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STUDY ON ANTIMICROBIAL CHARACTERISTICS OF SPICES' COMPOSITION

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Abstract. *In order to produce safe, high-quality milk and cultured milk foods with an extended shelf life it is important to inhibit the development of microorganisms. The research aims to study the effects of previously developed spice aromatic composition on test cultures of microorganisms (samples of contaminating microflora), such as Enterobactercloaceae, Micrococcus albus, Bacillus subtilis, Endomyceslactis. The spice composition containsginger, cinnamon,cloves, curcuma, sumac, anise, black pepper and sweetpepper, cardamom, fenugreek, nutmeg andbadiane (staranise).It is proved that all spicy compositions showed evident antimicrobial characteristics against the testing cultures. It has been established that all compositions of spices demonstrated expressed antimicrobial characteristics with respect to the typical representatives of the contaminating microflora of fermented milk products. Thus, developed compositions of spices can be recommended for using in receipts of fermented milk products without additional processing. The discovered bacteriostatic effect of spices compositions will provide deceleration of undesirable processes promoting the increase of products' stability during storage.*

Keywords: antimicrobial characteristics, fermented milk products, microbiology of dairy products, spices

1. Introduction

Application of natural ingredients in food is becoming increasingly important. Customers are interested in natural ingredients since synthetic additives may be harmful. Spicy aromatic substances represent a wonderful alternative.

Spices are promising ingredients not only in cooking, but they also can be used as enhancing components in formulas of manufactured foods .The application of spicy aromatic substances as components of milk product formulas is limited, thus the development of new kinds of milk products with spicy ingredients is currently an urgent task.

Spices are complex biologically active composition that not only impart vivid taste and scent to food, but also promote digestion, have bactericidal and antioxidant properties, and actuate metabolism.

They contain significant amounts of biologically active substances, such as volatile oils, vitamins, polyphenol, catechine, micro- and macro-elements. Thanks to volatile oils, spices canin hibit emergence and development of bacteria, thus promoting increased storage period of products. Biological activity of volatile oil depends on its composition and main components. For instance, phenols and terpenoids display antiseptic action; monoterpenoids have antiseptic and

bactericidal action; sesquiterpenoids and diterpenoids have fungicidal action, whereas aldehydes have antiviral action. Therefore, it is quite important to study germicidal qualities of spices applied in the manufacturing technologies of cultured milk products[1].

The spices compositions developed earlier by the authors were used as study subjects and include:

- compositionNo.1 – sweet pepper, ginger, cinnamon in proportion 1:1:1;
- compositionNo.2 – cloves, sweet pepper, ginger in proportion 0.75:1:1;
- compositionNo.3 – ginger, curcuma, sumacin proportion 1:1:8;
- compositionNo.4 – anise, cloves, ginger, black pepperin proportion 1:0.75:1:1;
- compositionNo.5 – sweet pepper, ginger, cardamon,fenugreek in proportion1:1:0.75:1.25;
- compositionNo.6 – anise, ginger, nutmeg, black pepperin proportion 1:1:1:1;
- compositionNo.7 – badiane, ginger, curcuma, sumacin proportion 1:1:1:8;
- compositionNo.8 – ginger, sumacin proportion 1:8[2].

Constituents (or components) of composition were selected following not only organoleptic indicators of obtained products, but also their antimicrobial properties based on information given in source of literature [1,3,4].

We took into consideration the detailed characteristics of each component of given compositions:

Ginger (*Zingiber*)is a well-known antiseptic, which activates metabolism, helps to normalize functioning of cardiovascular system, improve immunity, organism resistance to seasonal viral diseases. The content of essential oil in ginger is 3%, main component of which is ginger, zingiberene. This spice contains almost all the necessary for the human body such as amino acids, vitamins A, B_1, B_2, C, mineral substances – zinc, sodium, potassium, iron, calcium, phosphor etc [1,3].

*Curcuma*contains starch, curcumine colorant (0.6%, dissolved in fats and alcohol, does not dissolve in water),aromatic essential oil (1.5-5.5%), main component of which is zingiberene, tumeron. Curcuma contains vitamins K, Cand vitamins of group B, as well as calcium, magnesium, iodine, phosphor and iron[1,3].

