

COMPARATIVE ANALYSIS OF YOGHURTS OBTAINED WITH BIOACTIVE COMPOUNDS

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Abstract: *The yoghurt is a fermented milk product that offers many benefits to the health of the consumer and can be eaten from an early age. The bioactive compounds with which we decided to enrich the quality of yoghurt are different volatile oils that have antimicrobial and antioxidant effect. The objectives of the research are to examine the decision makers in the process of purchasing a dairy product obtained with bioactive compounds, the sensory analysis of several types of yoghurt by a team of tasters, the determination of lactic acid and pH determination. The volatile oils were encapsulated with sodium alginate and then were formed spherical capsules. Four types of yoghurt with bioactive compounds were obtained: yoghurt from cow's milk with the addition of mint volatile oil encapsulated in sodium alginate, yoghurt from cow's milk with the addition of basil volatile oil encapsulated in sodium alginate, yoghurt from cow's milk with the addition of fennel volatile oil encapsulated in sodium alginate and yoghurt from cow's milk with the addition of sea buckthorn volatile oil encapsulated in sodium alginate. The research was accomplished on the first day, on the seventh day and on the fourteenth day after the yoghurt was made. The obtained results have shown that the volatile oils with antimicrobial and antioxidant effects (mint oil, fennel oil and basil oil) increase the shelf life of yoghurt. Sea buckthorn volatile oil has a low antimicrobial activity and therefore it cannot increase the shelf life of yoghurt. All of these volatile oils offer many benefits to the health of consumers and the yoghurt fits perfectly into the current trend.*

Key words: *yoghurt, bioactive compounds, encapsulation, sensory analysis, questionnaire, lactic acid, pH.*

1. Introduction

The milk is a white - yellowish liquid, with sweet taste and characteristic smell secreted by the mammary gland of mammals, with a complex chemical

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composition that varies according to species, race, diet, age and health [2]. The milk is a very complex chemical and physicochemical system whose perfect knowledge is indispensable for understanding the principles of its preservation and transformation into derivatives products. Cow's milk is most commonly used in human food, because it is most similar to breast milk [10-11]. Since ancient times, a number of herbal substances have been extracted that have proven useful to humankind, both through nutritional surplus and for treating certain diseases. The extraction of oils from different plants has been made since antiquity in Egypt, Greece and Rome. Leaves, flowers and plant roots were placed in bottles and left in the sun to perform the extraction. These oils were used especially in religious ceremonies and aromatherapy. Sometimes plant flowers were soaked in wine, being used to treat digestive diseases [3]. The oldest description of a volatile oil produced by a method similar to classical hydro-distillation was made of Arnald de Villanova (1235-1311). This method was initially used to produce camphor distilled water from which essential oil was fractionated as a second product [6]. Following several studies conducted over the years, volatile oils extracted from various aromatic herbs have been found to have many health benefits for the human body. Most volatile oils have antimicrobial activity, especially on pathogenic bacteria such as *Listeria monocytogenes*, *Listeria innocua* and *Salmonella typhimurium* [9]. Another mode of action is the generation of irreversible damage to the bacterial cell membrane, which results in material loss (cytoplasm), ion leakage, loss of energy

substrate (glucose, ATP), leading directly to bacterial cytolysis and therefore to death. Another possibility of action is the inhibition of the production of amylase and protease that stops the production of toxins, the electron flow, leading to coagulation of the cell content [1]. Antiviral activity of volatile oils is mainly due to direct verucidal effects (by denaturing structural viral proteins or glycoproteins). Volatile oils interfere with the virus and inhibit the specific process of multiplication. Free radicals causes oxidation of biomolecules, including proteins, amino acids, DNA etc. and ultimately cause molecular changes related to aging, arteriosclerosis and cancer. Inside the human body, an imbalance between the production of free radicals and their removal through the antioxidant system leads to "oxidative stress". So, the external supply of antioxidants is necessary to achieve the balance between free radicals and antioxidants. Antioxidant activity is not only due to the presence of a phenolic group, whereas the ketones, aldehydes, hydrocarbons and ether also indicate the activity of removing free radicals. Volatile oils such as *Thymus caespitius*, *Thymus camphoratus* and *Thymus mastichina* exhibit high antioxidant activity of linalool and 1,8-cineols, while thymol and carvacol are almost absent. Therefore, to reduce oxidative stress, volatile oils need to be part of daily supplements [9].

