

The application of green tea Extract as a source of antioxidants in the processing of dairy products

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Abstract. Regular consumption of foods containing antioxidants reduces the bodily content of free oxygen radicals, which can cause pathological changes and premature organism aging. The aim of this work was the development of the formulations and determination of the parameters for the production of cottage cheese products with polyphenol fraction of green tea extract as a source of plant antioxidants. Parameters to obtain extracts with the high content of extracted substances and high antioxidant activity were determined. Optimal performance was achieved by brewing dry green tea leaves with (70 ± 2) °C water, followed by steeping at the same temperature for 10 minutes with continuous mechanical stirring. Optimal dry tea leaves to water ratio used for tea extracts' preparation was identified. The level of tea extract in cottage cheese products' recipes was determined. The flavour fillers which combine the best with green tea extract and taste were identified. The positive effect of tea extract component on shelf life of cottage cheese product was shown.

Key words: Antioxidant activity, polyphenols, the parameters to obtain extracts, cottage cheese products.

INTRODUCTION

Multiple studies conducted in different countries indicate that one of the reasons for early aging and many diseases is the oxidation stress, excessive concentration of free oxygen radicals in the physiological fluids of the body (Menshikova et al., 2006; Carlsen et al., 2010). Some adverse external factors can cause the level of active oxygen forms to rise. These forms can provoke damage of the macromolecules, which would cause the disruption of metabolic processes (Vladimirov & Archakov, 1971; Meerson, 1981).

To prevent the effects of the oxidation stress, it is necessary to consume products containing antioxidants, which can reduce the effects of free radicals. Bioflavonoids are one of the leading groups of antioxidants, which have anti-cancerogenic, anti-sclerotic, anti-inflammatory and hypoallergenic properties (Grek, 1999; Menshikova et al., 2006) and bactericidal activity (Gordon & Wareham, 2010, Stalnaja et al., 2014). Preventive application of antioxidants, especially during stress, increases general body resistance (Sorokina et al., 1997).

Main sources of bioflavonoids (polyphenols) for humans are drinks (tea, juices, wine), fruit and vegetables. Significant amount of phenol compounds are found in various tea breeds. The level of free glycolized and polymerized flavonoids reaches 30–40 mg l⁻¹ in drinks brewed according to the standard procedure (Manach et al., 2005). Polyphenols not only affect biological activity but also organoleptic properties, in particular colour and taste of plant raw materials.

One of the leading products with the highest content of water-soluble antioxidants is green tea (Fedoseeva et al., 2008). In non-fermented green tea 30–40% of dry weight is represented by phenol compounds which are mainly kachetins. Phenol compounds in green tea prevent consequences of oxidative stress (Nakagawa et al., 2002; Fedoseeva et al., 2008) have bactericidal (Gordon & Wareham, 2010) and anticarcinogenic activity (Lukin, 2015).

High antioxidant activity of polyphenols in body is manifested at low concentration of weaker synergists. In human body synergists are represented by reduced energy substrates (NADH and NADPH) formed during main catabolic processes and essential nutrients entering body with food (vitamins C, E, K, carotinoids, microelements) (Sorokina et al., 1997; Sharapova, 2008). In food products fortified with native plants, the components of food may be synergists.

Application of plant extracts assists in the extension of functional products' variety. This group includes such products as balsamic syrups (Pekhtereva, 2004), dairy drinks (Zabodalova et al., 2014), fermented whey-based drinks (Lazareva & Vysokogorsky, 2007; Palagina & Prikhodko, 2010), etc.

At the same time, the addition of antioxidant components of plant origin to the food products, including dairy, is one of the ways to prolong the products' shelf-life due to the inhibition of the oxidation processes (Bazarnova & Veretnov, 2004; Lazareva & Vysokogorsky, 2007; Bazarnova, 2010; Brosalin et al., 2013).

In dairy industry such antioxidants as tocopherols, salts of gallic and ascorbic acids, synergic antioxidants, lecithins and dihydroquercetin (DHQ) are used. For instance, DHQ is used in sour cream, yogurt, processed cheese, condensed milk, etc. for shelf life extension. Addition of DHQ at 0.02% of total fat enables extending dairy products' shelf life two to three times (Lazareva & Vysokogorsky, 2007; Palagina, 2010; Veretinskaya, 2011). Technology for the manufacture of fortified cottage cheese using antioxidant OriganoxWS (Frutarom) and dietary fibre complex Steyd Milk B-01 has been developed, optimal dose of the antioxidant being 0.03% (Ponomarev et al., 2011a; Ponomarev et al., 2011b).

Cottage cheese is one of the most popular protein dairy products. In spite of numerous examples of plant-derived components' application for the manufacture of dairy product, research in cottage cheese is limited to the addition of fruit fillers (Ostroumov et al., 2003; Capajeva & Suchkova, 2014) and commercial food additives based on them (Reshetnik et al., 2011).

The objective of this work was to develop the composition and identify technological parameters for the manufacturing of cottage cheese product using polyphenol fraction of green tea extract as the source of plant antioxidants.

MATERIALS AND METHODS

The objects of the study were green tea extracts and the samples of cheese products developed with them. Tea used in the study was Ahmad Tea (Nanchang Ltd).

