levels in colostrum and early mature milk were related to the protective effect in the development of IgE-associated with allergic disease in infancy [58].

An Asian study revealed that n-3 FA supplementation during pregnancy could reduce the chance of preterm birth. According to this article the intake recommendation to pregnant women is minimum of 200 mg (DHA) per day over and above the intake level recommended for adult general health, resulting in a total DHA intake of at least 300 mg/day. Likewise, PUFA supply and fish ingestion may positively influence the development of immune responses involved in allergic reactions, and reduce the risk of allergic diseases (asthma and eczema). It is recommended to women who breastfeed to achieve a minimum average daily supply of 200 mg DHA, to result in a milk with DHA content of 0.3% of FA [53].

A systematic review concluded that there is heterogeneity among studies in terms of presenting the association between PUFA and allergy, which could influence the results [59]. Some studies observed associations between n-3 and n-6 PUFAs and allergic disease [60], and the magnitude of this effect varied greatly. Otherwise it is known that breast milk contains different composition of PUFA, which could explain the variability of the results [61].

A cohort study has shown the ratio of n-6: n-3 FA in milk is associated with the risk of non-atopic eczema at 6 months, and perhaps the high level of n-6 may increases the risk of rhinitis [62]. Other authors hypothesized that variations in the lipid composition of milk could, in part, explain some of the controversies regarding the protective effects of breastfeeding against allergy, and concluded that the fatty acid composition of human milk is disturbed in atopic mothers having an effect on atopic sensitization in the primary 12 months of life [58].

2.5 Processing, composition in antioxidants and lipid stability in human milk

The methods of processing human milk employed in milk banks aim the preservation or inhibition of microbial growth, the prevention or delay of decomposition caused by the presence of enzymes, chemical reactions, and the preservation by grime, such as insects, hair, animals, etc. [63]. Generally, pasteurization and freezing techniques are employed. In addition, recent studies combine both processes with lyophilization in order to preserve the original characteristics of milk for an extended period [64].

However, food processing can cause nutritional loss and structural modifications. In milk banks, pasteurization is followed by freezing storage at −18°C for

up to 6 months. But, the thermal process causes modifications in the milk due to the inadequate intensity of the set time and temperature that has been applied. Consequently, proteins can be denatured, enzymes become inactive, lipids suffer oxidation and vitamins and minerals are unstructured [65].

The Holder pasteurization (30 minutes of heat at 62.5°C and frozen at −20°C) imposed by the global guideline was evaluated by studies demonstrating that the lipolytic activity increased, doubling the concentration of free fatty acids (FFA), while the low temperature reduced the lipolysis rate, even if it had been increased by the storage time under freezing [66].

The lyophilization process removes the water from food by sublimation, allowing its preservation at room temperature, with the addition of water the product returns to its original form without nutritional losses. This technique, applied in human milk, demonstrated to be effective, as it inhibits microbial contamination, preserves nutrients and oxidative markers, as well as ensures a prolonged conservation period in comparison to pasteurized human milk [67].

Lipids are the most compromised macronutrients present in milk during processing due to the autoxidation of the fatty acid. This degradation reaction can occur with or without oxygen, as well as be catalyzed by light, heat, irradiation and free radicals, forming toxic compounds, such as peroxides. According to the thermal processes, fatty acid may undergo structural isomers by generating trans molecules or losing their total or partially insaturations, damaging the product and causing nutritional loss. However, studies indicate that the PUFA is stable during pasteurization and it may be justified due to the high antioxidant activity of the human milk [68].

The milk naturally presents antioxidant compounds that delaying or preventing molecules from being affected by the oxidative processes [69]. These compounds operate according to diverse action mechanisms for cell protection, such as: (i) eliminate substances that initiate peroxidation, (ii) chelate metallic ions, turning it incapable of decomposing peroxides or forming free radicals, (iii) block the action of reactive species, (iv) interrupt the auto oxidizing chain reaction, and/or (v) reduce the local concentrations of O_2 [70].

Regarding the antioxidant category, it can be classified chemically by enzymatic and non-enzymatic, both perform synergistic actions in free radicals elimination [71]. Among the antioxidant enzymes, the milk is composed by the superoxide dismutase and the catalase, and the glutathione peroxidase that contains selenium. There are also other enzymes that catalyze the synthesis or regeneration of non-enzymatic antioxidants, named support enzymes, among which are glucose-6-phosphate dehydrogenase and glutathione reductase [72].

Non-enzymatic antioxidants present in breast milk are glutathione, amino acids arginine, citrulline and taurine, creatine, metallic ions selenium and zinc, ascorbic acid (vitamin C), carotenoids, flavonoids, coenzyme Q10, vitamins E and lactoferrin. Among these antioxidants that are three distinct classes: (i) antioxidants that performs as free radical abductor in the lipid milk portion, such as vitamin E and A, carotenoids and coenzyme Q10, (ii) antioxidants that performs in the aqueous phase, such as ascorbic acid, and (iii) antioxidants that performs in both cases, such as flavonoids [73].

Among the lipophilic antioxidants, the principals are: carotenoids, vitamin A and α-tocopherol. A vitamin E constituent is present in greater amount in colostrum, first phase of breast milk, providing it a yellowish color due to the intense presence of the pigment carotenoid β-carotene, and decrease from the beginning of lactation, despite the increase of total lipids [74]. The average level of vitamin A

on the third day of lactation comes to be three times superior than in mature milk. Similarly, the amount of vitamin E in colostrum can be the triple of that found in the mature milk and the carotenoids can present a level up to 10 times higher [75]. In this way, the precise balance of various antioxidants in breast milk, instead of any isolated factor, determines its oxidative stability [76].

2.6 Analytical methods for determination of fatty acids in human milk by gas chromatography: flame ionization detector (GC-FID)

2.6.1 Different extraction methods for total lipids (TL)

The fatty acid composition of human milk has been extensively studied over the last 25 years and almost all of the studies are obtained after lipid extraction.

Different methods of fat extraction have been proposed to determine the fat content in human milk by traditional method: crematocrit [77], esterified FA [78], Gerber method (butyrometer) [79], and gravimetric [80].

