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The unique characteristics of milky-wax ripe walnuts and their usage

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Abstract. The objective of the research is to obtain biologically valuable and safe food products, which have functional qualities and extended shelf life by using the antimicrobial properties of walnuts and walnut leaves of milky-wax ripeness. A study was conducted on the possibility of using walnuts of milky-wax ripeness (walnut materials) in the production technology of functional foodstuffs (cheese products, beverages, cheese, desserts, and bakery products). The study identified types and possible amounts of walnut additives into the developed products. Various additives were obtained during the research, such as extracts, tinctures, dry powders, and capsules. The most unique specific substance in raw nut is juglone. Juglone is a natural antibiotic that inhibits more than 100 kinds of pathogenic microorganisms. The developed products with the addition of walnuts and walnut leaves of milky-wax ripeness contain high amounts of vitamins (especially C, E, A, and group B) and minerals (iodine and manganese). For this reason, walnut contains the natural antibiotic juglone, which increases the shelf life of products up to 21 days without the use of stabilizers or preservatives. Because of the multifunctional properties of raw nut, more specifically the antibacterial, fungicidal and preservative properties of juglone, the additives ensure a sustainable microbiological state of the products. Juglone allows sparing technological heat treatment of raw milk (40 ± 2°C) that reduces energy costs for the operation and the equipment. Various population groups can successfully use the designed products as functional food; what is more, they can be used for medical purposes.

Key words: Walnut, fermented products, dairy products, juglone, preservative, milky-wax ripeness.

INTRODUCTION

Walnut is a unique vegetable raw material, all parts of which can be used by humans. Already the ancient Greeks and Romans mentioned walnut. Theophrastus, 'the father of botany', was one of the first to describe walnut. This plant is mentioned in the writings of Cicero, Pliny, Virgil, and Hippocrates (Derzhavina, 2000). Michuryn called walnut a tree, which is like an industrial complex, as absolutely all its parts are used by humans: ripe and unripe fruit, shells and partitions, green pericarp and leaves, bark, wood, and roots (Richter & Yadrov, 1985).

Curative and preventive properties of walnut have long been used by people in the areas of its growth – Moldova, Northern Caucasus, Romania, Tibet, Greece, Japan, China, France, etc. (Richter & Yadrov, 1985). The composition of milky-wax ripe raw walnuts includes essential oils, organic acids, alkaloids, glycosides, saponins,

coumarins, carotenoids, water-soluble vitamins, volatile, phenolic compounds, tannins, and trace elements (Yenikeeva, 2008). These natural complexes induce the therapeutic use of milky-wax ripe walnuts as supplements. The chemical composition of all parts of the walnut depends on the type, location, and environmental growing conditions.

All parts of walnut contain vitamins C, A, E, and group B, organic acids, minerals, and tannins. The unripe fruit of walnut contains up to 3–5% of vitamin C. The maximum content of vitamin C in unripe walnuts is observed at the beginning of endocarp solidification (Pilipenko & Orlova, 2009).

The main groups of minor bioactive components of food plants (quinones and hydroquinones) include juglone, which occurs in walnuts of milky-wax ripeness. Juglone (5-hydroxy-1,4-naphthoquinone) is a natural antibiotic with high bactericidal qualities. The German chemists Vogel and Reischauer first isolated it in 1856 from the green husk of walnut (Yenikeeva, 2008). Juglone inhibits the activity of *phosphatidylinositol-3-kinase*, which indicates its anticarcinogenic properties without marked toxicity inherent to other cytostatics (Babich & Stern, 1993). Juglone has high antimicrobial impact on both gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus mutans*), and gram-negative microorganisms (*Esherichia coli* and *Pseudomonas aeruginosa*), as well as on pathogenic yeast organisms (*Candida albicans*) (Polonik et al., 2004; Babula et al., 2005).

The aims of our work were to develop curd products using milky-wax ripe walnut, evaluate their chemical composition (fatty acids and amino acids), and monitor changes in sensory, physicochemical and microbiological properties of samples during storage.

MATERIALS AND METHODS

Materials

Non-fat curd, cream, starter for curd on pure cultures of lactic streptococci, calcium chloride, rennet powder, additives of fruit of walnut of milky-wax ripeness.

Additive preparation

For the production of supplements, we used different varieties of milky-wax ripe walnuts grown in the environmentally friendly Tsimlyanskii district of Rostov region (Russia). Harvesting of walnuts took place from 12 to 14 July in 2011 (average temperature was 28 ± 2°C) during fruit formation while they are rich in biologically active medicinal substances.