Evaluation of mass fraction of dry substances

Mass fraction of dry substances in aqueous extracts of green tea and in whey was evaluated by accelerated drying. Two layers of gauze were placed to the bottom of weighing bottle, dried with no lid in exsiccator at 105 °C for 20–30 min, followed by closing the lid and cooling in exsiccator for 20–30 min and weighing. 3 cm² of product studied was placed to the prepared weighing bottle, spread evenly on the gauze surface, followed by weighing with the lid closed. Next, open weighing bottle and the lid were placed to the exsiccator at 105 °C for 60 min, followed by closing the bottle, cooling and weighing. Drying and weighing was conducted after 20–30 min until the difference between two consequent weights was below 0.001 g. Mass concentration of dry matter DS was calculated as follows (1):

$$DS = (M_1 - M_0) 100 / V, \quad (1)$$

where: M_1 – weighing bottle with gauze and product before drying, g; M_0 – weighing bottle with gauze and product after drying, g; V – product volume, ml.

Determination of density

The density of the aqueous tea extracts was determined at 20 °C using aerometer type AOH-1 with the limit of absolute error 1.0 kg m⁻³.

Evaluation of active acidity

The active acidity of extracts was evaluated by potentiometric method using pH-meter pH-410. Sample extract amount of (40 ± 5) cm³ was placed to the 50 cm³ glass at (20 ± 2) °C, followed by immersing electrode pair into the glass.

Determination of water-soluble antioxidants' activity

Total antioxidant activity (TAA) in tea extracts was determined using FRAP method (Ferric Reducing Antioxidant Power) with indicator system Fe (III)/Fe (II) – o-phenanthroline (Temerdashev et al., 2006). Phenanthroline (NPF Ural Invest) and chloric iron (Rexant) were used. Optical density was measured using photoelectric colorimeter 'FEC H-57' (Medical Eng., light filter with transmission peak $\lambda = 507$ nm). The extract amount of 0.3 ml was placed to cuvette with light path of 1 cm followed by addition of 0.3 ml of 0.045 M o-phenanthroline solution and 1.5 ml of 96% ethanol. 30 min after the addition of 0.3 ml of 0.025 M FeCl₃, indication of the instrument was read. TAA was calculated as an ascorbic acid equivalent using standard calibration curve.

Manufacturing of cheese products

The test and control samples of cheese products were manufactured from fresh cow milk. Milk was heated to 40–45 °C followed by separation using electric separator Motor Sich CMS-80 to cream and skim milk with fat level below 0.05%. Skimmed milk was pasteurized at 76–78 °C with loading for 20 s, followed by cooling to 28–30 °C. For coagulation the following components were added into the milk: dairy starter cultures of

Lactococcus lactis subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*; calcium chloride; rennet powder.

The reagents were added to the mixture in the following quantities: starter – 2% v/v ; solution of calcium chloride on the basis of addition of 400 g of anhydrous salt per 1,000 kg of mixture (as 40% aqueous solution); rennet powder – 1 g with activity of 100,000 units per 1,000 kg of mixture (as 1% aqueous solution).

The mix was agitated and left for fermentation in thermostat at 28–30 °C. The curd obtained was pressed to remove whey to moisture level of 80%. The defatted cottage cheese was a control sample and was used as a base for cottage cheese products with green tea extract.

Preparation of green tea extract for the fortification of cottage cheese product

Tea sample of 28 g was placed to a glass container, followed by addition of 100 g of water at (70 ± 2) °C and exposure for 10 min in water bath at (70 ± 2) °C with continuous agitation. Next, the extract was filtered and cooled down to (20 ± 2) °C.

Preparation of cottage cheese product fortified with green tea extract

In order to obtain final product with moisture level of 80%, defatted cottage cheese was preliminarily pressed. The moisture content in the cottage cheese after the compression was calculated considering the amount of tea extract that will be added as well as the mass fraction of dry substances in the extract and original cottage cheese product according to the formula (2):

$$W_1 = 100 - (100 \cdot DS_1 - D DS_2) / (100 - D), \quad (2)$$

where: W_1 – moisture content in the cottage cheese product after the compression, %; DS_1 – mass fraction of dry substances in the cottage cheese product, %; DS_2 – mass fraction of dry substances in the tea extract, %; D – a dose of the tea extract, %.

The amount of whey which must be separated during compression was calculated by the formula (3):

$$M_2 = M_3 (DS_4 - DS_3) / (DS_4 - DS_5), \quad (3)$$

where: M_2 – the mass of separated whey during the compression, kg; M_3 – mass of the original cottage cheese product, kg; DS_3 – mass fraction of dry substances in the original cottage cheese product, %; DS_4 – mass fraction of dry substances in the cottage cheese product after the compression %; DS_5 – mass fraction of dry substances in the whey, %.

Green tea extract was added to preliminarily pressed defatted cottage cheese at a certain level and mixed with tea extract using blender.

Preparation of cottage cheese product fortified with green tea extract and fruit fillers

Fruit fillers were added to the pressed defatted cottage cheese fortified with green tea extract at the level of 15%. Experimental and control samples packed to glass jars with screwed metal lids were stored in refrigerator at (4 ± 2) °C.

Evaluating of titratable acidity

The cheese products were evaluated for titratable acidity. The method is based on neutralization of acids in the product with 0.1 mol·dm⁻³ sodium hydroxide solution with

phenolphthalein indicator. 5 g of product was placed to porcelain mortar, agitated thoroughly and ground with pestle. Next, 50 cm³ water at 35–40 °C was added with small portions followed by 3 drops of phenolphthalein addition. The mixture was agitated and titrated with alkaline solution till pink colour appearance was stable for 1 min. Acidity in Turner degrees (°T) was calculated by multiplication of volume (cm³) of sodium hydroxide solution used for titration by 20.

