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RESEARCH PAPER

Synthesis, Characterization and Physical Properties of Polyunsaturated Fatty Acids and Co Zero-Valent Nanoparticles/ Polyunsaturated Fatty Acids

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ABSTRACT

The aim of this work is to provide a brief introduction of antimicrobial lipids and their current status and challenges, and to present a detailed discussion of ongoing research efforts to develop nanotechnology formulations of fatty acids and monoglycerides that enable superior in vitro and in vivo performance. Examples of nano-emulsions, liposomes, solid lipid nanoparticles, and controlled release hydrogels are presented in order to highlight the potential that lies ahead for fatty acids and monoglycerides as next-generation antibacterial solutions. As a result of the research, the authors have developed a method for optimizing the composition and properties of the fat bases of milk and dairy products by mixing milk fat with non-dairy fats of various fatty acid groups. Studies on the chemical composition and physical and chemical properties of dietary fats have been carried out. The obtained data make it possible to theoretically substantiate the possibility of using animal and vegetable fats in the production of fat-containing dairy products with partial replacement of milk fat with vegetable oils, animal fats, or their mixtures, taking into account the formula for balancing the fatty acid composition. This method is available and convenient for use in production conditions.

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INTRODUCTION

The production of functional products, many of which contain added substances that have the desired physiological or health effect on the body, is actively developing all over the world. The use of polyunsaturated fatty acids (PUFAs) of the omega-3 and omega-6 groups for supplementation of dairy products seems to be the most promising both from a technological point of view and taking into account consumer preferences [1]. One of the key areas of this task is the development and implementation of products balanced in fatty acid

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composition. The ratio of fatty acids is one of the indicators of the biological and, accordingly, the nutritional value of fats. [2]. Saturated fatty acids (SFAs): lauric $C_{12:0}$, myristic $C_{14:0}$, palmitic $C_{16:0}$, and stearic $C_{18:0}$ increase the concentration of harmful (low-density) cholesterol [3]. Attention to PUFAs belonging to the omega-6 and omega-3 groups has increased after establishing their role in cholesterol metabolism and prevention of atherosclerosis [4,5]. The most important essential fatty acid of the omega-6 group is linoleic acid $C_{18:2}$, which is a part of cell membranes, participates in the metabolism and synthesis of prostaglandins, is necessary for cell growth and regeneration (the daily requirement is about 7 g). γ-linolenic acid $C_{18:3}$ omega-6 is formed in the body from linoleic acid; it is also necessary for the synthesis of prostaglandins. Plants contain α-linolenic acid $C_{18:3}$ omega-3, from which two omega-3 acids are synthesized: eicosapentaenoic acid $C_{20:5}$ and docosahexaenoic acid $C_{22:6}$. Both acids are found in the body of fish that live in the cold waters of the oceans and seas [1]. The aim of the present work is synthesis of cobalt nanoparticles and linking the material with the desired fatty acid. The antibacterial activity of the prepared compound is also studied in the present study.

MATERIALS AND METHODS

Separation and identification of fatty acid esters were performed by gas chromatography on a Chronos 1000 chromatograph. Gas chromatic analysis of omega-6 and omega-3 fatty acid concentrate was performed on a gas chromatograph Chronos 1000 with a flame ionization detector after converting them to methyl esters according to the method of the interstate standard (GOST 30418-96) by transesterification of fat using sodium methylate to methanol. The separation of methyl esters was performed on a capillary column with a length of 100 m and an internal diameter of 0.25 mm. The separation was performed in the polar stationary phase with a temperature increase from 60 °C to 180 °C at a rate of 20 °C per minute, the maximum column temperature was 230 °C. The polar stationary phase provides separation of fatty acid methyl esters by the number of carbon atoms and the degree of unsaturation. As a calibration standard mixture, a mixture of ethyl esters of individual fatty acids was used – saturated from $C_{4:0}$ (oleic) to $C_{21:0}$ (heneicosanoic), monounsaturated from $C_{10:1}$ (capric) to $C_{20:1}$ omega-9 (gondoic) and polyunsaturated $C_{18:2}$ omega-6 (linolenic), $C_{18:3}$ omega-6 (γ-linolenic), $C_{18:3}$ omega-3 (α-linolenic), C20:4 omega-6 (arachidonic). The fat of all samples was calculated using the internal normalization method.

