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Biochemical Transformations of Lipide and Carbohydrat-Protein Nano Complex in Liquid Foodstuff

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Abstract The process of enzymatic hydrolysis of dairy whey at presence of a polyfermental preparation pancreatin was investigated. The process of hydrolytic transformations of dairy proteins, fats and carbohydrates in comparison of a parallel estimation of changes of the sizes of the lipid, carbohydrates-protein formations which are being in nano region of area was studied.

Keywords Protein, Lipid, Carbohydrates Nano Complex, Liquid Foodstuff

1. Introduction

Fibers, fats and carbohydrates are the major components of foodstuff. They form internal structure, both liquid, and firm food compositions, and, in case of liquid products what milk and dairy whey, formation of internal structures is, for example, is reduced basically to emulsify to the condition of substance formed under laws of colloid systems[1]. Dairy whey – a valuable food stuffs, especially for older persons, because of presence in it the serum fibers containing in the structure greater, than in casein, quantities of irreplaceable amino acids. These fibers are high-grade and are used by an organism for a structural exchange, therefore dairy whey draws the increasing attention as raw material for reception of functional food stuffs[2,3].

By manufacture of dairy whey in it half of dry substances of milk, including the most part of lactose and mineral substances passes on the average. The basic component of dry substances of dairy whey is lactose which mass fraction makes more than 70 % of dry substances[4]. During fermentation and the subsequent biochemical transformations of components, the structure of an albuminous and carbohydrate part of a product undergoes a number of the transformations affecting nutritional value. Presence of components of milk and the common biological properties of whey allow to carry it to valuable industrial raw material which can be processed in various useful components[5].

Traditional dairy products represent steady or unstable-colloid-lipidic systems with protein-carbohydrate environments. The sizes of the basic formations in liquid structure of fresh dairy products, make, as a rule, less than 100 nanometers, that formally allows to carry such objects to systems with nano particles[6].

As definition of dispersiveness of liquid nano systems is enough a complex experimental problem, was of interest to estimate the possible changes of nano clusters in a liquid product– whey of milk during known biochemical transformations of the basic most valuable components.

The purpose of work consist in a quantitative estimation of the sizes carbohydrates- protein nano clusters during biotransformation of dairy raw material at a pseudo-molecular level.

2. Materials and Methods

In work used the milk of integral cow fat content of 3,5 %, dairy whey containing (%): protein – 0,8, fat – 0,3, carbohydrates – 4,2, mineral salts – 0,38, dried up pancreatic hydrolyzates wheys after 8 and 24 h of hydrolysis.

We used the pharmacopeia pancreatin with the common proteolytic activity of 12500 U/mg, lipolytic activity of 1000 U/mg and amilolytic activity of 12000 U/mg[7].

For the control of the sizes carbohydrates-protein nano clusters used the *Rayleigh* scattering data allowing on spectral characteristics of disperse system to estimate turbodimetric the linear sizes of particles in liquid system[8].

For suspension with spherical particles it is possible to write The *Rayleigh* equation in the form of $I_\Sigma / I_o = 24\pi^3/\lambda^4 \cdot [(n_1^2 - n_2^2)/(n_1^2 + n_2^2)]^2 \cdot C_V \cdot V$, where: I_Σ– full intensity of light disseminated of 1 sm^3 of system in 1 *sec*; λ– length of a wave of light, *sm*; n_1 – a parameter of refraction of a disperse phase (it is equal 1,333); n_2 – a parameter of refraction of a dispersive phase (accepted equal as for lipide dairy fat $n_D^{20} = 1,5$); C_V – a volume fraction of a disperse phase; V –

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the volume of a particle, sm^3. Considering, that the turbidity $\tau = I_\Sigma / I_o$ is numerically equal to the light of energy disseminated of 1 sm^3 of a solution in all directions, and also that the Rayleigh equation is carried out for very diluted systems, $[\tau] = \lim (\tau / C_V)$, $C_V \to 0$ in work used the diluted water dispersions of 1:2000 … 1:10000 at = 546 nanometers for construction of dependences of ratio of τ / C_V from C_V. In the given coordinates extrapolation at $C_V \to 0$ established τ / C_V and further calculated approximate diameter of investigated particles how it is described in works[9,10].

