

AN EPISTEMOLOGICAL BACKGROUND ON PARADIGM FORMATION OF LIPIDOMICS OF DAIRY INDUSTRY

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Abstract: The article considers post-genomic view on formation of scientific ideas about one of the major components of raw milk and products produced from it (for example, cheese). It is a lipid complex (milk fat) of the brand Lipidomics. The cluster structure of milk fat, its components and derivatives are described. The author shows the dynamics of milk fat transformation in the process of cheese production in logistic link to lipolytic activity of bacterial starters and enzyme preparations. The characteristic of lipid complex of milk whey is given. Information allows us to formulate Lipidomics positions of dairy products. The aim of the article is to attract researchers' attention to the object, and practitioners to a rational and careful use of milk fat in food products.

Keywords: raw milk, dairy products, cheese, lipids, milk fat, Lipidomics

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INTRODUCTION

Formulation of the problem. According to the principles of Lactoomics [1] (science about milk) and logistics of dairy industry [2], at the present level of knowledge [3, 4] Lipidomics of milk and dairy products is reduced to milk fat – monitoring of composition and properties, cooking and use. The perfect model is the production of butter [5] and spreads [6]. This subject is fully and systematically covered by works of Franz Adamovich Vyshemirsky, Doctor of Technical Sciences, Professor, Laureate of the State Prize of the Russian Federation, by his students and followers. There are more than 1.000 publications on this topic which give all the necessary information about the subject – milk fat, butter-making, dairies and a wonderful indispensable product – butter [7] – which is the indicator of dignity and well-being of human civilization.

Based on post-genomic biotechnology view on concepts of objects and phenomena knowledge, taking into account the experience of Glikoomics formation [8], it is useful to formulate epistemologically and as a paradigm our vision of one of the major components of milk – the fat phase – Lipidomics. The starting material and the base model of the article are numerous researches of Professor M.S. Umansky [9]. This article is dedicated to his blessed memory.

OBJECTS AND METHODS OF STUDY

The object under review is the unified scheme of milk lipids classification by M.S. Umansky, which is shown in Fig. 1.

The basic problem-target structure of research of raw milk lipids by M.S. Umansky with the interpretation of the whole range of milk and milk products, at the example of cheese-making is shown in Fig. 2.

Taking into account the lack or absence of identification procedures for simulation of milk lipids and its products, as well as bacterial cultures and enzymes, M.S. Umansky developed and modified 17 original methods of studying the objects of knowledge. A list of some of the information is provided:

- preparative TLC analysis of N-lipid;
- micro TLC analysis of N-lipid;
- preparative TLC analysis of P-lipid;
- Micro-TLC analysis of P-lipid;
- GLC analysis of fatty acid composition;
- GLC analysis of free fatty acids;
- GLC analysis of volatile fatty acids;
- esterase activity of mesophilic and thermophilic lactic acid bacteria;
- esterase activity of propionic acid bacteria.

Fig. 3 shows the logistic scheme of the system of complex analysis of milk lipids and milk products according to M.S. Umansky.