Honey was used as a preservative for the chopped nuts and as a sweetener. We used flower honey, which meets the requirements of the Russian standards (Russian consumer oversight agency, 2001).

Walnut additive technology consists of the following stages: receiving and sorting walnuts; washing walnuts and peeling them mechanically with a carborundum; chopping walnuts with a cutting-pulping machine, mixing chopped walnuts with honey heated to 38 ± 2°C, packaging and corking; storing at temperature 6 ± 2°C. The resulting additive is made of crushed milky-wax ripe walnuts (seed + partition + endocarp) mixed with honey; the color of the additives is homogenous and depends on the type of honey - from pale straw to dark brown; it tastes of honey and has a pronounced walnut flavour.

The preparation of curd products

Pasteurized cow cream (fat 25%) and non-fat curd were used for curd production. The cream should be homogenized (we used homogenizator Twin Panda Niro Soavi – 100 liters per hour) at temperature 60–70°C and pressure (8.0–10.0) MPa, then pasteurized at temperature 92 ± 2°C and incubated from 2 to 8 seconds, and then cooled to a temperature not exceeding 80°C (Orlova, 2007). The prepared curd and cream mixture was vacuumized and heated in a universal homogenizing module, after which it was stirred for 30 seconds at 1,500 rev min^{-1}. When the temperature reached 40 ± 2°C, the vacuum was turned off. Then we brought the supplement made of milky-wax ripe walnuts and honey. The additive was weighed, heated while mixing in the digester till 38 ± 2°C, after which it was added to the prepared curd and cream mixture. For removing air bubbles and distributing the additives uniformly, we started the stirrer and vacuum for 30 seconds. After that the product was packaged in polystyrene cups with a capacity of 200 grams. The packaged product was kept at irregular temperature for 2–3 h to prevent the formation of condensate, after which it was sent to the refrigerating chamber for cooling down till temperature 4 ± 2°C and the formation of structure for 6–8 h. The product should be stored at temperature 4 ± 2°C.

Manufacturing technology of curd products with the additive (15%) based on milky-wax ripeness walnuts is shown in Fig. 1.

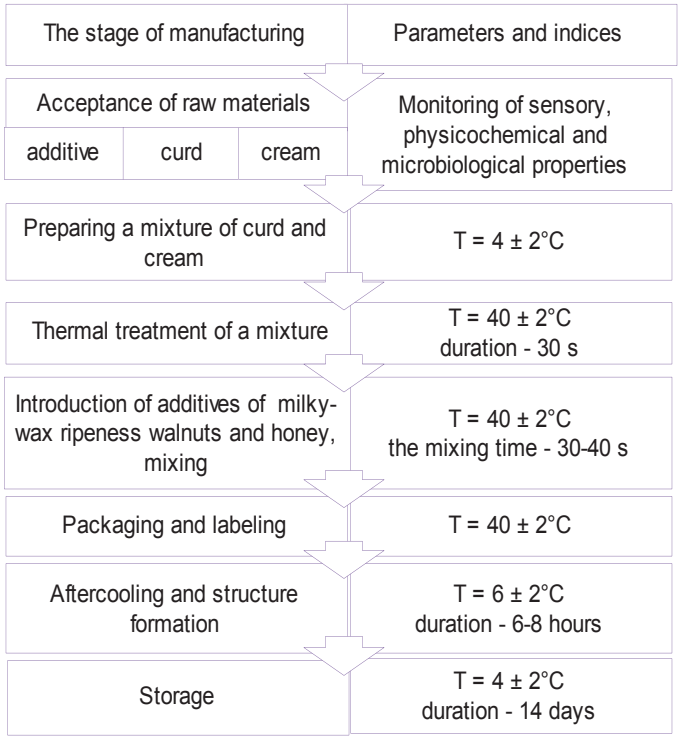

Figure 1. Curd products manufacturing technology.

Sensory evaluation

The taste, aroma, colour, and texture of all curd product samples were evaluated by a trained panel of 12 members using a five-point score system (5 excellent, 1 unacceptable).

Twelve panelists (eight women, four men; age 22–38 years) familiar with sensory evaluation techniques and regular consumers of curd products estimated the sensory properties of the samples.

Analysis

Juglone content of the walnut additives was determined by capillary electrophoresis using KAPEL 105 (sample injection 450 mbar*s, voltage 20 kV, temperature 20°C, detection 254 nm).

Amino acids, fatty acids, fat, solids, carbohydrates and titratable acidity were determined according to AOAC (1998).

