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Review article

Functional dairy products enriched with omega-3 fatty acids

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Abstract

A critical review was conducted on topics related to functional foods, omega-3 fatty acids, sources of omega-3 fatty acids, and types of dairy products fortified with omega-3 fatty acids. Functional foods are products enriched with special ingredients that give the food beneficial and healthy effects. Inadequate intake of functional foods is directly related to modern diseases, such as atherosclerosis, Alzheimer's, diabete type 2, cardiovascular and autoimmune diseases. Omega-3 and omega-6 are polyunsaturated fatty acids. They have an important biological role for the human body. High omega-6/omega-3 ratios and high amounts of omega-6 lead to a variety of cardiovascular, cancer, inflammatory and autoimmune diseases, while high levels of omega-3 suppress the development of these diseases. Sources of omega-3 fatty acids can be from plants, fish and algae. Dairy products can be enriched with omega-3 fatty acids by modifying the animal's diet or by directly adding vegetable or fish oils. Fresh and mature dairy products enriched with plant and animal sources of omega-3 fatty acids are found in the literature. From the literature review, it was found that there are not enough studies related to the enrichment of Bulgarian dairy products with omega-3 fatty acids.

Keywords

yoghurt, cheese, monounsaturated fatty acids, polyunsaturated fatty acids, fish oil, vegetable oils

Abbreviations

AA – archidonic acid; ALA – alpha-linolenic acid; DHA – docosahexaenoic acid; EFSA – European Food Safety Authority; EPA – eicosapentaenoic acid; LA – linolenic acid; MUFA – monounsaturated fatty acids; NA – not available; PUFA – polyunsaturated fatty acids

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Introduction

Functional food. The term "functional food" was first used in 1984 in Japan. These are food products enriched with special ingredients that give the food beneficial and healthy effects. In view of this, the relationship between nutrition, the pleasure of nutrition and strengthening of human physiological systems is investigated (Hardy 2000; Kwak and Jukes 2001). Fortified foods are foods to which one or more essential nutrients have been added. Their concentration should be higher than the traditional one. Fortified foods aim to reduce the deficiencies of certain substances in a certain group of people or of the whole society (Bonner et al. 1999).

There are different classifications for functional foods.

Makinen-Akula offers the following classification (Makinen-Aakula 2006):

- Functional foods that improve quality of life, such as probiotics and prebiotics;
- Functional foods that reduce an existing health risk, such as high cholesterol or high blood pressure;
- Functional foods that facilitate digestion, such as low-lactose or lactose-free products, gluten-free products.

Kotilainen et al. (2006), Sloan (2000) and Spence (2006) give the following classification for fortified foods:

- Foods fortified with additional substances, such as fruit juices with vitamin C, vitamin E, folic acid, zinc and calcium;
- Foods additionally enriched with components or nutrients not present in food, such as probiotics and prebiotics;
- Foods from which certain components have been removed, reduced or replaced by others with a proven positive effect on human health;
- Foods enriched with natural ingredients, for example a dairy product with omega-3 fatty acids.

Omega-3 fatty acids. From a chemical point of view, fatty acids are saturated and unsaturated. The classification of fatty acids is presented in Fig. 1.

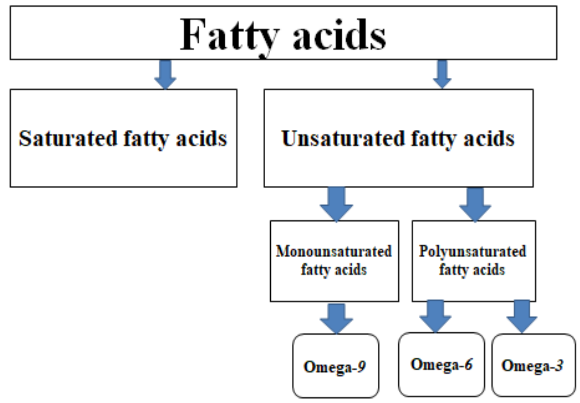

Figure 1. Classification of fatty acids

Saturated fatty acids don't contain a double bond in their hydrocarbon chain, for example (stearic acid C18:0). Unsaturated fatty acids are divided into ω-9 monounsaturated (with one double bond in the hydrocarbon chain) and polyunsaturated fatty acids (with more than one double bond in the hydrocarbon chain, ω-6, ω-3) (Fig. 2).