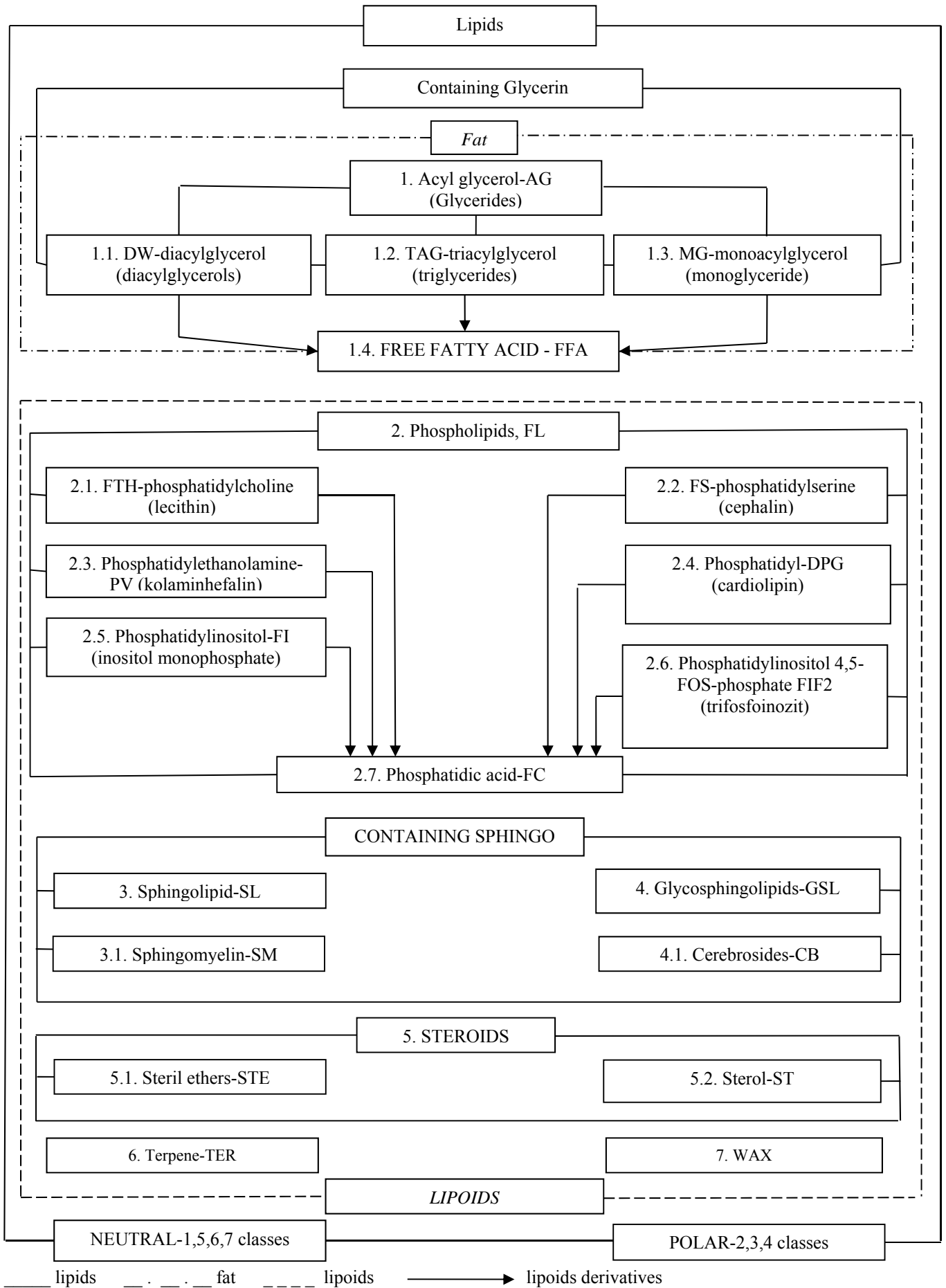


Fig. 1. A unified scheme of classification of milk lipids.

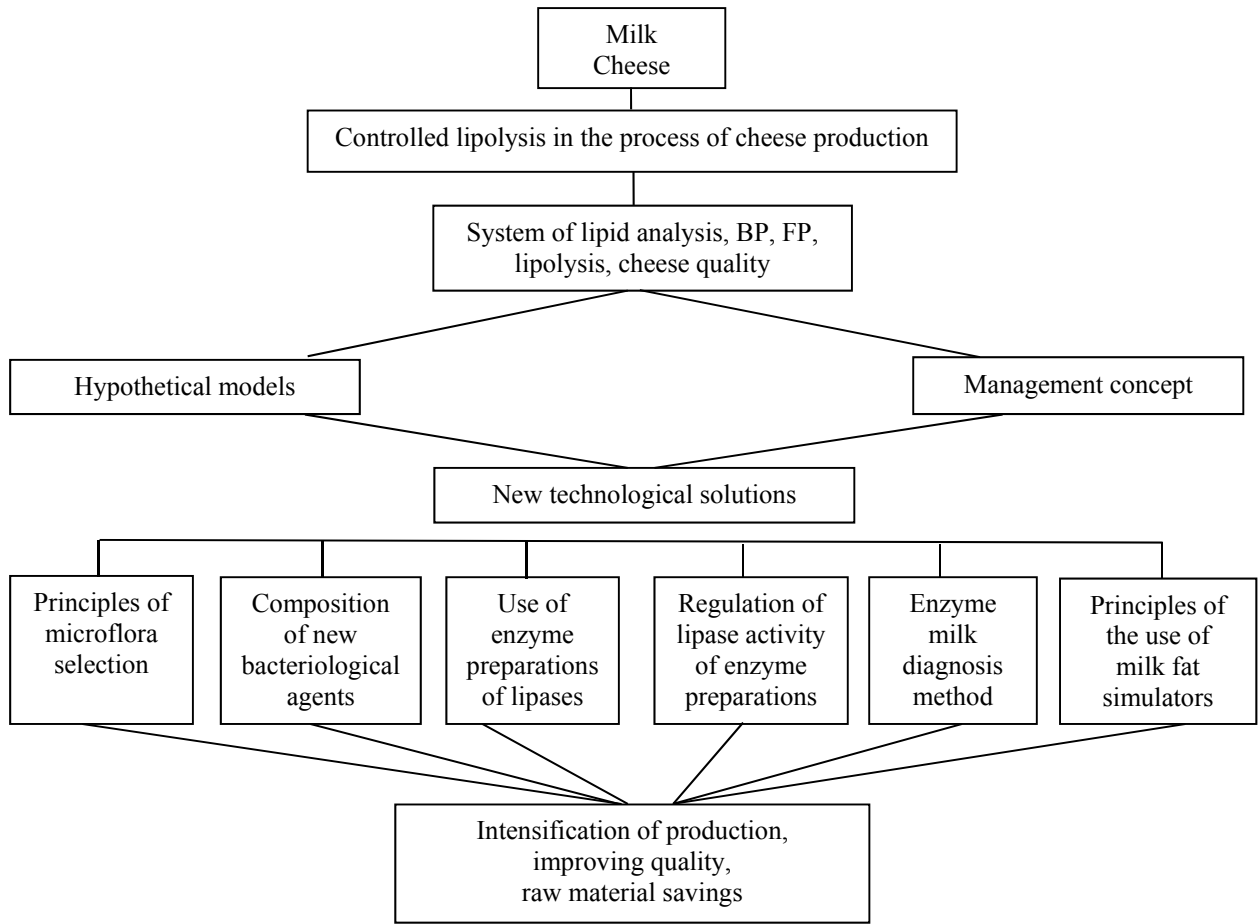


Fig. 2. Logistics of Lipidomics scheme research of raw milk and products.