The structure of carbohydrates (CH) was studied with use the BioLC chromatographic system including gradient pump GS50, electrochemical detector ED50, the the generator of eluent EG50 about 10 mm NaOH, chromatographic oven LC25 with column CarboPac PA20, manufactures DIONEX (Germany) [11,12]. Definition of the contents of free carbohydrates carried out in water extracts of 0,01 g of the sample (or 100 mkl of liquids) in 100 ml of HPLC water / 0,45mkm filter at 25℃. As standards of carbohydrates we used: arabinose (Ara, D-(−)-arabinose ≥99 %, A3131 Sigma), galactose (Gal, D-(+)- galactose ≥99 %, G0750 Sigma-Aldrich), glucose (Glc, D-(+)-glucose ≥99.5 %, G8270 Sigma), xylose (Xyl, xylose ≥99 %, G1270 Sigma), mannose (Man, D-(+)-mannose from wood, ≥99 % M2069 Sigma), fructose (Fru, D-(−)-Fructose ≥99%, F0127 Sigma), saccharose (Sug, α-D-Glc-(1→2)-β-D-Fru, sucrose ≥99.5% S9378 Sigma), ribose (Rib, D-(−)-ribose ≥99 % R7500 Sigma), lactose (Lac, β-D-Gal-(1→4)-α-D-Glc, α-lactose monohydrate L3625 Sigma-Aldrich), water solutions with concentration of 0,001 mg/ml.

The analysis of structure lipids was spent on modified *Folch* method[11,13] for this purpose the 1 − 5 ml of the whey, containing of 10 − 20 mg of lipids evaporated by means of using of the rotor evaporator dry and subjected the methylation. For comparison with lipid of milk the 20,0±0,1 ml of the cow milk mixed about 50 ml of a mix (1:1) of chloroform with methanol and further aliquot from a chloroformic layer subjected evaporation as is specified above.

For maintenance of completeness of definition of all fatty acids (FA), containing in lipid fractions the gas chromatography analysis, samples of the allocated fats subjected methylation on the modified method[11,14]. For this purpose 0,01 g of lipids in a mix of 15 % of a solution of 3 ml acetyl chloride in a metazero boiled at 100℃ during 2 h with the subsequent neutralization of a reactionary mix by addition of 1,25 ml of saturated solution of KOH of in CH_3OH up to pH 5.0 – 6.0. In a mix added 3 ml of saturated water solution of NaCl and 3 ml of hexane, mixed, defended during 30 mines and selected on the analysis of a solution of 0.5 ml from transparent hexane a layer containing methyl ethers of FA. Ethers analyzed on gas chromatograph HP6890 Hewlett-Packard (USA) with PID detector and capillary column HP-Innowax 30mmx0,32mmx0,5mkm in a current of nitrogen. The programmed rise in temperature with a speed 10℃·min^{-1} columns with 100 up to 260℃, injector and the detector – up to 250 and 300℃, input 1 mkl of tests, a stream of hydrogen from the generator – 35 ml· min^{-1}, nitrogen – 20 ml· min^{-1}, split mixture 1:100 was provided. Identification of peaks spent with use of the standard of FA (methy l) : *cis*-13,16- docosadienoate 2%, *cis*- 4, 7, 10, 13, 16, 19-docosahexaenoate 2%, *cis*-11,14-eicosadienoate 2%, *cis*-5, 8, 11, 14, 17- eicosapentaenoate 2%, *cis*-8, 11, 14- eicosatrienoate 2%, *cis*-11, 14, 17- eicosatrienoate 2%, *cis*-11- eicosenoate 2%, *cis*-10-heptadecenoate 2%, hexanoate 4%, γ -linolenate 2%, arachidate 4%, arachidonate 2%, behenate 4%, butyrate 4%, decanoate 4%, dodecanoate 4%, elaidate 2%, erucate 2%, heneicosanoate 2%, heptadecanoate 2%, linoleate 2%, linolelaidate 2%, linolenate 2%, myristate 4%, myristoleate 2%, oleate 4%, octanoate 4%, palmitate 6%, palmitoleate 2%, pentadecanoate 2%, *cis*-10-pentadecenoate 2%, stearate 4%, tricosanoate 2%, Supelco 47885U[15].

