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MICROBIOLOGICAL AND PHYSICOCHEMICAL ANALYSIS OF HONEY AND CINNAMON YOGURT

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Abstract: *In recent years, there has been increasing interest in the development of new functional fermented dairy products using natural antioxidants as additives that have a beneficial effect on the body. In this study, the effect of the amount of cinnamon added in 0,5%, 1.0% and 1.5% on the coagulation process was monitored. The addition of cinnamon in the amount of 1.5% by weight of milk, suppresses the activity of lactic acid bacteria and slows down the coagulation of milk. During storage of yoghurt, a gradual decrease in pH was observed in all samples, with the larger amount of cinnamon slowing the rate of acid formation from 5,0 to 4,4. High antioxidant activity was recorded in the sample with the highest amount of cinnamon during the whole yogurt storage period - 30.82%. The highest values of total lactic acid bacteria during storage were observed in sample S1 ($2{,}7.10^9$) at day 7 and the lowest in sample S3 ($1{,}1.10^9$). This trend persists throughout the storage period, but even on day 14 of storage, the amount of lactic acid bacteria in sample S3 is $3{,}1.10^8$, which complies with the requirements of Codex alimentarius.*

Keywords: *yogurt, cinnamon, coagulation, antioxidant activity, total lactic acid bacteria*

INTRODUCTION

Fermented dairy products have different nutritional and therapeutic properties. The lactic acid bacteria in them play a major role in determining the positive effects on human health. One of the benefits of fermented milk for human health is the prevention of gastrointestinal infections, the reduction of serum cholesterol levels and antimutagenic activity. Fermented products are recommended for consumption by people with lactose intolerance and patients suffering from atherosclerosis Shiby K. and N.Mishra (2013).

The demand for functional fermented dairy foods with improved nutritional properties has recently increased (Baycheva et al., 2016; Dimov et all., 2019). Adding functional additives to yogurt such as honey, cinnamon, herb extracts and more (Zlatev, 2016). leads to an improvement in its overall quality. Plain yogurt has a predominantly sour taste, but the addition of fruits, flavors and sweeteners to it enhances its aromatic balance and partially masks the effect of acetaldehyde in milk (Bills et al., 1972).

Cinnamon is one of the oldest food spices belonging to the genus Cinnamomum of the Lauraceae family. In addition to being a food spice, it has been used in traditional medicine since ancient times by the Egyptians. It has been found to have strong antioxidant, antibacterial, antipyretic, and anti-inflammatory properties that play an important role in tissue repair Elshafi e M., et al., (2012). Specific antioxidant phytochemicals identified in cinnamon include epicatechin, camphene, eugenol, gamma-terpin, phenol, salicylic acid, and tannins Prabuseenivasan S., et al., (2006).

Park Y et al., (1995) investigated the antifungal effects of cinnamon, including increasing the shelf life, when added to foods.

Efforts are now being made to replace synthetic antioxidants and the use of natural foods and beverages. Natural antioxidants can be formulated as functional foods that contribute to the processes in the human body in the fight against free radicals.

The purpose of this study is to perform microbiological and physicochemical analysis of yoghurt enriched with honey and cinnamon.

MATERIALS AND METHODS

Materials

The following materials have been used to make fortified yoghurt (yogurt) with Bulgarian honey and cinnamon honey: Cow's milk, obtained from the region of Yambol, Bulgaria, with constant composition and properties (fat content – 3,4%; active acidity – 6,6; dry non-fat residue – 8,7%; Bulgarian honey bee honey from the Veliko Turnovo region. The honey was harvested in 2018 and stored at room temperature without direct access to light; lyophilized (freeze-dried) yeast for the production of Bulgarian yoghurt by the company "LB Bulgaricum EAD", which is a lyophilized symbiotic combination of *Lactobacillus delbreukii ssp. bulgaricus* and *Streptococcus thermophilus* containing not less than 1,109 live cells from the specified microorganism strains in 1g of yeast; Cinnamon powder purchased commercially.

For the production of enriched yoghurt, honey is used in an amount of 10% by weight of milk, but different amounts of cinnamon – 0,5%, 1,0% and 1,5% (Sempe S1, S2, S3). The amount of honey added to yogurt and its influence has been traced in our previous studies.

The preparation of enriched yogurt with Bulgarian honey bee and cinnamon is presented in Fig. 1.