Synthesis of cobalt nanoparticles

For the synthesis of Co zerovalent nanoparticles, $CoCl_2$ raw material was dissolved into a minimum amount of distilled water and then the solution mixed with triethanol amine and then aniline solvents. The nanoparticles were formed as colloid. The nanoparticles were characterized by

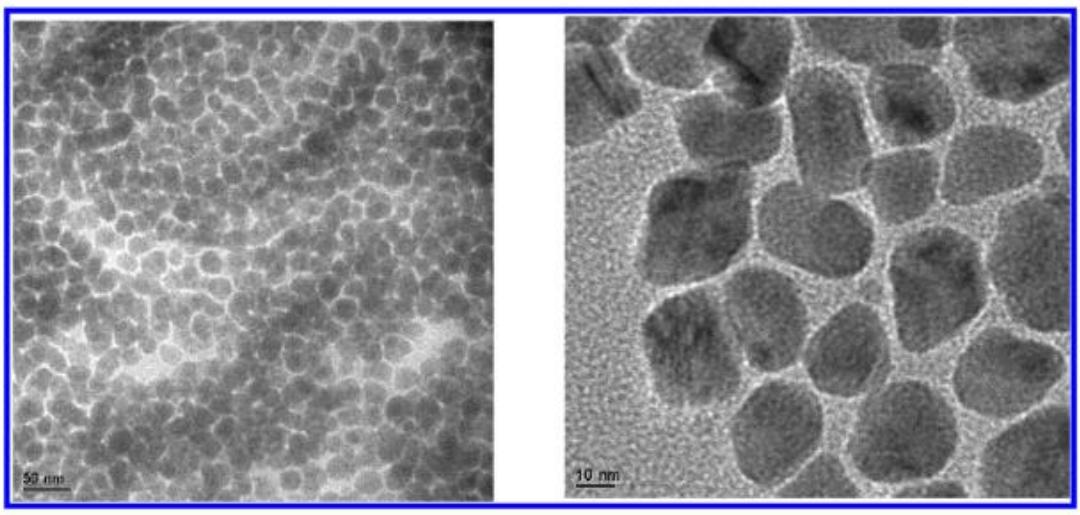

Fig. 1. TEM images of a) Co nanoparticles and b) functionalized fatty acid Co nanoparticles.

TEM images. The synthesized nanoparticles were mixed with the unsaturated fatty acids in a two neck flask at 50 ºC for 1 h.

RESULTS AND DISCUSSION

Fig. 1 shows TEM micro-graphs of cobalt nanoparticles coated with oleic acid. It can be seen that Co nanoparticles assemble very well. Each particle is separated from its neighbors by the organic ligand shell. The particle size is about 15~20 nm. The high-magnification TEM image (Fig. 1b) shows that the functionalized particles are not fully spherical.

DLS measurements were recorded for a time period of 12 months showing no significant changes in the size (inset of Fig. 2), confirming the remarkable stability of the particles in their organic dispersions for months. In summary, it is best to keep the concentrations of OA at the fivefold limit to obtain nearly monodisperse NPs.

To better understand the adsorption mechanism of the oleic acid on the surface of cobalt nanoparticles, Fourier transform infrared (FTIR) measurements were carried out on the pure oleic acid and the composite Co nanoparticles capped with oleic acid, respectively. Fig. 3 represents the typical IR spectrum of the pure oleic acid. The broad feature between 3500 and 2500 cm^{-1} was undoubtedly due to the O-H stretch of the carboxylic acid. No other functional group had such a broad and intense band at high wavenumber. Two sharp bands at 2924 and 2854 cm^{-1}, which were superimposed on the O-H stretch, were attributed to the asymmetric CH_2 stretch and the symmetric CH_2 stretch, respectively. The band at 1285 cm^{-1} exhibited the presence of the C-O stretch.13,14 The O-H in-plane and out-of-plane bands appeared at 1462 and 937 cm^{-1}, respectively.

