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CHEMICAL ANALYSIS OF SOFT MOLDY CHEESE REPINED WITH *Penicillium caseicolum*

Abstract. The influence of the *Penicillium caseicolum* mold to various concentrations on the process of moldy cheese ripening is studied. Analysis of mineral substances by using Mass spectrometry with inductively coupled plasma (ICP-MS) shows the increasing content of macro-elements such as: Mg, P, K, Ca and micro-elements: Fe, I, Mn, Cu, Se, Zn, Mo. Organoleptic characteristics in comparison with the requirements of GOST 33630— 2015 *Russian Camembert*, in particular: taste, odor, color, appearance, pattern and texture confirm that the obtained cheese corresponds to a soft moldy cheese. The taste of the studied cheese has a clean sour taste with a slightly mushroom flavor and a slight bitter taste, with milky-white and slightly creamy appearance. The microflora surface is properly distributed over the surface and covered with velvety edible white moldy rind. The taste is pleasant, fresh, mild and creamy with a delicate, homogeneous consistency throughout a whole mass. The developed moldy cheese has a number of advantages over the famous analogues: short ripening period, the ability to provide high levels of "biological value of the product", the presence of all essential amino acids.

Key words: bacteria, cheese, microflora, structure, mold, macro - and micro-elements, organoleptic assessment.

Introduction

Cheese is a common food product with a high nutritive value and pleasant organoleptic properties. Its wide range and unique composition makes this product highly demanded by all segments of the population [15].

Since ancient times, cheese has been an important component of human diet in many parts of the world. Cheese is a food product rich in protein, fat, minerals, and vitamins (all in general ten times more concentrated in cheeses than in milk), besides containing also biologically active amino acids, peptides, and lactic acid bacteria (LAB) that may be probiotic. New consumer's health perception is leading to development of new types of cheeses such as low-fat, low-salt, and probiotic cheeses. The microbial flora present from the formation of the curds to the ripened cheese, either as LAB starter culture or as nonstarter culture, associated with technological processing specificities and other milk native enzymes play a key role in the complex and dynamic biochemical process that leads to the varieties of the existing cheese types. Commonly, cheeses may be classified based on texture as 'soft,' 'semihard,' or 'hard' cheese or simply based on their origin and processing method as 'industrial' or 'traditional cheeses [8].

Probiotics are live microorganisms of LAB, most often LAB and bifidobacteria that belong to the natural human microflora, formed in the process of evolution. For the enhancing the growth of beneficial microflora, often prebiotics are used [16]. So, for the improving of the some physicochemical, microbiological and sensory properties, authors offer to use for fresh and matured soft cheeses - a mixture of cow milk and plant origin additives such as lupin milk that has a high content of the proteins. The incorporation of lupin milk at low concentration (25 mL/100 mL) significantly ($P \leq 0.05$) enhanced the taste, texture, flavor, and overall acceptability of both fresh and mature cheese [1].

Mold-ripened cheeses are divided into two distinct groups: surface-ripened cheeses (i.e., Camembert, Brie, Carrè de l Est ripened by Penicillium camemberti) and interior-ripened cheeses (i.e., blue cheeses,

Roquefort, Stilton, Danablu, Gorgonzola, Edelpikäse and Mycella cheeses where Penicillium roqueforti grows in the curd fissures) [11].

In the work [3] authors have studied the influence of three different variables on the sensory quality of Camembert-type cheese: type of lactic bacteria, type of ripener molds and inoculation method. Batches of Camembert-type cheese were produced using O or DL-type mesophilic starter culture, ripened with white *Penicillium camemberti* and *Penicillium candidum (or Penicillium caseicolum)* and mold inoculation was made directly into the milk or by spraying. These results demonstrate, therefore, that the combination of different ripener molds, inoculation methods and starter cultures directly influences the sensory quality of Camembert-type cheese, modifying significantly its texture, appearance, aroma and taste [2, 9, 18] .

According to the various sources, the growing demand has cheeses with a spicy and rich taste [4, 10]. Cheeses that are produced by using fungi are characterized of specific organoleptic characteristics, namely a good strong cheese and mushroom flavor and aroma with a spice, slightly salty, buttery or crumbly consistency and distributed streaked mold on the surface [5].