Determination of moisture level in cottage cheese

Moisture level in cottage cheese was determined using moisture analyzer Elex-7. Preliminarily single-layer filter paper bags 150 × 150 mm were prepared as follows: a bag was diagonally folded, a corner was turned up by 15 mm and enclosed to parchment of a slightly bigger size with no edges folding. Ready bags were dried in the analyzer between metallic plates heated to 102–105 °C for 3 min followed by cooling and storing in exsiccator. Ready bags were weighed with an error below 0.01 g, followed by placing 5 g of product, weighing it with an error below 0.01 g. Bags with product were closed, placed to the analyser between metallic plates heated to 102–105 °C and exposed for 5 min. Bags with dried samples were cooled in the exsiccator for 3–5 min and weighed.

Moisture level in the product W_2 , % was determined using equation (4):

$$W_2 = (M_4 - M_5) 100/5, \quad (4)$$

where: M_4 – bag weight before drying, g; M_5 – bag weight after drying, g; 5 – sample weight, g.

Examination of organoleptic characteristics

Organoleptic evaluation was conducted by 5-point scale using sensory analysis method (Kantere et al., 2001). The samples were evaluated by a trained panel of 12 members. Twelve panelists (age 22–38 years) qualified for sensory evaluation techniques and regular consumers of products estimated the sensory properties of the samples.

Microbiological analysis

Lactic acid bacteria (LAB) number, yeasts and moulds and colibacillus number were determined in the cottage cheese product during refrigerated storage.

The number of LAB in cottage cheese products was determined using plate method. Nutrient broth used was agar with hydrolyzed milk. For inoculation dilutions 10⁻⁶–10⁻⁹ were used. Inoculates were incubated at (30 ± 1) °C for 3 days followed by counting of LAB colonies' number and re-calculation per 1 g of product.

The number of yeasts and moulds was determined by inoculating of cottage cheese products diluted to 10⁻¹ on Petri dishes with wort agar. Plates were incubated at 20–23 °C for 5 days. Microorganisms were classified as yeasts and moulds based on characteristic growth on the nutrient broth and cell morphology. Colony numbers of yeasts and moulds were counted separately.

Method of coliform count determination was based on the ability of coliform bacteria to ferment lactose and acid formation at (37 ± 1) °C for 24 h on the nutrient broth. An indicator of coliform growth on Kessler medium is the formation of gas in the float. Inoculation of product on Kessler medium was conducted for dilutions 10⁻¹, 10⁻² and 10⁻³. 1 cm³ of each dilution was inoculated to the tube with 5 cm³ liquid Kessler

medium with floats. Tubes with inoculates were placed in thermostat at (37 ± 1) °C for 24 h, followed by examination and visual determination of presence or absence of gas in the floats.

All experiments were performed with at least three replications; data was processed by methods of mathematical statistics with 95% confidence level. Confidence interval was calculated according to standard procedure using Student coefficient for confidence level of 0.95.

RESULTS AND DISCUSSION

Preparation of green tea extracts

Preparation of green tea extract is crucial for its application as an ingredient of cottage cheese product. Extract components can fulfil various functions and perform as quality identifiers, deliver consumer quality attributes, including organoleptic properties. For instance, sugars, acids, salts and other tastings affect taste, colourings affect colour, flavourings affect flavour. In order to select the optimal way of preparing green tea extract, a comparative study of the effects of brewing conditions and exposure was conducted for physico-chemical and organoleptic properties of tea extracts. Selection of brewing temperature of (70 ± 2) °C was based on the data on the loss of useful properties of green tea at higher temperatures (U Vey Sin, 2005). This is related to bioflavonoids' instability. Parameters varied are given in Table 1.

Table 1. Technological parameters of tea extracts' preparation, brewing temperature (70 ± 2) °C

Method No	Temperature of exposure, °C	Exposure duration, min	Agitation
1	70 ± 2	5, 10, 15	–
2	20 ± 2	5, 10, 15	–
3	20 ± 2	5, 10, 15	+
4	70 ± 2	5, 10, 15	+

Properties of extracts

Comparative evaluation of extracts' properties was done for the following parameters: dry matter level, density, pH and antioxidant activity. Change of these characteristics reflects the efficiency of the process of transferring of substances from tea extracted under different brewing conditions. As Fig. 1 shows, when increasing the exposure duration in all tea brewing methods, the acidity values of tea extracts (pH) tended to decline.

The tendency is positive since it indicates more active transfer of organic acids – soluble compounds of tea leaves – to the extract. The extracts of tea brewed by methods 1 and 4 had the lowest pH values.

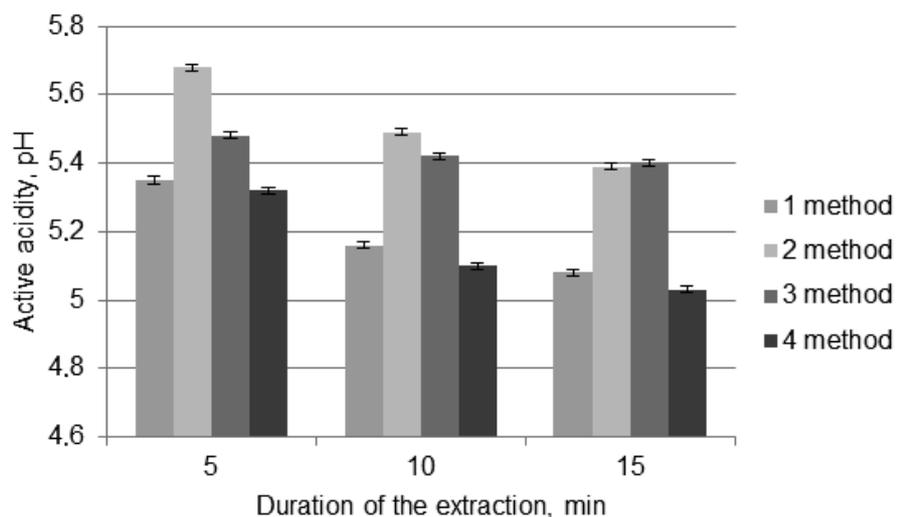


Figure 1. Active acidity of tea extracts.