Recent methods have been proposed in the direct quantification of FA in human milk by gas chromatography [81]. However, the gravimetric method is considered the gold standard for extracting the total lipid (TL) content in human milk, and Folch et al. [80] is one of the most recommended methods for it. This methodology extracts non polar, polar and neutral lipid using mixture of cold solvents for extraction and not compromising the chemical structure of the lipids.

2.6.2 Quantification of fatty acids in human milk by GC-FID

Prior to chromatographic analysis, a derivation step of the TL is required, converting the different lipid classes in fatty acids methyl esters (FAMEs). This step is necessary to enable the volatilization of the compounds of interest, and to allow the determination by GC; there are several methods for this purpose [82].

Normally, the quantitative determination of FA in the human milk is data generally normalized as g of FA per 100 g of FA or expressed as percentage of weight (area normalization) FA relative to all FA exposed in a chromatogram. In the normalization methods, all the FA of the sample must be considered and, in the case of omission of a component, the other components are affected. On the other hand, the results presented by the normalization present difficulties of interpretation and, therefore, in nutritional values of the human milk [81]. The main drawback about normalization methods is that the data set does give information on the amount of FA (in mass) per volume or mass in human milk.

Recent studies express the composition of FA in mass concentrations of FA by mass of liquid human milk, and direct quantification of FA in human milk by gas chromatography has been proposed [81].

The determination qualitative and quantitative of FAMEs by GC-FID is among the most commonplace analyses in lipid matrices. Quantification of FAMEs by GC-FID has been effectively performed whereas detection with GC tandem mass spectrometry (CG-MS) has been employed mainly for qualitative analysis of FA. Both detectors FID and MS, for chromatographic analysis, the derivation step of the lipids classes is required to conversion of TAG, DAG, MAG, phospholipids (transesterification process), FFA (esterification process) in FAMEs [83].

The American Oil Chemists Society (AOCS) and the Association of Analytical Chemists (AOAC) recommend parameters for accurate quantification of FA. Both sources indicate the use of internal standard (IS, methyl tricosanoate - 23:0) and

capillary columns. The IS are used to minimize the experimental errors, control extraction, transesterification and esterification, undesired saponification. The IS cannot be part of the composition of lipid sample or whole sample [83].

2.6.2.1 Relative response factor in the FID and methodology

The FID has become one of the most popular measuring devices employed in GC and it is the most sensitive detector for hydrocarbons, being the FA merely carboxylic acids with long chains of hydrocarbons.

As the FAMEs respond differentially in FID because of the combustion of carbon compounds that produces ions due to the chain size, presence of FA-substituted functional groups (carboxylics group, double bond) in a hydrocarbon, it reduces the combustion efficiency, and therefore, relative response factors in the FID depends on the effective carbons number. Thus, it is necessary to use correction factors for the FAMEs in relation to the IS. The applied factors are the experimental (empirical correction factor) and the theoretical correction factor (F_{CT}), theoretically determined from the number of effective carbons. It is also important the conversion factor from FAME to FA (F_{CEA}) [83, 84]. In this chapter, methodologies employing IS (23:0) and correction factors FCT, and FCEA for the FID response are described below [83].

The follow column and chromatography condition has been used with efficiency in separation process of the methyl esters (FAMEs) in total lipid of milk. The FAMEs are prepared by transesterification and esterification of total lipids. It is injected and separated into a CG-FID. The column Select FAME (part number CP-7420) fused silica capillary column 100 × 0.25 mm, and 0.25 μm of 100% cyanopropylpolysiloxane (high polarity) was employed. The carrier gas (H_2) flow rates are 1.2 mL min^{-1}; auxiliary gas (N_2) 30 mL min^{-1}; H_2 and synthetic air 35 and 350 mL min^{-1}, respectively. The volumes of the sample injection are 1.0 μL, split of 1:80. The injection temperature: 200°C, detector temperature: 240°C. The column temperature-programmed: 165°C for 7.00 min, the heating ramp of 4°C min^{-1} until 185°C (4.70 min.) after that another programming heating of 6°C min^{-1} until 235°C (5.00 min.). The FAMEs are identified by comparison of their retention times, determined by computer software analysis, with those of individual purified standards or secondary standards. The quantifications of FAMEs are performed with internal standard (IS 23:0) and the corrections factors for the FID response are utilized for the determination of concentrations [84]. The composition of fatty acids (FA) in the total lipids of samples is calculated in mg g^{-1} of total lipids (TL) using the Eq. (1) [83, 84].

$$Mx = \frac{A_x M_p F_{CT}}{A_p M_A F_{CEA}} \quad (1)$$

M_x is the concentration of FA "x" in mg g^{-1} of TL, A_x is the FA "x" peak area, A_p is the IS (FAME 23:0) peak area, M_p is the IS mass added to the sample in mg, M_A is the sample mass in grams, F_{CT} is the theoretical correction factor of the FID and F_{CEA} is the conversion factor from FAMEs to FA.

It is concluded that to determine the FA composition by GC-FID with high accuracy, and to express the composition of fatty acids in mass of FA per sample (by volume or mass) it is necessary to apply the correct derivation technique, internal standard and flame ionization detector relative response factors using the effective carbon number and conversion from FAMEs to FA.

2.7 Analytical techniques for analysis of lipids by electrospray ionization (ESI) and other techniques

2.7.1 Lipid extraction for analysis by liquid chromatography-mass spectrometry (LC-MS)

In order to determine TAG in food, initially, is essential to extract the lipids contained on it. Folch [80] and Bligh and Dyer [85] methods are extensively employed for the extraction of milk lipids. The addition of antioxidant, such as BHT is recommended prior to extraction to avoid lipid oxidation [86]. The internal standards for each lipid class are added to the matrix prior to extraction [87].

Preceding the LC–MS analysis, the extraction solvents (chloroform/methanol) must to be removed by evaporation, and the lipids are reconstituted with solvent compatible with the mobile phase of LC. Moreover, a pre-sample of SPE or TLC columns previous to LC–MS analysis may facilitate the lipid species identification due to improved resolution.