After the milk is graded, the specified amount of cinnamon is added, after which the milk is passeurized for 30 minutes at 65° C. The pasteurized milk is filtered to remove the undissolved cinnamon particles, then cooled to a temperature of 44 ÷ 45 °C and fermented with a lyophilized starter culture (Lactobacillus delbreukii ssp bulgaricus, Streptococcus thermophilus). The amount of yeast is 4% by weight of milk. The inoculated milk is thermostated at 42 ÷ 45 °C for 3 ÷ 4 hours. Upon reaching a pH of 4,8 ÷ 4,6, the milk is homogenized (stirred), cooled rapidly to 18 ÷ 20 °C and then honey is added. The resulting yogurt is cooled and stored at 0 ÷ 4 °C for further analyzes. Samples of yogurt obtained are analyzed both at the time of production and at 3, 7 and 14 days after storage.

Methods

Active acidity, pH potentiometric, pH meter (Model MS 2011, Microsyst, Plovdiv, Bulgaria) equipped with electrode (pH electrode Sensorex, Garden Grove, CA, USA); Titratable acidity (TA) - determined by the Turner method (BNS 1111-80) and expressed as a percentage of lactic acid.

$$L_{degr.lac} = T_{cur} - T_{beg}.0{,}009.0{,}95 \quad (1)$$

where $L_{degr.lac.}$ is digested amount of lactose; ΔT_{beg} - initial initial titratable acidity; ΔT_{cur} - current titratable acidity.

Antioxidant activity,% - Preparation of whey fraction from fermented milk by the method of Virtanen T., et al., (2007). Antioxidant activity was determined by inhibiting DPPH (1,1-diphenyl-2-picryl hydrazyl) radical, spectrophotometrically, by the method of Son S., and B. Lewis, (2002). A volume of 2 ml DPPH in ethanol (500 mM) was added to the 2 ml whey fraction, stirred vigorously and allowed to stand in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm. The antioxidant activity is expressed as% inhibition (I) of DPPH absorption:

$$I = \frac{A_c - A_t}{A_c} . 100 \quad (2)$$

where A_c is the absorbance of the control sample (DPPH solution without whey fraction); A_t is the absorbance of the test sample (DPPH solution plus whey fraction).

Microbiological analysis - Total lactic acid bacteria (Lactobacillus bulgaricus and Streptococcus thermophilus): sample preparation is carried out according to IDF standard (IDF 122C: 1996). Appropriate dilutions are inoculated into selective M17 and MRS agars as described in IDF Standard 117B: 1997 (IDF Standard 117B: 1997).

Statistical analysis. Statistica 8 software (Stat Soft Inc.) was used. Regression analysis was used to process the data. The residuals of the obtained regression model were analyzed. All data were processed at level of significance α=0,05.

RESULTS AND DISCUSSION

Fig. 2 shows the change in the values of total acidity (as a percentage of lactic acid) during storage of yogurt. It is seen that as the percentage of cinnamon added increases, the coagulation time slows down, but titratable acidity values continue to increase until fermentation is complete. Similar results for delayed coagulation were obtained by Bae H. and M. Nam (2006) in fermentation of ginseng milk.

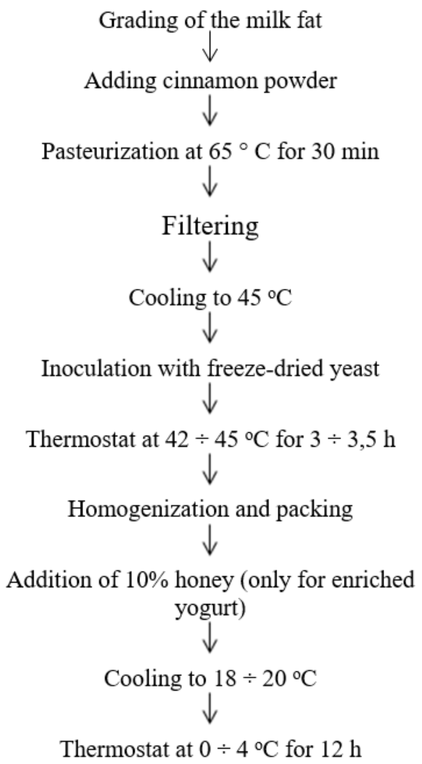


Fig. 1. Technological operations Scheme

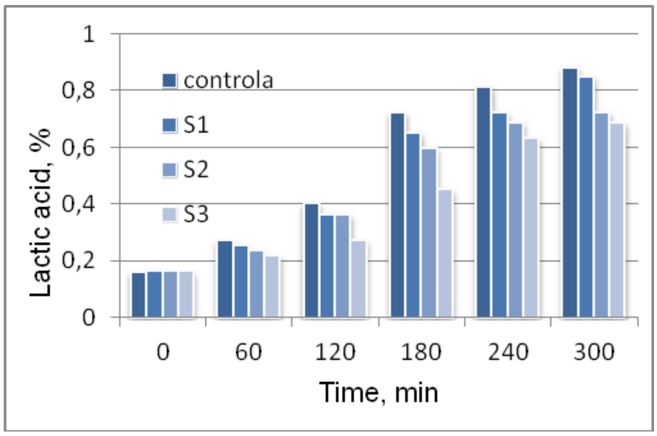


Fig. 2. Changes in total acidity (as a percentage of lactic acid) during storage of yogurt