Quantitative calculation spent with use of the automatic program of processing of chromatographic data for $C_6 - C_{24}$ FA Winpeak (Germany). Identified in three repetition peaks FA which maintenance of exceeded of 0,01% from a total sum.

Free FA analyzed a usual method by distillation of about the ferry, reextraction of components into hexan with the subsequent analysis of structure to method of a gas chromatography similarly specified above, but from chromato-weights-spectrometer detector MSD 5975 under control of Agilent MSD ChemStation and performance of library search for the quantitative analysis on database NIST08.L of Agilent (USA).

Degree of hydrolysis of fiber estimated on change of nitrogen of free amino groups[16]. Analyzed of lyophilic dried up hydrolyzates in comparison with milk and dairy whey.

3. Results and Discussion

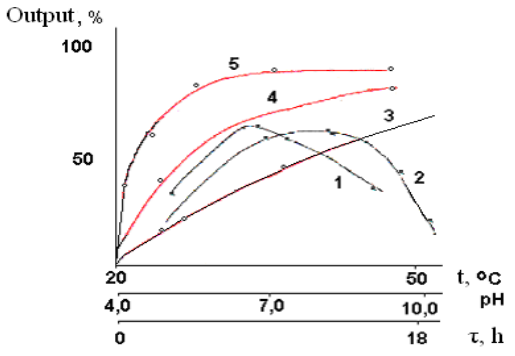

Figure 1. Dependence of a degree of hydrolysis of dairy whey from pH – 1, temperatures – 2, time of hydrolysis at concentration of pancreatin 0,5% – 3, 1% – 4, 2 % – 5

As is known, for improvement of biological properties of dairy whey apply the enzyme– galactosidase to transformation of lactose into more sweet both well soluble and acquired mix of monosugars (glucose and galactose), and also for hydrolysis of fibers up to peptide and amino acids (increase in biological value and quality – a transparency and absence of a deposit) proteolytic enzymes: protease *Actinomyces vulgaris*, tripsin, protophradin, pancreatine, chicken

pepsin, protoalbin, that provides 50−90% conversion of dairy fiber[17,18].

Earlier in our works, etc. researchers it has been shown, that pancreatine a pancreas of pigs and large horned livestock is the polyfermental preparation possessing the protease, lipase and amylase activity and actively participates in biotransformations of CH, fats and fibers in alive organisms[19–21].

On fig. 1 the curve of dependences of a degree of hydrolysis of dairy whey on parameters of process are presented. It is visible, that in case of used pharm. pancreatine the optimum degree of hydrolysis of albuminous components makes: pH 6.8, temperature 35–40℃, time 6 h at concentration of enzyme of 2% to weight of whey. In these conditions the degree of conversion of the basic components exceeds 80%.

In tabl. 1 data on hydrolyses are presented to disintegration of fibers of dairy whey. It is visible, that enzyme hydrolyzate of milk with a high degree of hydrolysis contains not only a greater share of free amino acids, but also parallel decrease in the sizes of dairy particles which as a result of hydrolysis decrease practically more, than twice is observed. The size of the size of particles which in dairy systems represent lipide carbohydrates-protein macrocomplexes, is important enough factor, especially at reception of functional foodstuff with the raised bioavailability in a treatment-and-prophylactic meal. With a high degree of dispersive ability of components, especially at a level nano, apparently, it is possible to use products for creation of substitutes traditional parenteral compoundings.

The research problem was attempt to track the transformation of nano claster, also interesting studying of change of a chemical compound of the basic components however was represented. As is known, in dairy products some hundreds FA which are connected in triglyceride, forming dairy fat contain[22–24].

However the basic is fat-acid structure includes no more than three tens FA, major of which (more than 80%) are palmitic, stearic and oleic (tabl. 2). The influence of polyfermental preparations with lipolytic activity leads to that the part of triglyceride breaks up with liberation corresponding FA. The traditional researches connected with hydrolysis of milk and dairy products are usually directed on transformation of dairy fibers and CH.

