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THE DETERMINATION OF THE BASE MATRIX OPTIMAL COMPOSITION WITH USE OF TEST ORGANISMS *TETRAHYMENA PYRIFORMIS*

Abstract. The use of accelerated biotesting in combination with chemical-analytical and microbiological methods allows evaluating quality and safety of dairy products fully and reliably without significant increase in costs. The relative biological value and the optimal range of fat and protein mass fractions ratio in normalized milk mixtures amounted to 0.36 - 0.89 were determined by means of the biotest analysis express method on protozoa. Milk normalization by fat mass fraction in indicated range contributed to the achievement the maximum relative pasteurized milk biological value (162±7) %. The efficiency protein assimilation was higher in the presence of a certain amount of fat. It was established the influence of methodological techniques during experimental work, in particular, the degree of samples dilution (0.05; 0.15; 0.3 mg nitrogen/ml) on the dynamics of number of test cultures depending on fat mass fraction in the medium was established and was selected the optimal milk dilution for testing up to 0.15 mg nitrogen/ml.

Key words: normalized milk, *Tetrahymena pyriformis*, relative biological value.

Introduction. Multicomponent food formulations development is aimed at ensuring optimal composition and ratio of the main components. At the same time, biological value and good product quality are undoubtedly one of the main criteria for formulations optimality and process efficiency. Formulation optimization is associated with restrictions introduction on finished product composition (for certain types of raw materials and ingredients used, protein, fat, carbohydrates content, etc.).

It is known that the quantitative proteins and fats ratio in product composition affects the digestibility of both. Fat and protein in diet should be balanced, because while reducing fat intake, the body uses excess protein for energy purposes. Fat lack in diet leads to energy malnutrition, exacerbates proteins deficiency in food: proteins begin spending on covering body's energy consumption. In addition, the protein digestion, received with food is worsening, protein metabolism products are accumulating - uric acid, etc., the acid-base balance is shifted to acid side [1].

The ratio of protein, fat and carbohydrates plays major role in food products biological value formation, which necessitates scientific approach to rational use of these nutrients in high-quality food products formulations development. The most reliable method of product quality integral assessment from nutritional point of view is biological method using higher animals [2, 3], application of which is very difficult for products continuous monitoring, raw materials evaluation, various processing methods and new technologies.

Recently, several authors [4–10] have developed the concept of "whole cell biosensors" (WCB) as an alternative to classical methods. Prokaryotic or eukaryotic cells are used as biosensors. Previous studies have shown that eukaryotic ciliates cells are similar to higher organisms in terms of genome and number of basic metabolic parameters [11,12], which allows interspecific extrapolation of assessing food products biological value results [13-15].

The *Tetrahymena pyriformis* ability to use intact proteins as nutrient substrate has practical importance in assessing food product biological value, and correlation coefficient of experimental results when comparing *T.pyriformis* ciliates and white rats is 0.93–0.98 and is estimated as high. The bioavailability of food product proteins in this case is characterized by rate of vital processes of indicator organism depending on food object quantity and quality, which can be estimated by increase dynamics in ciliates number [16].

Statement of the problem. The aim of the work is to study methodological aspects of accelerated dairy products biological assessment using test organisms *Tetrahymena pyriformis*. Based on the results of applying express method to determine indicators of dairy products relative biological value, it is planned to develop methodology for assessing technological processes effectiveness for dairy products production, which will exclude an empirical approach to biological value formation developed products.

The subject of this study was to determine effect of fat and protein ratios in the food matrix on dairy products relative biological value.

Methods. The object of the study was pasteurized normalized milk with fat mass fractions of 0.05% ч 8.0%; protein mass fractions 2.8% ч 3.6%. The normalized milk with various fat and protein mass fraction ratios (from 0.02 to 2.7:1) was obtained by mixing skimmed milk and cream. Normalized milk was homogenized at temperature of 60 °C and pressure of (17.5 ± 2.5) MPa, pasteurized at (92 ± 2) °C, cooled and prepared dilutions. The following parameters were measured in the normalized milk samples: the fat – by Gerber butyrometric method (ISO 2446:2008); the total protein - by Kjeldahl method with use of KJELTEC automatic system (ISO 8968-1:2014). The milk with various fat mass fraction was investigated in dilutions providing the amount of milk protein in the medium in terms of nitrogen: 0.05; 0.15 and 0.3 mg/ml. Reference samples in the analysis were samples containing skimmed milk.