Antibiotic-resistant bacterial infections caused by invasive medical devices (e.g., endotracheal tubes) represent a major challenge that requires new treatment strategies. In particular, there is demand for designing antibacterial surface coatings that prevent bacterial infections without relying on antibiotic solutions. To address this need, Taylor et al. developed a solid lipid nanoparticle (SLN) formulation that consisted of lauric acid, stearic acid, and oleic acid in the inner core which was surrounded by phosphatidylcholine lipid and sodium taurocholate (Fig. 4).

Fig. 5 illustrates the formation mechanism of the functionalized fatty acid. Upon the addition of

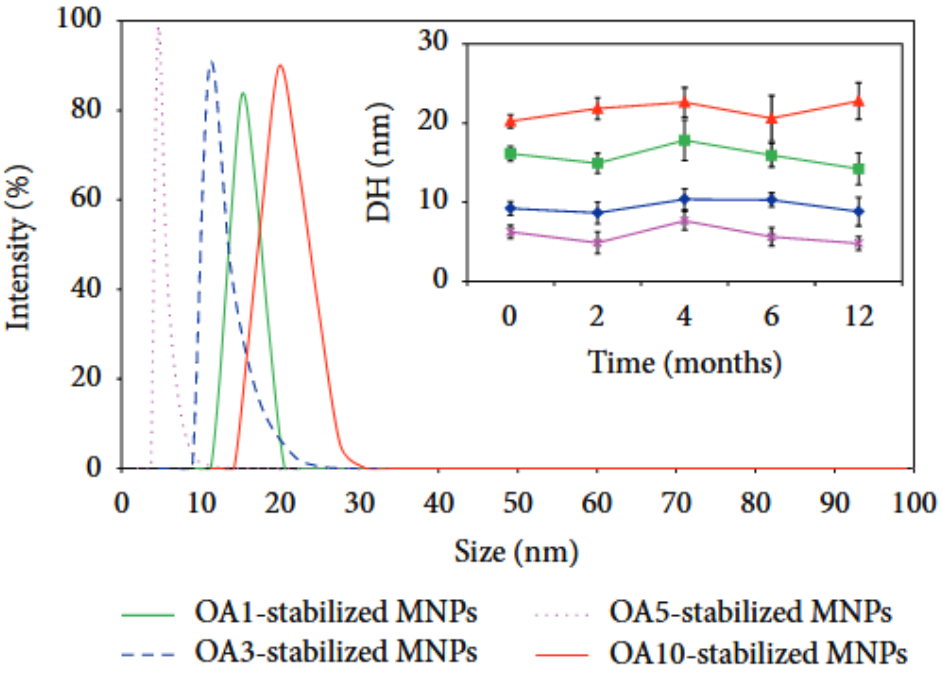

Fig. 2. DLS measurements of the different sized OA-stabilized M3NPs in hexane dispersions. The data clearly shows that as the (OA) molar ratios increase, the particle size decreases. However, once OA10x (i.e., 15 mmol OA) was reached, the size increases to ~20 nm, due to the worm-like streaks of aggregates formed.