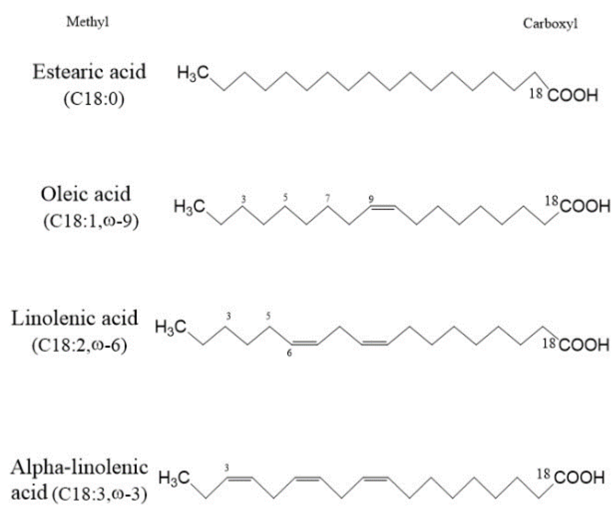

Figure 2. Chemical structures of saturated and unsaturated fatty acids

Monounsaturated fatty acids (ω-9) contain one double bond, and the 9 after ω indicates the position of the double bond in the hydrocarbon chain. Counting starts from the methylene end as shown in Fig. 2.

Polyunsaturated fatty acids are divided into two groups, omega-6 (ω-6) and omega-3 (ω-3). The difference between them is in the placement of the double bond in the hydrocarbon chain. In ω-6 the double bond is located at 6-th carbon atom, counting from the methylene end, while in ω-3 the double bond is located at 3-th carbon atom, (Fig. 2).

Different foods have different ratios of saturated and unsaturated fatty acids. Foods with a high content of saturated fatty acids are fats mainly of animal origin, such as cream, cheese, butter, etc. Food rich in unsaturated fatty acids are mainly vegetable oils, as well as fish oils and oils from algae. These peculiarities in their chemical structure lead to differences in their health effects on the consumer (Joordens et al. 2014). The human body produces monounsaturated (omega-9) fatty acids using saturated fatty acids. Omega-9 fatty acids play a role in carrying out various physiological processes in the body (Motulsky et al. 1989). Main sources of omega 9 fatty acids are almonds, walnuts, avocados and olive oil (Peou et al. 2016; Wang et al. 2015). Monounsaturated fatty acids increase HDL (good) cholesterol levels in the blood (Gogus and Smith 2010). Omega-6 and omega-3 fatty acids have various health effects on the human body - they play an important role in the body's biological and inflammatory processes, as well as those responsible for blood clotting. A team of scientists has found that omega-6 fatty acids incite inflammatory processes in the body, while omega-3 fatty acids have anti-inflammatory functions (Calder 2006). An increase in omega-6 fatty acids in the Western diet and a decrease in omega-3 fatty acids is associated with an increase in chronic diseases (Kaliannan et al. 2019). High omega-6/ omega-3 ratios and high amounts of omega-6 lead to various diseases, including cardiovascular diseases (Simopoulos 2008), cancer (Wang et al. 2017), inflammatory and autoimmune diseases (Simopoulos 2016; Kaliannan et al. 2019), while high levels of omega-3 suppress the development of these diseases. The recommended ratio of omega-6 to omega-3 is 1:1 to 4:1, which reduces the risk of chronic disease (Simopoulos 2016; Kaliannan et al. 2019). According Kolanowski and Laufenberg (2006), the daily intake of omega-6:omega-3 fatty acids should be in a ratio of up to 4:1 (Kolanowski and Laufenberg 2006). According to Simopoulos (2016), the optimal omega-6:omega-3 ratio ranges from 1:1 to 4:1, and it can vary depending on the individual's co-morbidities. (Simopoulos 2008). In a previous study, de Lorgeril et al. found that an omega-6: omega-3 ratio of 4:1 was associated with a 70% reduction in total mortality in the prevention of secondary coronary heart disease (De Lorgeril et al. 1994). Inflammation in rheumatoid arthritis patients may be reduced with an omega-6:omega-3 ratio of 2:1-3:1 (James and Cleland 1997). Radio omega-6: omega-3 5:1 helps relieve asthma symptoms in a subset of the asthmatic population. An omega-6:omega-3 ratio of 10:1 is considered unhealthy (Broughton et al. 1997).