The experiments repetition is 3-4 times. The obtained experimental data were processed by the methods of mathematical statistics (regression analysis) using applied service programs (Statistica, Mathcad). The confidence level of probability was taken equal to 0.95 with relative error of ±5%.

Biological experiment at *Tetrahymena pyriformis*. Comparative biological studies of the product were performed by biotesting methods on test organisms *Tetrahymena pyriformis* [17-22]. Cell counts were performed using the BioLaT-3 analytical complex (Europolitest LLC) according to the Operation Manual. The ciliates were counted 10 times in 0.02 ml of culture medium from each tube. Each milk sample was examined in triplicate. For biotesting, a less dense, pure tetrachimen culture was used (in the range of 50-100 thousand cells per 1 ml).

A pure culture of ciliates was subcultured into bacteriological tubes with 4 cm^3 peptone medium (2.0 g of peptone, 0.1 g of yeast extract, 0.5 g of glucose, 0.1 g of sodium chloride, distilled water (pH 7.1) up to 100 ml) every 7-10 days. Cultivation was carried out for 3 days in an incubator at temperature of 25 °C, shaking 2-3 times a day. Before culture introducing, milk samples were diluted with distilled water. In 1 ml of diluted milk, 1 ml of CSY (carbohydrate-salt yeast) medium was added (1.5 g of glucose, 0.1 g of yeast extract, 0.1 g of sodium chloride, distilled water (pH 7.1) up to 100 ml). Tubes with prepared samples were heat treated to inactivate microflora. After cooling to (25±2) °C, 0.02 ml of a 3-day-old tetrachimene culture, grown on peptone medium was added to each tube. The ciliates were cultured for 4 days in an incubator at 25 °C until stage of the stationary growth phase, shaking periodically 2-3 times a day. After 96 hours of cultivation, one drop of 5% alcohol solution of iodine was added to the vials, thoroughly shaken, diluted 2-4 times with water, and the grown cells were counted taking into account the dilution.

Relative biological value (RBV) was determined by cells number ratio, grown on test product to the ciliates number, grown on the control product, expressed as a percentage. Protein efficiency ratio (PER) was determined as ratio of the gain in live weight of test organisms to consumed protein, the protein relative effectiveness ratio (PRE) as ratio of protein efficiency of the experimental product to protein effectiveness of the control product, expressed in % (PRE_e x 100/PRE_c).

Results. A visual analysis of *T.pyriformis* population state, grown in cultivation media based on the studied milk did not reveal noticeable differences in these populations, as well as any morphological and functional disorders. Organisms death during 4-day cycle of population study was not observed.

Table 1 shows the results, obtained when testing pasteurized milk samples with different fat content and diluting samples to 0.05 mg nitrogen/ml.

From table 1 data it follows that in medium with 0.05 mg nitrogen/ml with an increase in fat mass fraction in range of 1-1.7%, the ciliates number increases by 2-2.3 times. In interval of fat mass fraction of 1.7-3.3%, an increase in test organisms was observed and, accordingly, an increase in RBV by 2.3-2.8 times. In range of fat mass fraction of 3.3–8%, the difference between their average abundance in almost all samples was statistically insignificant. The obtained results indicate that this concentration of protein in absence of fat in medium significantly limits reproduction of protozoa. The population increases when certain fat amount appears in culture medium. Thus, the use of milk dilution to protein concentration in terms of nitrogen of 0.05 mg/ml does not provide test organisms with sufficient amount of nutrients in all samples.

Table 1 – The number of *Tetrahymena pyriformis* cells grown on normalized milk with different ratio of fat and protein mass fractions, and RBV indicators (0.05 mg nitrogen/ml of medium)

i – number of factor level	Fat, %	The ratio of fat: protein in normalized milk, factor x	The number of living cells, parameter y (the number of repetitions of experience, n = 3)	Standard deviation, σ	Average error of the mean	RBV, %
1	0.05	0.02	2346	14	8	100
2	1.0	0.3	4668	913	528	198
3	1.7	0.6	5362	56	32	229
4	2.6	0.8	6381	110	64	272
5	3.3	1.1	6596	71	41	281
6	3.6	1.2	6563	244	141	280
7	4	1.3	5682	524	303	242
8	5	1.7	6548	789	456	279
9	6.6	2.3	6586	397	229	281
10	8	2.7	6431	131	76	274

Table 2 shows results, obtained when testing pasteurized milk samples with different fat content and diluting samples to nitrogen mass fraction of 0.3 mg/ml.