Alginate is a natural polysaccharide extracted from brown algae [5]. The entire range of alginate marketed is produced from marine algae including *Laminaria hyperborean*, *L. digitata*, *L. japonica*, *Lessonia nigrescence*, *Macrocystis pyrifera* and *Durvula Antarctica*. The main algae harvesting sites are the cold waters of

northern Europe, the western coast of South America, southern Australia and Tasmania and the surrounding area of Japan. A very large amount of brown algae is grown in China [8]. Sodium alginate is a natural product whose viscosity can vary greatly from lot to lot. For the formation of volatile oil capsules, it is necessary that the sodium alginate that has been used should have a fluid flowing viscosity. It is necessary to make the sodium alginate solution with one day before forming the alginate capsules. Preparation of the solution is done by adding 2% sodium alginate powder and water, but it is important not to mix immediately and to let it clear overnight [7].

2. Materials and Methods

For making the yoghurt with bioactive compounds, we have been used raw cow's milk that was pasteurized, cooled and enriched with lactic crops, and then the alginate capsules are added and left to thermostat for 3 hours. The alginate capsules were made from sodium alginate and a quantity of basil, mint, fennel and sea buckthorn volatile oil. The alginate solution was added gradually to the calcium chloride solution under centrifugation, thus obtaining the alginate capsules that were subsequently washed with distilled water.

In order to analyse the desire to purchase and the trends in the consumption of yoghurts, especially the yoghurt with the addition of volatile oils encapsulated in sodium alginate, we conducted a market survey. To realize this market survey, we used a questionnaire. A questionnaire is a research tool that consists of a series of questions in order to gather certain information from people.

The questionnaire was invented by Sir Francis Galton and was designed for statistical analysis of responses. As a research tool, the questionnaire has a number of advantages: it is cheap; it does not require much effort on the part of the interviewer and often has standardized answers that make it easy to fill in [4]. At this market survey, were participated 200 persons between the ages of 18 and 60 years and the questions were related to the frequency of buying dairy products, the reason for the purchase, the yogurt fat content and the reason to purchase the yoghurt with bioactive compounds.

Sensory analysis will be realize by a team of 5 amateur tasters, regular consumers, on the first day after making the yogurt, on the 7th day and on the 14th day. In order to perform the sensory analysis was used the method by comparison with unit scoring scales. In this method, the taster examines the sensory qualities of cow's milk yoghurt with the addition of volatile oils encapsulated in sodium alginate by comparison with unit scoring scales (0 to 5 points). For each sensory characteristic (colour, consistency, taste, odor and viscosity), a table with score scales is drawn up. The score for assessing the sensory features is as follows: 5 points – very good; 4 points – good; 3 points – satisfactory; 2 points – unsatisfactory; 1 point – inappropriate; 0 points – altered.

Lactic acid determination was performed using enzymatic tests and the use of semiautomatic clinical biochemistry analyser Stat Fax 1904. The principle of this enzymatic test consists in the quality of the results obtained by enzymatic determinations based on the enzyme specificity, the accuracy of the enzyme-catalysed reactions and the recognition of

the extinction coefficients of the light-absorbing substances. The enzymatic tests to which we refer are UV methods based on the measurement of the increase or decrease in the absorption of the NADH or NADPH coenzyme at 340 nm. The result is expressed in grams / 100 grams of product.

pH determination was performed with Hanna Instruments model pH meter.

3. Results and Discussions

3.1. Market Survey

To conduct the market survey, we have opened a questionnaire because it is the simplest and best-known method of analysing the desire to purchase and trends in the consumption of yoghurts from cow's milk, especially the one with the addition of volatile oils encapsulated in sodium alginate. The questionnaire used contained nine questions and the results obtained for each individual question are the following:

1. Do you consume yogurt from cow's milk?

As can be seen in the Figure 1, most interviewees consume yoghurts from cow's milk and only 4 people said they did not consume any yoghurt.