Experimental design of shelf-life

Samples stored at 4 ± 2°C were evaluated on days 1, 7, 14, and 21 taking into account the factor of safety. The shelf-life of the developed products was evaluated according to MUK 4.2.1847–04 'Control methods. Biological and microbiological. Sanitary-epidemiological assessment of terms of consumption and suitability for food storage. Methodology.' (Russian consumer oversight agency, 2004).

Microbiological counts

The amount of *S. Aureus*, coliforms, yeasts and molds, and pathogens was determined by the methods described by Neusely et al., 2012.

RESULTS AND DISCUSSION

We have identified the possible amounts of walnut additives used for the developed products.

We have found out the dependence of the estimated organoleptic characteristics of the final product on the amount of contributed additive, which is expressed by the following equation 1:

$$Y = (28.026 - 0.962\ x)/(1 - 0.065\ x + 0.0013\ x^2) \quad (1)$$

Approximating function, which expresses the dependence of the organoleptic characteristics on the amount of additive and its deviation from the experimental values, is presented in Fig. 2.

The samples with 5, 10, 15% of additive had the most acceptable sensory properties. When we added more supplements, the taste of the final product was too sweet, and the consistency too liquid. The samples with the addition of less than 5% were not sweet and the taste of the additives was almost imperceptible. The resulting products had a smooth consistency without whey separation, a fermented taste of nuts and honey, and the colour varied from light cream to cream.

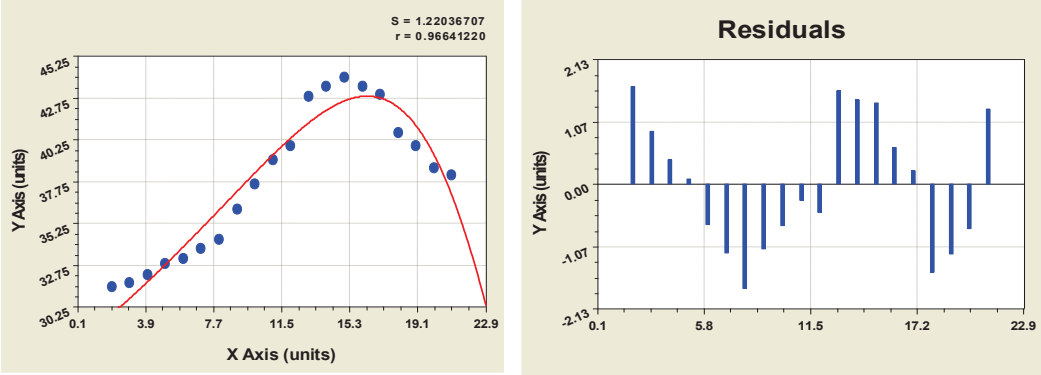

Figure 2. Approximation between organoleptic characteristics and the percentage of additives: X- the percentage of additive components, Y – organoleptic characteristics, points.

Table 1 shows the results of a study of the chemical composition of the samples of curd products. The maximum value of solids was observed in the sample with 15% additive, being 36.08%. The quantity of juglone had maximum value in the sample with 15% additive (Pilipenko & Orlova, 2009).

Table 1. Chemical composition of test samples

Sample	Mass fraction of dry substances,%	Mass fraction of protein,%	Fat mass fraction,%	Mass fraction of carbohydrates,%	Mass fraction of juglone,%
Base	36.03	14.23	10.0	11.8	0
5%	32.43	16.26	10.0	6.17	0.24
10%	34.23	15.30	10.0	8.93	0.47
15%	36.08	14.39	10.0	11.69	0.71

In order to characterize the biological value of the investigated products, the composition of amino acids was determined and the amino-acid score was calculated. The results are presented in Table 2. The results of the study of biological values indicate that the proteins of the new types of curd products are characterized by a complete set of essential amino acids. It was found out that the additive has increased the content of essential amino acids, which were especially limited in the base sample. The sample with 15% of additive had the greatest number of them. It can be explained by the fact that walnut protein has increased the content of lysine (up to 12.4 g/100 g of protein), methionine (5.6 g/100 g of protein), and tryptophan (to 3.4 g/100 g protein).

The fat of the product consisted of a mixture of milk and vegetable fats. The lipids of milk fat have 70.0% of saturated fatty acids, and about 4.0% of polyunsaturated fatty acids. The lipids of milky-wax ripe walnuts consist of 84.5% of unsaturated fatty acids, the content of linoleic and α-linolenic acid can be up to 56.0% and 11.0% respectively.