Cinnamon has a very tender aroma, due to its content of (4-10%) eugenol, phellandrene. The taste is sweetish, a bit burning, warming. Its basic components arecinnamicaldehyd 65-76% (80%). *Cinnamon* shows antiseptic effect and improves digestion[1,3].

*Clavus*is a perfect antiseptic, its content of essential oil is up to 22% and eugenol is the basic component. Clavus contains vitamins A, E, K, Cand vitamins of group B, as well as micro- and macro-elements[1,3].

*Sweet pepper*as a spicy combines aromas of clavus, cinnamon, nutmeg and black pepper and as medicine it is used for therapy of stomach diseases. Its content of essential oil is up to 5% and eugenol is thebasic component[1,3].

Black pepper has got its aroma due to the essential oil which content is up 15%. Basic components of this oil arealkaloid piperine, terpenes and sesquiterpenes, depentenes. *Black pepper* has general strengthening, bactericidal, anti-inflammatory effect. It contains vitamins of group B and A, C, E, K, as well as magnesium, calcium, potassium, phosphor, iron, zinc etc[1,3].

Sumac contains a number of acids, vitaminC, apple, citric, tartaric, amber, maleic, fumaric, ascorbic acids. Sumac also contains volatile oils, aldehydes, terpenoids, and fatty oils. Sumac leaves contain a number of tanning substances, basic part of which is tannin, and the rest – derivatives of gallic acid[1,3].

Anise. Essential oil – anethol is used in medicine, perfumery, and liquor and spirit production, food industry. Anise fruit contains2-3% of essential oil, 4-23% of fatty oil, 18% proteins, 3-5% sugar, furfurol, caffeic and chologenic acids and other wholesome substances. Anise essential oil contains 80-90%of anethol, estragon

10%,anise aldehyde and anise alcohol [1,3].
Badiane (*Staranise*) contains polysaccharides, resins, essential oil (5-10%), tanines, terpenes, vitamins, micro- andmacro-elements and other biologically active substances. Badiane has anti-inflammatory, general strengthening effect, stimulates functioning of digestive system [1,3].
*Cardamom*is recommended for improving appetite, having a general strengthening effect. Its content of essential oil is up to 10% and the basic components are borneol, cineol, sabinene andcitrene. Cardamom contains vitamins group B and vitamin C, micro-andmacro-elements(iron, zinc, potassium, calcium, magnesium, phosphor)[1,3].
Fenugreek contains small amounts of alkaloid trigonelline, nicotinic acid, steroid sapogenin, flavonoids, glair and bitter substances, essential oils (main component coumarine), proteins, tanines, vitamins, phosphor, iron [1,3].
Nutmeg has stimulating effect. It contains vitamins A, C, B_1, B_2, B_3, B_6, B_9, B_4andmicro- and macro-elements. Its content of essential oil is up to 15% (the basic components are dipentene, borneol)[1,3].
Milk and dairy products microflora is conditionally divided into the following groups:

1) microorganisms instrumental in the technology of milk and milk products. Useful lactobacilli are applied in the manufacturing of various milk products. Instances of such lactobacilli are homofermentative and heterofermentative streptococci and bacilli, which facilitate lactic fermentation. They are applied in the manufacturing of cultured milk products and cheeses, as well as in forage siloing, leavening of vegetables and fruit[5,6,7].

2) microbeswhich are harmful to the technology and cause deterioration of milk and milk products. In case they are present in milk, they develop taste, scent and consistence defects and their sanitary characteristics deteriorate. The consumption of such products can be harmful and cause disturbance of the function of food system. Such harmful microbes are micrococcus, colibacillus, hay bacillus, proteus, and fluorescent bacteria[5,6,7].