2. How often do you buy yogurt from cow's milk?

In terms of purchasing frequency (Figure 2), most people buy weekly yogurts from cow's milk, followed by those who buy daily. The fewer responded that they occasionally buy yogurts, and those who buy monthly occupy the penultimate place.

3. What is the main reason for purchasing cow's milk yoghurts?

Most people interviewed chose the main reason for buying yogurts from cow's milk (Figure 3) is the benefits they bring to the health of the consumer. Another quite appreciated feature is taste, followed by packaging, price and advertising.

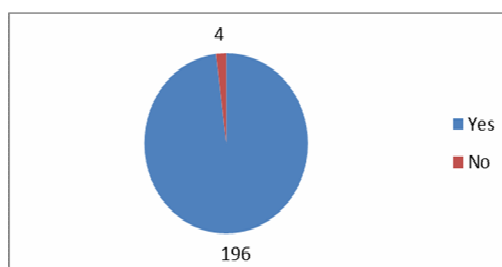


Fig. 1. Evaluation of the results of question number 1

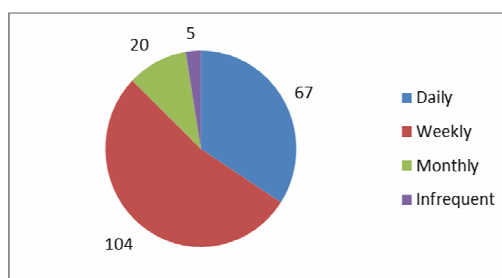


Fig. 2. Evaluation of the results of question number 2

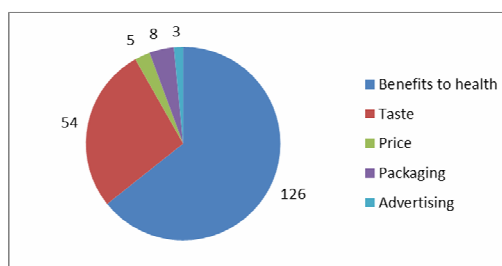


Fig. 3. Evaluation of the results of question number 3

4. What is the fat content you want to contain the yogurt you are going to buy?

As can be seen in Figure 4, most of the consumers surveyed chose the most fat content of 3.5%. The second place was 0.1%, followed by 10% and finally by 2%.

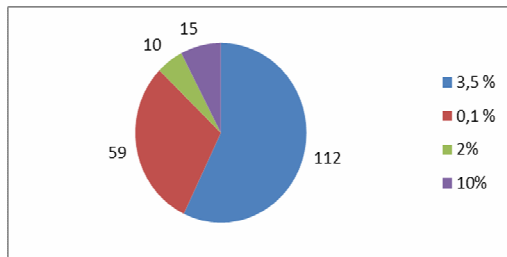


Fig. 4. Evaluation of the results of question number 4

5. *Would you like to try a new type of yoghurt, namely cow's milk yoghurt with the addition of volatile oils encapsulated in sodium alginate?*

Regarding the desire to purchase the new assortment of cow's milk yoghurt with the addition of volatile oils encapsulated in sodium alginate, most people decided to purchase it in order to try it, but 34 of the people were reluctant (Figure 5).

6. *What are the main characteristics of cow's milk yoghurt with the addition of volatile oils encapsulated in sodium alginate?*

The most important feature that consumers want to have cow's milk yogurt with the addition of volatile oils encapsulated in sodium alginate is to prevent the development of contamination microorganisms (Figure 6). This feature is closely followed by the prevention of food poisoning and ultimately increasing the shelf life.

7. *What amount of yogurt do you want to contain a pack?*

As can be seen in Figure 7, most people want a pack to contain 125 g of yogurt. Much less want to contain 350g, 500g, and fewer have chosen a different amount.

8. *In what environment do you live?*

Most interviewed people live in urban areas and only 42 people live in rural areas (Figure 8).