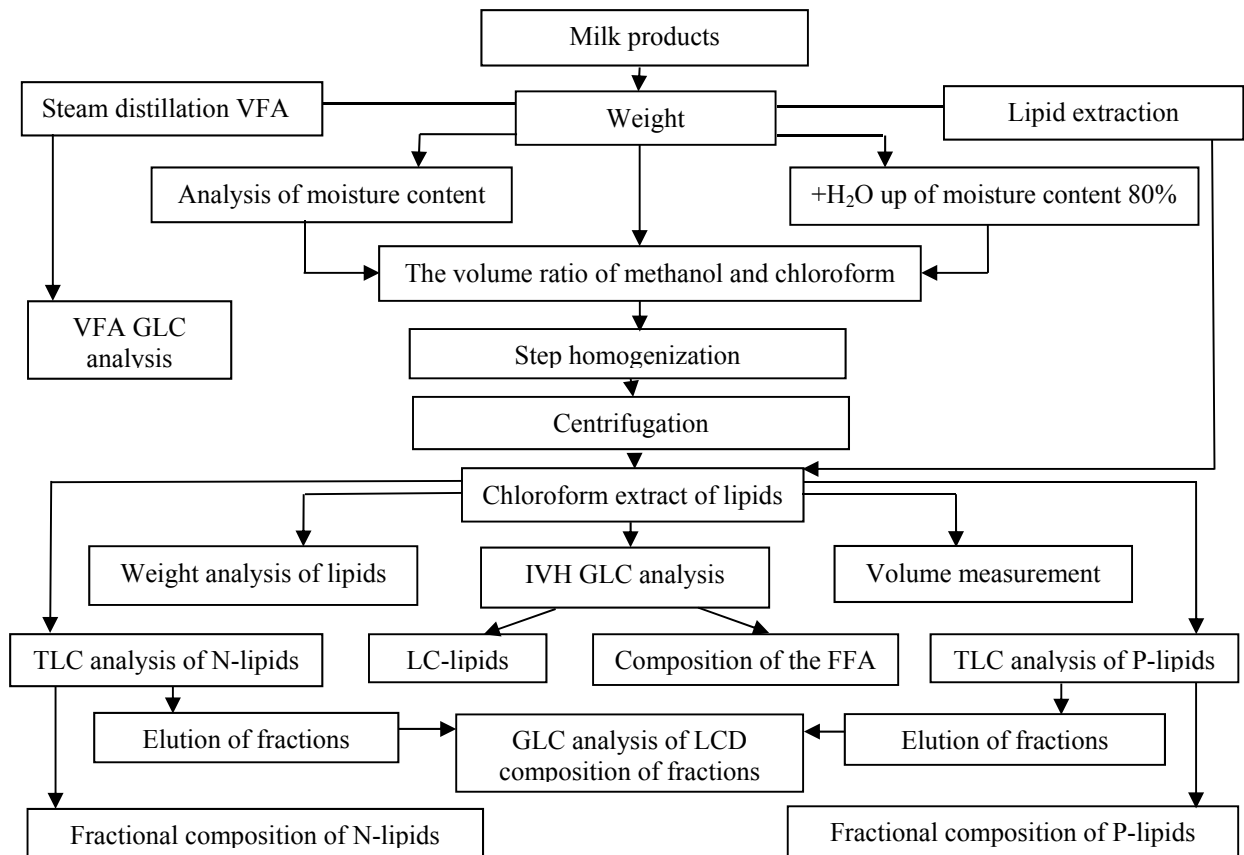


Fig. 3. Scheme implementation of the system of lipid complex analysis of milk and dairy products.

As a result of the above system of cognition research object, at the example of an idealized model of the most sophisticated biotechnological object – cheesemaking, we can formulate principles of Lipidomics of dairy industry: lipid complex and lipase of raw milk, analytics of lipids and lipase, enzymes-builders, biogenic enzymes of bacterial cultures, lipolysis in the cheese during its production, storage and maturation. A single technique aimed to establish laws governing the presence of somatic cells in raw milk and active lipoprotein (which is not considered in industry) needs a separate independent study and practical implementation. Then the problem is in a logical sequence.

RESULTS AND DISCUSSION

Lipids (*Greek* Lipos – fat) are hydrophobic (insoluble in water but soluble in organic solvents) chemical compounds with different composition and structure [10]. In everyday practice we know animal fats, such as milk fat, vegetable oil and microbial oil (there are six product groups with more than 20 items). Among lipids (fats) of animal origin, and in particular in vegetable oils, a unique place is taken by the subject

of this article – milk fat, the main indicator of the value of raw milk in Russia. The uniqueness of this bioenergy power given by nature at the disposal of “homo-sapies” can not be overestimated, and to understand it is very simple. Just have a portion of the natural (preferably fresh) and skim milk. Everyone will understand the difference! Milk fat generates a dairy product – its taste, aftertaste, color and even “childhood memories” about your nurse-mother, “fairy tales and songs” (Alexander Pushkin).

This systematic file represents identified and isolated milk lipids according to A. Tepel [3] as neutral fat (acyl glycerol), lipoids (fat-like compounds), isoprenoid lipids and substances related to lipids (Fig. 4).

The main component of the lipid complex of milk-milk fat (glycerides) – occupy more than 98%. The remaining 2% of the lipids (related substances) are phospholipids, glycolipids, sterols, vitamins, pigments; they are usually a part of the lipoprotein membranes of the fat globules – formalized milk fat. Milk fat is a complex hierarchical system which according to Mikhail Umansky includes all partially lipid compounds. It is shown in Fig. 5.