Table 2. Contents of essential amino acids in test samples

Name of amino acids	Base		5% additive		10% additive		15% additive	
	X^*, mg g^{-1}	A^*, %	X^*, mg g^{-1}	A^*, %	X^*, mg g^{-1}	A^*, %	X^*, mg g^{-1}	A^*, %
Valine	50.28	100.56	55.91	111.82	57.31	114.62	58.64	117.28
Isoleucine	48.10	120.25	54.93	137.33	57.31	143.28	59.64	149.10
Leucine	79.69	113.84	93.51	133.59	98.53	140.76	103.51	147.87
Lysine	63.77	115.95	73.67	133.95	76.77	139.58	79.82	145.13
Methionine + cystine	27.97	79.91	30.55	87.29	33.13	94.66	35.71	102.03
Threonine	36.46	91.15	50.28	125.70	60.42	151.05	70.52	176.30
Tryptophan	9.83	98.30	10.97	109.70	12.11	121.10	13.25	132.50
Phenylalanine + tyrosine	82.83	138.05	101.43	169.05	111.36	185.60	121.24	202.07

X^* is amino acid content in 1g protein product; A^* is amino-acid score of the amino acid.

The results of determining the fatty acid composition of curd products are presented in Table 3. PUFA ratio ω_6, ω_3 is 9.886 : 1, and recommended by the Institute of Nutrition, the ratio is 10 : 1 for a healthy person.

Table 3. Fatty acid composition of products

Name of acid	Fatty acid content in the test samples, g			
	Base	5% additive	10% additive	15% additive
Sum of saturated acids	**7.047**	**6.526**	**6.142**	**5.563**
Butyric	0.211	0.198	0.185	0.149
Capric	0.254	0.217	0.198	0.162
Myristic	1.384	1.354	1.347	1.331
Palmitic	3.722	3.526	3.282	3.213
Stearic	0.946	0.938	0.721	0.583
Arachic	0.090	0.070	0.056	0.049
Unidentified	0.440	0.223	0.353	0.276
Amount of unsaturated acids	**2.953**	**3.474**	**3.858**	**4.437**
Myristoleic	0.113	0.075	0.053	0.060
Palmitoleic	0.248	0.252	0.211	0.228
Oleic	2.090	2.141	2.159	2.214
Linoleic (**ω_6**)	0.189	0.698	1.124	1.523
α-linolenic (**ω_3**)	0.081	0.121	0.147	0.168
Arachidonic (**ω_6**)	0.153	0.144	0.141	0.139
Unidentified	0.078	0.043	0.023	0.105
ω_6/,ω_3	**4.275**	**7.020**	**8.687**	**9.886**

The results of organoleptic evaluation of the curd products during storage are presented in Fig. 3. The best results on the first day of storage have samples No 1 and No 4, since by all indications they have received the highest score – 20 points.

Sample No 4 got the highest score after 21 days of storage. There was no change in the appearance of the sample; the surface remained smooth and glossy with evenly distributed patches of additive. The consistency of sample No 4 was the same as that of

the fresh sample: rather viscous, thick, and slightly fluffy. The colour was cream and uniform throughout the mass, a little more intense than that of the fresh sample. The taste and smell of sample No 4 on the 21st day of storage were weaker, having a less pronounced taste of nuts and honey.

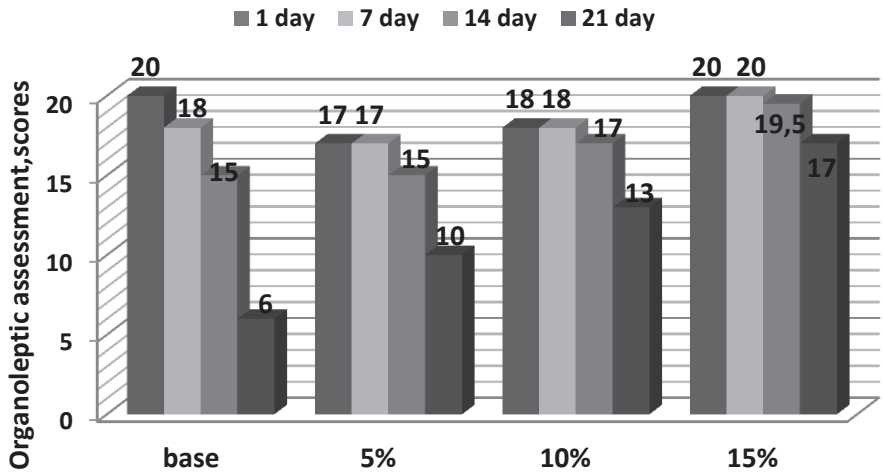

Figure 3. Organoleptic assessment during the storage of different percentages of additive components.