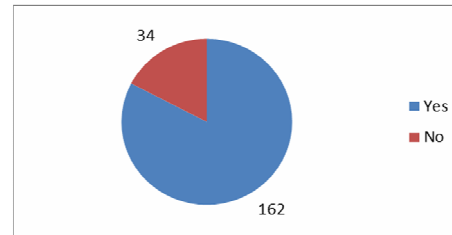


Fig. 5. Evaluation of the results of question number 5

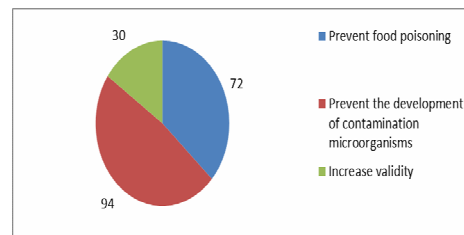


Fig. 6. Evaluation of the results of question number 6

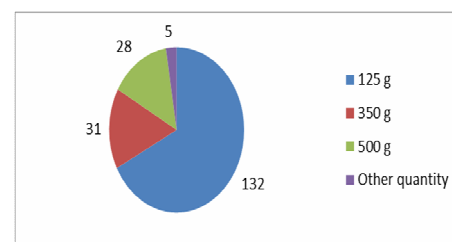


Fig. 7. Evaluation of the results of question number 7

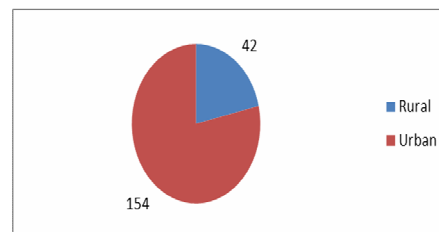


Fig. 8. Evaluation of the results of question number 8

9. How old are you?

As you can see in Figure 9, most respondents are between the ages of 26 and 40, followed by those aged 18-25. The smallest are between the ages of 41 and 55 and over 55 years.

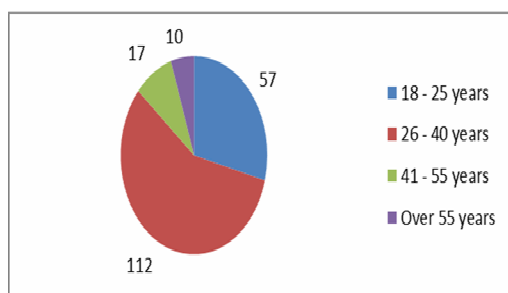


Fig. 9. Evaluation of the results of question number 9

At this study was participated 200 people and were obtained the following results: most consumers chose the main reason for purchasing dairy products, health benefits from consumption, most young people interviewed buy dairy products about 2- 3 times a month, and over 40 about once a week. Most interviewees prefer to purchase yogurt in approximately 125g and 350g glasses. The fat content of the most appreciated yogurt is 3.5% fat, followed by 0.1% fat. Regarding the desire to purchase a yogurt with the addition of volatile oils encapsulated in sodium alginate, most consumers have decided to consume it because its ability to prevent food poisoning and the development of microorganisms is a very important benefit for them.

3.2. Sensory Analysis

The tasters analysed the sensory characteristics of cow's milk yoghurt with the addition of volatile oils encapsulated

in sodium alginate by comparing with score scales from 0 to 5 points set in the sensory analysis standards.

The sensory analysis of the four assortments of yoghurt with bioactive compounds was carried out on the first day after the yogurt, on the seventh day and on the fourteenth day after its realization. The results obtained from each assortment are shown in the figures 10-21.

From the Figure 10, we can see that the most appreciated sensory characteristic on the first day after the realization of the yogurt is the taste. This feature obtained the maximum score; more exactly note 5 from each of the tasters. After taste, was appreciated the colour, smell and viscosity. The least appreciated feature was consistency, with only a score of 5.