From the data of table 2 it follows that when diluting milk to 0.3 mg/ml in terms of nitrogen, nutrients concentration in medium did not limit growth of ciliates in all samples. At the same time, milk fat did not play a significant role in overall metabolism, judging by the fact that with an increase in its content in medium, change in cells number did not exceed the maximum standard deviation. Thus, milk dilution to 0.3 mg N/ml in conditions of our experiment did not allow us to reveal the effect of fat mass fraction in environment for protozoa growth.

Table 2 – The number of *Tetrahymena pyriformis* cells grown in normalized milk with different ratio of fat and protein mass fractions, and indicators of RBV (0.3 mg nitrogen/ml of medium)

i – number of factor level	Fat, %	The ratio of fat: protein in normalized milk, factor x	The number of living cells, parameter y (the number of repetitions of experience n = 3)	Standard deviation, σ	Average error of the mean	RBV, %
1	0.05	0.02	6526	1498	866	100
2	1.0	0.3	6142	774	447	94
3	1.7	0.6	5854	392	227	90
4	2.6	0.8	6426	158	91	98
5	3.3	1.1	5047	499	288	77
6	3.6	1.2	6450	665	384	99
7	4	1.3	6471	519	300	99
8	5	1.7	7147	80	46	110
9	6.6	2.3	6672	60	35	102
10	8	2.7	6583	267	154	101

Table 3 shows results, obtained by testing pasteurized milk samples with various fat mass fractions and diluting samples to nitrogen content of 0.15 mg/ml. Judging by total cells number, grown in media with different milk concentrations, dilution to 0.15 mg N/ml contributed to the best growth of test culture. From the data of table 3 it follows that in an environment with milk diluted to 0.15 mg N/ml, with an increase in fat content, the number of protozoa increases by 1.5 times, and with ratio of fat and protein mass fractions 0.3ч1.1 exceeds cells number of grown in medium with high milk protein content (0.3 mg N/ml). An increase in fat: protein ratio to 1.2 and higher led to decrease in grown cells number. This allows to conclude that protein use efficiency is higher in presence of certain fat amount.

Table 3 – The number of *Tetrahymena pyriformis* cells grown on normalized milk with different ratio of fat and protein mass fractions, and indicators of RBV (0.15 mg nitrogen/ml of medium)

i – number of factor level	The ratio of fat: protein in normalized milk, factor x	The number of living cells, parameter y (the number of repetitions of experience n = 4)	Standard deviation, σ	Average error of the mean	RBV, %
1	0.02	4714	1509	755	100
2	0.3	6929	932	466	147
3	0.6	7784	656	328	165
4	0.8	7518	499	250	159
5	1.1	7312	640	320	155
6	1.2	6350	210	105	135
7	1.3	6318	557	279	134
8	1.7	6606	105	53	140
9	2.3	6604	505	253	140
10	2.7	6475	1538	768	137

The values of PER and PRE for various dilutions were also calculated and analyzed (table 4, figure 1).

Table 4 – The number of test culture cells, grown on normalized milk with different ratio of fat and protein mass fractions and PER, PRE indicators

The ratio of fat: protein in milk	Milk dilution to mg nitrogen/ml:					
	0.05		0.15		0.3	
	The number of cells, units	PER/PRE, %	The number of cells, units	PER/PRE, %	The number of cells, units	PER/PRE, %
0.02	2346	0.28/100	4312	0.17/100	5466	0.11/100
0.3	4002	0.47/170	6589	0.26/153	6689	0.13/118
0.6	5322	0.63/228	7685	0.3/176	6131	0.12/109
0.8	6459	0.76/275	7402	0.29/171	6314	0.12/109
1.1	6546	0.77/279	7206	0.28/165	4694	0.09/82
1.2	6735	0.79/286	6592	0.26/153	5980	0.12/110
1.3	5311	0.62/225	6642	0.26/153	6838	0.13/118
1.7	5990	0.7/254	6663	0.26/153	7203	0.14/127
2.3	6305	0.74/268	6360	0.25/147	6714	0.13/118
2.7	6524	0.77/279	6656	0.26/153	6394	0.13/118