2.7.2 Direct infusion analysis in MS

Sample direct infusion into the mass spectrometer was one of the first techniques employed in TAG analysis [88]. Its main advantage is the rapidity. However, despite the progresses in the last 15 years, there are three main problems associated with the technique: (1) ion suppression, (2) isotopic interference and (3) differentiation of isomers. Consequently, a chromatographic separation is crucial to avoid ions suppression and to differentiate the isomeric species, being the direct infusion technique rarely employed for characterize lipid in milk sample.

2.7.3 TAG determination by LC-MS

Nowadays, milk TAG are characterized in three levels: (1) carbon chain size, (2) level of composition in FA and (3) level of FA position; providing information, respectively, on the composition of milk fat TAG, FA composition of a lipid species and regiospecific distribution of FA on TAG molecules.

2.7.4 Identification of the carbon chain size

TAG is defined in this chapter as a series of species with the equal total number of acylated carbons (CN) and the equal number of double bonds (DB), regardless of its constitution in FA. So each TAG group has a unique chemical formula and a precise mass. Ammonium salt is added to the mobile phase so the TAG are detected as ammoniated adducts (to become more stable and avoid the formation of different adducts with the same molecule) in the positive ionization mode ESI+. The most abundant TAG groups contain 26–54 acyl CN and 1–8 DB, with molecular mass ranging from 500 to 1000.

2.7.5 Composition of FA contained in the TAG

Aiming the determination of the FA contained in the TAG, the diacylglycerols (DAG+) are formed after neutral loss of one of the three FA chains. For each TAG molecule, three DAG+ ions correspond to the loss of each of the three FA, so the FA composition of any TAG molecule can easily be deduced by the mass difference (for example, neutral loss of 245, 273 and 301 corresponding to the loss of FA 14:0, 16:0 and 18:0, respectively). As expected, each TAG group may contain different

FA compositions (referred as isomeric species), being the liquid chromatography with reverse phase (RP-LC) combined to a C18 column, non-aqueous mobile phase (generally acetonitrile and isopropanol) and a surface gradient (up to 150 min) the greatest recognized method to separate species of TAG isomers from the same group or even ECN [89].

2.7.6 Determination of FA position in the TAG

Determining the position of FA in the TAG (sn-1, sn-2 or sn-3) is more challenging. Two protocols are commonly employed to achieve it, both based on the partial hydrolysis of TAG to cleave FA at sn-1 and sn-3 with Grignard reagent [90] or pancreatic lipase [91], being the last one, the method approved by the AOCS [92]. It is followed by MAG TLC isolation and lastly the GC determination of the FA that was at the sn-2 position after transesterification of the MAG-sn-2-FA. Both methods may reveal the percentage distribution of the different FA at sn-2 position and also for a particular FA, the percentage at sn-2 position compared to sn-1/sn-3 position. In other words, this approach generates information on the overall percentage of FA in sn-2, the FA composition of a mixture and not for each individual TAG species.

It is essential to mention that the precision of these two methods depends on the complete conversion of TAG and DAG into MAG, which needs to be carefully monitored. This is a rather time-consuming procedure, so a simplified protocol combining lipase digestion with direct LC–MS and quantification of MAG is urgently needed.

2.8 Determination of TAG, phospholipids and sterols from human milk by HPLC analysis

The first approaches for the analysis of TAG in lipid-rich matrices have been based on GC coupled with FID and MS. However, in recent years, to simplify sample preparation, *i.e.*, to avoid time-consuming preliminary treatments, TAG analysis have been carried out by high performance liquid chromatography (HPLC) [93, 94]. TAG analysis by HPLC can be performed by normal phase (NP) and reversed phase (RP). Among it, RP with non-aqueous (NA) mobile phases is the most extensively used mode. In NARP-HPLC the separation is based on the equivalent carbon numbers (ECNs); TAG with the same ECN can be separated based on the position, configuration of the double bounds, the length and unsaturation of FA. In NP separation mode it has been used silver ions impregnated columns, and this mode is named as silver ion (Ag)-HPLC. In (Ag)-HPLC the separation is based on specific silver ion/double bond interactions and the retention times depends on the position, on the cis/trans double bonds configuration and increases with the unsaturation number of the chains [93, 95].

Several detection systems have been used for TAG analysis, such as ultra-violet (UV) detector, evaporative light scattering detector (ELSD) and refractive index detector (RID) which are suitable for quantitative analysis by means of reference, but it does not permit structural information. Thus, HPLC coupled to MS is the currently preferred TAG analysis technique, being electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) the favorite ionization sources [93, 96].

For phospholipids analysis, HPLC is the most commonly used chromatographic technique. PLs are primary determined by NP-HPLC, but RP-HPLC and, in the last years, hydrophilic interaction liquid chromatography (HILIC) has also been

used. In NP and HILIC modes the separation is based on the different polarity of the "headgroups" and in RP mode the separation is based on the features of the chain lengths, number of unsaturation and geometry of acyl chains [97, 98]. Among the detection systems used for phospholipids determination, low-wavelength UV detectors, ELSD, RID and most recently, charged aerosol detector (CAD), ELSD is probably the most extensively reported for phospholipids class analysis in the food matrices. The determination of phospholipids by HPLC coupled to MS has been also increased in the last years, being ESI and matrix-assisted laser desorption ionization (MALDI) the preferred ionization sources. Despite the advantages of MALDI, ESI is more used due to the difficulty in coupling HPLC to MALDI-MS [99–101].

Finally, for sterols analysis the most conventional used chromatographic techniques are GC coupled to FID or MS detection systems and HPLC coupled to UV detection systems. However, the high temperatures achieved during GC methods can cause degrade some sterols and HPLC-UV methods have relatively poor sensitivity and selectivity towards sterol molecules [102–104]. Thus, during the last decade, based on the accurate identifications and good selectivity and sensitivity of MS detectors, the use of HPLC coupled to MS for sterols analysis gained ground. Because sterols are highly lipophilic and have few polar groups, APCI is the most widely used ionization technique, although conventional ESI methods have also been applied. In the same way, RP-HPLC is the preferred analysis mode, and the analyte interactions with the stationary phase increase with increasing molecular sizes and decreasing number of double bonds in sterol molecules [104, 105].