Comparative evaluation of dry matter levels in extracts showed that methods 1 and 4 are more preferable (Fig. 2). Increasing the exposure duration in all brewing methods resulted in increased dry matter levels.

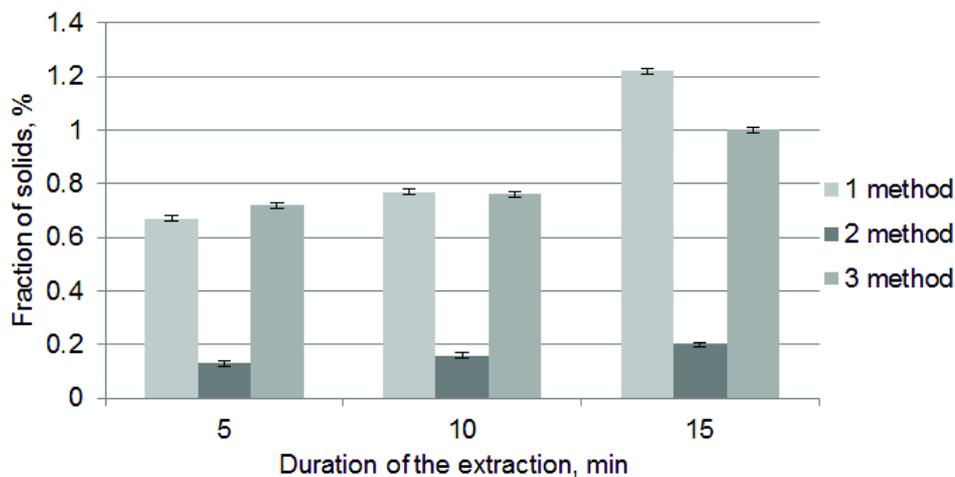


Figure 2. Dry matter level in tea extracts.

Determination of antioxidant activity of tea extracts revealed the advantage of method 4 (Fig. 3). However, it was noted that increasing exposure over 10 min is unreasonable since for the extract with maximal antioxidant activity obtained in methods 1 and 4, it leads to decline of this parameter. Apparently it is linked with the instability of antioxidants at elevated temperatures.

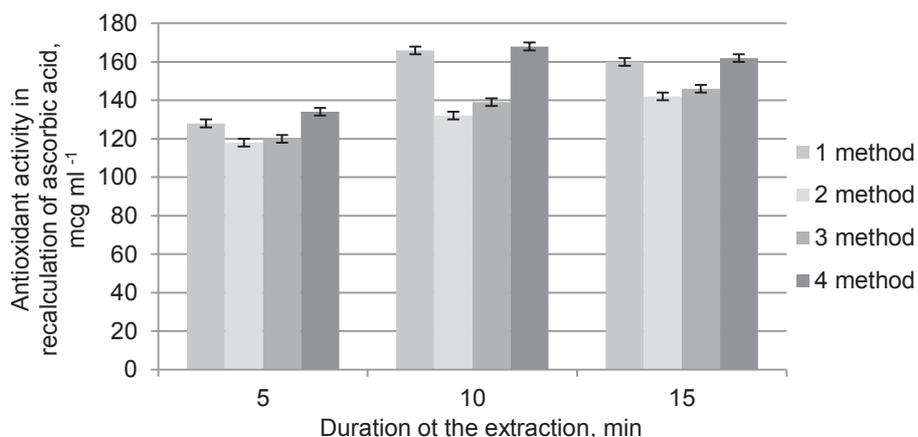


Figure 3. Antioxidant activity of tea extracts.

Organoleptic properties of the tea extracts agree with their physico-chemical properties. Exposure in water bath at brewing temperatures was accompanied with increased taste and flavour in the tea extract. Agitation during exposure intensified the process insignificantly.

Thus, brewing dry tea leaves followed by exposure in water bath at $(70 \pm 2)^\circ\text{C}$ for 10 min with continuous agitation appears to be the most reasonable method of tea extract's preparation. This method enables to obtain extracts with the highest level of extractive compounds and antioxidant activity.

Addition of tea extracts to cottage cheese product was conducted after preparation of cottage cheese base with lowered moisture level. Addition of the green tea extract to normalized mix leads to a loss of tea components during syneresis together with whey.

It is reasonable to use the tea extract with maximal level of dry matter. Dry matter level in tea extract can be increased by elevating tea amount during brewing. The effect of extraction hydromodule (dry tea to water ratio) in the range 1:25 to 1:2.5 (tea amount from 4 g to 40 g per 100 g water) on extract's properties was studied. Brewing was conducted using method 4. Physico-chemical properties of extracts are given in Table 2.

Table 2. Study of extract properties at different dry tea levels

Dry tea levels (g per 100 g of water)	pH	Density, kg m^{-3}	Dry matter, %	Antioxidant activity in ascorbic acid equivalents, $\mu\text{g}\cdot\text{ml}^{-1}$
4.0	5.29 ± 0.02	$1,001 \pm 2$	1.2 ± 0.2	162 ± 3
8.0	5.24 ± 0.02	$1,003 \pm 1$	2.1 ± 0.1	169 ± 2
12.0	5.19 ± 0.01	$1,008 \pm 3$	3.3 ± 0.2	176 ± 3
16.0	5.16 ± 0.01	$1,013 \pm 2$	4.5 ± 0.3	187 ± 3
20.0	5.14 ± 0.01	$1,018 \pm 2$	5.8 ± 0.2	197 ± 1
24.0	5.12 ± 0.01	$1,021 \pm 1$	6.5 ± 0.3	206 ± 2
28.0	5.11 ± 0.01	$1,024 \pm 1$	7.3 ± 0.2	209 ± 3
40.0	5.02 ± 0.02	$1,033 \pm 2$	9.6 ± 0.1	210 ± 3

Ten times increase in dry tea level resulted in 3% increase of dry matter in extracts and 5% decline in active acidity. Antioxidant activity in ascorbic acid equivalent was 29.6% higher. Most considerable changes in the majority of parameters analyzed (mainly dry matter and antioxidant activity) were noted when increasing dry tea level till 28 g per 100 g water. Further elevation of dry tea level was accompanied by decline in dynamics of change.