From the data in table 4 and figure 1, it can be seen that with an increase in samples dilution degree, the values of PER and PRE are increase. When milk is diluted to 0.3 mg nitrogen/ml, the values of PER and PRE are minimal and increase to 1.0–8.0% in presence of fat in 1.1ч1.3 times. Correlation with fat mass fraction is practically absent, which indicates protein excess in the medium and, obviously, insignificant fat consumption.

When milk is diluted to 0.05 mg nitrogen/ml, the values of PER and PRE were maximum and most dependent on fat presence in medium. At the same time, the number of cells, grown in medium with

comparison sample (skimmed milk) was the lowest and with an increase in fat mass fraction in the medium it increased most significantly (1.7ч2.8 times). With further fat mass fraction increase (over 3.6%), the values of PER and PRE are slightly decreased. Obviously, protein lack in the medium was compensated by consumption of fat certain proportion.

The use of milk dilution up to 0.15 mg nitrogen/ml made it possible to reveal a certain dependence of PER and PRE on fat mass fraction in the medium, maximally expressed at a ratio of fat and protein mass fraction of 0.3ч1.1.

Thus, dilution of milk for testing up to 0.15 mg nitrogen/ml provides a fairly balanced intake of protein and fat by infusoria in all samples, which allows to identify the effect of their ratio on growth of test organisms and determine its optimum.

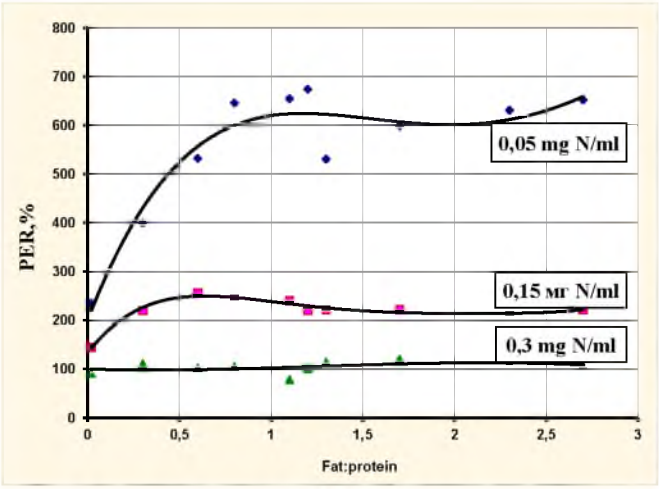

Figure 1 – PER dependence on the ratio of fat and protein mass fraction in normalized milk when using different dilutions

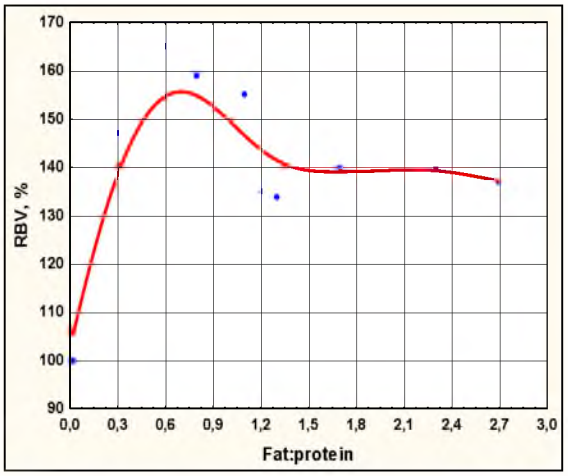

Figure 2 – Dependence of normalized milk RBV on the ratio of fat and protein mass fraction

Considering the obtained data, further studies were carried out using samples dilution to protein content in terms of nitrogen of 0.15 mg/ml.

Analysis of the data in table 3 for presence of gross errors showed, that calculated values of maximum relative deviation are less than table quantile value distribution of maximum relative deviation, therefore, there are no grounds for dropping out the extreme results of the sample.