3. Conclusions

The human milk fat contains 98% of neutral lipids (TAG, DAG and MAG) and the fatty acid composition of these constituents is directly related to the nutritional and physico-chemical properties of human milk fat. Especially, the most significant fatty acids in human milk are LCPUFA, including EPA, DHA and AA, essential for proper growth and development. Analytical techniques such as gas chromatography with flame ionization detector (GC-FID) can be employed to evaluate the fatty acid composition of human milk fat. Prior the chromatographic analysis, the lipids derivatization is essential to allow volatilization of the interest compounds. Moreover, TAG analysis can be carried out by mass spectrometry and high performance liquid chromatography (HPLC), in order to determine the FA contained in the TAG, as well as TAG's FA position. Conclusively, breastfeeding is an incomparable ideal food for the healthy growth and development of infants and offers numerous short- and long-term health benefits for breastfed newborns.

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Conflict of interest

None.

Author details

Jesui Vergilio Visentainer[1], Oscar Oliveira Santos[1], Liane Maldaner[1], Caroline Zappielo[1], Vanessa Neia[2], Lorena Visentainer[3], Luciana Pelissari[2], Jessica Pizzo[1], Adriela Rydlewski[2], Roberta Silveira[2], Marilia Galuch[1] and Jeane Laguila Visentainer[4]*

1 Department of Chemistry, Maringa State University, Maringa, Brazil

2 Postgraduate Program in Food Science, State University, Maringa, Brazil

3 Faculty of Medical Sciences, Department of Dermatology, State University of Campinas, Campinas, Brazil

4 Department of Basic Health Sciences, Maringa State University, Maringa, Brazil

*Address all correspondence to: jelvisentainer@gmail.com

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Chapter 7

Phenolic Compounds in *Hibiscus mutabilis* Seeds and Their Effects on the Oxidative Stability of DHA-Enriched Goat Milk Emulsion

Adela Mora-Gutierrez, Rahmat Attaie and Maryuri Núñez de González

Abstract

Food emulsions undergo oxidative deterioration during production and storage, which is usually initiated from the unsaturated fatty acids. Synthetic antioxidants are frequently used to retard lipid oxidation in food emulsions. Most plants and their seeds are rich sources of natural antioxidants such as the carotenoids and polyphenols. The most abundant fatty acids found in the oil from the seeds of *Hibiscus mutabilis* (HM) are oleic acid (C18:1*n*-9, 16.3%), linoleic acid, (C18:2*n*-6, 64.7%), and palmitic acid (C16:0, 18.8%). The total tocopherols in HM seed oil were at an average concentration of 187.0 μg/g, which included α-tocopherol (21.4%), γ-tocopherol (78.2%), and δ-tocopherol (0.4%). The HM seed oil can be incorporated into food emulsions such as in DHA-enriched goat milk emulsion to stabilize added oil from oxidation. The HM seed oil was mixed with algae oil, a rich source of omega-3 docosahexaenoic acid (DHA; C22:6*n*-3), before emulsification and storage of goat milk. The addition of HM seed oil containing phenolics to algae oil at 1:1 ratio prior to goat milk emulsification significantly ($p < 0.05$) protected the goat milk emulsions against oxidative deterioration. In goat milk emulsions, the addition of ascorbyl palmitate retarded oxidation as was determined by the peroxide values and anisidine values.

Keywords: *Hibiscus mutabilis* seeds, phenolics, algae oil, milk emulsion, stability

1. Introduction

Human breast milk contains both docosahexaenoic acid (DHA, C22:6*n*-3) and arachidonic acid (AA, C20:4*n*-6) [1], which are essential for health. Studies in animals and humans indicate that DHA is essential for normal visual and brain function in the premature infants and possibly in the full term infants [2, 3]. In some breast-fed infants, colic has been related to the mother's consumption of cow milk [4, 5]. In older infants, the incidence of cow milk protein intolerance was encountered in 5–15% of cases [6]. A popular therapy among pediatricians is to change from cow milk to vegetable protein soy-based formula; however, infants with cow milk protein intolerance will also react adversely to soybean proteins [7]. When the problem is allergy to cow milk proteins (casein, whey), goat milk is a suitable substitute to cow milk [8].

The omega-3 fatty acids in the milk of grass-fed goats are predominantly linolenic acid (C18:3*n*-3), or alpha (α)-linolenic acid (ALA). DHA can be synthesized from dietary ALA, but the human body can only make very small amounts of DHA from ALA [9]. Therefore, there is a need to supplement foods with DHA. The addition of DHA from algae oil in food emulsions such as in goat milk emulsion requires the need for antioxidants. Antioxidants increase the shelf life of emulsions, but a clean label ingredient is required when added to milk. Oxidation in oil-in-water emulsions is thought to occur at the interface region between the oil and the aqueous phases [10]. In the oil phase of the emulsions, fatty acids are the target of free radicals i.e., hydroxyl radicals, which stimulate lipid peroxidation. Nonpolar antioxidants such as tocopherols and ascorbyl palmitate have been shown to be highly effective in protecting oil-in-water emulsions [11]. Tocopherols are free-radical terminators thereby, interrupting the free-radical chain of oxidative reactions by contributing hydrogen from the phenolic hydroxyl groups [12]. Ascorbyl palmitate, a lipid-soluble antioxidant, exhibits antioxidant activities that include single oxygen quenching and free-radical scavenging [13–15]. Ascorbyl palmitate has been shown to work synergistically with tocopherols by donating a hydrogen to the tocopheroxyl radical, formed as a result of tocopherol donating a hydrogen to the lipid radical [16, 17].

The oxidative stability of DHA-enriched emulsion may also be accomplished by the addition of vegetable oil to algae oil. In this context, one of the strategies developed to protect fish oil in a cow milk emulsion against oxidation was the mixing of rapeseed oil with fish oil prior to emulsification of cow milk [18]. The authors found that tocopherol isomers in concentrations similar to those found in natural rapeseed oil, and added to rapeseed oil stripped of natural tocopherols, significantly inhibited oxidation in cow milk emulsions enriched with fish oil [18].