The studying of results of action lead by us of pancreatin on dairy components shows, that the basic lipide structure, both milk, and the dairy whey received from it are similar enough, however the mass fraction of free FA during enzyme processings increases. Traditionally processes connected with liberation of free FA simplistically supervise on so-called acid number which grows out the acid-core of titration driven away with water the ferry from analyzed tests of these acids. For natural foodstuff border of a condition of the validity of production, i.e. for fresh foodstuff in which the mass fraction of free FA makes less than 0.1–0.5 %, usually size of acid number (AN) makes 2–4 mg KOH/g lipid parts of the sample[11]. At processing dairy whey pancreatin, the size of AN monotonously increased with 1.8 up to 8.5 mg KOH/g that can testify that during enzyme processings of a dairy product by a polyfermental preparation there is also a hydrolysis of dairy fat to liberation of fat acids which are determined by the acid-core titration.

Table 1. The contents of the general and free amino acids in pancreatic hydrolyzates of milk

№	AA	Milk		Pancreatic hydrolyzates,6h		Pancreatic hydrolyzates, 24h	
		Sum AA, mg/100 g	Free AA, mg/100 g	Sum AA, mg/100 g	Free AA, mg/100 g	Sum AA, mg/100 g	Free AA, mg/100 g
1	Asp	389	1,5	8393	808	8712	1347
2	Thr	192	0,8	4200	1352	4100	2356
3	Ser	140	0,6	3100	1720	3050	2415
4	Glu	477	1,9	10256	2968	11336	5044
5	Gly	185	0,7	4020	224	3683	349
6	Ala	289	1,2	7067	1144	7212	16621
7	Cys	89	0,4	893	328	539	789
8	Val	115	0,5	2269	1648	2081	2214
9	Met	68	0,3	452	1280	356	1477
10	Ile	135	0,5	2911	2488	2430	3495
11	Leu	385	1,5	8306	5712	8739	7898
12	Tyr	144	0,6	3105	1576	2912	1366
13	Phe	180	0,7	4047	2456	3803	2857
14	His	119	0,5	2397	768	2818	1171
15	Lys	451	1,8	9840	5256	8243	7572
16	Arg	105	0,4	2086	2232	2652	2753
17	Pro	13	0,1	235	2264	2186	3194
Σ		3476	14	73577	34296	74852	62918
Conversion,%		–		45		84	
The average size of nano claster, nm		120		60		40	

Table 2. Fatty-acid composition

Designation of a fatty acid (FA)	Milk	Initial whey	Pancreatic hydrolyzates,6h	Pancreatic hydrolyzates,24h	Time of peak,min
Butyric C4:0	2,07	2,04	1,55	1,16	4,10
Caproic C6:0	1,53	1,46	1,04	0,71	4,42
Octanoic C8:0	2,04	1,92	1,72	0,92	5,14
Decanoic C10:0	3,22	3,12	2,83	0,84	7,39
Decenoic C10:1	0,36	0,23	0,26	0,12	7,68
Undecanoic C 11:0	3,3 1	0,15	2,53	2,43	8,42
Dodecanoic C 12:0	3,74	3,01	0,41	0,31	9,29
Tridecanoic C 13:0	0,25	0,24	0,12	0,11	10,65
Tetradecanoic C14:0	8,35	10,1	12,5	13,7	11,39
cis-9-Tetradecenoic C14:1	0,62	0,72	0,6	0,53	11,55
Pentadecanoic C15:0	3,13	3,13	3,05	3,21	12,37
cis-10-Pentadecenoic C15:1	0,42	0,54	0,32	0,53	13,02
Hexadecanoic C16:0	23,7	23,1	25,6	27,4	13,59
cis-9-Hexadecenoic C16:1	2,02	1,67	1,88	1,75	13,73
Heptadecanoic C17:0	2,11	1,49	1,46	1,25	14,31
cis-10-heptadecenoic C17:1	0,35	0,15	0,16	0,14	14,60
Octadecanoic C18:0	9,54	12,5	13,5	13,2	15,52
cis-9-Octadecenoic C18:1n9c	23,3	22,6	20,4	11,4	16,43
trans-9-Octadecenoic C18:1n9t	0,23	0,2	0,13	0,15	16,66
cis-9,12-Octadecadienoic C18:2 n6	3,61	4,2	1,53	2,53	16,97
cis-6,9,12-Octadecatrienoic C18:3 n6	0,52	1,05	1,14	1,27	17,95
cis-9,12,15-Octadecatrienoic C18:3 n3	0,63	0,35	0,46	0,32	18,35
Nonadecanoic C19:0	0,53	0,04	0,46	0,48	18,5
Eicosanoic C20:0	0,41	0,5	0,71	0,76	19,29
cis-9-Eicosenoic C20:1 n9	0,12	0,41	0,33	0,23	18,65
cis-11,14-Eicosadienoic C20:2 n6	0,12	0,17	0,15	0,09	20,20
cis-8,11,14-Eicosatrienoic C20:3n6	0,15	0,12	0,1	0,11	20,55
cis-11,14,17-Eicosatrienoic C20:3n3	0,05	0,03	0,03	0,02	21,50
cis-5, 8,11,14-Eicosatetraenoic) C20:4w6	0,36	0,34	0,66	0,45	21,75
cis-5,8,11,14,17-Eicosapentaenoic) C20:5w3	0,07	0,21	0,15	0,18	22,25
Heneicosanoic C21:0	0,05	0,04	0,11	0,15	22,87
Docosanoic C22:0	0,05	0,15	0,62	0,85	23,30
cis-13-Docosenoic C22:1n9	0,4	0,13	0,14	0,15	23,96
cis-13,16-docosadienoic C22:2 n6	0,1	0,08	0,06	0,06	25,1
cis-4,7,10,13,16,19-Docosahexaenoic) C 22:6 n3	0,1	0,14	0,12	0,15	26,01
Tricosanoic C23:0	0,06	0,11	0,15	0,14	26,25
Tetracosanoic C24:0	0,04	0,14	0,24	0,22	26,83
cis-15-Tetracosenoic C24:1	0,33	0,87	0,54	0,71	28,13
Not identified FA	5,32	2,55	2,24	11,27	4,0-30,0
In total	100	100	100	100	