Observed trends reflect change in extract components' ratio. Since the purpose of green tea extract's addition was not only to obtain cottage cheese product with new sensory properties but also to increase shelf life, antioxidant activity was considered as the most important physico-chemical parameter. Thus 28 g of green tea per 100 g was selected as the most reasonable level.

Cottage cheese enrichment with tea extracts and taste fillers

Sensory analysis plays an important role in the evaluation of food quality. Sensory analysis conducted based on scientific ground excels some laboratory methods especially in terms of such parameters as taste, flavour and texture. For products such as wine and tea, organoleptic (sensory) analysis happens to be the only way to determine quality so far. Sensory characteristics enable to determine how the product is perceived by consumer. The disadvantages of organoleptic analysis include subjectivity of evaluation, relative expression of its results in dimensionless units, incomparability and insufficient reproducibility of results. In order to reduce the disadvantages mentioned, it is necessary to train panelists of basic organoleptic parameters for evaluation and to follow the conditions of organoleptic analysis. Taste perception plays the most important role in organoleptic analysis of foods (Nikolaeva 2006; Shepeleva, 2008).

The influence of green tea extract level on the organoleptic parameters of cottage cheese product was studied. Green tea extract has a specific taste and flavour. It was required to determine the maximal extract level which enables to obtain product with best organoleptic characteristics.

Cottage cheese samples with various green tea extract levels and taste fillers were evaluated. Green tea extract with dry matter level (7.3 ± 0.2)% to defatted pressed cottage cheese at the level of 1 to 17% (1% interval) was added and mixed using a blender. Tea taste was not detected or was considered too weak at the levels up to 7% (inclusively) in the cottage cheese. At the levels of 8% and 9%, the cottage cheese product had a pleasant moderately expressed green tea taste and flavour. Such a product can be recommended for manufacture without extra taste fillers. In cottage cheese samples with extract levels from 10% to 16%, an increase of bitter taste was observed and correction with taste fillers was required. In the sample with 17% extract, tea taste was described as 'too bitter, unpleasant'.

At the next stage, taste fillers were selected. Right choice of taste fillers enables to preserve the pleasant tea taste in the cottage cheese product while neutralizing the bitter taste. Moreover, various taste fillers and their compositions enable to extend variety and obtain new products with original taste characteristics.

The following taste fillers were used: sugar, chocolate syrup, rosehip syrup, cranberry syrup, lemon juice, raspberry jam, honey, pine nuts, walnuts and raisins. The selection of filler was performed according to criteria such as compatibility with tea taste, neutralization of bitter tea taste and cost. Results of the evaluation are given in Fig. 4.

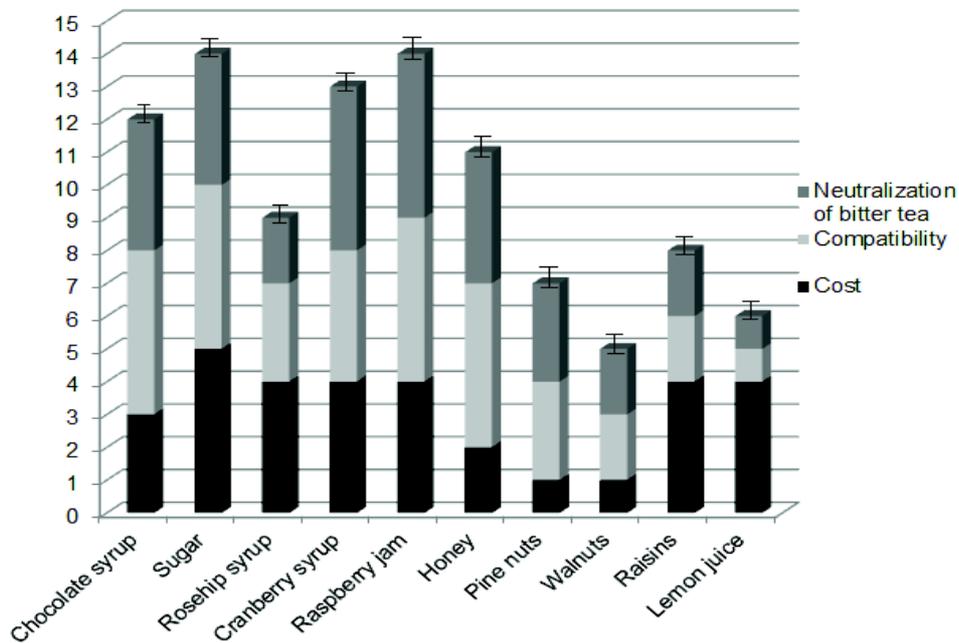


Figure 4. Organoleptic and cost analysis of cottage cheese products with taste fillers.

Evaluation of each criterion was done using a 5-point scale. Higher value for taste evaluation meant better organoleptic parameters: 0 – not perceived, 1 – somewhat perceived, 2 – weak intensity, 3 – moderate intensity, 4 – strong intensity, 5 – very strong intensity. In terms of cost, the higher was the taste filler’s cost, the lower was the score.