The cheeses range is a sufficiently large. It includes soft cheeses that ripen under the influence of fungi proliferating on the surface of cheese (Russian Camembert, White dessert wine, Camembert, brie etc.) and soft cheeses with mold growing throughout the mass of cheese (Roquefort, blue, blue projloc - tion, Stilton, etc.). Cheeses of this group are known under different names in many countries: Roquefort, Bleu du Velay, Bleu D'Auvergne (France), Stilton (UK), Normana (Norway), Mycella (Denmark), Aura (Finland), Gorgonzolla (Italy), Maytag Blue Cheese (USA), Trikatas rokforas siers (Latvia)[12,13].

In the moldy cheese production one of the significant values is a reducing of the food safety hazards and modern solutions for that.

The process of risk analysis consists out of three components, risk assessment, risk management and risk communication. These components are internationally well spread by Codex Alimentarius Commission as being the basis for setting science based standards, criteria on food safety hazards, e.g. setting maximum limits of mycotoxins in foodstuffs [7].

In this regard chlorine dioxide (ClO_2) as a strong oxidizing agent can be applied in the bactericidal, fungicidal and viricidal treatment of the food raw materials that consist of food-related microorganisms, including Gram-negative and Gram-positive bacteria, yeasts, mould spores and Bacillus cereus spores [17].

The work purpose is to study the influence of *Penicillium caseicolum* in various concentrations on the ripening process of the moldy cheese surface microflora, chemical analysis of the content of macro - and micronutrients, organoleptic assessment in accordance with the requirements of GOST.

Scientific novelty: scientifically justified the selection of the components in the recipe of the soft moldy cheese and biological value of the developed food product.

Materials and methods

In the present study it was used "Evitaliya" probiotic enzyme, developed by "Probiotic" NPF (Moscow, Russia) that is a freeze-dried, but retained the ability to multiply in the digestive tract, special strains of lactic acid and other microorganisms (*Lactococcuslactis, Streptococcusthermophilus, Lactobacillusacidophilus, Lactobacillushelveticus, Propionibacteriumfreudenreichiissp. shermanii*), as well as producers of vitamins: B1, B2, B6, B12, A, E, C, folic acid, minerals iron, calcium, magnesium and others. The main feature of this composition of microorganisms is their ability to carry out the reaction of fermentation of milk with no off-gassing, but with the formation of acids that are beneficial to the digestive system and thereby inhibit the growth of putrefactive and conditionally pathogenic microbes, reduce the load on the liver by reducing the formation of amines, enterotoxins and other substances of microbial origin. In one leaven "Evitaliya" contains (2x109 + 2x109) CFU (colony forming units), more than 4 billion live microorganisms. The clinical efficacy confirmed at the Central research Institute of Gastroenterology of the Department of Health (Moscow, Russia) [19].

As a starter culture used a mesophilic starter for cheese named "BK-Uglich-S" production of "Experimental Biofactory"(Uglich, Russia), consisting of pure dairy cultures: *Lactococcuslactissubsp. lactis; Lactococcuslactissubsp.cremoris; Lactococcuslactissubsp.diacetilactis* et al [20].

Cow milk acidity not more than 18-21°T pasteurized at a temperature of (73±3)°C during 15-20 seconds, cooled till 34°C, carried out fermentation by "Evitaliya" probiotic enzyme for up to 24 hours,

then sediment at a temperature of 30-32°C for 30-35 min. by introducing with stirring a 40 % solution of $CaCl_2$ and the mesophilic starter culture [21]. Further, whey was removed, a batch of cheese was formed, self-pressing and salting were conducted.

For the study of the microstructure, a sample was fixed, stained by methylene blue dye and photos studied by using the "Prima expert digital microscope" (Russia). The samples of fresh cheese were sprayed with *Penicillium caseicolum* mold [22] at various concentrations (%): 0; 1; 3 and 5. Cheese ripening was carried out at a temperature of 10-15°C for 10-12 days and at a humidity of 90-95% [23].

The number of mesophilic aerobic and facultative anaerobic microorganisms was determined by the method of limiting dilutions on a nutrient medium of agar, for the determining the total number of mesophilic aerobic and facultative anaerobic microorganisms on a Petri dish and deep sowing according to the TU 9229-026-04610209-94[24].