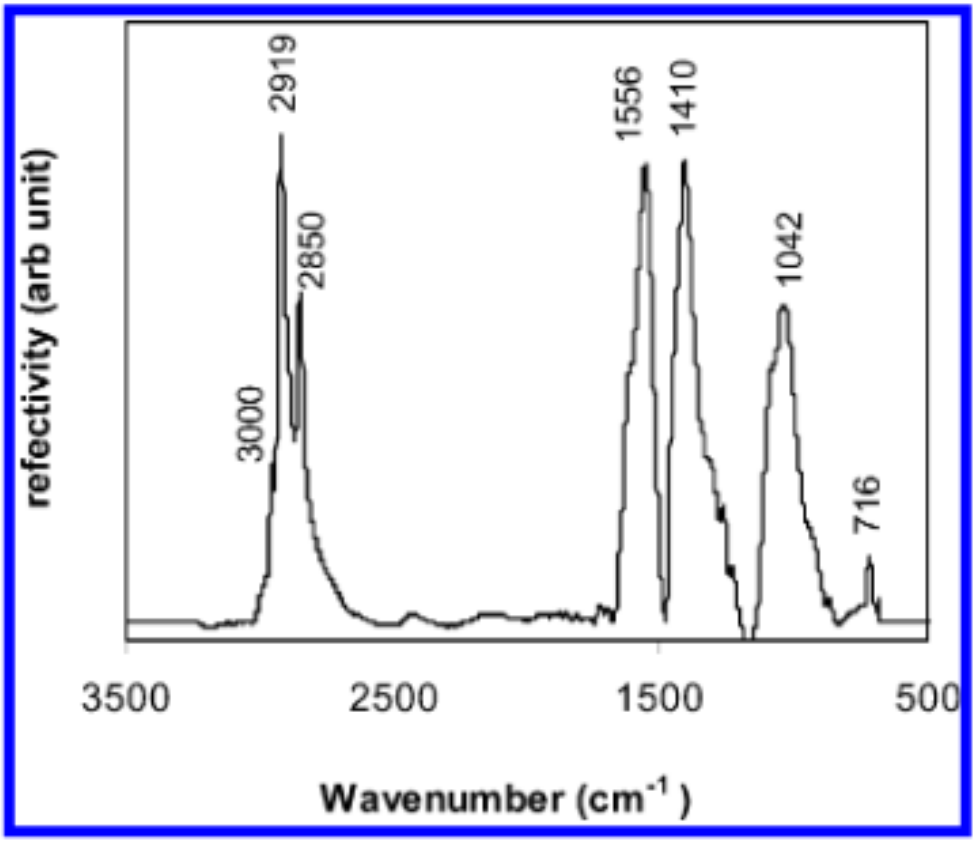

Fig. 3. FTIR spectrum obtained from oleic acid coated Co nanoparticles. The main resonances are identified in the figure and discussed in the text with reference to n-alkane vibrations on Co solid surfaces.

the alkylamine HA, a color change into brownish emulsion with a phase transfer of the metal cations into the oily phase is observed. Phase transfer of metal ions from an aqueous to an organic phase using alkylamines has been elegantly demonstrated. Weak adsorption of alkylamine to the iron/iron oxide surface was proposed and recently proven, indicating an amine-surface interaction via electron donation from the nitrogen lone pair to the positively charged iron ions. With the aid of the HA agent, the ionic metal components become compartmentalized and isolated by the fatty acids, acting as steric barriers and protective stabilizers. Coprecipitation by the base generates tiny oxide nuclei at the interfaces, which subsequently form well-ordered dispersions of fatty acid-capped black metal oxide nanocrystals. Since the precursors become spatially separated at the water-oil inter-face, crystal growth can be limited and agglomerations can be inhibited in favor of small, well-dispersed particles.

The authors used gas-liquid chromatography to determine the fatty acid composition of dairy (mare, cow, camel, and goat) and food animal (beef, horse, mutton, goat, and camel) fats. Chromatograms of vegetable oils (sunflower and flax) containing the maximum amount of omega-6 and omega-3 PUFAs were recorded [6-11].

Comparative data on the fatty acid composition (group and mainly individual acids, determination of biological and nutritional value) of dairy, food animal fats, and vegetable oils (which will be used as sources of essential omega-6 and omega-3 acids to obtain a fat additive) are shown in Tables 1, 2.

To form a balanced fatty acid composition of the finished product, the authors conducted studies of animal fats, sunflower and olive vegetable oils, and studied the possibility to supplement dairy products with PUFAs, in particular of the omega-3 and omega-6 groups.

Thus, the combination of milk fat with vegetable oils and fats of a particular group makes it possible to bring the fatty acid composition of the created