From a nutritional point of view, omega-3s are unsaturated essential fatty acids that cannot be synthesized in the human body because of the lack of the desaturase enzyme which is responsible for incorporating double bonds at the omega-3 position (West et al. 2003). There are three omega-3 fatty acids (Fig. 3) that are involved in human biochemical processes.

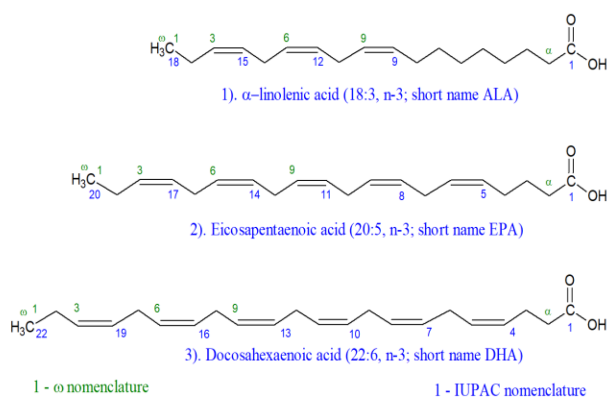

Figure 3. Chemical structures of α-linolenic acid, eicosapentaenoic acid, decoxaheptaenoic acid

These are α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA is the shortest chain of the omega-3 fatty acids and it is mainly found in vegetable oils and nuts. The other two important fatty acids EPA and DHA are found in fish and microalgae (Chew et al. 2008; Kalogeropoulos et al. 2010; Guedesb et al. 2011; Ward and Singh 2005). ALA can be transformed in the human body to EPA and DHA, but conversion is very limited and inefficient (Kalogeropoulos et al. 2010). Therefore, omega-3 should be taken in addition, as a dietary supplement or in the form of foods rich in omega-3 fatty acids. Humans are highly dependent on an omega diet to be healthy and maintain good function and growth. Regular intake of docosahexaenoic and eicosapentaenoic acids reduces the risk of type 2 diabetes, Alzheimer's, bipolar disorder, heart disease and schizophrenia (Patel et al. 2021a). The human body needs periodic intake of eicosapentaenoic, decoxahexaenoic, α-linolenic acid to stay healthy.

Globally, the intake of omega-3 fatty acids is not sufficient (Sioen et al. 2009). According the Bulgarian legislation on the physiological norms for feeding the population, the recommended daily intake is as follows:

- the adequate dietary intake of alpha-linolenic acid (an essential omega-3 polyunsaturated fatty acid) for all age groups is 0.5% of the energy value of food;
- the adequate intake of omega-3 long-chain polyunsaturated fatty acids (eicosapentaenoic acid plus docosahexaenoic acid) for children over 2 years of age and adults is 250 $mg.d^{-1}$; during pregnancy and breastfeeding, an additional intake of 100-200 mg docosahexaenoic $acid.d^{-1}$ is required.

According EFSA panel from 2012 opinion on acceptable upper intake limits for eicosapentaenoic, docosahexaenoic and docosahexaenoic acids, the additional intakes of these acids do not raise public safety concerns. As a result of this opinion, in Regulation 1925/2006 there are no restrictions on the intake of omega-3 fatty acids