Cottage cheese samples with cranberry syrup, raspberry jam, honey, sugar and chocolate syrup obtained the highest scores. These fillers can be used both individually and in combination. The selected fillers were varied by their dose of adding.

Organoleptic evaluation of test samples was conducted. Such analytical descriptors as tea taste, taste filler, bitter taste, colour and consistency were used. Each parameter was evaluated using a 5-point scale. Score increase for each parameter except for ‘bitter taste’ indicated product improvement. By contrast, the higher the ‘bitter taste’ score was, the lower was the perception of it.

As an example, the results of organoleptic evaluation of cottage cheese product with tea extracts (15%) and chocolate syrup are given in Fig. 5.

It was noted that the higher was the filler level in the cottage cheese product, the lower the bitter taste was and the stronger chocolate taste was perceived. When chocolate syrup was added above 19% level, tea taste became less strong which resulted in score decline for this descriptor. Filler quantity level did not have noticeable influence on the colour of cottage cheese product, whereas texture remained different at chocolate syrup levels up to 19% inclusively.

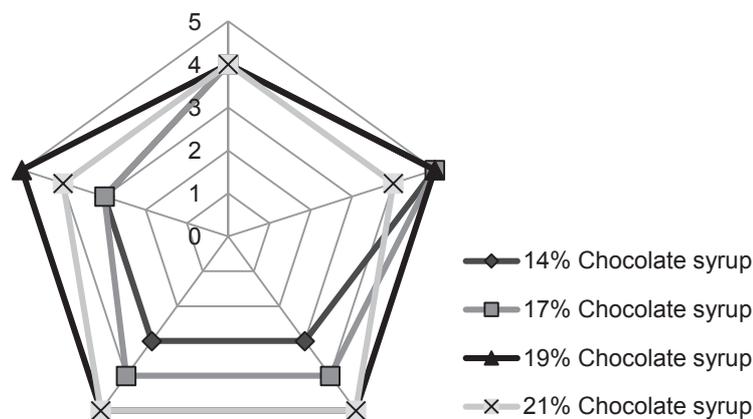


Figure 5. Organoleptic parameters of cottage cheese products with chocolate syrup.

The following taste fillers for cottage cheese product with green tea extracts are recommended: 19% chocolate syrup; 11% raspberry jam and 4% sugar; 12% honey; 14% cranberry syrup and 5% sugar.

The study of the cottage cheese with green tea extract was conducted. Defatted cottage cheese was prepared. First half of it was used as a control sample; the second half was used to prepare the product with 9% green tea extract.

Control and test samples were placed for storage at 0–4 °C. During storage acidity, organoleptic and microbiological parameters of cottage cheese were determined.

Acidity of green tea extract was lower than acidity of cottage cheese, thus acidity of the test sample was lower than in the control sample (Table 3). It was found that acidity of the sample with green tea extract increased by 6 °T during 10 days, whereas in control sample it increased by 22 °T. Elevation of acidity resulted from the formation of acid by microorganisms, thus the results indicate the bactericidal activity of green tea and the shelf life improvement of fortified cottage cheese product.

Table 3. Acidity of cottage cheese products during storage

Shelf life, days	0	1	3	5	7	10
Control sample, °T	220 ± 2	230 ± 1	234 ± 1	236 ± 1	238 ± 1	242 ± 2
Test sample, °T	204 ± 1	206 ± 1	206 ± 1	208 ± 1	208 ± 1	210 ± 1

The results of organoleptic evaluation of cottage cheese products indicate that change of taste properties in control sample during storage happened faster and more profoundly when compared to test sample (Figs 6 and 7).

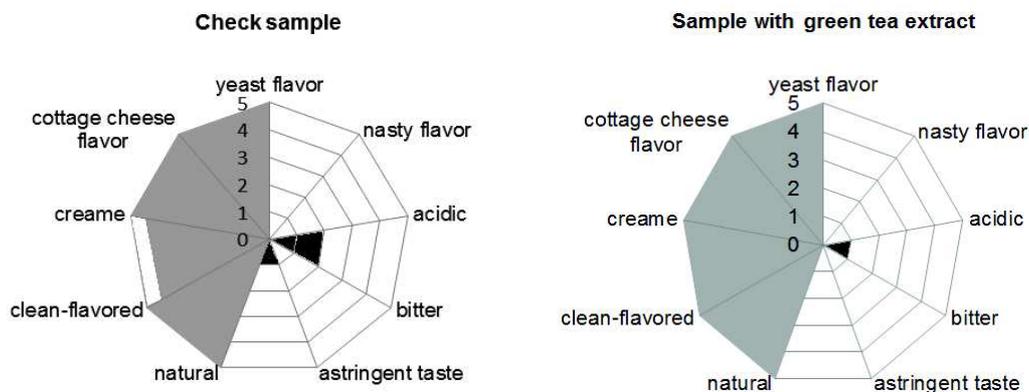


Figure 6. Taste scores in cottage cheese products after 7 days of storage.