The formalization of obtained experimental data (Table 3) by least-squares method allowed obtain an empirical mathematical dependence:

$$y = 239.65x^5 - 2927.5x^4 + 12059x^3 - 20942x^2 + 14284x + 4388.4$$

Determination factor R^2 = 0.923; standard error of the regression equation – 354.3. $F_{fact.}$ = 9.6; $F_{theor.}$ (0.05, 5, 4) = 5.2. Since $F_{fact.} > F_{theor.}$, the equation adequately describes the identified dependence.

Thus, a close relationship was found between the number of grown test organisms and the ratio of fat and protein mass fraction in culture medium and nonrandomness of their joint change.

Based on obtained experimental data, the RBV of normalized milk was calculated. The dependence of milk RBV on the ratio of fat and protein mass fraction was correspondingly similar (figure 2).

Regression equation:

$$y = 5.18x^5 - 62.69x^4 + 256.76x^3 - 444.3x^2 + 302.46x + 93.14$$

where y – RBV, %; x – the ratio of fat and protein mass fraction in normalized milk.

R^2 = 0.925. Standard error of the regression equation – 7.36. $F_{fact.}$ = 9.97; $F_{theor.}$ (0.05, 5, 4) = 5.2. Since $F_{fact.} > F_{theor.}$, the equation is adequate.

Determination of maximum function:

$$dy/dx = 25.9x^4 - 250.76x^3 + 770.28x^2 - 880.6x + 302.46 = 0.$$

When x_o = 0.6 maximum function value y_{max} = 162±7. Given the calculation error, the optimal value x_o = 0.36ч0.89.

Conclusion. During experimental work, the influence of samples dilution degree on dynamics of *Tetrahymena pyriformis* test culture number dependence on the ratio of fat and protein mass fraction in the medium and the value of protein efficiency coefficient was established. The optimal milk dilution for testing was selected - up to 0.15 mg nitrogen/ml. By accelerated biotesting method, the indicators of relative biological value and optimal ratio of fat and protein mass fraction in normalized milk mixtures were determined, intended for whole milk products production, amounting to 0.36ч0.89, contributing to an increase in their relative biological value to (162±7) %. The obtained data will allow to evaluate the effectiveness of technological regimes for whole milk products production and to choose the optimal parameters that provide high degree of products usefulness that contribute to solving the problem of maintaining human health. It should be noted that the obtained values of protein efficiency coefficient did not characterize protein biological value. Obviously, to characterize protein biological value, it is advisable to use nitrogen metabolism coefficient as the ratio of consumed nitrogen amount by test organisms to amount of total nitrogen introduced into medium. To determine the long-term effect of medium composition on the nature of tetrachimene growth curve and culture life span, longer observations are required.

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БАЗАЛЫҚ МАТРИЦАНЫҢ *TETRAHYMENA PYRIFORMIS* ТЕСТІН ҚОЛДАНУ АРҚЫЛЫ ҚҰРАМЫН ОҢТАЙЛЫ АНЫҚТАУ

Аннотация. Нутрициологияның дамуы мен азық-түлік нарығындағы бәсекелестіктің артуы өнімді түзету және жаңа рецептура әзірлеу арқылы өнім түрлерін үнемі жақсарту қажеттілігіне әкеледі. Көп компонентті тамақ құрамын жасау негізгі компоненттердің оңтайлы құрамы мен арақатынасын қамтамасыз етуге бағытталған. Сонымен қатар, оңтайлылықтың негізгі өлшемдерінің бірі өнімнің биологиялық құндылығы мен жоғары сапасы болып саналады.

Рецептураны оңтайландыру дайын өнімнің құрамына шектеу енгізуге байланысты (шикізат пен қолданылатын ингредиенттердің белгілі бір түрлері, ақуыз, май, көмірсулар және т.б.). Ақуыз, май және көмірсудың қатынасы тамақ өнімінің биологиялық құндылығын қалыптастыру барысында үлкен рөл атқарады әрі жоғары сапалы тағам рецептурасын жасауда осы қоректік заттарды ұтымды пайдалануға ғылыми көзқарасты қажет етеді.