In samples S1 and S2, the percentage of lactic acid at the start of coagulation approaches the control level – 0,16%. From the second to the fifth hour of coagulation, as the percentage of cinnamon in milk increases, the percentage of lactic acid decreases. The amount of lactic acid accumulated in the fourth hour of coagulation in the control was 0,88% and for sample S3 0,68%. The reason for the lower percentage of lactic acid may be the greater amount of cinnamon added to the sample (1,5%), which probably suppresses the activity of the lactic acid bacteria and slows down the coagulation of the milk. Similar results were obtained by Choi Y et al. (2016) in obtaining milk with lactic acid bacteria isolated from kimchi (traditional Korean cuisine) and cinnamon ethanol extracts.

The change in the values of active (pH) during storage of the obtained yoghurts was also observed. During storage, a decrease in pH was observed in all yogurt samples. In the control, the change in active acidity during storage was 4,6 to 4,1 and in the sample S1 4,6 to 4,2, respectively. The low temperature and the content of more cinnamon in sample S3 slows the rate of acid formation from 5,0 to 4,4. In the yoghurt samples containing more cinnamon (S3), changes in the active acidity were moderate and smaller than those in the control. These results suggest that the shelf life of yogurt samples can be effectively extended by suppressing the acidification process of lactic acid accumulation.

The addition of cinnamon increases the antioxidant activity of yogurts compared to the control during all storage periods. The highest antioxidant activity was recorded on day 7 for sample S3 (30,82%) followed by sample S2 (28,9%) and control (22,0%). The high antioxidant activity is due to both the added cinnamon and honey in the yogurt samples.

Table 1 shows the dynamics in the development of lactic acid bacteria during sample storage. The results obtained show that the total number of lactic acid bacteria at the beginning of the process was $5,5.10^7$, CFU/g, for samples S1 and S2, and $5,4.10^7$, CFU/g for sample S3. These values suggest a good development of the starter lactic acid bacteria in the earlier stages of the technological process. The highest values of total lactic acid bacteria during storage were observed in sample S1 ($2,7.10^9$, CFU/g) on day 7, and the lowest in sample S3 ($1,1.10^9$, CFU/g). This trend persists on the 14th day of storage - for sample S1 - $3,9.10^8$, CFU/g and for sample S3 - $3,1.10^8$, CFU/g.

Table 1. Total number of lactic acid bacteria (CFU/g) in yogurt with honey and cinnamon

Sample	Storage time (days)			
	0	3	7	14
Control	$5,6\ .10^7$	$2,2.10^9$	$1,3.10^9$	$3,4.10^7$
S1	$5,5.10^7$	$2,6.10^8$	$2,7.10^9$	$3,9\ .10^8$
S2	$5,5.10^7$	$3,4.10^8$	$1,4.10^9$	$3,3.10^8$
S3	$5,4.10^7$	$2,1.10^8$	$1,1.10^9$	$3,1.10^8$

The experimental data obtained on the antioxidant activity of yoghurts were used to derive a regression model to describe the relationship between antioxidant activity, the amount of cinnamon and the storage period, and to study its suitability. Multiple regression was sought between the antioxidant activity of the yogurt obtained to the amount of cinnamon added to the samples as a function of response and storage period. After analyzing the coefficients of the model and excluding the insignificant ones, it was found that a model of the type suitable for describing the relationship between the antioxidant activity, the amount of cinnamon and the storage period:

$$z = b_0 + b_1 x + b_2 y + b_3 x^2 + b_4 y^2 \quad (3)$$

where z is the antioxidant activity,%; x - the amount of cinnamon added,%; y - storage period, d. After the statistical data processing, the determination coefficient was determined, R^2=0,97, which means that 97% of the change in antioxidant activity is due to the factors controlling the amount of cinnamon and the storage period of the product, described by the model used. Of all the models studied, the coefficient of certainty is the highest. The statistically significant coefficients of the model are as follows: b_0=27,0045; b_1=-1,86453; b_2=0,2247; b_3=2,79148; b_4=-0,38382. The resulting regression model describes the surface z=f(x,y), which can be represented in a three-dimensional coordinate system. A general view of the model is shown in Fig.3. On the x-axis is the amount of cinnamon, on the y-axis the storage period is applied, on the z-axis the antioxidant activity is applied.