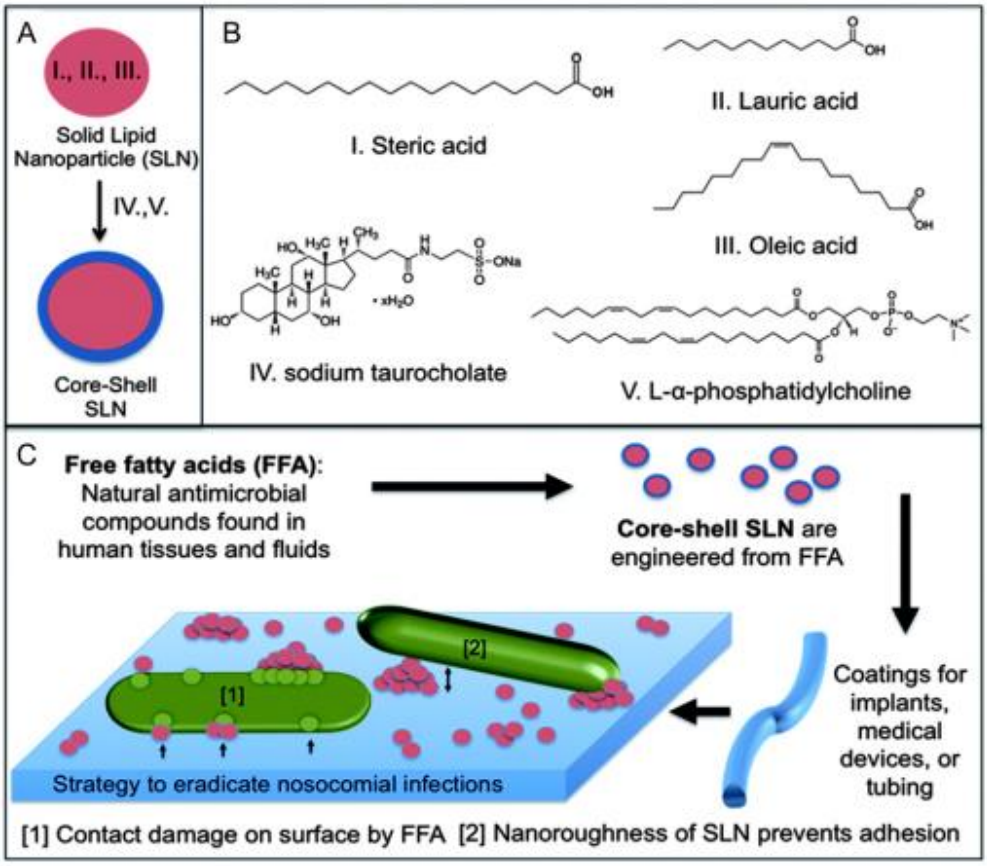

Fig. 4. (A) Core-shell solid lipid nanoparticles are prepared which contain antimicrobial free fatty acids; (B) Lauric acid and oleic acid are antimicrobial lipids which are encapsulated in the inner core; (C) Deposited SLNs on the tube surface inhibit bacterial adhesion and damage bacteria.

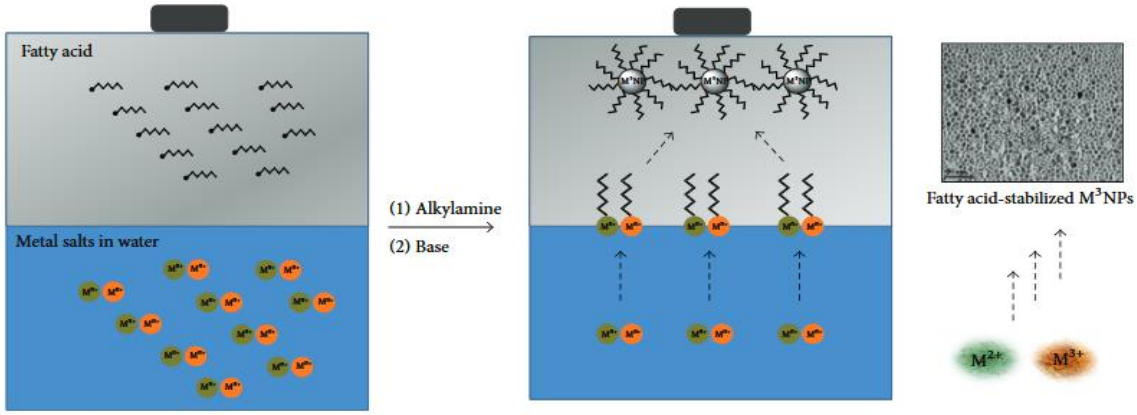

Fig. 5. Schematic representation of the formation mechanism of the fatty acid-linked with Co nanoparticles, aided by the presence of alkylamine as the structure-directing and pulling agent.

product closer to the "hypothetically ideal fat". When adjusting the optimal ratio of milk fat: vegetable oil, it is important to take into account not only the structural and rheological characteristics of the produced product but also the medical and biological requirements for the consumption of

Table 1. Fatty acid composition of animal fats and vegetable oils.