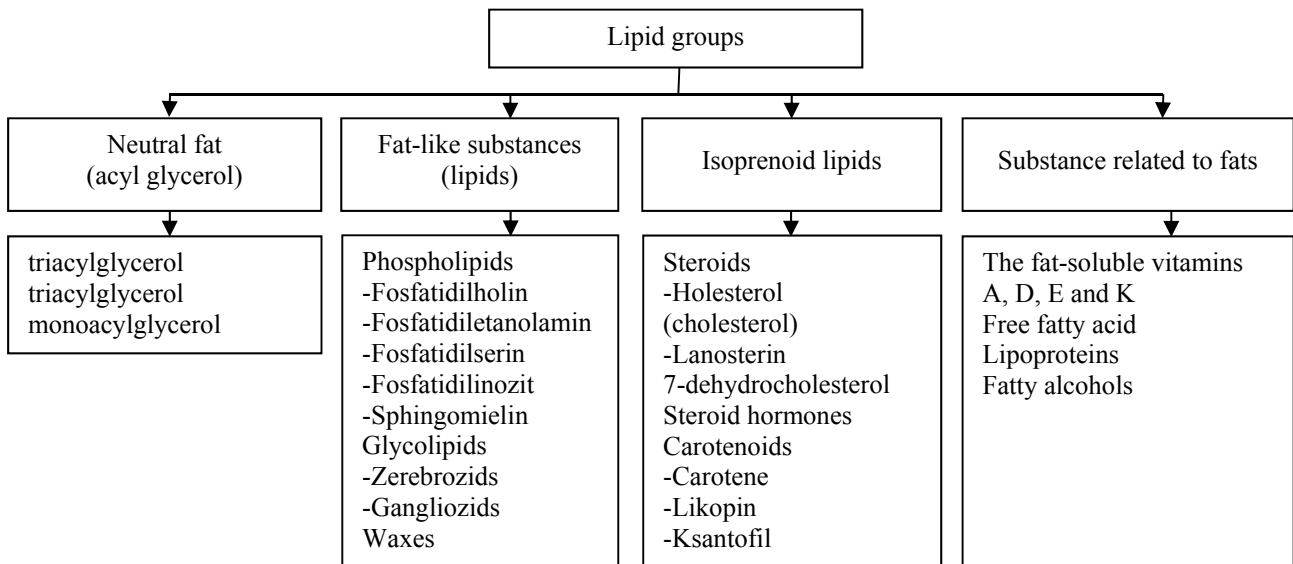


Fig. 4. Milk lipids groups.

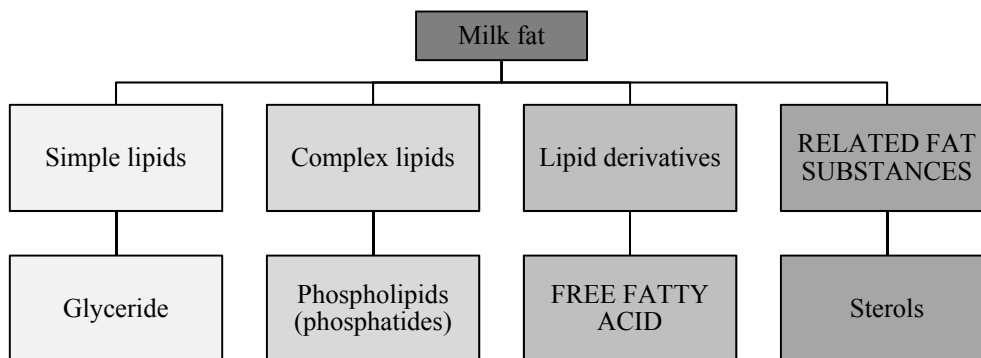
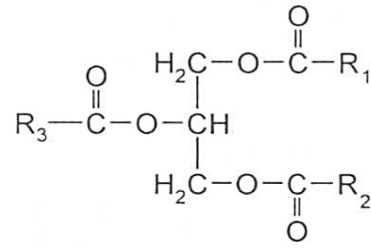


Fig. 5. Systemology of milk fat by M.S. Umansky.

Thus, milk lipid complex shown in Fig. 1 and 2 is unique and is reduced in practice to milk fat cluster structures – globules. Biotechnology fat-containing milk products (cream, sour cream, and of course, butter) is connected with the fat globules. They also play a significant role in the formation of the entire range of protein-fat products – drinks, canned milk, condensed and dried milk, cottage cheese, all kinds of cheeses, etc. A common scientific base of the whole range of milk fat modification and possible derivatives is Lipidomics (science about lipid complex – milk fat). Its principles are formed for all assortment groups and individual milk and milk products. Lipidomics of cheese in complete technological cycle – cheese and whey is considered as a basic example (analog).

The general structural formula of milk fat triglycerides is shown below (R, R1 and R2 are hydrocarbon radicals of higher carboxylic acids).



Schematically fat globules of cow's milk at the present level according to A.Tepel [3] are shown in Fig. 6.

The starting components of the milk fat-structural elements of acyl glycerol – are fatty acids, characteristic of which is shown in Table 1.

The complexity of the structure of one of the groups of milk lipids is shown in Fig. 7.