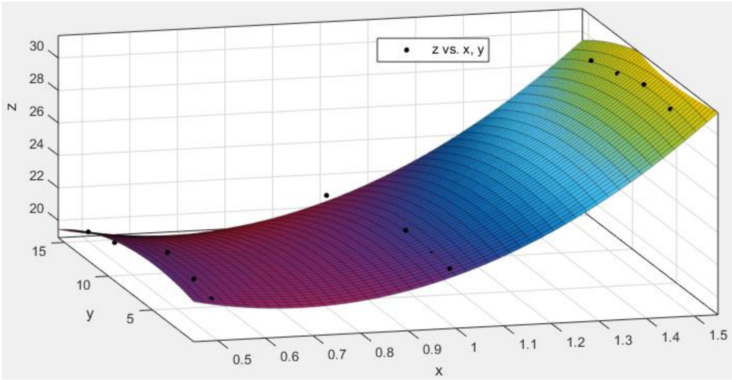
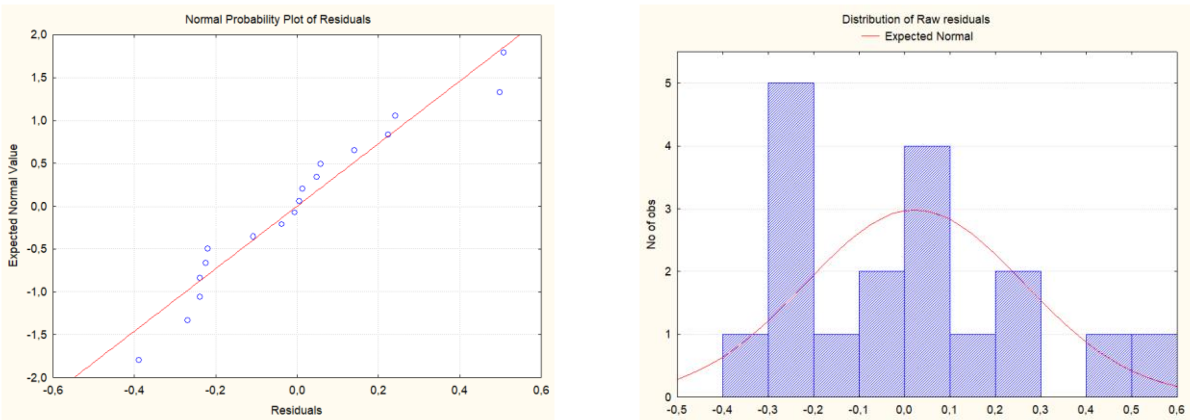


Fig. 3. Resulting model of the form z=f(x,y) – general view

The analysis of the residuals and their graphical representations are shown in Fig.4 as a normal probability graph. To the extent that the points are in a straight line, the residuals have a distribution close to normal and the preconditions for the regression analysis can be considered to be fulfilled. As can be seen from the distribution of the residues and their location around the normal line, under the normal probability graph, they are close to the normal distribution and the assumptions of the regression analysis can be considered to be fulfilled. The analysis of residuals shows a lack of systematic deviations of the actual data from the theoretical curve, which indicates their normal distribution.



a) normal probability plot of residuals b) Frequency histogram of residuals

Fig.4. Graphic analysis of residuals

CONCLUSION

The study found that the addition of cinnamon in an amount of 1,5% by weight of milk, suppresses the activity of lactic acid bacteria and slows down the coagulation of milk. Delayed coagulation enhances the aromatic balance, masks the action of acetaldehyde in yogurt, giving it a soft and balanced taste.

The addition of honey and cinnamon to the milk makes it possible to expand the range of lactic acid products with prophylactic and curative properties on the human body.

The model obtained describing the antioxidant activity of yoghurts, to the amount of cinnamon added to the samples and the storage period, is quadratic and describes with sufficient accuracy the experimental data obtained. The model has a coefficient of certainty of R^2=0,97 and low error values (below 3%). The residuals have a distribution close to normal and the preconditions for the regression analysis are fulfilled.

The results of this study suggest that the addition of cinnamon powder in yogurt production slows down the processes of lactic acid formation and hence its acidification. Cinnamon is a natural antioxidant with a wide range of beneficial nutritional properties, making it a suitable supplement for the production of functional foods.

The results obtained could be used in the development of cinnamon-added yogurt production technology.

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