Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XV, Issue 4- 2016, pag. 334 - 340

THE INFLUENCE OF BERRY PUREE ON MICROBIOLOGICAL INDICATORS OF CHEESE PRODUCT DURING STORAGE

Olena GREK[1], Tetiana PSHENYCHNA[1], *Olena KRASULYA[1], Yuliia PAKHOMOVA[1], Kateryna IVASHCHENKO[2]

[1]*Educational and Research Institute of Food Technology, National University of Food Technologies, 01601, Volodymyrska str.68, Kyiv, Ukraine, olena_krasulya@ukr.net*
[2]*Odessa Institute of Postgraduate education NUFT*
65110 Balkovskaya 54 str., Odessa, Ukraine
*Corresponding author
Received 14th October 2016, accepted 27th December 2016

Abstract: *A contemporary trend is providing people with wholesome foods enriched with vitamins and microelements by improving food products' composition by addition of functional ingredients. This trend may be successfully achieved by innovative solutions related to the technologies of cheese products. One of the most promisive ways to increase significantly nutrition value and consumptional properties of cheese product is based on the enrichment of cheese by dairy-protein and berry raw materials. The modernization of the technical process consists in the fact that the process of milk-protein clot coagulation is carried out by processed berries. The process needs to be carried out under the strict observation of microbiological parameters. Similarly the product stability should be rigorously kept during its storage. Since the products' organoleptic, physico-chemical properties and their shelf-life depend on initial contamination, composition and state of bacterial flora, these indicators should also be controlled. Taking into account the above mentioned aspects, the effect of black currant as coagulant in the form of frozen milled berries in homogenized and sterilized puree on development of bacterial flora (lactic acid microorganisms, yeasts and moulds) in dairy-protein products during storage was examined. Safety and compliance with the regulatory requirements of received samples are proven. The positive impact on dairy-protein foundation' stability and quality of berry coagulant is proven.It was confirmed that the use of black currant in homogenized puree which has stable indicators is more appropriate and safe.*

Keywords: *cottage cheese, black currant, heat treating, thermo acid coagulation.*

1. Introduction

The task to provide consumers with balanced nutrition requires relevant approaches and solutions in terms of milk processing. Modern food products have to supply human organism with vitamins, macro- and microelements and other essentials for normal functioning of substances. The development of modern food product technologies capable of the above mentioned qualities is an essential problem. Such technologies might be based on the addition of various ingredients in the process of cheese production in some stages. Such technologies involve the addition of not only traditional ingredients but raw berry materials which contain biologically active substances. The analysis of the information sources shows that not only the enrichment of dairy ready products by raw berry materials is possible, but also the addition

of berry materials on a stage of protein component coagulation as well [1].
Berries contain from 80 to 90 % moisture. Carbohydrates are represented by fructose and sucrose; among minerals we mention ferrum, potassium, sodium, magnesium, phosphorus, calcium, zink, cuprum, etc. The biochemical composition of berry crops is shown in table 1 [2-11].

Table 1.

Biochemical composition of berry crops

Berry	Carbohydrates content, g/100 g	Saccarides content, %	Vitamin C content, mg/100 g	Micro- and macroelements content, mg/100 g
Black currant	7.30…15.38	4.50…11.02	157.70…181.00	464.15…478.48
Red currant	7.70…15.80	7.02…10.75	41.00…74.80	367.52…374.35
Rasberry	8.30…11.97	5.00…11.00	21.80…35.00	229.87…239.14
Blackberry	4.40…9.61	6.20…6.90	21.50…28.40	235.96…242.87
Bilberry	8.60…15.49	6.00…7.18	9.70…21.30	101.83…108.24
Cranberry	3.70…6.80	4.40…6.80	13.30…23.80	113.77…118.50
Guelder	7.90…12.3	7.31…9.56	15.00…27.60	20.20…28.60

According to the table data, black currant berries have the biggest amount of minerals (464.15…478.48 mg/100 g) and vitamin C (464.15…478.48 mg/100 g). High content in vitamin C, in comparison with other berry crops, determines its high antioxidant properties. Nevertheless, black currant berries fall short of the carbohydrates amount to bilberry (8.6…15.49 g/100 g) and red currant (7.7…15.8 g/100 g).
Black currant is one of the most widespread berry crops that grow in Ukraine. Medicinal-and-prophylactic properties are determined by fact that the berries contain vitamins, macro- and microelements, polysaccharides (pectin), polyphenols and others which are necessary for humans. Berries contain a big amount of iron, phosphorus and calcium salts in the form of organic compounds, which are easily digestible by the human body. The physico-chemical indicators of black currant berries are given in table 2 [1, 12].