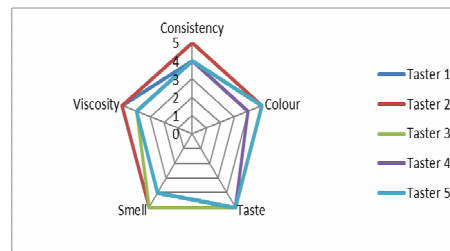


Fig. 10. Evaluation of the characteristics of yogurt from cow's milk with the addition of mint volatile oil encapsulated in sodium alginate on the first day of storage, according to the scores

It can be seen that on the seventh day after the product was manufactured (Figure 11), the highest scores were obtained by colour, smell and viscosity. The least was taste and consistency.

As you can see (Figure 12), on this day the lowest notes were obtained. However, the most appreciated characteristic was smell, and then colour, taste, viscosity and the lowest scores were obtained by

consistency.

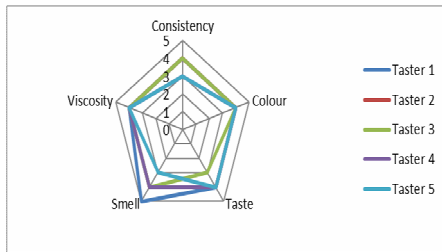


Fig. 11. Evaluation of the characteristics of yogurt from cow's milk with the addition of mint volatile oil encapsulated in sodium alginate on the seventh day of storage, according to the scores

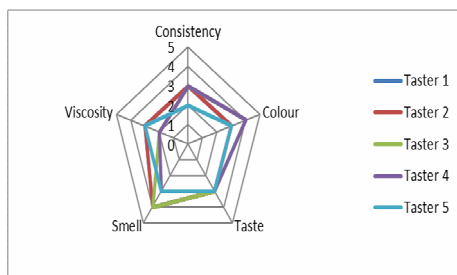


Fig. 12. Evaluation of the characteristics of yogurt from cow's milk with the addition of mint volatile oil encapsulated in sodium alginate on the fourteenth day of storage, according to the scores

As can be seen from Figure 13, the most appreciated feature on the first day after making the cow's milk yoghurt with the addition of fennel volatile oil encapsulated in sodium alginate was the colour. This feature obtained the maximum score; more exactly note 5 from each of the tasters. The viscosity and consistency were equally appreciated, with three ratings of 5 and the less appreciated were the taste and the smell, these characteristics having only two points of 5.

The grades obtained on the seventh day after making the yoghurt were lower than those obtained on the first day of

realization (Figure 14). No taster gave the maximum score, note 5. However, most note 4 were given to consistency and colour, followed by viscosity and smell. Taste has the lowest ratings, the only tasters who awarded the note 4, being the taster 1 and 2.

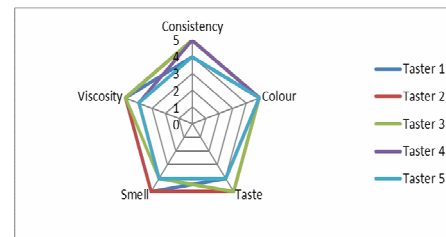


Fig. 13. Evaluation of the characteristics of yogurt from cow's milk with the addition of fennel volatile oil encapsulated in sodium alginate on the first day of storage, according to the scores

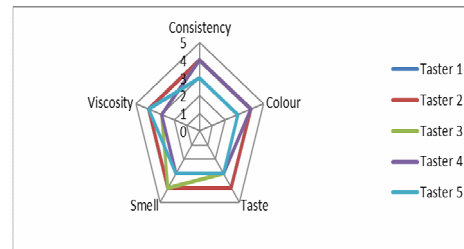


Fig. 14. Evaluation of the characteristics of yogurt from cow's milk with the addition of fennel volatile oil encapsulated in sodium alginate on the seventh day of storage, according to the scores

As with yogurt in cow's milk with the addition of mint volatile oil encapsulated in sodium alginate, the lowest scores were given on the fourteenth day (Figure 15). Only one note of 4 was given by the taster 2 for colour. The lowest ratings were given to taste and viscosity, with only two notes of 3.

As can be seen in Figure 16, the most appreciated feature on the first day of

making cow's milk yogurt with added basil volatile oil encapsulated in sodium alginate was the smell. This feature obtained the maximum score; more exactly note 5 from each of the tasters. The taste and colour were equally appreciated, with four notes of 5, and less appreciated were consistency and viscosity, these characteristics being only three points of 5.