Acid Code	Classification	Content of fatty acids, % by weight						
		beef	horse	mutton	goat	camel	sunflower oil	flax oil
Saturated fatty acids								
$C_{8:0}$	N-	1.43	-	0.11	-	-	-	-
$C_{10:0}$	N-	2.32	0.30	0.62	-	-	-	-
$C_{11:0}$		2.02	0.21	-	-	-	-	1.04
$C_{12:0}$		1.82	0.14	0.18	-	-	-	2.26
$C_{13:0}$		-	-	0.66	-	-	-	-
$C_{14:0}$		2.81	3.76	7.09	3.91	4.76	0.15	-
$C_{15:0}$		0.40	0.27	0.66	0.98	0.67	0.02 (weak)	-
$C_{16:0}$		22.72	29.67	23.80	27.11	26.27	8.01	6.08
$C_{17:0}$		1.04	0.45	0.93	0.53	1.33	0.02 (weak)	0.18
$C_{18:0}$		30.45	6.10	10.97	36.43	25.78	3.17	4.19
$C_{20:0}$	N-	0.09	-	0.06 (weak)	0.20	0.17	0.39	0.39
$C_{21:0}$	N-	-	1.20	-	-	-	-	-
Branched (iso-, anteiso-) saturated fatty acids								
$C_{14:0}$	Iso-	0.57	0.13	-	-	-	0.20	-
$C_{15:0}$	Iso-	0.27	-	0.14	0.33	0.30	-	-
$C_{15:0}$	Anteiso-	0.24	-	0.17	0.31	0.09	-	-
$C_{16:0}$	Iso-	-	0.11	0.18	0.41	-	-	-
$C_{17:0}$	iso-	0.52	-	0.55	0.53	1.33	0.03 (weak)	-
$C_{17:0}$	anteiso-	0.16	-	0.47	0.99	0.26	0.28	0.18
$C_{18:0}$	Iso-	0.31	-	0.20	-	0.27	-	0.09
Monounsaturated fatty acids								
$C_{14:1}$	ω5	0.40	0.30	0.66	-	1.37	-	-
$C_{15:1}$	ω5	0.34	-	0.03 (weak)	0.11	0.54	0.24	0.23
$C_{16:1}$	ω9	0.21	-	0.73	0.69	1.08	0.10	0.07 (weak)
$C_{16:1}$	ω7	1.18	4.89	2.58	0.68	1.50	-	0.14
$C_{17:1}$	ω7	0.35	0.45	0.78	0.41	0.48	0.04 (weak)	0.09 (weak)
$C_{18:1}$	ω9	23.89	36.24	38.89	15.91	23.89	26.85	17.38
$C_{18:1}$	ω7	5.04	1.50	1.93	4.61	5.17	1.39	0.80

$C_{20:1}$	ω9	0.20	0.51	0.34	0.07 (weak)	0.15	0.14	0.04 (weak)
Polyunsaturated fatty acids								
$C_{18:2}$	ω6	4.28	6.59	5.45	3.26	2.76	58.06	15.02
$C_{8:3} - \gamma$	ω6	0.40	0.12	1.33	0.91	0.75	0.24	0.93
$C_{18:3} - \alpha$	ω3	0.20	6.80	0.48	0.02 (weak)	0.94	0.68	51.10
$C_{20:4}$	ω6	-	0.20	-	-	-	-	-

a particular essential acid. The design of binary and multi-component compositions to regulate their fatty acid composition should be carried out in two stages: determining the optimal ratios of ingredients and evaluating the effectiveness of the lipid component of the designed composition. At the first stage of practical development, binary compositions consisting of milk fat and liquid vegetable oils of various fatty acid groups were considered as raw components in the design of fat bases to optimize their fatty acid composition. To develop fat additives, fat emulsions based on vegetable sunflower and flax oils with different percentages: Lynol-1, Lynol-2, and Lynol-3 were developed initially (Table 3).

Sunflower and flax oils were used in the composition of different fatty acid groups. The proportion of vegetable oil in the binary composition varied from 20 to 35%. Adding 20–25% of vegetable oil to the composition allows getting a fat base characterized by a fairly soft consistency and having a hardness of 25–42 g/cm, depending

Table 2. Content of fatty acids in animal fats.