Hibiscus mutabilis (Malvaceae) are shrubs with peach color flowers and originally native of China. The seeds of *Hibiscus mutabilis*, which do not have economic applications yet, are a source of vegetable oil. Although not widely reported in the literature, a high content of phenolic compounds, tocopherols are found in *Hibiscus mutabilis* seed oil. The seeds of *Hibiscus mutabilis* are also a source of lectin. Lectin from the seeds of *Hibiscus mutabilis* has carbohydrate-binding specificity to galactonic acid, which potently inhibited HIV-1 reverse transcriptase [19]. HIV, the RNA virus that causes AIDS, gradually disrupts the immune system in humans. Since a recent study suggested that DHA in high DHA-concentrated fish oil positively contributed to certain aspects of immune function in middle-aged obese adults [20], DHA-enriched goat milk stabilized by *Hibiscus mutabilis* seed oil potentially can be used as immune stimulator for the adjunctive therapy of HIV.

In the present work the suitability of *Hibiscus mutabilis* seed oil for enhancing the oxidative stability of DHA-enriched goat milk emulsion was studied. Based on the potential synergistic effects of ascorbyl palmitate with tocopherols, it was assumed that the most efficient oxidative stabilization during homogenization and storage of DHA-enriched goat milk may be achieved by combining both of these lipophilic antioxidants.

2. Materials and methods

2.1 Materials

Raw milk from French-Alpine goats, raised at the International Goat Research Center, Prairie View A&M University, Prairie View, Texas, USA, was obtained. Raw milk with a fat content of 4.1% (wt/wt) that was determined according to the

method of Kleyn et al. [21] was collected during the early lactation period. Iron and copper contents in raw goat milk were determined by atomic absorption spectrometry using a Varian SpectrAA 55 (Varian Analytical Instruments, Inc., Walnut Creek, CA, USA). Raw goat milk was dry-ashed in a muffle furnace (Barnstead/Thermolyne Corp., Dubuque, IA, USA) at 550°C. Ashes were dissolved in 0.2% nitric acid solution. The concentrations of iron and copper in raw goat milk were determined from the calibration curves that were produced under the same experimental conditions with known standards.

Algae oil was provided by Nutrinova Inc. (Somerset, NJ, USA). Algae oil was subjected to chromatography to remove peroxides, carotenoids, tocopherols, and other antioxidants, as previously described [22]. The chromatographically purified algae oil has a DHA concentration of 42.9% (**Table 1**). The fatty acid composition of chromatographically purified algae oil was determined by preparation of methyl esters [23], which were analyzed by gas chromatography–mass spectrometry (GC–MS). For the fatty acid profile of raw goat milk, the samples were centrifuged at 10,000× g for 1 h to harvest milk fat. Fatty acids of milk fat (% wt/wt) were directly methylated by *in situ* transesterification as described [24] and analyzed by GC-MS (Agilent model 7890A GC system attached to an Agilent model 5975C mass detector; Agilent Technologies Inc., Santa Clara, CA, USA) on a 30 m × 0.25 mm internal diameter, 0.25 μm film thickness capillary column. Methyl ester of 10,

Fatty acid (% wt/wt)	GM	PAO	PHMO	NHMO
C4:0	1.2			
C6:0	1.5			
C8:0	2.1			
C10:0	7.7			
C11:0	0.1			
C12:0	3.2			
C13:0	0.1			
C14:0	8.2	2.8		
C14:1(*n*-5)	0.1			
C15:0	0.4	1.2		
C16:0	21.1	30.1	18.7	18.8
C16:1(*n*-7)	0.6			
C17:0	0.2	0.3		
C18:0	9.0	0.9		
C18:1 (*n*-9)	23.3		16.0	16.3
C18:2 (*n*-6)	4.1		64.6	64.7
C18:3 (*n*-6)	0.2			
C18:3 (*n*-3)	2.8			
C20:0	0.1			
C20:1 (*n*-9)	0.3			
C20:4 (*n*-6)	0.2			
C20:4(*n*-7)		0.9		
C20:4 (*n*-3)		0.9		
C22:1(*n*-9)				
C22:5(*n*-6)		10.5		
C22:5 (*n*-3)		0.7		
C22:6 (n-3)		42.9		
C24:1	0.1			
Others	13.4	8.8	0.7	0.2
Natural tocopherols (μg/g oil)				
α-tocopherol	17.8			40.0
β-tocopherol				
γ-tocopherol				146.2
δ-tocopherol				0.8
Peroxide value (meq/kg)	0.1			0.1
Anisidine value	0.2	0.01	0.01	0.1
Free fatty acids (% wt/wt)	0.1	0.01	0.01	0.01

Table 1.
Chemical composition, peroxide value and anisidine value of goat milk (GM), chromatographically purified algae oil (PAO), chromatographically purified Hibiscus mutabilis seed oil (PHMO), and natural Hibiscus mutabilis seed oil (NHMO).

13-nonadecadienoate (Nu-Chek-Prep U-58M, Elysian, MN, USA) was used as an internal standard. The concentrations of tocopherols in the raw goat milk and the chromatographically purified algae oil were determined by reversed-phase high-pressure liquid chromatography (HPLC) [25], and the results were expressed as μg/g of oil. The content of free fatty acids in the raw goat milk and the chromatographically purified algae oil was determined by AOAC method [26]. The fatty acid composition, the concentrations of tocopherols, the peroxide value (PV), the anisidine value (AV), and the content of free fatty acids in the raw goat milk and chromatographically purified algae oil samples are presented in **Table 1**.

The lipid-soluble antioxidant, ascorbyl palmitate, was purchased from DSM Nutritional Products, Inc. (Parsippany, NJ, USA). All reagents used were of analytical grade, ACS certified or HPLC grade, from Sigma-Aldrich (St. Louis, MS, USA). Deionized water was prepared by passing distilled water over a mixed bed of cation-anion exchanger and was used throughout this study.