The grades obtained on the seventh day (Figure 17) after making the yoghurt were lower than those obtained on the first day of realization. No taster gave the maximum score, note 5. However, only four grades were awarded for colour, and the smell and viscosity obtained four notes of 4. The taste and consistency obtained from the five tasters only three notes of 4.

As with the two previous yogurt varieties, the lowest grades were obtained on this day. No notes of 4 and 5 were awarded (Figure 18). Only the smell received scores of 3 from all five tasters. The colour, taste and viscosity achieved four points of three and the consistency obtained only two notes of 3.

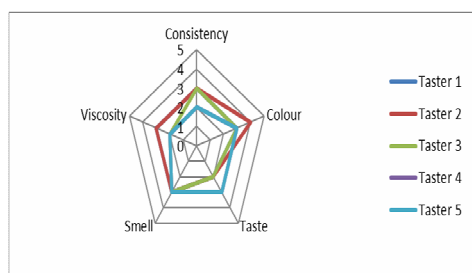


Fig. 15. Evaluation of the characteristics of yogurt from cow's milk with the addition of fennel volatile oil encapsulated in sodium alginate on the fourteenth day of storage, according to the scores

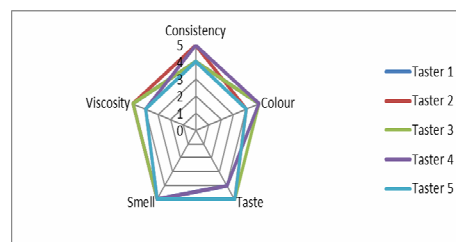


Fig. 16. Evaluation of the characteristics of yogurt from cow's milk with the addition of basil volatile oil encapsulated in sodium alginate on the first day of storage, according to the scores

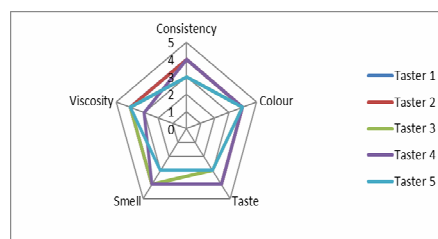


Fig. 17. Evaluation of the characteristics of yogurt from cow's milk with the addition of basil volatile oil encapsulated in sodium alginate on the seventh day of storage, according to the scores

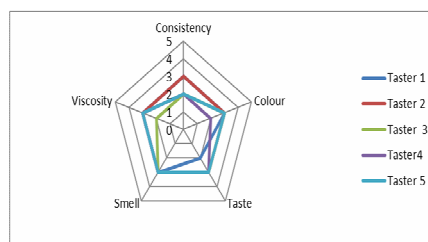


Fig. 18. Evaluation of the characteristics of yogurt from cow's milk with the addition of basil volatile oil encapsulated in sodium alginate on the fourteenth day of storage, according to the scores

As can be seen in Figure 19, the most appreciated characteristics of cow's milk yoghurt with the addition of sea buckthorn volatile oil encapsulated in sodium alginate are consistency, colour and taste. All of

these features received three 5 of the five tasters. The viscosity and smell were less appreciated by the five tasters, who received only two notes of 5.

The highest grades obtained on the seventh day after the yoghurt was obtained by colour (Figure 20). This feature received the score 5 from the second taster. The consistency and the taste were less appreciated, and the least appreciated were the viscosity and smell.