No.	Fatty acids groups	Content of fatty acids, %						
		beef	horse	mutton	goat	camel	flax	sunflower
1	Saturated, incl.:	63.50	42.37	46.80	75.28	61.44	14.21	12.27
	n-acids	61.43	42.13	45.0	72.71	58.19	13.94	11.76
	branched iso-, anteiso-	2.07	0.24	1.71	2.57	3.25	0.27	0.51
2	Unsaturated, incl.:	36.50	57.63	53.20	24.78	38.56	85.79	87.73
	monounsaturated	31.62	44.00	45.94	20.59	34.11	18.74	28.73
	monounsaturated incl. ω9	24.21	36.79	39.96	16.67	25.05	17.49	27.09
	polyunsaturated, incl.:	4.88	13.63	7.26	4.19	4.45	67.05	58.98
	diene	4.28	6.60	5.45	4.17	2.76	15.02	58.06
	trienoic	0.60	6.83	1.81	0.02	1.69	52.03	0.92
	Essential polyunsaturated fatty acids							
3	ω 6, incl.:	4.68	6.91	6.78	4.17	3.51	15.95	58.30
	linoleic ω6 $C_{18:2}$	4.28	6.59	5.45	3.26	2.76	15.02	58.06
	γ-linolenic ω6 $C_{18:3}$	0.20	0.12	1.33	0.91	0.75	0.93	0.24
	arachidonic ω6 $C_{20:4}$	-	0.20	-	-	-	-	-
	ω 3, incl.:	0.40	6.80	0.48	0.02	0.94	51.10	0.68
	α-linolenic ω3 $C_{18:3}$	0.40	6.80	0.48	0.02	0.94	51.10	0.68

Table 3. Content of fatty acids content in vegetable oils and fat emulsions.

Fatty acids	Lynol-1, %	Lynol-2, %	Lynol-3, %
SFA	12.5	12.87	12.3
Essential unsaturated fatty acids	87.5	87.1	87.7
MUFA	22.7	25.7	20.7
PUFA	64.8	61.4	67.0
W6	52.1	45.6	48.6
W3	12.7	15.8	18.4
W9	22.1	24.2	20.0
$C_{18:1}$ W9	22.1	27.1	19.8
$C_{18:2}$ W6	52.0	45.2	48.1
$C_{18:3}$ gamma-W6	-	0.4	0.45
$C_{18:1}$ alpha-W3	12.7	15.8	18.5
$C_{14:0}$	0.45	0.83	0.9
$C_{16:0}$	7.5	7.3	6.6
$C_{18:0}$	4.2	3.5	4.1

on the used oil. The highest hardness value is in the base that used partially hydrogenated oils. Reducing the proportion of vegetable oil to 5–10 % allows getting a product of a dense consistency with a hardness of 80–98 g/cm. The best quality characteristics are in the compositions of fat with sunflower and flax oils "Lynol-1".

Fat additives containing omega-6 (linoleic) and omega-3 (α-linolenic) PUFAs for the supplementation of dairy products were obtained by mixing animal food fats (beef, horse, goat, camel) and fat emulsion "Lynol-1" (sunflower and flax oils) taken in different proportions. Thus, the authors developed the composition of new fat additives with a balanced composition of PUFAs of the omega-3 and omega-6 groups. The developed fat additives are added to skim milk in such an amount that the content of the mass fraction of fat in milk or dairy product is the standard amount – 1, 2.5, 3, or 6%. To obtain cow milk with a fat content of 2.5%, with a balanced fatty acid composition and supplemented with omega-6 and omega-3 PUFAs, experimental studies were conducted in the laboratory. In skimmed cow milk with a temperature of 30–32 °C, an equal amount of 15.2 g of fat additive "Lynol 1" was added. The milk was intensively mixed in s JKA ultra-tarrent-18 disperser – 6 min until a uniform thick foam and the added fat disappeared. Then, it was treated with 20-kilohertz ultrasound for 2 minutes (Sonoplus HD 2200 ultrasonic homogenizer) and finally mixed in a disperser for 5 minutes until a thick foam was formed. The mass fraction of fat in the experimental milk was 2.5%. The fatty acid composition of supplemented milk lipids obtained by adding fat additives in comparison with the fatty acid composition of the control sample is shown in Table 4.