Bacteria E.coli were determined by seeding the dilutions of the product in the Kessler medium, followed by their incubation in a thermostat at $(37\pm1)^0$C for 18-24 h, and the number of foreign microorganisms by culturing on agar with the aging at $(37\pm1)^0$C for 48 h.

Mineral content in the experimental cheese was determined by using Scanning Electron Microscope (SEM) and Mass spectrometry with inductively coupled plasma (ICP-MS). A method of mass spectrometry with inductively coupled plasma (ICP-MS) allows you to define a number of metals and several nonmetals at concentrations up to 10-10%, i.e. one particle of 10^{12}, with an atomic weight of 7 to 250, that is, from Li to U. It is able to determine the content from nanograms per liter to 10-100 milligrams per liter. The method is based on the use of inductively-coupled plasma as the ion source and mass spectrometer for separation and detection in an argon gas environment. Unlike of the atomic absorption spectroscopy that determines at a time only one element, the ICP-MS device can detect all elements simultaneously that can significantly speed up the measurement process [25].

Results and discussion

The study of the cheese surface by the "Prima expert digital microscope"

In the sample 1 (Fig.1) without the addition of the mold, after pressing, absent of any significant changes were observed, mainly a white cheese without pathogenic changes.

Figure 1 - Cheese without the addition of *Penicillium caseicolum*

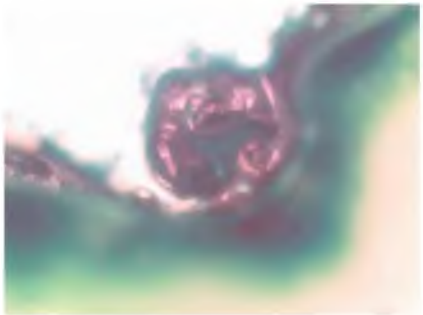

Figure 2 - Cheese with the addition of 1% *Penicillium caseicolum*

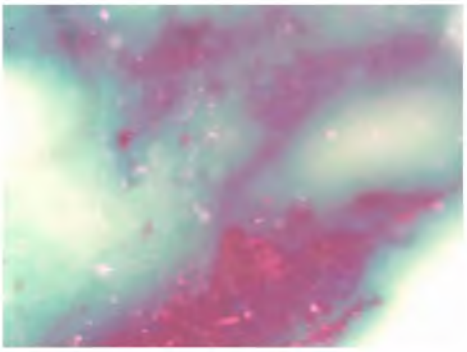

Figure 3 - Cheese with the addition of 3% *Penicillium caseicolum*

Figure 4 - Cheese with the addition of 5% *Penicillium caseicolum*

In the sample 2 (Fig.2) with the addition of 1% of mold is seen a weak formation of covered mold on the outer surface that is visible after 10-12 days of treatment.

In the sample 3 (Fig.3) with the addition of 3% mold after 10-12 days, there is intensive development of mold on the outer and inner surfaces of the cheese, in the form of thin filamentary structures without external pathogenic microflora.

In the sample 4 (Fig.4) with increasing of a mold doses to 5 %, the structure of cheese significantly changes; pathogenic organisms appear that testify to the active development of microorganisms.

In this regard, the recommended dose of addition of *Penicillium caseicolum* mold is around 1-2%. In this case a proportional development of mold on the outer surface without pathogenic organisms is observed.

The study of the mineral substances by using Mass spectrometry with inductively coupled plasma (ICP-MS)