Table 3. Change of the contents of the basic carbohydrates at pancreatic processing dairy whey (%)

Designation	Time of peak, min	Milk	Whey dairy	Pancreatic hydrolyzates, 6h	Pancreatic hydrolyzates,24h
Ara	5,2	0,025	0,0003	0,0190	0,011
Gal	6,8	0,0033	0,009	0,0185	0,006
Glc	7,4	0,013	0,021	0,0260	0,012
Xyl+ Man	8,5	0,026	0,031	0,0170	0,014
Fru+ Sucrose	9,8	0,0071	0,0087	0,0036	0,002
Rib	10,8	0,0035	0,004	0,0017	0,0006
Lac	17,2	4,5 (91% от Σ CH)	4,2 (97% от Σ CH)	1,53 (50% от Σ CH)	0,15 (33% от Σ CH)

The structure of flying FA differed from general structure FA in the sample a little, mass fraction of $C_{18} – C_{24}$ FA was on 34–45% less, than the fraction of dairy whey from which they have been received, that, apparently, speaks greater volatility the lowest FA.

From tabl. 2 it is visible, that the mass fraction of low fat acids C6 – C12 during biotransformation decreases, and the contents of higher limiting fat acids C14 – C22 increases. Presence of C4 – C8 FA in total amount more than 5 – 8% is prominent feature of dairy products and the cow milk and these FA, apparently are most subject to influence of enzymes. Transformations of nonsaturated fatty acids carry more complex, as a rule, extreme character. Similar character in change of is fat-acid structure of animals lipids a various origin, we observed of investigating process of storage of production on the basis of meat raw material[25,26].

In tabl. 3 the results of definition of amount of minor CH in processable products are presented.

Tabl. 3 shows the analysis of data, that a carbohydrate component dairy nano clasters, apparently, is the most unstable group of substances. Their contents in an analyzed mix during enzyme processings decreased practically in thirty times. Thus the mass fraction of the core dairy disaccharide – lactoses decreased from 91–97 % more, than three times that can specify sufficient activity of pancreatin during hydrolysis of dairy sugars.

4. Summary

Thus, the enzyme processings of dairy components leads to degradation of all making substances of milk and change of their componental structure with simultaneous reduction of the sizes of lipid, carbohydrate and protein formations, which size are in nano scale areas.

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