The optimal ratio between SFAs:MUFAs:PUFAs, according to various sources, is considered to be 1:1:1, the ratio of omega-3 to omega-6 is from 1:2 to 1:4 (Tutelyan et al., 2009). New supplemented dairy products, as can be seen from Table 5, are as close as possible to this ratio, and in the control sample, it is exceeded, for example, in cow milk fat, omega-3:omega 6 is 1:8.

Table 4. Comparative table of the content of fatty acids in lipids of the supplemented milk of different animal species (% by weight)

Cn	Mare		Camel		Goat		Cow	
	control	experiment	control	experiment	control	experiment	control	experiment
Saturated fatty acids								
$C_{10:0}$	3.6	-	0.1	0.42	7.2	-	1.7	0.02
$C_{12:0}$	4.6	-	0.7	-	3.7	-	2.1	0.02
$C_{14:0}$	5.2	2.73	8.5	2.53	9.3	1.21	9.3	0.97
$C_{16:0}$	18.2	20.61	27.5	17.56	24.7	13.21	25.7	15.58
$C_{18:0}$	1.0	4.80	16.1	15.29	4.5	17.75	11.4	21.60
Monounsaturated fatty acids								
$C_{14:1}$	0.5	0.11	0.4	0.18	0.2	0.24	0.7	0.14
$C_{16:1}$	6.1	2.24	5.4	0.91	0.6	0.65	1.0	1.43
$C_{18:1}$	17.0	24.50	18.0	20.84	14.8	19.99	21.1	20.25
Polyunsaturated fatty acids								
$C_{18:2}$ ω 6	8.2	35.78	2.7	27.97	1.7	35.32	1.9	32.04
$C_{18:3}$	12.9	-	2.1	0.49	0.5	0.29	1.2	0.25

Table 5. Total fatty acid content in oil extract of some vegetables.

Fatty acids	Mare		Camel		Goat		Cow	
	control	experiment	control	experiment	control	experiment	control	experiment
Saturated fatty acids (SFAs)	40.2	28.3	61.4	41.5	72.9	33.5	67.2	38.9
Essential unsaturated fatty acids	59.6	71.7	38.5	58.5	27.0	66.5	32.8	61.1
Monounsaturated fatty acids (MUFAs)	31.3	27.7	33.1	23.1	23.6	21.7	29.2	21.5
Polyunsaturated fatty acids (PUFAs)	28.4	44.0	5.5	35.3	3.4	44.8	3.6	39.6
Omega-6 (W6)	28.1	36.1	4.7	28.4	3.1	35.6	2.8	32.3
Omega-3 (W3)	0.3	7.9	0.8	6.8	0.3	9.2	0.7	7.3
Omega-9 (W 9)	20.1	24.5	20.3	21.3	20.7	20.1	23.1	20.4
W6:W9:W3	93.6:1:67	4.6:3.1:1	5.8:1:25.3	4.1:1:3.1	10.3:1:69	3.9:2.2:1	4:1:33	4.5:1.75:1
SFAs:MUFAs:PUFAs	1.4:1.1:1	1:1:1.6	11.2:6:1	1.8:1:1.5	21.4:6.9:1	1.5:1:2.1	18.7:8.1:1	1.6:1:14

CONCLUSION

Supplementation of all types of milk (cow, goat, mare, camel) with new developed fat additives allows achieving an optimal ratio of fatty acids, improves the balance of the fatty acid composition, and improves the anti-atherogenic, lipotropic, and anti-sclerotic properties of milk in comparison with control samples (Table 5). The content of SFAs and MUFAs in the supplemented milk has been changed: in cow milk – by 8.5 and 12.3%, in goat milk – by 7 or 14.5%, in camel milk – by 16 and 16.7% in mare milk – by 28.5 and 9%, respectively, lower than in the control samples, and the PUFA content in all types of milk was from 1.5 to 2 times higher compared to the control. It indicates a higher biological efficiency of such supplemented milk. This research is funded by the Ministry of Agriculture of the Republic of Kazakhstan (BR10764998).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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