2.2 Preparation of *Hibiscus mutabilis* seed samples

Fresh harvested seeds of *Hibiscus mutabilis* (**Figure 1**) were obtained from The Village Botanica, Inc. (Waller, TX, USA). The seeds were immediately frozen with liquid nitrogen until analysis. The frozen seeds were thawed, dried by air blower and then milled using a blender. The ground samples that passed through a 35-mesh sieve were used for oil extraction.

2.3 Oil extraction of *Hibiscus mutabilis* seeds

The ground fractions of *Hibiscus mutabilis* seeds were placed in a filter paper (Whatman No. 42) and introduced in a cartridge and they were extracted in a Soxhlet extractor (Southern Labware, Inc., Cumming, GA, USA) using hexane at 65–70°C during approximately 5 h, the time necessary to extract most of the oil from the seeds. The solvent was then evaporated by a vacuum dryer (Columbia International Tech, Irmo, SC, USA), and the oil yield was 9.0 g from 100 g of seeds. The extracted *Hibiscus mutabilis* seed oil was transferred into glass tubes, centrifuged at 12,000× g for 30 min at room temperature, and then stored at 4°C in the dark until analyses. This oil is referred to as the natural *Hibiscus mutabilis* seed oil (**Table 1**). The natural *Hibiscus mutabilis* seed oil was subjected to chromatography to remove naturally occurring antioxidants such as tocopherols and carotenoids [22]. Thus, this

Figure 1.
Image of seeds of Hibiscus mutabilis.

Hibiscus mutabilis seed oil is void of antioxidants and peroxides. The percent fatty acid composition of the chromatographically purified *Hibiscus mutabilis* seed oil and the natural *Hibiscus mutabilis* seed oil were determined according to procedures described [23] by GC–MS. The fatty acid composition of the chromatographically purified *Hibiscus mutabilis* seed oil was similar to the natural *Hibiscus mutabilis* seed oil (**Table 1**). The content of free fatty acids in the chromatographically purified *Hibiscus mutabilis* seed oil and the natural *Hibiscus mutabilis* seed oil was determined by AOAC method [26]. The concentrations of tocopherols, the PV, the AV, and the concentrations of free fatty acids of the natural and the chromatographically purified *Hibiscus mutabilis* seed oil samples are presented in **Table 1**.

2.4 Preparation of emulsions

Three liters of raw goat milk was pasteurized by heating at 72°C and holding milk at this temperature for 15 s. Chromatographically purified algae oil (0.25 wt%), chromatographically purified *Hibiscus mutabilis* seed oil (0.25 wt%) or natural *Hibiscus mutabilis* seed oil (0.25 wt%) with and without ascorbyl palmitate (200 μg/g of oil) were added to goat milk. Goat milk samples were then cooled to 50°C and immediately homogenized at 22.5 MPa (3263.35 psi) through a high-pressure TC5 homogenizer (Stansted Fluid Power, Harlow, UK). The goat milk emulsion samples were transferred to sterile 100-ml Pyrex dark brown glass bottles, which were flushed with nitrogen and then stored at 2°C in the dark for 14 days. The goat milk emulsions, with added oils, were labeled as follows: PAO = chromatographically purified algae oil, PHMO = chromatographically purified *Hibiscus mutabilis* seed oil, NHMO = natural *Hibiscus mutabilis* seed oil, and LAAP = lipid-soluble antioxidant ascorbyl palmitate.

2.5 Droplet size measurement

The particle size of the oil droplets in the goat milk emulsions was measured at day 1 and day 14 at 21 ± 1°C with a SALD-2101 laser diffraction particle size analyzer (Shimadzu Corporation, Columbia, MD, USA). The emulsion samples were diluted 100 times with double deionized water before they were transferred into the chamber of the instrument. Particle size measurements in μm were carried out in triplicate.

2.6 Measurement of peroxide value

Lipids from the DHA-enriched goat milk emulsions were extracted by chloroform:methanol (1:1 wt/wt), using a small volume of solvent [27, 28]. The PV was measured directly on the oils or fats extracted from the DHA-enriched goat milk emulsions by colorimetric determination of iron thiocyanate [29]. This method measures primary oxidation products of oils or fats i.e., hydroperoxides of oils and fats. The mean measurements in meq/kg of three replicates were reported.

2.7 Measurement of p-anisidine value

The para (*p*)-anisidine value was determined in the DHA-enriched goat milk emulsions by AOAC method [30]. This method determines the amount of aldehydes (principally 2-alkenals and 2,4-dienals) present in the emulsion samples. The mean measurements of three replicates were reported.

2.8 Statistical analysis

The results of triplicate analyses were expressed as means ± standard deviations. The data were analyzed by ANOVA using PRO GLM procedure of SAS (version 8.2, SAS Institute, Cary, NC, USA). The least significant difference test was used to determine significant differences among treatment means at $p < 0.05$.

3. Results and discussion

The rate and extent of oxidation of marine oils i.e., algae oil, fish oil depends on the matrix of the food to be fortified. Milk relative to some other foods offers good protection against oxidation, since these marine oils are emulsified and stabilized by the casein micellar structure [18]. Casein adsorbs to the newly formed interface thereby, providing enhanced protection by forming a physical barrier [31, 32]. Although milk is stored in refrigerators (2–4°C) and has a relatively short life (21 days), it is still subject to overall stress due to UV and visible light, temperature fluctuations, and handling abuse.

Lipid oxidation proceeds from the interface to the oil droplet interior in oil-in-water emulsions i.e., goat milk emulsion, therefore, the susceptibility of lipids to oxidation at the interface is the most important factor affecting the oxidative stability of lipids in food and beverage emulsions. It is generally accepted that the attack of free radicals and trace metals on lipids at the interface increases with the increase in the area of interface. Thus, the oxidative stability of DHA in goat milk emulsions should decrease with decreasing droplet sizes. The results of the droplet size determinations (**Table 2**) showed that the droplets did not change in size from day 1 to day 14 of storage at 2°C, indicating that the goat milk emulsions were physically stable during the 2 weeks of storage. The average droplet size in all goat milk emulsions containing 0.5% oil was from 1.20 ± 0.01 to 1.25 ± 0.03 μm, while the droplet size in the original goat milk sample was 0.89 ± 0.02 μm. These results showed that the sizes of droplets in goat milk emulsions containing 0.5% added oil, irrespective of the oil type, were significantly ($p < 0.05$) larger than the droplets in the original goat milk sample. The decrease in the oil droplet size induces the increase in the droplet interface [33], from which the oxidation proceeds to the oil droplet interior.