Sources of omega-3 fatty acids

Oils from fish origin. Fish are the main source of omega-3 fatty acids (eicosapentaenoic EPA and docosahexaenoic DHA (Fig. 4). Their fatty acid composition is one of the key factors in evaluating the nutritional value of fish and can vary according to species, habitat, diet, etc. Fish can be both marine and freshwater. Barbel fillets have the highest amount of monounsaturated fatty acids (MUFA) (18.51%), while river mullet fillets have the highest amount of polyunsaturated fatty acids (PUFA) (1.87%). Rayfin fillets have the most omega-6 (1.1%), while river mullet fillets have the most omega-3 (1.87%) (Gaffari and Khoshnood 2021), salmon (EPA-0.8%, DHA-1.6%, polyunsaturated fatty acids-12.4%), pussian sturgeon (EPA-11.3%, DHA-0.7%, polyunsaturated fatty acids-46.8%) (Kyosev and Dragoev 2008). The results in the study conducted by Gaffari and Khoshnood show that the studied freshwater fish are a good source of omega-3 fatty acids and can be considered as a valuable food source (Gaffari and Khoshnood 2021).

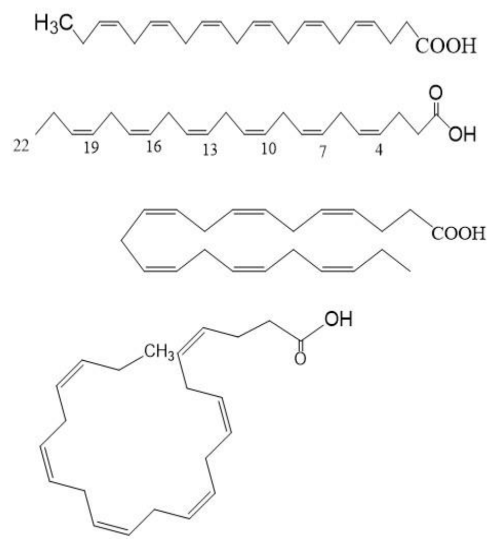

Figure 4. Various images of the structure of DHA

However, marine fish such as salmon and pussian sturgeon remain one of the best and most affordable sources of omega-3 fatty acids. Most fish oils are derived from marine fish. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most important polyunsaturated fatty acids known for their health benefits and positive effects, such as reducing cardiovascular disease, preventing some tumor diseases and some inflammatory conditions of the immune system (Gowda et al. 2018; Munoz-Tebar et al. 2019; Goksen et al. 2020). DHA plays a vital role in the structure and function of the brain and eyes, and is suitable for intake during pregnancy and early childhood, and is also essential to ensure optimal development (Calder 2016). EPA has a unique chemical structure and important biological effects. By replacing the omega-6 fatty acid arachidonic acid (AA; 20:4, omega-6) in the membrane of the phospholipids, EPA can alter the physical properties of cell membranes. It also has an anti-inflammatory and antithrombotic effect, contrary omega-6 fatty acid, which has a proinflammatory and prothrombotic effect (Adkins and Kelley 2010; Jump et al. 2012; Larsson et al. 2004).

Table 1 describes the EPA and DHA content of fish oils and other omega-3 sources. It can be seen that the highest content of EPA and DHA is found in tuna oil and 60% tuna oil concentrate. Enriching various foods with fish oil is an opportunity to increase fish oil consumption. However, the biggest technological barrier to effectively incorporating amounts of EPA and DHA into food is the resulting oxidation and fishy flavor due to lipid breakdown (Feizollahi et al. 2018; Gumus and Gharibzahedi 2021).

Table 1. EPA and DHA content of fish oil and other n-3 sources. Adapted from Calder (2016) Copyright 2016, S. Karger AG

Type of oil	EPA, $g.100g^{-1}$	DHA, $g.100g^{-1}$	EPA + DHA, $g.100g^{-1}$
Cod liver oil	11	9.0	20
Krill oil	14	6.5	20
Tuna oil	11	35	46
Algae oil used in infant formula	0	> 40	> 40
60% fish oil concentrate from tuna	36	24	60

Fish oils rich in omega-3 fatty acids are characterized by a specific taste and aroma, which they also impart to enriched products, which limits their use (Piombo et al. 2006; Botelho et al. 2013; Waraich et al. 2013; Ganesan et al. 2014). Using highly refined and odorless fish oil in the production of foods enriched with omega-3 fatty acids or encapsulated fish oil in the production of foods enriched with omega-3 fatty acids may be an alternative option to mask the undesirable sensory characteristics and thus to protect the oil during the production process (Iafelice et al. 2008).