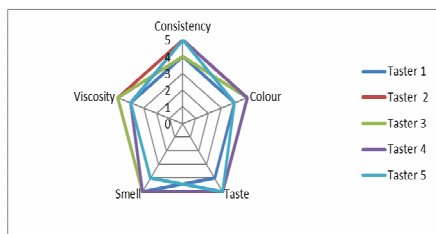


Fig. 19. Evaluation of the characteristics of yogurt from cow's milk with the addition of sea buckthorn volatile oil encapsulated in sodium alginate on the first day of storage, according to the scores

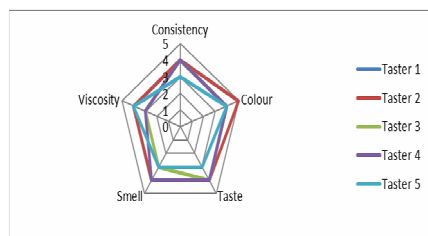


Fig. 20. Evaluation of the characteristics of yogurt from cow's milk with the addition of sea buckthorn volatile oil encapsulated in sodium alginate on the seventh day of storage, according to the scores

As with other previous yogurt varieties, the lowest grades were obtained on this day. No notes of 4 and 5 were awarded (Figure 21). Only the colour received scores of 3 from all five tasters. The taste and the smell obtained four grades of 3

and the viscosity obtained three points of 3. The least appreciated was the consistency that received from the tasters only two notes of 3.

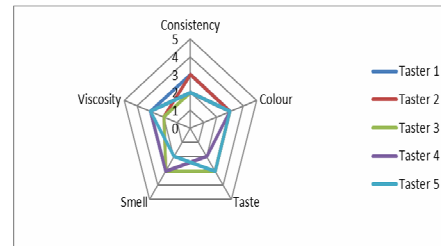


Fig. 21. Evaluation of the characteristics of yogurt from cow's milk with the addition of sea buckthorn volatile oil encapsulated in sodium alginate on the fourteenth day of storage, according to the scores

The highest scores were obtained on the first day of tasting, that is, one day after yoghurt preparation. The most popular assortment of yoghurt with the addition of volatile oil encapsulated in sodium alginate is the yoghurt with mint oil. It obtained the highest scores for taste and colour. Then too much was appreciated and the yoghurt with the addition of sea buckthorn volatile oil encapsulated in sodium alginate, which obtained great scores for taste and smell. Less appreciated was yoghurt with the addition of volatile basil oil encapsulated in sodium alginate, the most appreciated feature being the smell. The least appreciated was the yoghurt with the addition of volatile oil of fennel encapsulated in sodium alginate, the colour obtaining the highest score.

3.3. Lactic Acid Content

To determine the lactic acid content of the five samples, more exactly for the blank sample - simple cow's milk yoghurt, the cow's milk yoghurt sample with the

addition of mint volatile oil encapsulated in sodium alginate, the sample of cow's milk yoghurt with addition of fennel volatile oil encapsulated in sodium alginate, the sample of cow's milk yoghurt with addition of basil volatile oil encapsulated in sodium alginate and the sample of cow's milk yoghurt with addition of sea buckhorn volatile oil encapsulated in alginate sodium, determinations were made on the first day, on the seventh day and on the fourteenth day after storage and the results obtained are shown in Figure 22.

As can be seen in Figure 22, the lactic acid content of the blank sample grows over the storage period. The highest lactic acid content of the blank sample was recorded on the fourteenth day and the lowest content on the first day.

Regarding the lactic acid content of the yoghurt samples with volatile oils, the highest value of the lactose content of the first day was recorded for the sample of yoghurt with fennel volatile oil and the lowest lactose content was recorded for the sample of yoghurt with sea buckhorn volatile oil. On the fourteenth day, the lowest lactose content was recorded for the sample of yoghurt with sea buckhorn volatile oil, and the highest value was recorded for the sample of yoghurt with basil volatile oil. Sea buckhorn volatile oil has a low antimicrobial activity and therefore, after the seventh day of storage, the lactic acid content do not remains constant. The lactic acid content for yogurt samples with the addition of basil, mint and fennel volatile oils stabilized after the seventh day of storage. By the addition of volatile oils with antimicrobial activity, lactic fermentation is reduced and the acidity of the yogurt remains constant, which increases its quality the shelf life.

As regards the determination of lactic

acid, the best scores were obtained from the yogurt samples with the addition of volatile oil. The comparison was made with a blank test, more exactly a yogurt without volatile oils. On the seventh day, the results began to stabilize on samples of yogurt with the addition of volatile oils, obtained more favourable result comparative with blank sample. All these results show that volatile oils improve the finished product.