Goat milk emulsion[3]	Diameter (μm)[1,2]	
	Storage day 1	Storage day 14
PAO + PHMO	1.25 ± 0.03[a]	1.23 ± 0.01[a]
PAO + NHMO	1.21 ± 0.01[a]	1.22 ± 0.03[a]
PAO + NHMO + LAAP	1.20 ± 0.01[a]	1.23 ± 0.03[a]
Goat milk (no oil)	0.89 ± 0.02[b]	0.86 ± 0.01[b]

[1]Mean ± standard deviation.

[2]Means with different letters (a or b) are significantly different ($p < 0.05$).

[3]PAO = chromatographically purified algae oil, PHMO = chromatographically purified *Hibiscus mutabilitis* seed oil, NHMO = natural *Hibiscus mutabilitis* seed oil, and LAAP = lipid-soluble antioxidant ascorbyl palmitate.

Table 2.
Droplet sizes of goat milk emulsions prepared by mixing chromatographically purified algae oil (0.25 wt%), chromatographically purified Hibiscus mutabilis seed oil (0.25 wt%) or natural Hibiscus mutabilis seed oil (0.25 wt%) with and without added ascorbyl palmitate (200 μg/g of oil) during 14-day storage at 2°C.

The results of **Table 2** suggest that DHA in goat milk emulsions containing 0.5% added oil is expected to be less oxidized in the droplet interior.

When supplementing foods with algae oil or fish oil, it is important to consider the initial quality of the raw material. In this work, we have ensured that all oils tested had similar variables such as age and storage temperature. The results of **Table 1** showed that all oils had low initial values of PV, and very low content of free fatty acids. Rancid flavors in goat milk is usually associated to the release of short-chain free fatty acids and is a persistent problem in dairy goat farms due to mishandling procedures starting from the farm until it reaches the consumers. The content of free fatty acids in the goat milk used in this study was very low (0.1%) and likewise, the PV content of goat milk was also low (0.1 meq/kg) suggesting that the quality of goat milk was acceptable from the sensory perspectives.

As to the goat milk emulsions, the chromatographically purified *Hibiscus mutabilis* seed oil (PHMO) together with the chromatographically purified algae oil (PAO) had a significantly ($p < 0.05$) higher PV than the other two goat milk emulsions at each storage time at 2°C (**Figure 2**). On the other hand, the goat milk emulsion with the natural *Hibiscus mutabilis* seed oil (NHMO) and the chromatographically purified algae oil (PAO) exhibited good oxidative stability as inferred from a low PV (**Figure 2**). The goat milk emulsion containing the chromatographically purified algae oil (PAO) and the natural *Hibiscus mutabilis* seed oil (NHMO) with added ascorbyl palmitate (LAAP) had the lowest PV during the study (**Figure 2**). These results suggest that the presence of antioxidants i.e., γ-tocopherol, ascorbyl palmitate improved the oxidative stability of goat milk emulsions under storage at 2°C for 14 days, which may contribute to the shelf life of goat milk emulsions.

The key challenge in formulating food products with marine oils is their sensitivity to iron and copper, catalysts to oxidation that exists in even the cleanest water, foods, and other ingredients. The goat milk used in this study, contained approximately 138 ppb iron and 27 ppb copper, and these values were not affected by the addition of oils to the goat milk. The presence of trace metals in goat milk

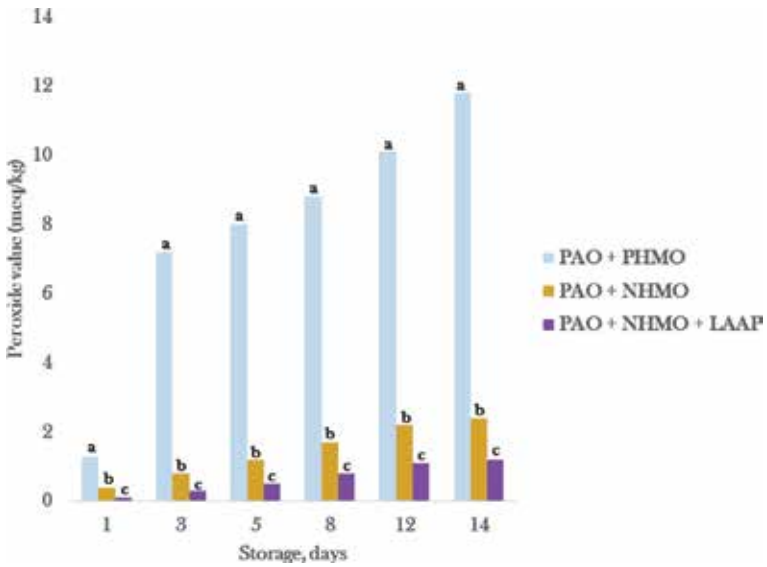

Figure 2.
Peroxide values of goat milk emulsions containing the different oils with and without added ascorbyl palmitate during 14-day storage at 2°C. Means (n = 3) within each storage day with different letters (a–c) are significantly different ($p < 0.05$).

is expected to accelerate the degradation of lipid hydroperoxides as well as the degradation of the secondary oxidation products into shorter chain volatiles.

The results of **Figure 3** showed that the goat milk emulsions containing a mixture (1:1) of the chromatographically purified algae oil (PAO) and the chromatographically purified *Hibiscus mutabilis* seed oil (PHMO) were more oxidized than the goat milk emulsions containing a mixture (1:1) of the chromatographically purified algae oil (PAO) and the natural *Hibiscus mutabilis* seed oil (NHMO). The protective effect of the natural *Hibiscus mutabilis* seed oil (NHMO) may be partially ascribed to the high content of tocopherols, especially γ-tocopherol. As pointed out earlier, the tocopherols are free-radical terminators, which donate a hydrogen to the peroxyl radical [12]. Goat milk contains citric acid [34], and citric acid is recognized as a metal chelator. The chelating properties of citric acid have been proposed to protect tocopherols during oxidation [35]. Therefore, citric acid in goat milk could enhance the antioxidant activity of tocopherols in the emulsions containing the natural *Hibiscus mutabilis* seed oil (NHMO) and the chromatographically purified algae oil (PAO) at 1:1 ratio (**Figure 3**).