The organoleptic characteristic of the product depends very much on the amount of oil added, as well as on the type of fish used (Gibney 1997; Kolanowski et al. 1999; Roche and Gibney 1994). However, due to the high sensitivity of fish oil to oxidation, fortified food products must be manufactured, packaged, stored and delivered in conditions that eliminate all factors leading to oxidation of omega-3 fatty acids and in particular the presence of oxygen, UV light, increased temperature and humidity, as well as the content of metal ions in the product, mainly iron, copper and manganese (Jacobsen 1999).

From a technological point of view, an opportunity to overcome the disadvantages described above, such as oxidation, atypical aroma and taste, when adding fish oils to omega-3 enriched foods, is the oil encapsulation technology. It is possible to add natural antioxidants such as rosemary (Siejak 2021), chamomile (Avula et al. 2014; Guimarães et al. 2013; Lin and Harnly 2012; Matić et al. 2013; Mulinacci et al 2000; Nováková et al. 2010; Raal et al. 2012; Roby et al. 2013; Xie et al. 2014; Zielinski et al. 2014), beetroot and ginger extract (Srivastava et al. 2015). Another disadvantage can be the spreadable consistency of the products, which occurs as a result of the lower melting point of fish oils compared to the melting point of other animal fats.

Oils from plant origin. Vegetable oils do not impart off-flavors and aromas when added to dairy products, unlike fish oils. Given this, a number of researchers are searching ways to enrich foods with omega-3 fatty acids through vegetable oils. Many plants are a rich source of omega-3 fatty acids - flaxseed, soy, kiwi, raspberries, canola, camelina, walnut (Piombo et al. 2006; Botelho et al. 2013; Waraich et al. 2013; Ganesan et al. 2014). Dal Bello B. and his team enrich yogurt with omega-3 fatty acids from plant sources, using linseed oil, camelina oil, raspberry oil, *Echium plantagineum* oil and blackcurrant oil. The α-linolenic acid content of the above oils is respectively: linseed oil (71%), camelina oil (36%), raspberry oil (29%), *Echium plantagineum* oil (33%) and blackcurrant oil (14%) (Dal Bello et al. 2015). Linolenic (LA) and α-linolenic acid (ALA) are the biosynthetic precursors of ω-6 and ω-3, respectively, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Jakobsson et al. 2006; Sprecher et al. 1995). The most consumed fatty acids, LA, ALA attract attention for their influence on inflammatory processes. Omega-3 fatty acids from plant sources are of great interest because they are known to be able to metabolized to EPA, DHA, which have anti-inflammatory and immunostimulating effects (Czarnowicki et al. 2017; Harbige 2003). All these healthy fats have physiological benefits for the body. Their consumption contributes to the maintenance of normal levels of triglycerides and blood pressure, reducing the risk of cardiovascular diseases, protecting against certain types of tumors and increasing the beneficial effects on the brain, retina and nervous system. (Arterburn et al. 2007; Harris et al. 2008; Gogus and Smith 2010). Both fish oils and vegetable oils must be protected from oxidation so that the finished product does not acquire after taste and aroma. Antioxidants can be added to protect the product from oxidation. It is possible to add natural antioxidants to food products enriched with vegetable sources of omega-3 fatty acids, such as: rosemary (Siejak 2021), chamomile (Avula et al. 2014; Guimarães et al. 2013; Lin and Harnly 2012; Matić et al. 2013; Mulinacci et al. 2000; Nováková et al. 2010; Raal et al. 2012; Roby et al. 2013; Xie et al. 2014; Zielinski et al. 2014), beetroot and ginger extract (Srivastava et al. 2015) and others.