3) pathogenicmicroorganisms which are dangerous to human/animal health. They do not alter the composition and properties of milk and milk products, but they act as causative agents of human/animal infectious diseases. When contaminating milk, some bacteria (staphylococcus, proteus) can secrete toxins which cause food poisoning [5,6,7].
The characteristics of some contaminants in the production of milk products are examined as follows:
Micrococcus is immovable and does not produce spores. During reproduction in milk it secretes rennet and lactic acid. Certain types decompose fat, producing bitter taste of milk. In addition to sour taste, clot also has a bitter taste which is caused by an increasing amount of peptones [5,6,7].
Enterobacter is a genus of facultative anaerobic, rod-shaped bacteria. It is a part of a normal intestinal microflora of humans and animals, thus it is used as a bacterial indicator of sanitary quality of soil, water and foods; it is considered an opportunistic pathogen bacterium. In milk products, acid is produced as a result of fermenting lactose; other carbohydrates and alcohols can also be fermented with or without gas production. When consuming food products containing such bacteria, consumers with weakened immunity may develop serious diseases[5,6,7].
Bacillus is a genus of gram-positive aerobic soil bacteria, and, as all species of the genus, it can produce endospores. It grows quickly in milk and can cause severe protein disintegration producing methane, indole, hydrogen sulfide, mercaptans and other musty-smelling substances. When growing in milk and milk products it produces protein disintegration products

and toxic substances. Striking examples of the genus are hay and potato bacillus, which develop quickly in milk and disintegrate protein producing albumin, peptides, amino acids and ammonia and imparting a bitter taste to the milk. After a long time in milk it can accumulate harmful substances[5,6,7].

Endomyces is an aerobe; it reproduces in cultivated milk products, leavened vegetables, malt and on damp walls. When developing in milk and milk products it produces protein disintegration products and toxic substances. Such bacteria travel into milk using particles of forage and soil [5,6,7].

2. Materials and methods

The research aimedto study the effects of spicy aromatic composition on microorganisms which are species of contaminating microbiota characteristic of production of cultivated milk products.

Previous researches based on cultured cheese have shown that during holding cultured milk products, which contained 2.75-11% of spice composition, there were no significant alterations of organoleptic or technological quality characteristics[8]. During the first 3 days of holding, the actual acidity characteristic did not change. During the next 5 days of holding there was a gradual decrease in the actual acidity characteristic, an average from 4.5 to 4.2 pH units accordingly. During the holding period a test sample showed a decrease of the actual acidity characteristic from 4.4 to 3.8 pH units. The organoleptic properties did no undergo any changes during the abovementioned time. It may be assumed that the applied spice composition have shown sufficient germicidal action. Therefore, a decision was made to look into the influence of spice composition on pure growth of microorganisms that are typical contaminants of cultured milk products.

The following typical contaminants of cultured milk products were used as test cultures: *Enterobactercloaceae*, *Micrococcus albus*, *Bacillus subtilis*, *Endomyceslactis*. Pure growth of bacteria and yeast, used in the research, are kept in the Collection of viable cultures of microorganisms in the Department of Biotechnology and Microbiology of the National University of Food Technologies.

In order to determine germicidal properties of the tested spice composition, suspensions of twenty-four-hour test cultures, cultivated in agar growth medium (meat peptone (MPA) for bacteria and wort agar for yeast at the temperature of 30°C) were prepared. The number of viable cells was calculated by the KOH method (colony-forming units/sm^3). Then suspensions from each test culture were moved into 8 vials (putting 10 sm^3 of each suspension) and 0.1 g of each tested spice composition was added into the vials; the produced substances were kept at the optimal temperature for growth (30 °C) for 2 hours. After the exposure, the number of living cells was calculated by the KOH method. The microorganism survival rate was expressed by a percentage ratio between the number of viable microorganisms in exposed samples and the number of viable microorganisms in the original suspension.

3. Results and discussion

The number of cells in the original suspensions: $8.9 \cdot 10^5$ – *Enterobactercloaceae*, $9.3 \cdot 10^5$ – *Micrococcus albus*, $1.2 \cdot 10^6$ – *Bacillus subtilis*, and $7.4 \cdot 10^4$ – *Endomyceslactis*. Figures 1 and 2 illustrate the survival rate of cells of the cultures tested.

Based on the data given in Figure 1, all tested spice compositionshave shown significant germicidal properties against the test cultures of bacteria. However, the germicidal action of composition against

different cultures was not identical, e.g. the composition No.6 showed themaximal germicidal properties against the cultures of *Enterobactercloaceae*, the degree of survival of which was 3.3%, whereas its germicidal properties against cultures of *Micrococcus albus* were only third highest. The most stable germicidal properties against the inhibition of alltested cultures of bacteria were demonstrated by the composition No.1 and No.2: the degree of survival of bacterial cultures was 7.2-34.8%.