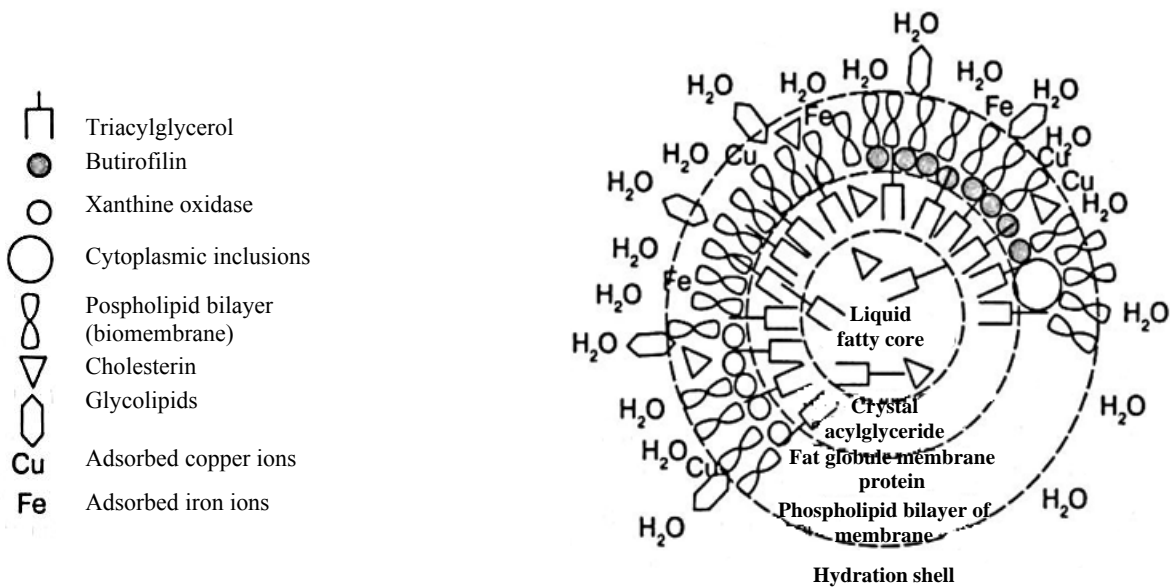


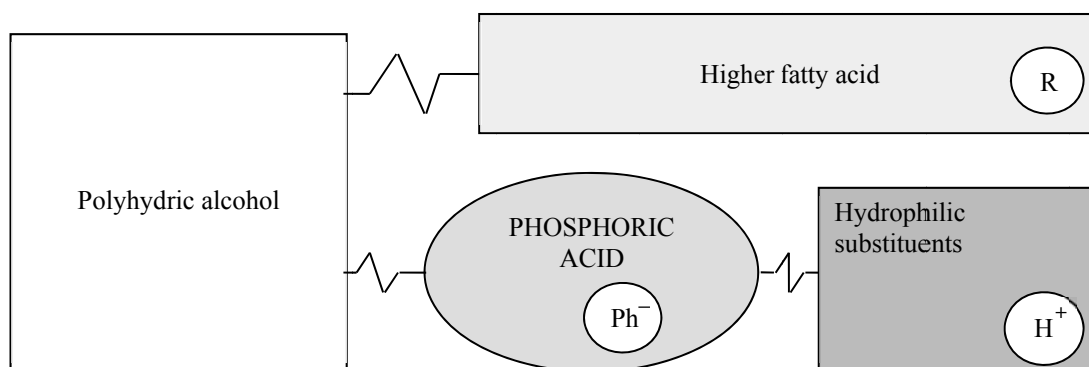
Fig. 6. Schematic representation of fat globules and the membrane.

Table 1. Fatty acid composition of milk fat

Index	Acid		Amount, %
	Systematic	Every day	
Saturated acids			
4:00	butane	oil	3.60
iso-4:0	2-methylpropanoic	isobutyric	0.20
5:00	pentane	valeric	0.20
6:00	hexane	kapron	1.80
7:00	heptane	heptyl	0.01
8:00	octane	caprylic	1.00
9:00	nonanoic	pelargonic	0.30
10:00	decanoic	capric	2.10
11:00	Gendekanoic	undecyl	0.30
12:00	dodecanoic	lauric	2.70
13:00	tridecanoic	tridecyl	0.05
iso-13:0	11-metildodekanoic	izotridecyl	0.01
14:00	tetradecanoic	myristic	11.00
iso-14:0	12-metiltridekanoic	izomyristic	0.20
15:00	pentadecanoic	pentadecyl	1.10
iso-15:0	12-metiltetradekanoic	izopentadecyl	0.08

Table 1. Ending. Fatty acid composition of milk fat

Index	Acid		Amount, %
	Systematic	Every day	
16:00	hexadecanoic	palmitic	27.50
iso-16:0	13-methyl pentadecanoic	izopalmitic	0.20
17:00	heptadecanoic	margarine	1.00
iso-17:0	14-metilgeksadekanoic	izomargarine	0.01
18:00	octadecanoic	stearic	10.20
19:00	nonadecanoic	nonadedecylic	0.10
20:00	eicosanoic	arachidic	1.80
21:00	Geneycosanoic	-	0.01
22:00	docosanoic	behenic	0.08
23:00	Tricosanoic	-	0.01
24:00:00	Tetracosanoic	lignoceric	0.05
26:00:00	Geksacosanoic	tseritonic	0.07
28:00:00	Oktacosanoic	montanic	0.01
Unsaturated acids			
$\Delta 9-10:1$	9-decene	kaprolenoic	0.20
$\Delta 10-11:1$	10-undecyl	-	0.06
$\Delta 9-12:1$	9-dodecenoic	lauroleinoic	0.30
$\Delta 9-14:1$	9-tetracenoic	myristoleic	1.00
$\Delta 9-16:1$	9-geksadecenoic	palmitoleic	4.00
$\Delta 9-18:1$	9-octadecenoic	oleic	29.80
trans- $\Delta 11-18:1$	trans-11-octadecenoic	vaccenic	0.80
$\Delta 9-20:1$	9-eykozsnooc	gadoleic	0.10
$\Delta 13-22:1$	13-dokochnoic	erucic	0.05
trans- $\Delta 13-22:1$	trans-13-docosenoic	brassicidic	0.02
$\Delta 9,12-18:2$	9,12-okgadekadienoic	linoleic	3.20
$\Delta 9,12,15-18:3$	9,12,15-ktadekatrnoic	linolenic	1.40

**Fig. 7.** Block diagram of the structure of the phospholipids.

Systemology of raw milk lipids can be illustrated in details by the example of fat fraction of milk whey in my monograph “Milk whey phenomenon” [11]. This combination of objects of knowledge seems logical in terms of the formation of a Lipidomics paradigm in dairy industry according to the scheme: milk = cheese + whey.

Lipid complex of milk whey is associated with the milk fat in the form of globules in the form of emulsion or suspension, depending on the temperature. The native (fresh) milk whey is emulsion that should be taken into account when organizing processing such as separation.

Study of lipid complex of milk whey in classical science Lipidomics has not only informative, but also practical importance to replenish food and software technology, for example, in the preparation of milk sugar.

The cluster structure of milk fat as an object of nanotechnology is quite clearly demonstrated in the works of T. Smykova [12, 13]. Figure 8 shows the cluster structure of the system of lipid complex of milk whey.