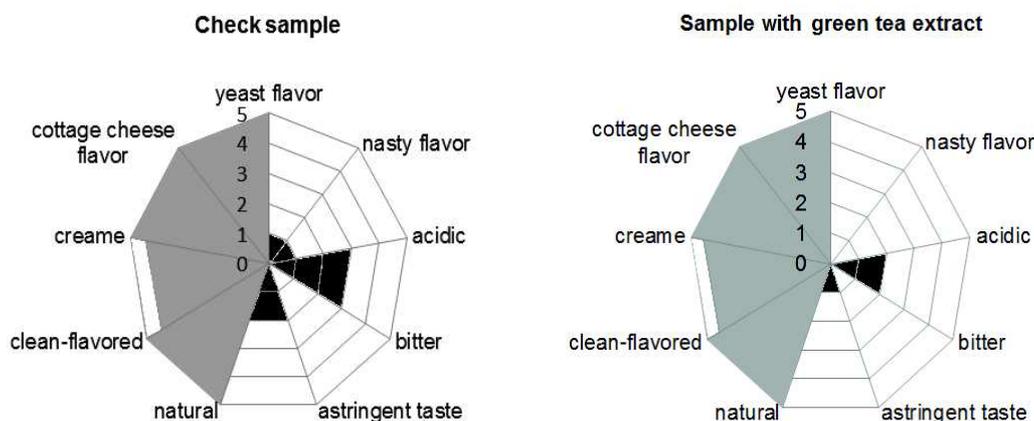


Figure 7. Taste score in cottage cheese products after 10 days of storage.

Microbiological parameters of cottage cheese products during storage are given in Table 4.

Table 4. Microbiological parameters of cottage cheese products during storage (0 – fresh sample; C – control sample, T – test sample with tea extract)

Parameter	Limit	Storage duration, days							
		0		5		7		10	
		C	T	C	T	C	T	C	T
Lactic acid bacteria, CFU g ⁻¹ *	At least 10 ⁶	7×10 ⁷	7×10 ⁷	–	–	–	–	2×10 ⁶	1×10 ⁶
Yeasts, CFU g ⁻¹	100	<10	<10	<10	<10	40	<10	110	40
Mould, CFU g ⁻¹	50	<10	<10	<10	<10	<10	<10	<10	<10
Coliforms in 0.01 g	Not allowed	Not detected							

* CFU g⁻¹ – colony-forming units in 1 g.

Lactic acid bacteria (LAB) number was determined in fresh samples (0) and at different points during storage (5, 7, 10 days). The slightly higher LAB number in control sample resulted in faster increase in acidity during storage.

During storage in both samples coliforms in 0.01 g were not detected, mould did not exceed 10 CFU g⁻¹. Yeasts' number in control sample till the end of shelf life did not exceed limit, whereas in test sample it was 40 CFU g⁻¹.

The study of control and test samples during storage showed that the addition of green tea extract to cottage cheese products increases their stability during storage. The results obtained agree well with the data of bactericidal properties of flavonoids (Stalnaja, 2014) and green tea (Lukin, 2015).

CONCLUSIONS

Based on the conducted studies, the following conclusions have been made:

Technological parameters for the preparation of tea extracts with highest levels of extractive substances and antioxidant activities were established. Optimal parameters were achieved by brewing dry green tea leaves in water at (70 ± 2) °C, followed by exposure for 10 min with continuous agitation.

Optimal green tea leaves to water ratio was 28:100.

The tea extract level should be 9% for the preparation of cottage cheese product without taste fillers, whereas for products with taste fillers it should be 16%.

The following taste fillers for cottage cheese products with green tea extracts are recommended: 19% chocolate syrup; 11% raspberry jam and 4% sugar; 12% honey; 14% cranberry syrup and 5% sugar.

Positive effect of green tea extracts on shelf life of cottage cheese products was established.

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REFERENCES

- Bazarnova, Ju.G. & Veretnov, B.Ja. 2004. Inhibition of radical oxidation of nutrition fats with flavonoid antioxidants. *Voprosi pitanija* **3**, 35–42 (in Russian).
- Bazarnova, Ju.G. 2010. Phytoextracts – natural inhibitors of food spoilage (electronic resource). *Nauchnij zhurnal NIU ITMO. Serija: Processi I apparati pishevih proizvodstv.* **2**. URL: <http://www.processes.ihbt.ifmo.ru/ournal/308> (in Russian).
- Brosalin, S.B., Evstigneeva, T.N., Luzgina, E.V. 2013. Development of the process details for the preparation of watermelon concentrate as an antioxidant in fermented dairy products. *VI Mezhdunarodnaja nauchno-tehnicheskaja konferencija 'Nizkotemperaturnije i pishevije tehnologii v XXI veke' Materiali konferencii*, Saint-Petersburg, NIU ITMO, pp. 600–602 (in Russian).
- Capajeva, M.A. & Suchkova, E.P. 2014. Creation of functional cottage cheese with cranberry pulp. *Materiali nauchnoj shkoli-seminara dlja molodih uchenih 'Kachestvo I bezopasnost proizvodstva: problem I puti reshenija'*, Saint-Petersburg, Lema, pp. 195–198 (in Russian).
- Carlsen, M.H., Halvorsen, B.L., Holte, R., Dragland, S., Sampson, L., Willay, K., Senoo, Y., Umesono, Yu., Sanada, Ch., Barikmo, I., Berhe, N., Willet, W.C, Phillips, K.M., Jakobs, D.R. & Blomhoff, R. 2010. The total antioxidant content of more than 3100 foods,