From the analysis of results, illustrated by the diagram below (Figure 2), it is clear that all spice compositions have displayed germicidal properties against the cultures of *Endomyceslactis.* Compositions no. 3, 4, 6, 7 and 8 demonstrated stronger inhibitory action; notably the application of compositionNo.6 resulted in a 9% survival rate.

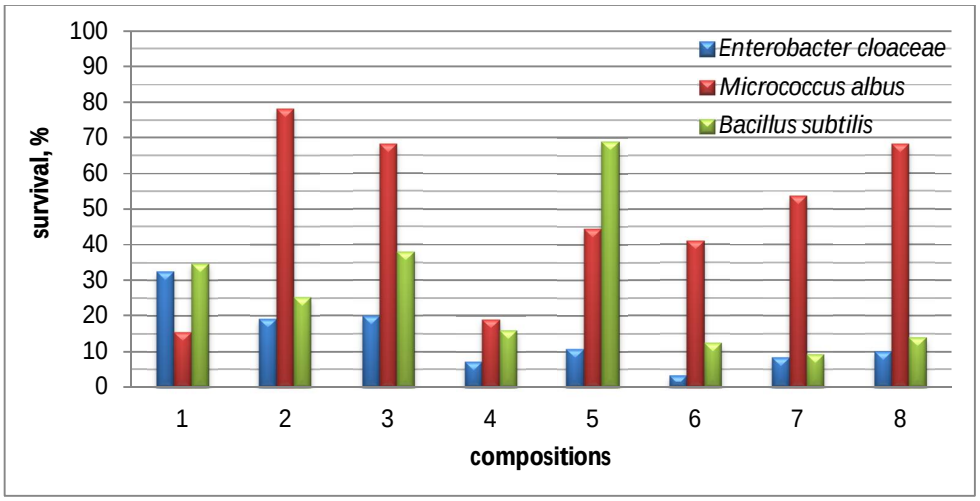

Fig.1.Antimicrobial effect of spices on bacteria test-cultures

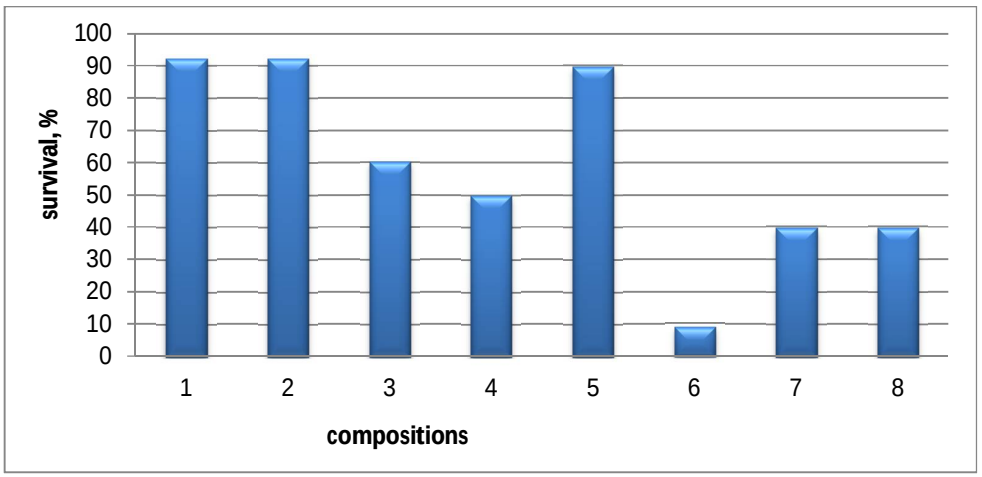

Fig.2.Antimicrobial effect of spices against the test-culture *Endomyces lactis*.

4. Conclusion

The conducted research demonstrated germicidal action of spice composition against test cultures of microorganisms which cause deterioration of milk products. The spice composition that displayed the strongest germicidal properties contained anise, cloves, ginger, black pepper and nutmeg, due to their higher levels of volatile oils and phenol compounds. Notably, the spice compositions were introduced into suspensions in amounts, specified by the formulations of cultured milk products.

Therefore, the applications of the developed spice composition in the technology of cultured milk products will result in bacteriostatic effect, thus increasing the storage periods of produced milk products and stabilizing the physicochemical, organoleptic and microbiological properties of the products during holding.

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