Соңғы уақытта классикалық әдістерге балама ретінде прокариоттық немесе эукариотты жасушаларды қолданатын «тұтас жасушалы биосенсорлар» (WCB) тұжырымдамасы жасалды. Алдыңғы зерттеулер көрсеткендей, эукариотты инфузорий жасушалар геномы және бірқатар негізгі метаболиттік көрсеткіштері бойынша жоғары ағзаларға ұқсас, бұл тамақ өнімдерінің биологиялық құндылығын бағалау нәтижелерін спецификалық экстраполяциялауға мүмкіндік береді. Азық-түлік өнім ақуыздарының биологиялық құндылығы тағам нысанының саны мен сапасына байланысты индикаторлар ағзасының тіршілік әрекетінің маңызды үрдістерінің жылдамдығы негізінде сипатталады, оны инфузорий санының көбею динамикасымен бағалауға болады.

Аталған зерттеудің пәні сүт өнімдерінің салыстырмалы биологиялық құндылығына өнім матрицасындағы май мен ақуыздың қатынасының әсерін анықтау болып саналады.

Зерттеу нысаны ретінде май мен ақуыздың массалық үлесі 0,02-ден 2,7: 1-ге дейін пастерленген қалыпқа келтірілген сүт алынды.

Құрамындағы түрлі массалық үлесі бар сүттің ақуыз мөлшерін 0,05; 0,15 және 0,3 мг/мл. азотпен қайта есептеу арқылы сұйылтыла отырылып зерттелді. Талдауда салыстыру үшін құрамында майсыздандырылған сүті бар үлгілер бар сынамалар қолданылды.

Өнімді салыстырмалы биологиялық зерттеулер *Tetrahymena pyriformis* тест-ағзасымен биотесттеу әдісі арқылы жүргізілді. Тест-ағзаларының саны мен салыстырмалы биологиялық құндылықты (СБҚ) пайызбен өрнектелетін сынақ үлгілерімен бір ортада өсірілген жасуша санының салыстырылатын үлгілерде өсірілген инфузорий мөлшерінің қатынасымен анықтадық.

Тәжірибелік жұмыс барысында оқыту әдістерінің, атап айтқанда, ортадағы майдың массалық үлесіне байланысты сынама дақылдар санының динамикасына сұйылтылу дәрежесі (0,05; 0,15; 0,3 мг азот / мл)

анықталды. Ақуызды ассимиляциялаудың тиімділігі белгілі бір май мөлшерінің қатысуы негізінде жоғарылады. Сынаққа қажетті сүтті 0,15 мг азот /мл дейін сұйылту жағдайы барлық үлгілерде инфузориядан ақуыз мен майдың жеткілікті теңгерімді қабылдауын қамтамасыз етті, бұл олардың арақатынасының тест-ағзаның өсуіне әсерін анықтауға мүмкіндік береді.

Тәжірибелік мәліметтерді минималды квадраттар әдісімен ресімдеу тестік дақылдардың өлшемі, салыстырмалы биологиялық құндылық СБҚ және өнім матрицасының құрамы арасындағы қатынасты дәл сипаттайтын эмпирикалық математикалық тәуелділікті алуға мүмкіндік берді (анықтау коэффициенті 0,92). Функцияны саралай отырып, қалыпқа келтірілген сүт қоспаларында май мен ақуыздың массалық фракцияларының оңтайлы арақатынасы 0,36-0,89 құрады. Көрсетілген диапазонда майдың үлес салмағы бойынша сүттің нормалануы пастерленген сүттің максималды салыстырмалы биологиялық құндылығына (162±7) % қол жеткізуге ықпал етті.

Жеделдетілген биотестілеуді қолдану химиялық-аналитикалық және микробиологиялық әдістермен бірге шығын өсуінсіз сүт өнімдерінің сапасы мен қауіпсіздігін негұрлым толық және сенімді бағалауға мүмкіндік береді. Алынған мәліметтер бізге сүт өнімдерін өндірудің технологиялық режимдерінің тиімділігін бағалауға және адамның денсаулығын сақтау мәселесін шешуге ықпал ететін өнімдердің жоғары дәрежелі пайдалылығын қамтамасыз ететін оңтайлы көрсеткіштерді таңдауға септігін тигізеді.

Түйін сөздер: қалыпқа келтірілген сүт, Tetrahymena pyriformis, салыстырмалы биологиялық құндылық.

