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 bio-transformation 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 turbidimetric 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 $I_\Sigma = I_o \frac{24\pi^3 I^2}{\lambda^4 \left( \frac{n_1^2 - n_2^2}{n_1^2 + n_2^2} \right)^2 \cdot C_v \cdot V}$, where: $I_\Sigma$– full intensity of light disseminated of 1 sm³ of system in 1 sec; $\lambda$– 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 disperse phase (accepted equal as for lipide dairy fat $n_{20}^{\text{DL}} = 1,5$); $C_v$ – a volume fraction of a disperse phase; $V$ –
the volume of a particle, $\text{sm}^3$. Considering, that the turbidity $\tau = I_o / I$ is numerically equal to the light of energy disseminated of 1 $\text{sm}^3$ 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 $1:2000 \ldots 1:10000$ at $= 546$ nanometers for construction of dependences of ratio of $\tau / C_V$ from $C_V$. In the given coordinates extrapolation at $C_V \to 0$ established $\tau / C_V$ 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 generator of free amino groups[16]. Analyzed of lyophilic dried up hydrolyzates in comparison with milk and dairy whey. 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 chromatogramspectrometer detector MSD 5975 under control ofAgilent 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 $\text{pH}$ – 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 a capacity of 1 ml 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 (methyl) : 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- eicosenoate 2%, cis-10-heptadecenoate 2%, heptadecanoate 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%, tricosenoate 2%, Supelco 47885U[15].

Quantitative calculation spent with use of the automatic program of processing of chromatographic data for $C_6 – C_{34}$ 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 chromatogramspectrometer 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).

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

Earlier in our works, etc. researchers it has been shown, that pancreatin 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 enzyme hydrolysis of dairy fat to liberation of fat acids 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 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.

| №   | 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 |
| 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,%

The average size of nano claster, nm

Table 1. The contents of the general and free amino acids in pancreatic hydrolyzates of milk.
### 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 C11:0                | 3.3  | 0.15         | 2.53                        | 2.43                          | 8.42             |
| Dodecanoic C12:0                | 3.74 | 3.01         | 0.41                        | 0.31                          | 9.29             |
| Tridecanoic C13:0               | 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-Octadecanoic C18:1n9c     | 23.3 | 22.6         | 20.4                        | 11.4                          | 16.43            |
| trans-9-Octadecanoic C18:1n9h   | 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 C20:3 n6 | 0.15 | 0.12      | 0.1                         | 0.11                          | 20.55            |
| cis-11,14,17-Eicosatrienoic C20:3 n3 | 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| 0.0009     | 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% or Σ CH) | 4.2 (97% or Σ CH) | 1.53 (50% or Σ CH) | 0.15 (33% or 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 $C_6 – C_{12}$ during biotransformation decreases, and the contents of higher limiting fat acids $C_{14} – C_{22}$ increases. Presence of $C_4 – C_8$ 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].

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.

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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