Table 2.

Physico-chemical indicators of black currant berries

Moisture mass fraction, %	Pectin substances mass fraction, %	Tanning and coloring substances mass fraction, %	Organic acids mass fraction, %	Flavonoids mass fraction, mg %	P – active substanses, mg%	Titratable acidity,%	Sugar-acid index
85.60… 86.99	7.400… 11.100	0.70…0.90	1.90…3.66	245… 1047	319…560	2.0…4.3	2.0…4.8

Black currant berries may be provided in the native or frozen state and in the form of paste. They are widely used in food production: jam, jelly, juice, syrup, wine etc. In cheese product manufacturing we recommend to use frozen and sterile berries, homogenized LiQberry puree. Black currant puree is recommended for use as an additional source of biologically active, pectin substances, vitamins, micro and macroelements, fatty saturated and unsaturated

acids. Nutrition and energy value of the homogenized puree per 100 g of the product, should not be less than: proteins – 1.0 g; carbohydrates – 8.0 g, fibers – 3.0 g; 36.0 kcal/150.6 k. The physico-chemical indicators of the homogenized black currant puree are given in table 3.

Table 3.

Physico-chemical indicators of the homogenized black currant puree

Dry soluble substanses, % not less than	pH value, no higher than	Polyphenols, mg/100 g	Organic acids, g/100 g	Vitamin C, mg/100 g	Pectins, g/100 g
10.0	3.5	350…400	2.0…2.5	20…40	0.9…1.1

There have been data reported about the use of berries not only as cheese products filler, but as a coagulant in the process of coagulum obtaining [13]. In our opinion, the use of berry coagulant is a promising area of combination in the dairy-protein products manufacturing and requires further research. According to the classical technology of cottage cheese production, coagulation of the casein occurs at pH 4.6 at a temperature (30±2) °C. The duration of fermentation lasts for (8…10) hours. In the process of berry-protein foundation processing, it is expedient to approach the pH of fermented milk mixture to the corresponding value by correction of the process by adding berry raw materials. According to the regulatory documents, shelf life period of the cottage cheese without additional treatment is of 72 hours at a temperature (0…6) °C.

2. Matherials and methods

During the development of technology for cheese product based on dairy-protein, the characteristics of all components providing nutritional and biological value, safety requirements including microbiological parameters of the product were considered.
The aim of the research is to study the effect of different types of black currant on the shelf life and microbiological parameters of berry-protein foundation.

Object of research – cheese product, which is received by using black currant as coagulant.
The following conventional methods are used in the work: identification of viable lactic microorganisms and their most probable number; determination of coliform bacteria; identification of yeasts and fungi; determination of the amount of mesophilic aerobic and facultative anaerobic microorganisms.
Dairy-protein clot was produced by the classical technology from whole milk with the following indicators: solids mass fraction – 12.3 %, fat mass fraction – 2.6 %, protein mass fraction – 2.8 %, active acidity – 6.9 units. pH, density – 1029 kg/m^3 [14]. Besides, frozen berries or homogenized black currant puree produced by LiQberry (*Ukraine Technical Conditions 15.3-24110704-003:2011*) were added to the milk.
The amount of berry coagulant was determined by leading up normalized milk with fermented compound to the classical pH value. On the average, the mass of pasty currant was (10±1) % from the mass of normalized mixture. For cottage cheese production Vivo ferment was used, which contains the following strains of microorganisms: *Lactococcus lactis subsp. Lactis, Lactococcus lactis subsp. Cremoris, Lactococcus lactis subsp. lactis biovar. diacetylactis*. Fermentation control (monitoring) was carried out at a