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ОПРЕДЕЛЕНИЕ ОПТИМАЛЬНОГО СОСТАВА БАЗОВОЙ МАТРИЦЫ С ИСПОЛЬЗОВАНИЕМ ТЕСТ-ОРГАНИЗМОВ *TETRAHYMENA PYRIFORMIS*

Аннотация. Развитие нутрициологии, усиление конкуренции на продовольственном рынке приводит к необходимости постоянного совершенствования ассортимента выпускаемой продукции путём коррекции существующих и разработки новых рецептур.

Разработка многокомпонентных рецептур пищевых продуктов имеет целью обеспечение оптимального состава и соотношения основных компонентов. При этом одним из основных критериев оптимальности, несомненно, является биологическая ценность и доброкачественность продукта. Оптимизация рецептур связана с введением ограничений по составу готового продукта (по отдельным видам используемого сырья и ингредиентов, содержанию белка, жира, углеводов и пр.). Соотношение белка, жира и углеводов играет основную роль в формировании биологической ценности пищевых продуктов, что обуславливает необходимость научного подхода к вопросам рационального использования этих нутриентов при разработке рецептур высококачественных пищевых продуктов.

В последнее время в качестве альтернативы классическим методам была разработана концепция «цельноклеточных биосенсоров» (WCB), в качестве которых используются прокариотические или эукариотические клетки. Проведенными ранее исследованиями показано, что эукариотические клетки инфузорий имеют сходство с высшими организмами по геному и ряду основных параметров обмена веществ, что допускает межвидовую экстраполяцию результатов оценки биологической ценности пищевых продуктов. Биологическая доступность белков пищевого продукта при этом характеризуется скоростью протекания процессов жизнедеятельности индикаторного организма в зависимости от количества и качества пищевого объекта, что может быть оценено по динамике прироста количества инфузорий.

Предметом данного исследования являлось определение влияния соотношений жира и белка в продуктовой матрице на относительную биологическую ценность молочных продуктов.

Объектом исследования являлось молоко, нормализованное, пастеризованное с соотношением массовых долей жира и белка от 0,02 до 2,7:1.

Молоко с различной массовой долей жира исследовали в разведениях, обеспечивающих количество молочного белка в среде в пересчете на азот: 0,05; 0,15 и 0,3 мг/мл. Образцами сравнения в анализе служили пробы, содержащие обезжиренное молоко.

Сравнительные биологические исследования продукта выполняли методами биотестирования на тест-организмах *Tetrahymena pyriformis*. Определяли количество тест-организмов и относительную биологическую ценность (ОБЦ) по отношению количества клеток, выросших на среде с опытными образцами, к количеству инфузорий, выросших на образцах сравнения, выраженному в процентах.

В ходе экспериментальных работ установлено влияние методических приемов, в частности, степени разведения проб (0,05; 0,15; 0,3 мг азота/мл) на динамику численности тест-культуры в зависимости от массовой доли жира в среде. Эффективность ассимиляции белка была выше в присутствии определенного

количества жира. Разведение молока для тестирования до 0,15 мг азота/мл обеспечивало достаточно сбалансированное потребление белка и жира инфузориями во всех образцах, позволяющее выявить влияние их соотношения на рост тест-организмов. Формализация экспериментальных данных методом наименьших квадратов позволила получить эмпирические математические зависимости, адекватно описывающие взаимосвязь численности тест-культуры, ОБЦ и состава продуктовой матрицы (коэффициент детерминации 0,92). Путем дифференцирования функции был определен диапазон оптимального соотношения массовых долей жира и белка в нормализованных молочных смесях, составивший 0,36-0,89. Нормализация молока по массовой доле жира в указанном диапазоне способствовала достижению максимальной относительной биологической ценности пастеризованного молока (162±7) %.

Применение ускоренного биотестирования в сочетании с химико-аналитическими и микробиологическими методами дает возможность более полной и достоверной оценки качества и безопасности молочных продуктов без существенного увеличения затрат. Полученные данные позволят оценить эффективность технологических режимов производства цельномолочных продуктов и выбрать оптимальные параметры, обеспечивающие высокую степень полезности продукции, способствующей решению проблемы сохранения здоровья человека.

Ключевые слова: нормализованное молоко, *Tetrahymena pyriformis*, относительная биологическая ценность.

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