The formation basis of lipid complex clusters of milk whey is volatile fatty acids (VFA) in the form of chromatograms, which is shown in Fig. 9.

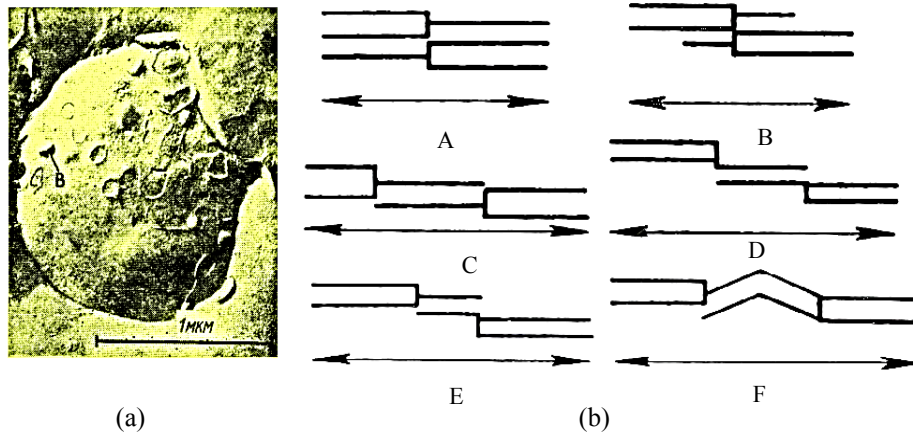


Fig. 8. Lipid complex of milk whey: (a) fat globules, and (b) structure glycerides of milk fat: saturated monoacids (A), saturated diacid with a difference in the length of the acid chain by two carbon atoms (B), saturated diacid with a difference in length of the acid chains of more than two carbon atoms (C, D), saturated different acids (E), oleodisaturated acids (F).

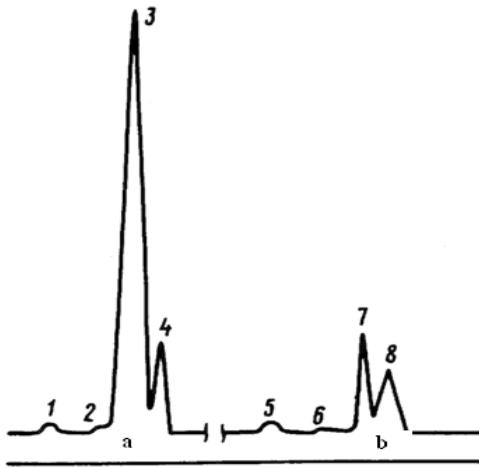


Fig. 9. The chromatogram of volatile fatty acids of curd (a) and cheese (b) whey: butyric acid (1, 5); propionic acid (2, 6); acetic acid (3, 7); formic acid (4, 8).

It should be noted that based on the VFA of E.I. Melnikova, Professor of VGUIT [14] a new perspective is formed on the original component of milk whey – osmophoric compounds that deserve special consideration.

Milk fat has only about 5% in dry milk whey, but it plays an important role in industrial processes, even indirectly – phospholipids, lipoproteins and even fat sugar. Lipid complex is certainly separated from milk whey in the identified complex, separate fractions or together with other components. It should be noted that the component of milk fat globular membrane whey cream (MFGM), shown in Fig. 8 (on the left), attracted the attention of physicians. It is proved by the special report on the Sixth International Conference on milk whey (US, 2011) [15].

A special place in M.S. Umansky’s researches [9] is given to lipolytic enzymes of raw milk, which classification is shown in Table 2.

Table 2. Classification of lipolytic enzymes

Systematic number	Systematic title	Everyday title
3.1.1.3	Triacylglycerol-atsilgidrolase (glycerol ester hydrolase)	Lipase
3.1.1.34	Triacylglycerol-atsilgidrolase (glycerol ester hydrolase)	Lipoprotedlipase
3.1.1.13	Sterol ester hydrolase	Cholesterol esterase
3.1.1.4	Phosphatide-2-atsilgidrolase	2 phospholipase (PLA2)
3.1.1.4.-	Phosphatide-1-atsilgidrolase	Phospholipase 1 (phospholipase A1)
3.1.1.5	Lysolecithin-atsilgidrolase	lysophospholipase
3.1.3.4	Phosphatidate-phosphogidrolase	Phosfatidatfosfatase
3.1.4.3	Fosfatidilholin- choline phosphogidrolase	Phospholipase 3 (phospholipase C)
3.1.4.-	Sphingomyelin-N-atsilsfingozingidrola za	sphingomyelinase

In [9] it is shown that lipolysis and milk quality are interrelated, especially in mechanically activated raw materials (mixing) (Fig. 10) and abnormal (not cheesable) milk.

As it was mentioned above even more important is M.S. Umansky's dependence of the content pattern of somatic cells in raw milk-enzyme on lipoprotein lipase activity (Fig. 11).

This fundamentally new (Patent, AS USSR № 1010946) method for the determination of cheesable milk by the number of somatic cells is unique and requires implementation.

A separate topic of M.S. Umansky research regarding Lipidomics is devoted to the study of the lipolytic activity of lactic and propionic acid bacteria. It fully solves a complex problem – the emergence of biogenic elitors in cheesemaking in vitro and in vivo, which requires separate consideration. Further, according to logistics principles lipolysis of milk fat in cheese is discussed. For example, the identification sheet may be composed of phospholipids content in cheese (Table 3).