On the 21st day of storage, the control sample had a too acid taste and yeast flavour. Separation of serum and unpleasant odour were observed. We concluded that the product was not suitable for consumption.

The change in titratable acidity is shown in Fig. 4.

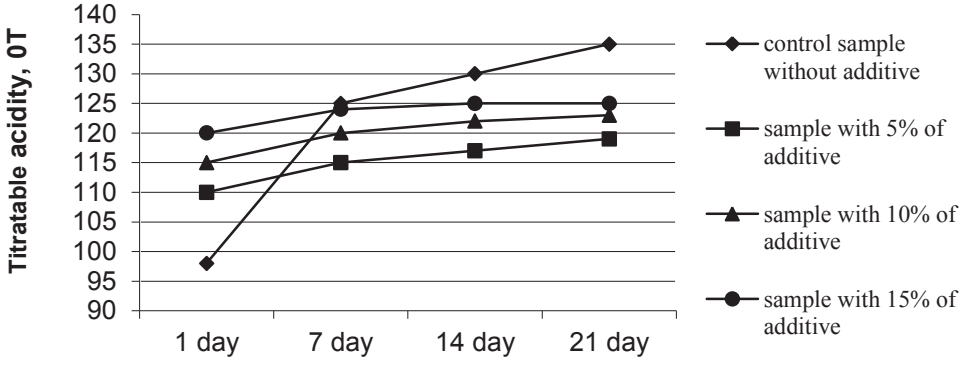

Figure 4. Change in titratable acidity of curd products during the storage.

In the control sample without the additive, quite a rapid growth of it can be observed at the beginning of the storage. After 14 days of storage, titratable acidity of the control sample did not meet the requirements of Russian legislation (Rosstandart, 2008). Introduction of supplement to the curd product allowed halting the growth of acidity in the product, especially in the sample with 15% of the additive. It is obvious that the preservative effect of juglone slows the formation of acids. It is known that juglone has properties, which partially inhibit the growth of lactic acid bacteria even in low concentrations (Babula et al., 2005).

We have investigated microbial indicators of samples in accordance with Russian legislation (Rosstandart, 2008). The results of microbiological tests (on day 14) are shown in Table 4.

In the studied samples of curd products molds, coliform bacteria in 0.001 g of product and pathogens including salmonella in 25 g were not detected. Colony-forming units (CFU) *Staph. aureus* in 1 g were not detected. The amount of yeast in the control sample did not meet the requirements of Russian legislation. Samples No 1, 2 and 3 are within the expected microbiological levels for this type of product and present no food safety concern (Rosstandart, 2008).

Table 4. The result of microbiological tests

Name of indicator	Product weight (g, cm^3), which is not allowed	Sample No1 control sample without additive	Sample No 2 sample with 5% of additive	Sample No 1 sample with 10% of additive	Sample No 1 sample with 15% of additive
CGB (coliforms), in 0.01 cm^3	0,001	not detected	not detected	not detected	not detected
S. aureus, in 0.1g	0,1	not detected	not detected	not detected	not detected
Pathogenic, including *salmonella*	25	not detected	not detected	not detected	not detected
Yeast, CFU/g	100	110	45	22	20
Molds, CFU/g	50	not detected	not detected	not detected	not detected

We assume that the reduction in yeast amount is related to the increase in additive percentage. It may be explained by the presence of naphthoquinone juglone.

CONCLUSION

Milky-wax ripe walnut is a unique product. Its amazing properties have been used since ancient times. The use of the unique characteristics of milky-wax ripe walnut (especially juglone) enables to develop fortified foods, increase the shelf life of products due to their content of naftachinon – juglone in walnuts. These studies have shown only a small part of the results of introducing walnut based additives into curd products.

Introduction of the additives allows obtaining a cream-colored product with a pronounced walnut and honey taste and odour and a smooth and pleasant consistency. The additive of milky-wax ripe walnut slows acid formation.

In contrast to the control sample, the samples with the additive displayed an increase in:

- The amount of essential amino acids, thus improving the biological value of the final product;
- The ratio of ω_6/ω_3 from 4.275 to 9.886.

The results of monitoring changes in sensory, physicochemical and microbiological properties of samples during storage allow us to recommend the shelf life of 14 days (4 ± 2°C) for curd products with additive.

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