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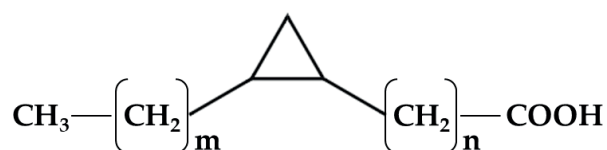
n,m	Common name
9,5	lactobacillic acid
6,7	dihydromalvalic acid
7,7	dihydrosterculic acid

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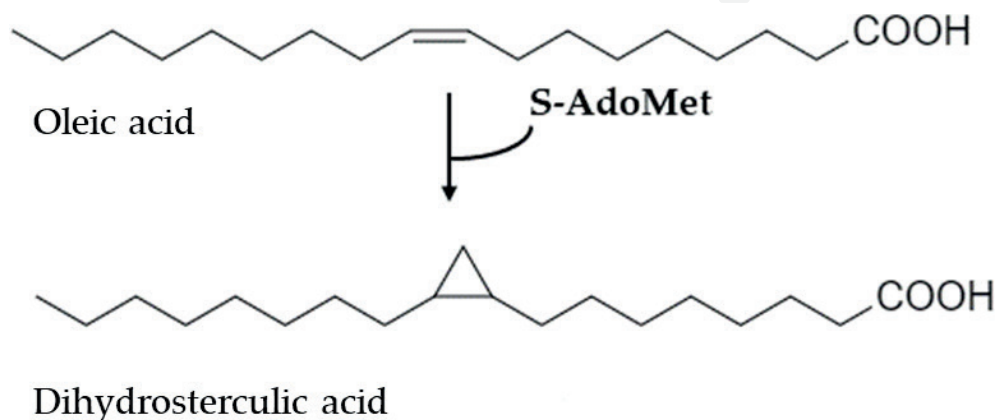
Figure 2.
Biosynthesis of dihydrosterculic acid from oleic acid by CPFA synthase.

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.
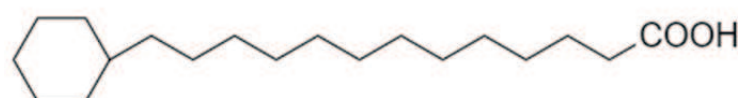
2.2 Omega-cyclohexyl fatty acids

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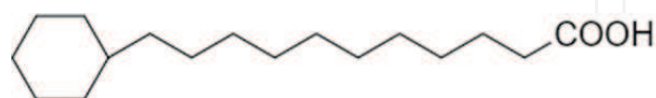
Figure 3.
Chemical structure of 11-cyclohexylundecanoic and 13-cyclohexytridecanoic acids.

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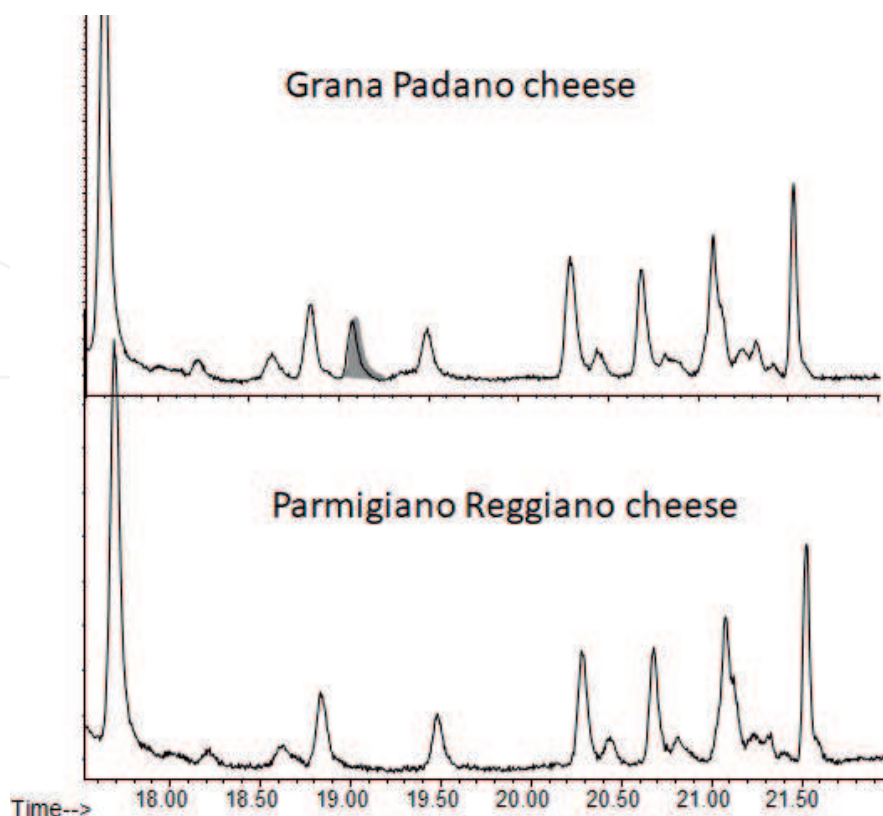
Figure 4.
Enlarged view of CPFA peak elution zone and comparison between a sample of Grana Padano cheese positive to CPFA and a sample of Parmigiano-Reggiano cheese negative to CPFA [43].