- beverages, spices, herbs and supplements used worldwide. *Nutritional Journal* **9**(3). URL: <http://nutritionj.biomedcentral.com/articles/10.1186/1475-2891-9-3>
- Fedoseeva, A.A., Lebedkova, O.S., Kanibolockaja, L.V., Shendrik, A.N. 2008. Antioxidative Activity of Tea extracts 'Himiya rastitel'nogo syrya' **3**, 123–127, (in Russian)
- Gordon, N.C. & Wareham, D.W. 2010. Antimicrobial activity of the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) against clinical isolates of *Stenotrophomonas maltophilia*. *Int J Antimicrob Agents* **36**(2), 129–131.
- Grek, O.R. 1999. Plant flavonoids and their biological and pharmacological properties. In: Gichev Yu.P., Oganova E. (ed) *Introduction to specific micronutrientology*. Novosibirsk, Medicina, pp. 212–239 (in Russian).
- Kantere, V.M., Matison, V.A. & Fomenko, M.A. 2001. *Organileptic analysis of food products*. Izdatelskiy kompleks MGUPP, Moscow, 151 pp. (in Russian).
- Lazareva, O.N. & Vysokogorskiy, B.E. 2007. Plant extracts lowering oxidation in dairy products. *Molochnaya Promyshlennost'* **10**, 83–84 (in Russian).
- Lukin, A.A. 2015. Prospective direction of green tea application as a bioactive compound in food technology // *Vestnik Yuzhno-Uralskogo gosudarstvennogo universiteta. Seriya: Pischeviye I biotekhnologii* **2**. URL: <http://cyberleninka.ru/article/n/perspektivnye-napravleniya-ispolzovaniya-zelenogo-chaya-v-kachestve-biologicheskii-aktivnogo-veschestva-v-tehnologii-produktov> (date: 19.03.2016).
- Manach, C., Williamson, G., Morand, Ch., Scalbert, A. & Remesy, Ch. 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **81**(1), 2305-2425.
- Meerson, F.Z. 1981. *Adaptation, stress, prevention*. Nauka, Moscow, 279 pp. (in Russian).
- Menshikova, E.B., Lankin, W.Z. Zenkov, N.K., Bondar, I.A., Krugovih, N.F. & Trufakin, V.A. 2006. *Oxidative stress. Prooxidants and antioxidants*. Slovo, Moscow, 556 pp. (in Russian).
- Nakagawa, T., Yokozawa, T. & Terasawa, K. 2002. Protective Activity of Green Tea against Free Radical- and Glucose-Mediated Protein Damage. *J. Agric. Food Chem.* **50**(8), 2418–2422.
- Nikolayeva, M.A. 2006. *Theory of food products understanding*. Norma, Moscow, 288 pp. (in Russian).
- Ostroumov, L.A., Prosecova, O.E. & Prosecov, A.Yu. 2003. Technology of dairy textured dairy products based on protein-carbohydrate and plant-derived raw materials. *Izvestiya vuzov. Pischevaya tekhnologiya* **1**, 28–30 (in Russian).
- Palagina, M.V. & Prikhodko, Yu.V. 2010. Substantiation of Far Eastern plans application as functional ingredients in food technology. *Izvestiya vuzov. Pischevaya tekhnologiya* **4**, 24–26 (in Russian).
- Pekhtereva, I.P. 2004. Functional drinks based on plant-derived raw materials. *Izvestiya vuzov. Pischevaya tekhnologiya* **1**, 79–80 (in Russian).
- Ponomarev, A.N., Merzlikina, A.A. & Golubeva, L.V. 2011a. Evaluation of shelf life of cottage cheese fortified with 'OriganoxWS'. *Pererabotka moloka* **1**, 58 pp. (in Russian).
- Ponomarev, A.N. & Merzlikina, A.A. 2011b. Fortified cottage cheese. *Molochnaya Promyshlennost'* **3**, 73 pp. (in Russian).
- Reshetnik, E.I., Maksimyuk, V.A., & Utochkina, E.A. 2011. Evaluation of possibility to create protein product with functional additives based on plant raw materials of the Far East. *Tekhnika I tekhnologiya of food production* **4**, 1–5 (in Russian).
- Sharapova, E. 2008. *Natural antioxidants and their use in production of dairy products*. OmEi, Omsk, 143 pp. (in Russian).
- Shepeleva, E.V., Mitaseva, E.V. & Radaeva, I.A. 2008. Quantitative criteria of sensory evaluation of fermented dairy product. *Molochnaya Promyshlennost'* **8**, 70–73 (in Russian).
- Sorokina, I.V., Krisin, A.P., Hlebnikova, T.B., Kobrin, V.S. & Popova, L.N. 1997. Role of phenol antioxidants in increasing organic systems stability to free radical oxidation:

- Analytical review/SO RAN GPNTB, Novosib in-t organ. khim. Novosibirsk, Ser. Ecologiya* 46 pp. (in Russian).
- Stalnaja, M.I., Arutyunova, G.Yu. & Boyko, I.G. 2014. Biological and therapeutical effects of broth and extracts derived from the plants of shrub Kuril tea. *Новые технологии* 1. URL: <http://cyberleninka.ru/article/n/biologicheskoe-i-terapevticheskoe-deystvie-otvarov-i-ekstraktov-iz-rasteniy-kurilskogo-chaya-kustarnikovogo>.
- Temerdashev, Z.A. Chrapko, N.V., Tsyupko, T.G., Voronova, O.B. & Balaba, A.N. 2006. Determination of antioxidant activity of food products using indicator system Fe (III) / Fe (II) - Organic reagent. *Zavodskaja laboratorija. Diagnostika materialov* 72(11), 15–19 (in Russian).
- U Vey Sin. 2005. *Encyclopedia healing tea*. Neva, Sankt-Peterburg, 320 pp. (in Russian).
- Veretinskaya, E. 2011. Application of digydrroquercetine for milk shelf life extension. *Pererabotka moloka* 4, 44 (in Russian).
- Vladimirov, Ju.A. & Archakov, A.I. 1972. *Lipid peroxidation in biological membranes*. Nauka, Moscow, 252 pp. (in Russian).
- Zabodalova, L., Ishchenko, T., Skvortcova, N., Baranenko, D. & Chernjavskij, V. 2014. Liposomal beta-carotene as a functional additive in dairy products. *Agronomy Research* 12(3), 825–834.