temperature 32 °C for 4.5 hours to reach the value of pH 4.5…4.6.
Before introducing in normalized mixture, frozen currant berries needed an additional processing. They were defrosted and milled in a blender for 2.5...3.0 min to the puree consistence, with a particle size of 200…250 microns.
In parallel, the samples with homogenized blackcurrant puree were prepared. It was produced in industrial conditions by advanced technology using hydrodynamic (cavitation) processing of raw materials on a TEK-SM device. The berries were treated in the above mentioned setup according to designed regime to a necessary level of homogenization and industrial sterility [15-16]. The samples of cheese product obtained were kept at the temperature (4±2) °C. Microbiological parameters in as-prepared product, after the storage for 72 and 144 hours were determined.
The microbiological research to assess the safety of cheese products with increased biological value was carried out by standard methods on the base of subsidiaries and dependent companies "Odessa Institute of Postgraduate Education NUFT" laboratory [17].

Microbiological analysis of berry-protein foundation includes the determination of:
- coliform bacteria – by signs of growth (turbidity, gas formation, change of colour) in the Kessler broth and growth of colonies on Endo fueler;
- moulds and yeasts – by the growth of colonies on nutrient Saburo agar fueler;
- mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM) by counting the colonies which grew up on meat-and-peptone agar.

3. Results and discussion

The research results of lactic acid bacteria amount in cheese products during storage are shown in the table 4.

Table 4.

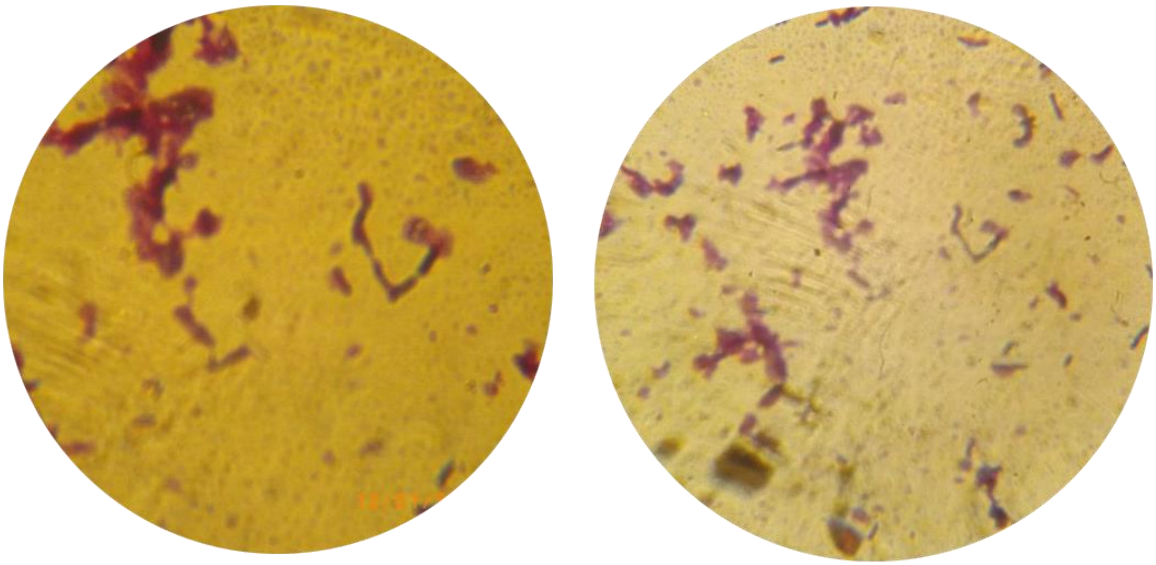
The research results of lactic acid bacteria amount in cheese product samples during storage, CFU in 1 g of product