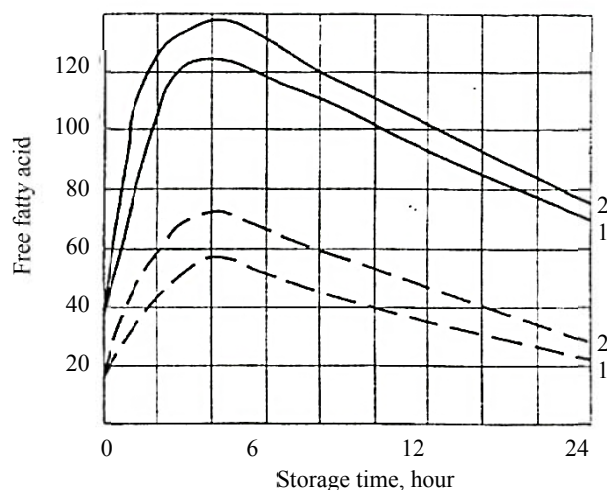


Fig. 10. Changes in the amount of free fatty acids in mechanically active milk (---) and cream (—) depending on the duration of storage temperature: 1 – at 4°C, 2 – at 18°C.

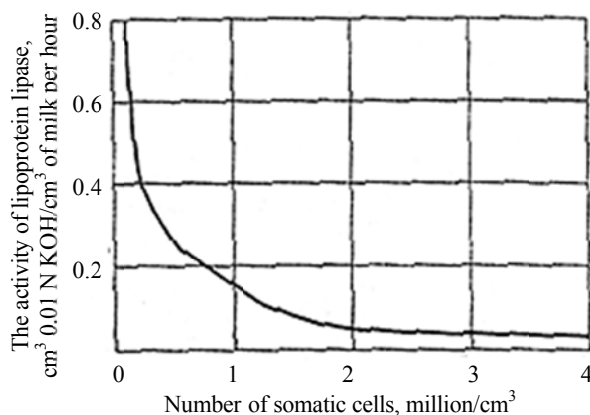


Fig. 11. Dependence of lipoprotein lipase activity on somatic cells in milk.

Table 3. The content of phospholipids in the cheese

Type of cheese	Phospholipids, % 10 ⁻³	Type of cheese	Phospholipids, % 10 ⁻³
Roquefort	2.0	Krestsentsa	82.6
Parmigiano	60.4	Russian	83.0
Emmental	76.8	Dutch	115.0
Lithuanian	77.0	Kostroma	124.0
Fontana	80.7	Cottage	376.0

Seven guidelines of lipolysis cheese formulated by M.S. Umansky have no analogues and are waiting for research. Below (Fig. 12) you can see a hypothetical scheme of bioconversion of lipid complex in cheese milk.

On the basis of the postulates and revealed laws, using original research methods, we obtained unique results (criteria optimization and neural network modeling) on the regulation of lipolysis in cheeses with low (Table 4) and high (Table 5) temperatures of the second heat.

A simple analysis of Tables 4 and 5 shows the depth and practical value of cheese lipidology as a “launching pad” for Lipidomics of dairy industry.

The most valuable research made by M.S. Umansky [9] are studies of activity of lipase of milk coagulated enzymes (Table 6) and original research methods of lipolysis. They are unique and should be (as innovative) the intellectual property of the Siberian Institute of Cheesemaking (Barnaul, Altai Territory), where M.S. Umansky worked for many years.

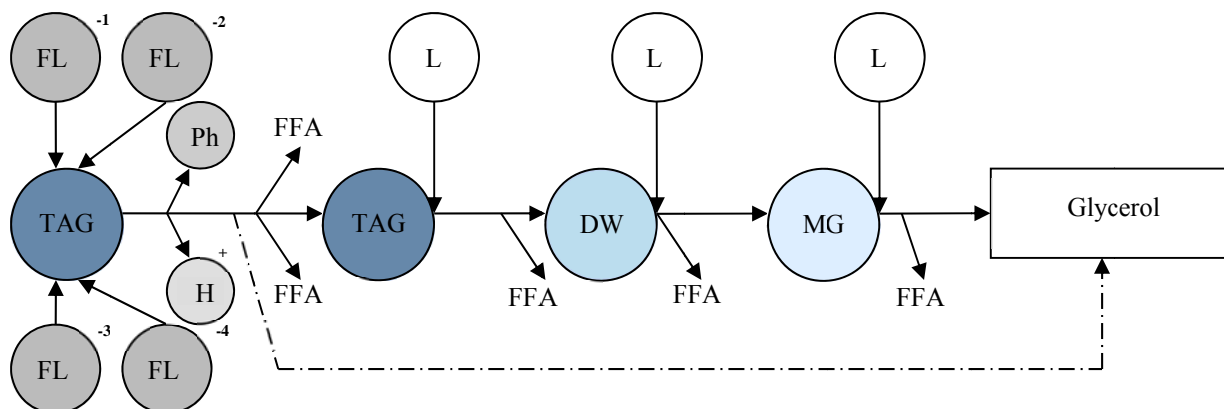


Fig. 12. Schematic diagram of a hypothetical sequential enzymatic hydrolysis of lipids in cheese (Fig. 1).