quantitative 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.
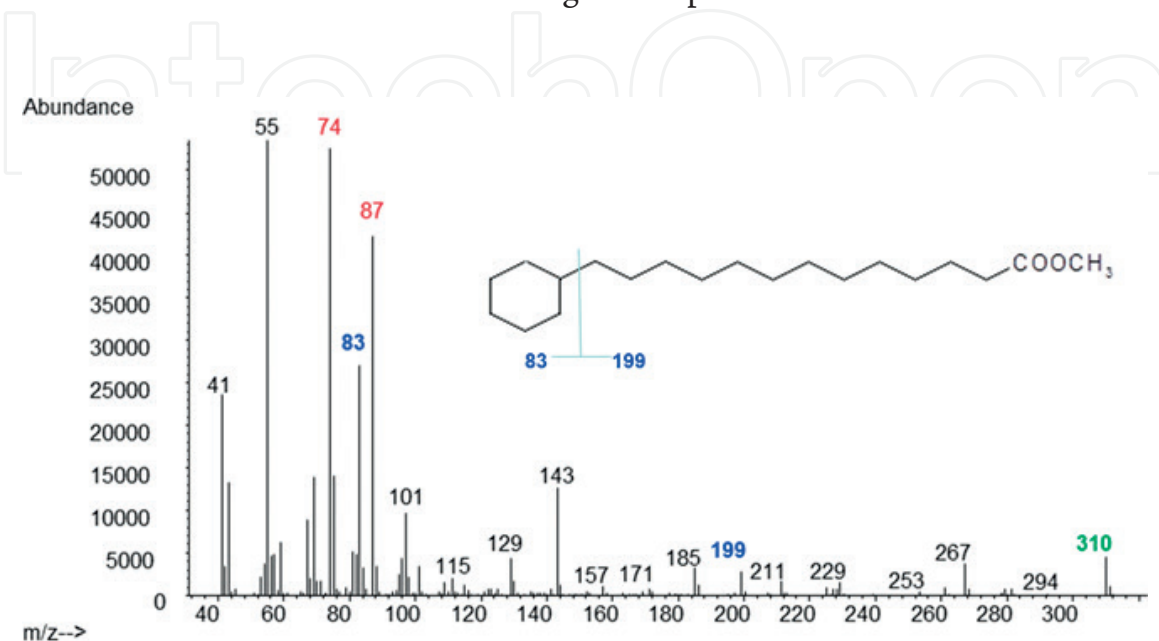
13-cyclohexyl tridecanoic fatty acid methyl ester was detectable in the GC-MS profile as it elutes just before the eicosanoic acid (arachidic acid) and after eicosenoic acid. However, due to the presence of interfering signals, the identification of 13-cyclohexyl tridecanoic was confirmed by the mass 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.

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