The norm, according to the state standard, not less than (for cottage cheese)	**Cottage cheese (control) Sample 1**			**Cheese product with frozen milled currant berries Sample 2**			**Cheese product with homogenized black currant puree Sample 3**		
	As-prepared	After storage		As-prepared	After storage		As-prepared	After storage	
		72 hours	144 hours		72 hours	144 hours		72 hours	144 hours
$1 \cdot 10^6$	$1.1 \cdot 10^{10}$	$0.7 \cdot 10^9$	$1.1 \cdot 10^9$	$1.1 \cdot 10^{10}$	$0.7 \cdot 10^8$	$1.1 \cdot 10^7$	$1.1 \cdot 10^{10}$	$0.9 \cdot 10^7$	$1.1 \cdot 10^7$

According to the data shown in table 4, the amount of lactic acid bacteria in cheese product has decreased to ($1.0 \cdot 10^3$) CFU with the addition of berry puree. After 72 hours of storage, the amount of lactic acid bacteria in all samples has decreased as compared to as-prepared product. This confirms that currant puree is not a nutritious substrate for lactic acid microorganisms, as it inhibits their growth during product storage.
To determine the final characteristics and morphological properties of dairy products' microflora, the research of preparations with their dilution under the microscope after Gram staining were conducted. When investigating smear **sample 1** (dairy-protein foundation), a

big amount of rod bacteria and few cocci were determined, namely:

- Microorganisms painted in blue (Gr +), oval, which have a capsule and are grouped in pairs. (Fig. 1a);
- Microorganisms painted in blue (Gr +) in the form of long rods placed in pairs. (Fig. 1b).



a **b**

Fig. 1. Photomicrographs of preparations with the breeding of dairy- protein foundation (1000x magnification): a – photomicrograph of oval microorganisms; b – photomicrograph of microorganisms in the form of long rods

During microscopic examination of **sample 2** (cheese product with frozen milled currant berries) a big amount of long rods (Gr^+) with rounded ends, which are located singly or in pairs were found. In sight of sample, single oval cells, which have a capsule, are visible. Microscopy of the **sample 3** smear (cheese product with homogenised currant puree) showed the presence of long sticks in large numbers placed in chains or pairs.

The next task of research was to identify E. coli bacteria, which mainly belong to the Escherichia genus, ferment lactose in culture medium with acid and gas creation. According to the regulatory document requirements, the presence of coliforms in the cottage cheese and cheese products are not allowed in 0.01 g of product with shelf life of more than 72 hours. During the research no coliform bacteria in the berry-protein foundation samples obtained by different methods were found.

To confirm the samples accordance to the State Standard of Ukraine, the presence of yeast and mold fungi were tested. When present in food products moulds produce mycotoxins that cause the development of various toxicosis in humans. The presence of these microorganisms in berry-protein foundation leads to its rapid deterioration and has negative impact on the level of safety for human consumption.

The results of microbiological research of cheese products samples safety are presentedin the table 5.

Table 5.

The content of moulds and yeasts in model samples of cheese products

Storage time, hours	Mould amount, CFU in 1 g				Yeasts amount, CFU in 1 g			
	The norm according to DSTU 4554: 2006, no more than	Control	Cheese product with black currant berries	Berry-protein foundation with blackcurrant puree	The norm according to DSTU 4554: 2006, no more than	Control	Cheese product with black currant berries	Berry-protein foundation with blackcurrant puree
Immediately after production	$5{,}0 \cdot 10^1$	Not found	$18 \cdot 10^2$	Not found	$1 \cdot 10^2$	Not found	Not found	Not found
72			Not found					
120			Not found					

During the whole period of storage in sample 1 (cottage cheese without currant berries) and sample 3 (with homogenized puree), no colonies of yeast and moulds were found; as-prepared sample 2 (berry-protein foundation with black currant berries milled to a paste state) contained $18 \cdot 10^2$ CFU of mould fungi, which significantly exceeded the norm. The obtained results prove that the product contamination by mould fungi happened as a result of using defrosting of crushed currant berries without additional heat treatment. This has been confirmed in the process of determining the microbiological quality indicators of raw material (black currant). The number of mesophilic aerobic and facultative anaerobic microorganisms, yeasts and mould fungi in black currant were verified. Research showed the following: a significant contamination of defrosted berries - QMAFAnM was $45 \cdot 10^2$, mould fungi - $27 \cdot 10^3$ CFU, yeast – were not found. The research of sample 2 after 72 and 144 hours of storage has shown the absence of these microorganisms. Such results may be explained by the formation of nisin antibiotic in the process of Streptococcus lactic vital activity, in the presence of which the number of outside microorganisms, except of lactic acid, decreases.