Types of dairy products with omega-3 fatty acids

In recent years, a number of companies have focused on the production of functional dairy products, including those enriched with omega-3 fatty acids. However, there are a number of challenges in the production of dairy products enriched with omega-3 fatty acids. The possibility of increasing the content of omega-3 fatty acids in milk is the subject of much research (Lai et al. 2020). More and more scientific developments are aimed at regulating animal nutrition in order to increase the content of omega-3 fatty acids in milk (Tzora et al. 2022). A team of scientists suggests feeding the animals with foods enriched with linseed and lupine seeds. In the conducted studies, triple increased values of omega-3 fatty acids in milk were found. Most often, linseed, lupine seeds, rapeseed, etc. are included in animal feed (Tzora et al. 2022). Also, the ratio of omega-6 to omega-3 is approximately 2:1. Although studies have been conducted to enrich animal diets with forages rich in omega-3 fatty acids, no universal diet has been found to achieve optimal amounts and ratios of omega-3 to omega-6 fatty acids. It is necessary to consider the season, the type of feeding of the animals (pasture or manure), the age of the animals, etc. Given this, direct fortification of dairy products with sources of omega-3 fatty acids remains the better option, as feeding animals with omega-3 fatty acids, the only source is of plant origin. It is more appropriate to directly enrich the milk from which dairy products will be produced with omega-3 fatty acids. In this way, a higher amount of omega-3 fatty acids is achieved and their content in the finished product is dosed more precisely.

Dairy products are suitable for fortification with omega-3 fatty acids due to their high frequency of consumption and storage under refrigerated conditions (Kolanowski and Weibbrodt 2007).

Table 2 presents fresh dairy products enriched with omega-3 fatty acids. From the table it can be seen that the sources of omega-3 used are from animal and vegetable origin. One of the studies is the development of yogurt enriched with omega-3 fatty acids, using different plant sources. According to the data provided, it is reported that when linseed, blackcurrant and *Echium plantagineum* oil is added to the milk, the finished product contains the highest amount of omega-3 fatty acids. In the sensory analysis of the products, the yogurt enriched with blackcurrant is characterized by after taste, while the one with flaxseed has the highest overall organoleptic evaluation (Dal Bello et al. 2015).

Another study is directed to a large group of fresh dairy products fortified with omega-3 fatty acids from fish sources. The conclusion of this study is that it is possible to enrich certain dairy products with omega-3 fatty acids by adding fish oil, but in limited quantities. It has been found that for yogurt and cream, fish oil fortification is appropriate to be in amounts of 1 to 5 $g.kg^{-1}$, while for fresh cheese spread, butter and processed cheese, fish oil fortification is appropriate to be from 20 to 60 $g.kg^{-1}$, without affecting the sensory qualities of the product. The highest level of enrichment appears to be possible in the case of hard and high-fat dairy products, such as butter, processed cheese and cheese spread, and especially in cases where additional flavoring has been added.

Fresh dairy products maintain stable sensory qualities during 4-week storage. A serving (about 30 g) of spreadable cheese, butter and processed cheese enriched with fish oil at levels found in the study can provide 180-360 mg of omega-3 fatty acids. This significantly increases the level of average intake of omega-3 in the human diet.

Table 2. Fresh dairy products enriched with omega-3 fatty acids

Product	Source of omega-3 fatty acids	Added oil, g.100 g^{-1}	Omega-3 fatty acids in the final product, g.100 g^{-1}	Source
Natural drinkable yoghurt	Fish oil	0.1-0.5	NA	(Kolanowskiand and Weibbrodt 2007)
Yoghurt, drinkable, flavoured with strawberry	Fish oil	0.1-0.5	NA	(Kolanowskiand and Weibbrodt 2007)
Cream, reduced fat	Fish oil	0.1-0.5	NA	(Kolanowskiand and Weibbrodt 2007)
Cream, full fat	Fish oil	0.1-0.5	NA	(Kolanowskiand and Weibbrodt 2007)
Processed fresh cheese, not flavoured	Fish oil	2-6	NA	(Kolanowskiand and Weibbrodt 2007)
Processed fresh cheese, vanillin flavoured	Fish oil	2-6	NA	(Kolanowskiand and Weibbrodt 2007)
Spreadable fresh cheese, not flavoured	Fish oil	2-6	NA	(Kolanowskiand and Weibbrodt 2007)
Spreadable fresh cheese, with garlic	Fish oil	2-6	NA	(Kolanowskiand and Weibbrodt 2007)
Yoghurt	Flaxseed oil	0.3	0.7	(Dal Bello et al. 2015)
Yoghurt	*Camelina sativa*	0.5	0.2	(Dal Bello et al. 2015)
Yoghurt	Raspberry oil	0.7	0.2	(Dal Bello et al. 2015)
Yoghurt	Black current oil	1.4	0.4	(Dal Bello et al. 2015)
Yoghurt	Echium plantagineum	0.6	0.6	(Dal Bello et al. 2015)
Yoghurt	Flaxseed oil	0.2	NA	(Almasi et al. 2021)
Queso fresco	Flaxseed oil	1	0.2	(Bermúdez-Aguirre and Barbosa-Cánovas 2011)
Queso fresco	Fish oil	1	0.8	(Bermúdez-Aguirre and Barbosa-Cánovas 2011)