Table 4. The content of the lipid fraction in cheese

Lipid fractions	The content of the fractions in cheeses with different starter cultures (% of total)	
	Strong lipolytic activity	Weak lipolytic activity
Phospholipids	1.44	1.86
Mono + 1,2-diacylglycerols	8.47	6.65
Sterols + unidentified fraction	8.06	6.60
Free fatty acid 1,3-diacylglycerols + triacylglycerol	9.01	7.34
	67.84	72.72
Steridy + hydrocarbons	5.18	6.03
lipolysis ratio (CI)	0.34	0.26

Table 5. The mass proportion of phospholipid component in the mature cheese, 10³ %

Phospholipid component	Variants of test cheese		
	I	II	III
hospho sphingosine	16.2 ± 1.8	15.0 ± 2.6	18.4 ± 3.1
hosphatidylcholine	18.6 ± 1.4	18.2 ± 1.5	18.9 ± 1.3
phosphatidylethanolamine	18.8 ± 1.2	19.9 ± 1.7	19.6 ± 1.2
phosphatidylserine	3.3 ± 2.6	3.6 ± 1.9	4.4 ± 1.4
phosphatidylinositol	1.7 ± 1.5	2.0 ± 2.1	2.6 ± 1.8

Table 6. Lipolytic activity of milk coagulated enzymes on different substrates (10⁻⁹ mol. sec⁻¹)

Enzyme preparations	Olive oil		Synthetic milk fat emulsion		Cream	
	A ₁	A ₁ ^{rel}	A ₂	A ₂ ^{rel}	A ₃	A ₃ ^{rel}
Rennet calves	0.12 ± 0.00	1.0	0.12 ± 0.00	1.0	0.27 ± 0.01	1.0
Pepsin beef	0.09 ± 0.00	0.8	0.10 ± 0.00	0.9	0.29 ± 0.01	1.1
Pepsin porcine	0.13 ± 0.01	1.1	0.08 ± 0.01	0.7	0.41 ± 0.01	1.5
Pepsin chicken	0.15 ± 0.01	1.3	0.11 ± 0.00	0.9	0.79 ± 0.02	2.9
Pepsin duck	0.02 ± 0.00	0.2	0.23 ± 0.01	1.9	0.29 ± 0.01	1.1
Fromaza	0.05 ± 0.00	0.4	0.08 ± 0.01	0.7	0.24 ± 0.01	0.9

Table 6. Ending. Lipolytic activity of milk coagulated enzymes on different substrates (10^{-9} mol. sec $^{-1}$)

Enzyme preparations	Olive oil		Synthetic milk fat emulsion		Cream	
	A ₁	A ₁ ^{rel}	A ₂	A ₂ ^{rel}	A ₃	A ₃ ^{rel}
Rennilaza	0.04 ± 0.00	0.3	0.23 ± 0.01	1.9	0.41 ± 0.02	1.5
Rennie-nomiin	1.28 ± 0.04	10.7	2.02 ± 0.06	16.8	1.76 ± 0.04	6.5
Meyto- rennet	0.12 ± 0.00	1.0	0.16 ± 0.01	1.3	0.27 ± 0.01	1.0
Mucor	0.14 ± 0.00	1.2	0.17 ± 0.02	1.4	0.29 ± 0.01	1.1
Mezen-terrine	0.20 ± 0.01	1.7	0.16 ± 0.01	1.3	0.60 ± 0.02	2.2
Kazorus-Sulin	0.27 ± 0.01	2.3	0.43 ± 0.03	3.6	0.35 ± 0.01	1.3

CONCLUSION

In general, a brief analysis of M.S. Umansky's contribution to the development of selective lipolysis in cheese in the author's edition "lipidology of cheese" underlines the greatness of his work and the possibility of forming Lipidomics of dairy industry. Moreover, it allows us to formulate the following conclusions.

(1) An independent scientific direction – lipidology of cheeses (initiators and leaders are Professor H.H. Dilanyan and L.A. Ostroumov) began to form in biotechnological research in milk production and dairy products in 70 years of the last century. Its development was promoted by the appearance of such research methods as gas-liquid and thin-layer chromatography, spectrophotometry, alkalimetry and others.

(2) Methodological framework for the implementation of the management ideas of lipolysis in cheese is created. It includes the development and modification of 17 original analytical methods of milk research, culture media, bacterial whey and starter cultures at different stages of cheese production. The main processes of lipolytic in milk are studied. It is shown that lipolysis scale depends on the presence of lipoprotein lipase in the milk and other lipolytic enzyme, which is the main producer of psychrophilic microorganisms. Lipid and fatty acid composition of different kinds of cheese is investigated. It is found that each of them is characterized by a certain content of ester compounds and their hydrolysis products. To assess the level of enzymatic catalysis of lipid fractions an integral indicator – ratio of fatty acid specificity – is proposed.

(3) We proved experimentally the ability of lactobacilli to produce enzymes of lipolytic orientation that hydrolyze triacylglycerols of milk fat forming diacylglycerols, monoacylglycerol and fatty acids. The degree of lipase and phospholipase activity of

Lactococcus and Lactobacillus has interspecific and intraspecific differences.

(4) We prove the existence of lipase and phospholipase activity in the majority of the representatives of propionic acid bacteria involved in the maturation of cheeses with a high temperature of second heating. The intensity of these symptoms at propionic acid bacteria is higher than at lactic acid bacteria.

(5) The hypothesis is put forward that there is a connection between specificity and degree of lipolytic activity of lactic and propionic acid bacteria used as starter cultures, and the intensity and direction of enzymatic hydrolysis of lipids in ripening cheeses. Selection of microflora in such a way is an effective quality management tool and the length of cheese ripening.

(6) The dependence of the formation processes of cheeses with a low temperature of second heating on the level of lipolytic activity of the lactic microflora starter cultures is set.

(7) We determined lipolytic activity of 12 different kinds of milk-products and proposed the criterion system of evaluation on this indicator of their suitability in cheese making.

(8) Original solution for the management process of cheese lipolysis is created, the novelty of them is protected by 15 copyright certificates for inventions (patents).

All above mentioned allows us to start the formation of Lipidomics postulates system form within Lactomics of dairy industry, starting with raw milk and its products in dairy products with a mandatory full and rational use of byproduct of raw milk-skim milk, milk whey and buttermilk. The legality of the implementation of systematic research is convincingly proved by Professor M.S. Umansky. The field of activity here is huge – all product groups and separate types of dairy products.

*To the blessed memory of Professor Mark Solomonovich Umanskiy
(04.07.1941 – 13.02 2011)*

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