4. Conclusion

According to the research results of microbiological indicators in the process of berry-protein foundation storage, no negative dynamics was found. Cheese product with homogenized blackcurrant puree complies with the cheese product regulatory requirements at the beginning and end of storage. The sample, which is produced by addition of milled defrosted black currant berries, turned out to be contaminated with mould fungi, making thus the product unsafe for consumers and requiring mandatory additional heat treatment. Therefore, it is more appropriate and safe for the given technology to use sterile, homogenized blackcurrant puree. In the case of fresh berries or defrosted raw berry material use, preparatory operations should be carried out carefully.

5. References

[1] MAKARKINA M.A., YANCHUK T.V., The characteristics of the black currant grades by sugars and organic acids content, *Modern gardening*, 2: 9–12, (2010).
[2] MAKAROV V.N., VLAZNEVA V.N., Bioactive substanses in berry crops and products of their processing, *Storage and processing of the agricultural raw materials*,12: 75–82, (2008).
[3] YUSHKOV A. N., SAVELYEV N.I., AKIMOV M.Y., Antioxidant activity and biochemical content of berry crops*, Science and APK technique achievements,*8: 5–6,(2010).
[4] ISAEVA E.L., Cranberries and blueberries. The overcoming bacteria berries*, Healthy Living and Longevity,* 64p., (2010).
[5] POPOVA E.I., KHROMOV N.V., VYNNYTSKAYA V.F., Biochemical evaluation of viburnum accessions and prospects of it use in the manufacture of functional foods, *Scientific sheets: ser. Natural Sciences,* 21: 127–131, (2012).
[6] SIMAKHINA G.A., Basic bio-components of raspberry and their behavior during freezing, *Food & Ingredients,* 4: 44–46, (2009).
[7] PETROVA S.N., Composition of black currant's fruits and leaves Chemistry *of plant raw materials,* 4: 43–50, (2014).
[8] STRELTSINA S.A., TIKHONOVA O.A., Nutrient and bioactive substances of black currant berries and leaves in the conditions of the North-West Russia, *Agricultural Russia*, 1: 1–16, (2010).
[9] KAMZALOVA A.M., DMITRIEVA V.T., GUMENYUK S.L., LIPSKAYA O.I., The biochemical composition of currant berries in the conditions of Belarus, *Fruit growing,* 215p., (2005).
[10] ARTEMOVA E.N., Physico - chemical properties of red currant jelly, *Food Industry,* 7: 58–59 (2006).
[11] HUSEYNOVA B.M., DAUDOVA T.I., Environmental aspects of the biocomplex formation in fruits as wild blackberries and blackthorn, *Biological resources: flora, 675–678 (2010).*
[12] SHEVCHUK L.M., The quality of black currant in the Carpathian region, *Ukrainian Academy of agricultural Sciences*, 10: 24–26, (2008).
[13] SHCHETININ M.P., KOLTYUGINA O.V., KOSYNKINA A.A., Cheese product with berry ingredients, *Dairy industry* 10: 58 (2011).
[14] DSTU 4837:2007 Quick-frozen fruits and berries, *State Committee for Technical Regulation and Consumer Policy*, 28 p. (2009).
[15] Ivashchenko K.Y., Improving of homogenized fruit products technology using hydrodynamic processing: synopsis of a thesis..phd,speciality 05.18.13. «Technology of canned and chilled products» NUFT: 20 p., (2015).
[16] BESSARAB O.S., OSYPENKO S.B., STOYANOVA L.O., PAKHOMOVA K.Y. Innovative technology for homogenized fruit products with increased biological value based on hydrodynamic processing of raw materials, *Equipment and technology of food production,* 32: 74–19, (2014).
[17] DSTU 7357:2013 Milk and dairy products, *State Committee for Technical Eegulation and Consumer Policy*, 39 p. (2014).