Table 1 - Macro - and micro-element composition of cheese

Elements	Content, mg/kg			
	Sample 1	Sample 2	Sample 3	Sample 4
Mg	0,00054231	0,00060808	0,00045314	0,00131322
Al	0,00003354	0,00003931	0,00002064	0,00001584
P	0,00042759	0,00056883	0,00112975	0,00945801
K	0,01600329	0,01988739	0,00691212	0,00266662
Ca	0,00040363	0,00054239	0,00099536	0,00860307
Ti	0,00000466	0,00000573	0,00000247	0,00000459
Mn	0,00002677	0,00003374	0,00000871	0,00000198
Fe	0,00006591	0,00010834	0	0
Co	0	0,00000002	0	0,00000010
Cu	0,00000770	0,00001017	0,00000425	0,00002110
Zn	0	0,00001138	0	0,00007383
As	0	0,00000018	0	0,00000183
Rb	0,00000397	0,00000492	0,00000160	0,00000215
Sr	0,00002764	0,00003394	0,00001181	0,00001833
Mo	0,00000088	0,00000113	0,00000036	0,00000143
Cd	0,00000003	0,00000005	0,00000002	0
Sn	0,00000198	0,00000227	0,00000104	0,00000113
Sb	0,00000004	0,00000005	0,00000001	0,00000006
Cs	0,00000001	0,00000001	0	0
Ba	0,00000676	0,00000796	0,00000377	0,00009004
Tl	0	0	0	0

Analysis of the data shows that the sample 1 cheese without of the addition of *Penicillium caseicolum* mold is rich in content of macronutrients such as Mg (magnesium), P (phosphorus), K (potassium), Ca (calcium), Mo (molybdenum).

In samples 2, 3 and 4, the content of Mg (magnesium) P (phosphorus), Ca (calcium), and Zn (zinc) significantly increase. However, markedly reduce the content of the following elements: K (potassium), Mn (manganese) and Fe (iron). The degree of variation of the remaining values is within acceptable limits. It is possible that changes in the composition of inorganic elements are associated with biochemical reactions between amino acids and phospholipids cheese with probiotic microorganisms.

The study of organoleptic assessment

Comparison of organoleptic properties of the investigated cheese with and without the application of *Penicillium caseicolum* was analyzed in accordance with the requirements of GOST 33630— 2015 *Cheeses and processed cheeses* and GOST 32263-2013 *Soft cheeses. Specifications.* As the standard for the comparison the soft cheese – *Russian Cam*embert was chosen. The analysis is considered sample 1(cheese without any additives) and sample 2 (cheese with the addition of *Penicillium caseicolum* 1%) (table 1).

Table 1 - Organoleptic properties of the experimental soft moldy cheese

Name of the indicator	According to the requirements GOST 33630— 2015 *Russian Camembert*	The data obtained, a description	
		Sample1	Sample 2
Taste and smell	Pure fermented milk with a slightly mushroom or strong mushroom flavor. A slight bitter taste.	Medium sour and salty, with a specific taste and smell of pasteurization, and a slight taste of whey protein.	Cheese has a clean sour taste with a slightly mushroom flavor and a slight bitter taste.
Color	Light from white to yellow.	Milky white and with a shade of golden-cream.	Milky-white and slightly creamy.
Appearance	The cheese is packed in a lacquered or matted foil. The outer layer is compacted, elastic, covered with a mycelium of white mold, soft to the touch. It is allowed a small deformation.	The cheese has a shape of low cylinder with rounded edges and a convex surface	The cheese has the shape of a low cylinder, wrapped in lacquered foil. Velvety edible white moldy rind is covered.
Pattern	Absent of drawing. Allowed the presence of a small number of small holes and voids of irregular shape	Eyes of irregular shape	Absent of drawing.
Texture	Gentle, homogeneous throughout the mass. May be slightly spotting in the subcortical layer, with a small engine (no more than 1.5 cm) in the center of the more dense cheese dough	Homogeneous, soft mass	Gentle, homogeneous throughout the mass. Slightly spotting in the subcortical layer, with the presence of a small nucleus in the center of the cheese test

Comparative analysis of organoleptic indicators confirms that the obtained cheese corresponds to the soft moldy cheese. The paste is soft, homogeneous and buttery. The surface microflora is properly distributed over the surface, covered with velvety edible white moldy rind. The taste is pleasant, fresh, mild and creamy with a delicate, homogeneous consistency throughout the mass.