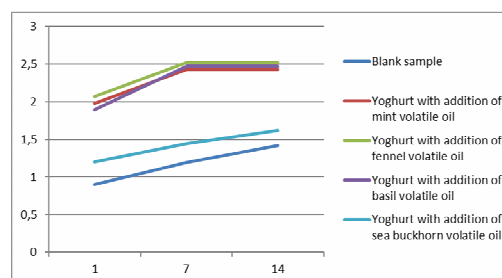


Fig. 22. Variation of lactic acid content for the five yoghurt samples from cow's milk in the first, seventh and fourteenth day of storage

3.4. pH Determination

To determine the pH of the five samples, more exactly for the blank sample - simple cow's milk yoghurt, the cow's milk yoghurt sample with the addition of mint volatile oil encapsulated in sodium alginate, the sample of cow's milk yoghurt with addition of fennel volatile oil encapsulated in sodium alginate, the sample of cow's milk yoghurt with addition of basil volatile oil encapsulated in sodium alginate and the sample of cow's milk yoghurt with addition of sea buckhorn volatile oil encapsulated in alginate sodium, determinations were made on the first day, on the seventh day and on the fourteenth day after storage and the results obtained are shown in Figure 23.

As can be seen (Figure 23), the pH of the blank sample decreases over the storage

period. The highest pH of the blank sample was recorded on the first day and the lowest content on the fourteenth day.

Regarding the pH of the yoghurt samples with volatile oils, the highest value of the pH on the first day was recorded for the sample of yoghurt with sea buckhorn volatile oil and the lowest pH was recorded for the sample of yoghurt with fennel volatile oil. On the fourteenth day, the lowest pH was recorded for the sample of yoghurt with

fennel volatile oil and the highest value was recorded for the sample of yoghurt with sea buckhorn volatile oil. Sea buckhorn volatile oil has a low antimicrobial activity and therefore, after the seventh day of storage, the pH do not remains constant. Adding basil, mint and fennel volatile oils, the pH is no decreasing during storage and from a qualitative point of view the finished product is superior to simple yogurt and the shelf life will increase.

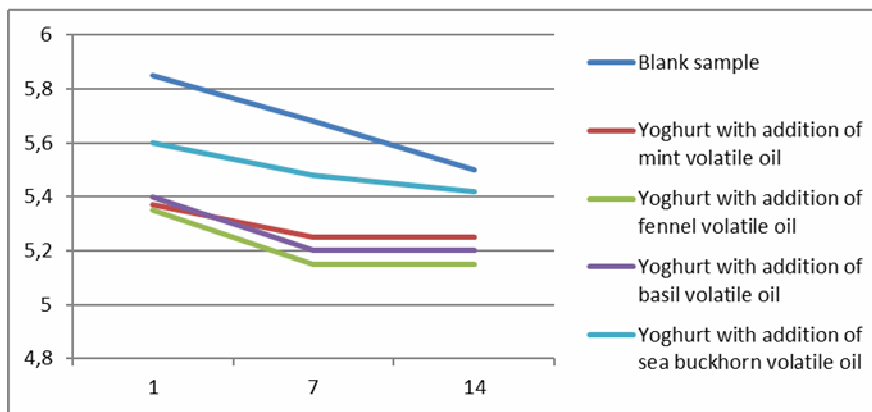


Fig. 23. Variation of pH for the five yoghurt samples from cow's milk in the first, seventh and fourteenth day of storage

4. Conclusions

As a result of the analysis, it is observed that cow's milk yoghurt with the addition of volatile oils encapsulated in sodium alginate corresponds qualitatively. Volatile oils of mint, basil and fennel have antimicrobial and antioxidant effect, thus helping to reduce the growth of pathogenic microorganisms during storage and to increase the shelf life of the finished product. In addition to these benefits to the health of the consumer, cow's milk yoghurt is a highly appreciated dairy product due to its nutritional properties, its high calcium content and the possibility of

its consumption at an early age.

All these aspects show that the chosen product fits perfectly into the current trend due to the benefits to the consumer's health and to increasing the shelf life of the product by incorporating bioactive components.

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