NA – not available

Table 3. Mature dairy products enriched with omega-3 fatty acids

Product	**Source of omega-3 fatty acids**	**Added oil, g.100 g^{-1}**	**Omega-3 fatty acids in the final product, g.100 g^{-1}**	**Sources**
Cheddar	Flaxseed oil	1	0.5	(Bermúdez-Aguirre and Barbosa-Cánovas 2011)
Cheddar	Fish oil	1	0.9	(Bermúdez-Aguirre and Barbosa-Cánovas 2011)
Mozzarella	Flaxseed oil	1	0.08	(Bermúdez-Aguirre and Barbosa-Cánovas 2011)
Mozzarella	Fish oil	1	0.3	(Bermúdez-Aguirre and Barbosa-Cánovas 2011)
Processed cheese, not flavoured	Fish oil	2-6	NA	(Kolanowski and Weibbrodt 2007)
Processed cheese with garlic	Fish oil	2-6	NA	(Kolanowski and Weibbrodt 2007)
Butter	Fish oil	2-6	NA	(Kolanowski and Weibbrodt 2007)
Soft cheese (curd), reduced fat	Fish oil	2-6	NA	(Kolanowski and Weibbrodt 2007)
Soft cheese (curd), full fat	Fish oil	2-6	NA	(Kolanowski and Weibbrodt 2007)

NA – not available

For people with a low intake of fish and fish products, a dairy product enriched with fish oil can be a useful and safe source of adequate intake of omega-3 fatty acids (Kolanowskia and WeiXbrodtb 2007).

Table 3 presents mature dairy products enriched with omega-3 fatty acids. In one of the studies, mature dairy products were evaluated physicochemically, microbiologically and sensorially. Three types of cheese enriched with omega-3 fatty acids (from plant and fish sources) Queso fresco, cheddar, mozzarella are evaluated. The results show that in the sensory evaluation, the samples of all three types of cheese enriched with a vegetable source of omega-3 fatty acids were better accepted by the tasters compared to the samples enriched with an animal source (fish oil) of omega-3 fatty acids. The overall study suggests that omega-3 fatty acids are easily incorporated into products such as cheese to ensure adequate intake through the consumer's diet (Bermúdez-Aguirre and Barbosa-Cánovas 2011).

Conclusions

The most recent studies on the fortification of dairy products with omega-3 fatty acids are presented in this review article. Direct fortification of milk through animal feed, although giving good results, is limited because fortification is only with sources of omega-3 fatty acids from plant sources. In addition, the amount of omega-3 fatty acids in milk is more accurate and the same at each production point when the product is directly fortified with omega-3 fatty acids. There is a preponderance of dairy products enriched with omega-3 fatty acids from plant sources, compared to those enriched with omega-3 fatty acids from fish and algae. This is probably due to the fact that using omega-3 fatty acids from a plant source limits the possibility of an unpleasant (fishy) taste in the product. This drawback can be avoided by the method of encapsulating the oils. Some of the undesirable sensory characteristics can be overcome by adding a natural antioxidant to limit the oxidation of the oil. In this way, it is possible to mask the taste and smell of fish. Little research has been done on the possibilities of enriching bulgarian dairy products with omega-3 fatty acids, both from plant and animal sources.

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