Conclusion

The study results of the surface microstructure of the moldy cheese by the digital microscope showed that the recommended dose of the adding of *Penicillium caseicolum* is around 1-2%, in this case a homogeneous mold is developed on the outer surface, no pathogenic organisms. Analysis of mineral substances by using Mass spectrometry with inductively coupled plasma (ICP-MS) shows the increased content of macroelements such as: Mg, P, K, Ca and microelements: Fe, I, Mn, Cu, Se, Zn, Mo. Organoleptic characteristics in comparison with the requirements of GOST 32263— 2013 *Russian Camembert*, in particular: taste and odor, color, appearance, pattern and texture confirm that the obtained product is a soft moldy cheese. The taste of the studied cheese has a clean sour taste with a slightly mushroom flavor and a slight bitter taste, milky-white and slightly creamy. The surface microflora is properly distributed over the surface and covered with velvety edible white moldy rind. The taste is pleasant, fresh, mild and creamy with a delicate, homogeneous consistency throughout the mass. Moldy cheese has a number of advantages over well-known analogues: short ripening period, the ability to provide high levels of "biological value of the product", the presence of all essential amino acids.

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ХИМИЧЕСКИЙ АНАЛИЗ МЯГКОГО СЫРА С ПЛЕСЕНЬЮ СОЗРЕТОГО С *Penicillium caseicolum*

Аннотация. Изучено влияние плесени *Penicillium caseicolum* в различных концентрациях на процесс плесневого созревания сыра. Анализ минеральных веществ с использованием масс-спектрометрии с индуктивно связанной плазмой (ICP-MS) показывает увеличение содержания макроэлементов, таких как: Mg, P, K, Ca и микроэлементов: Fe, I, Mn, Cu, Se, Zn, Mo. Органолептические характеристики по сравнению с требованиями ГОСТ 33630- 2015 «Русский камамбер», в частности: вкус, запах, цвет, внешний вид, структура и текстура подтверждают, что полученный сыр соответствует мягкому плесневому сыру. Вкус изучен-

ного сыра имеет чистый кислый вкус с небольшим грибным вкусом и легким горьким вкусом, с молочно-белым и слегка сливочным вкусом. Поверхность микрофлоры правильно распределена по поверхности и покрыта бархатистой съедобной белой заплесневелой коркой. Вкус приятный, свежий, мягкий и сливочный с тонкой, однородной консистенцией по всей массе. Разработанный плесневый сыр имеет ряд преимуществ перед известными аналогами: короткий период созревания, способность обеспечивать высокий уровень «биологической ценности продукта», присутствие всех незаменимых аминокислот.

Ключевые слова: бактерии, сыр, микрофлора, структура, плесень, макро - и микроэлементы, органолептическая оценка.

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PENICILLIUM CASEICOLUM ЗЕҢІМЕН ДАЙЫНДАЛҒАН ЖҰМСАҚ ІРІМШІКТІ ХИМИЯЛЫҚ ТАЛДАУ

Аннотация. *Penicillium caseicolum* зеңінің әртүрлі концентрацияларда ірімшікте зеңнің пісіп жетілу процесіне әсері зерттелді. Минералдық заттарды талдау барысында масс-спектрометр индукциялық плазмамен байланысын (ICP-MS) пайдалана отырып, мынадай макроэлементтердің артқандығын көрсетеді: Mg, P, K, Ca және микроэлементтер: Fe, I, Mn, Cu, Se, Zn, Mo. Органолептикалық көрсеткіштері ГОСТ 33630 - 2015 «Русский камамбер» талаптарымен салыстырғанда, атап айтқанда: дәмі, иісі, түсі, сыртқы түрі, құрылымы алынған жұмсақ зеңді ірімшікпен сай екенін растайды. Зерделенген ірімшік таза қышқыл, зеңді және аздап ащы, сонымен қатар сүтті, кілегейлі дәм береді Микрофлораның беті беткі жағы бойынша дұрыс орналасқан және жеуге жарамды үлпілдек ақ зеңмен жабылған. Дәмі жағымды, балғын, жұмсақ және кілегейлі барлық массасы бойынша біртекті консистенциялы. Әзірленген зең қосылған ірімшіктің белгілі баламаларга қараганда бірқатар артықшылықтары бар: қысқа пісіп жетілу қабілеті, жогары деңгейде «өнімнің биологиялық құндылыгын» қамтамасыз ету, барлық ауыстырылмайтын амин қышқылдары құрамында болуы.

Түйін сөздер: бактериялар, ірімшік, микрофлора, құрылым, зең, макро және микроэлементтер, органолептикалық көрсеткіш.