Ascorbyl palmitate (LAAP), which was added (200 μg/g of oil) to the natural *Hibiscus mutabilis* seed oil (NHMO) and the chromatographically purified algae oil (PAO) at 1:1 ratio, significantly reduced ($p < 0.05$) the extent of oxidation in this goat milk emulsion at 7-day and 14-day storage at 2°C (**Figure 3**). This protective effect of added ascorbyl palmitate (LAAP) was not observed in goat milk emulsions containing the chromatographically purified *Hibiscus mutabilis* seed oil (PHMO) and the chromatographically purified algae oil (PAO) at 1:1 ratio during 14-day storage at 2°C (data not shown). Ascorbyl palmitate (LAAP) had a more pronounced protective effect on the goat milk emulsion prepared with the chromatographically purified algae oil (PAO) and the natural *Hibiscus mutabilis* seed oil (NHMO) at 1:1 ratio by working synergistically with the γ-tocopherol isomer at 7-day and 14-day storage at 2°C (**Figure 3**). It is likely that ascorbyl palmitate retarded oxidation during storage of oil-in-water emulsions by direct scavenging of free radicals and tocopherol regeneration [18].

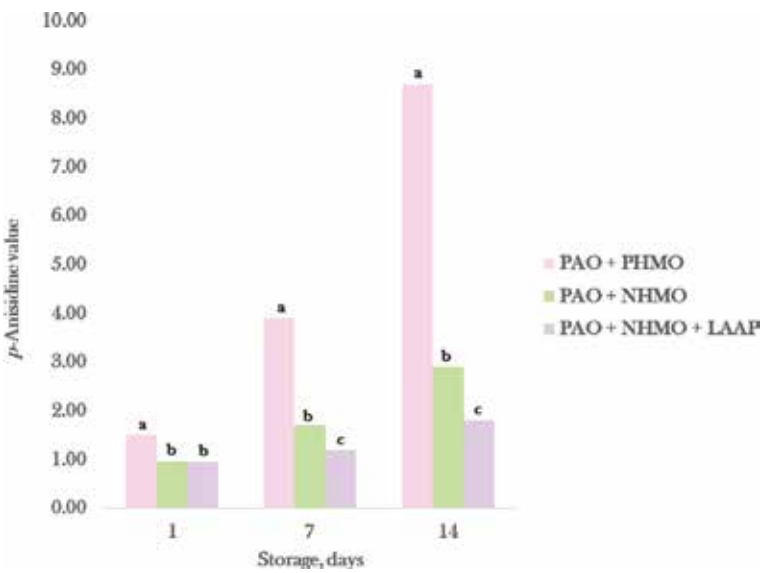

Figure 3.
p-Anisidine values of goat milk emulsions containing the different oils with and without added ascorbyl palmitate during 14-day storage at 2°C. Means (n = 3) within each storage day with different letters (a–c) are significantly different ($p < 0.05$).

There is a direct relationship between the level of oxidation and sensory deterioration and even at times when there are no detectable oxidation parameters, the taste of the finished products could be displeasing. The decomposition of lipid hydroperoxides from marine derived *n*-3 polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA, C22:6*n*-3) and eicosapentaenoic acid (EPA, C20:5*n*-3), produces undesirable rancid and fishy off-flavors. Future work will evaluate the sensory attributes of DHA-enriched goat milk emulsion stabilized by the natural *Hibiscus mutabilis* seed oil and ascorbyl palmitate.

4. Conclusions

This work showed the suitability of using *Hibiscus mutabilis* seed oil to protect marine-derived *n*-3 PUFAs in oil-in-water emulsions i.e., DHA-milk from oxidative degradation for 14 days at 2°C. The natural *Hibiscus mutabilis* seed oil efficiently protected the chromatographically purified algae oil from oxidation during emulsification and storage of DHA-enriched goat milk emulsion. The addition of ascorbyl palmitate to the natural *Hibiscus mutabilis* seed oil and the chromatographically purified algae oil prior to goat milk emulsification had a significant ($p < 0.05$) protective effect on DHA-enriched goat milk emulsion. The combination of differences in fatty acid composition and concentration of tocopherols for the natural *Hibiscus mutabilis* seed oil seems to affect the oxidative stability of the goat milk emulsions prepared with this oil. This study provides a useful precedent for understanding the antioxidant activity of *Hibiscus* seed oils in food and beverage emulsions containing marine *n*-3 PUFAs.

Complementary work is currently being performed in our laboratory to optimize the oxidative stability of DHA-enriched goat milk emulsions with added seed oils from *Hibiscus* species such as *Hibiscus moscheutos* and *Hibiscus dasycalyx* to be able to withstand the thermal and mechanical stresses of industrial processes.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author details

Adela Mora-Gutierrez*, Rahmat Attaie and Maryuri Núñez de González
Cooperative Agricultural Research Center, Prairie View A&M University,
Prairie View, Texas, USA

*Address all correspondence to: admora@pvamu.edu

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Fatty acids are considered as a very important category of chemical compounds to human health as well as from an industrial perspective. This book intends to provide an update on fatty acid research, their methods of detection, quantification, and related diseases such as cardiovascular disease and diabetes. Cyclic fatty acids are also covered, along with short chain fatty acids, which are important to the human gut microbiota. Fatty acids are important in the chemical structure of the cell membrane and its pivotal role in this aspect is reviewed herein. The book also contains a chapter that deals with some unpublished molecular aspects concerning the roles of fatty acids in depression and bipolar disorder. All in all, the book provides a brief overview of both highly explored as well as overlooked perspectives of fatty acids, while highlighting its significance as a biochemical molecule, which is imperative to the livelihood of unicellular and multi-cellular organisms alike.

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