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OBTAINING OF A MILK DRINK WITH MUSHROOMS AND BIOACTIVE COMPOUNDS

Mihaela A. TIȚA[1] Maria A. CONSTANTINESCU[1] Cecilia GEORGESCU[1] Oana POPA[2] Ovidiu TIȚA[1] Loreta TAMOŠAITIENE[3] Vijole BRADAUSKIENE[4]

Abstract: *Fermented dairy products are a rich source of nutrients. Buttermilk is part of fermented dairy products and is highly valued by consumers due to its sensory and nutritional qualities. The study aims to capitalize on buttermilk by adding mushroom powder and volatile oil of dill and caraway encapsulated in sodium alginate. The research was performed over for 20 days using sensory and physicochemical analysis methods. The samples analyzed were plain buttermilk, buttermilk with encapsulated dill volatile oil, buttermilk with encapsulated caraway volatile oil, buttermilk with added mushroom powder, buttermilk with added mushroom powder and encapsulated dill volatile oil and buttermilk with the addition of mushroom powder and encapsulated caraway volatile oil. The results of the physicochemical analyzes were statistically processed using the Minitab program. The addition of mushroom powder in buttermilk has positively influenced its sensory characteristics, and the results obtained from physicochemical analyzes were superior to plain buttermilk. The addition of volatile oil of dill and caraway has positively influenced both the sensory and physical-chemical characteristics of buttermilk.*

Key words: *buttermilk, yellow mushrooms, volatile oils, bioactive compounds, dill, caraway.*

[1] Department of Agricultural Sciences and Food Engineering, *Lucian Blaga* University of Sibiu, Doctor Ion Rațiu No.7, 550012 Sibiu, Romania;
[2] S.C. Scandia Food S.R.L., Podului No.133, Sibiu, Romania;
[3] Klaipeda State University of Applied Sciences, Klaipeda, Lithuania;
[4] Kaunas University of Technologie, Food Institute, Kaunas, Lithuania; Klaipeda State University of Applied Science, Klaipeda, Lithuania;
Correspondence: Maria A. Constantinescu; email: adelina.constantinescu@ulbsibiu.ro.

1. Introduction

Proper nutrition ensures the correct development and regeneration of tissues and cells, thus producing the energy needed by the body for both physical and rest. Most studies have found that raw materials used in food industry processes have a lower nutrient content. By processing them, finished products are obtained with an excused content of bioactive compounds [5]. Lately, consumer behavior has changed, as it is increasingly oriented towards nutritious, organic foods and a balanced diet has helped to maintain or increase health. At present, the attention of processors has begun to focus on obtaining functional foods that bring certain health benefits to consumers. Such products are constantly growing and developing in the USA, Canada, Japan and Western European countries [4].

Fermented dairy products are a rich source of nutrients. The nutritional characteristics of fermented dairy products are determined by the nutrients present in the milk, the nutrients derived from other ingredients and the nutrients resulting as metabolites from the fermentation of lactic acid bacteria. Also, during the fermentation process milk undergoes transformations that can change its nutritional and physiological value [10], [13], [15], [17], [19].

Buttermilk is part of fermented dairy products and is highly valued by consumers due to its sensory and nutritional qualities. It is obtained only from cow's milk with high fat content and by using a production starter culture consisting only of lactic streptococci. Buttermilk enriched with cultures is a lightly salted fermented product made from low-fat or low-fat milk using mesophilic cultures [9].

In the Romanian territory, there is a wide range of edible mushrooms, these being widely distributed in forests [3. Mushrooms are consumed for their nutritional and functional properties in fresh or dried form. Various bioactive compounds such as polysaccharides, proteins, phenols and flavonoids have been isolated from different species of edible fungi. Numerous studies have reported that some edible mushrooms have antioxidant, antitumor, antiallergic, anti-inflammatory, antiviral and antibacterial effects. In oriental medicine, many edible mushrooms are widely used to prevent chronic diseases. Mushrooms are also recommended in the diet of diabetic patients because they have a low glycemic index and a high content of dietary fiber [20].

Vegetable protein obtained from mushrooms ranks second in the world after soy. Edible mushrooms are generally considered foods with high nutritional value. The edible part of a mushroom represents more than 3/4 of the product used by the consumer. The chemical composition of mushrooms differs from one species to another depending on the stage of development, the nutrient substrate, the morphological appearance, the growing period and the microclimate. Laboratory tests have shown that 100-200 g of dried mushrooms consumed daily can replace the consumption of meat in a person's diet [7].

Chanterelles (*Cantharellus cibarius*) are edible mushrooms commonly found on the Romanian market. It is a species of the *Basidiomycota* cluster, from the

Cantharellaceae family and of the cohabiting genus *Cantharellu*s. The fungus grows in Romania, especially in deciduous forests in the area of Moldova and Bucovina. It is often found among blueberries, blackberries and raspberries in May – October [14]. Chanterelles have a high dose of glutamic acid that helps strengthen the immune system and is an important source of vitamin D needed to fix calcium and phosphorus in the body. The high content of iron, copper and potassium helps to form the structure of proteins in the connective tissue and maintain the health of the cardiovascular system. They have a high content of B vitamins that help break down fats, carbohydrates and proteins, and essential amino acids have the role of strengthening the immune system [16].

Dill (*Anethum graveolens*) is an annual plant, native to southwest and central Asia and is a species of the genus *Anethum*. Dill contains many compounds and chemical derivatives, which are known for their antioxidant and disease prevention properties. It contains several types of antioxidants and vitamins, such as niacin, pyridoxine (B-complex vitamins) and dietary fibre. Dill leaves and seeds are excellent sources of volatile oil. The main compounds of volatile dill oil are eugenol, limonene, terpene and myristin. The volatile oil extracted from dill has been used for therapeutic purposes, as a local anaesthetic, antiseptic and has digestive and disinfectant properties [2].

Caraway (*Carum carvi*) is a biennial plant of the *Apiaceae* family that grows in Europe and western Asia. Caraway seeds are rich in minerals such as iron, copper, calcium, potassium, manganese, selenium, zinc, magnesium, but also vitamins such as the complex of vitamins A, C, E and vitamin B. They are an excellent source of volatile oil, being a natural adjuvant for strengthening the immune system [8].

2. Materials and Methods

2.1. Overview

The main purpose of this research is to create a milk drink with the addition of mushrooms and bioactive compounds. The mushroom species was chosen because of the variety of ingestions it gives and the high protein content compared to most vegetables. Volatile oils are an excellent source of antioxidants, and their use in food production can be a great direction for the current situation.

The encapsulation of the volatile oil was performed in the laboratory in sodium alginate. In carrying out the proposed research, the following samples were used for analysis:

- BM: control buttermilk sample;
- DI: buttermilk sample enriched with dill volatile oil: 0.5g dill capsules for 50g buttermilk;
- CU: buttermilk sample enriched with caraway volatile oil: 0.5g caraway capsules for 50g buttermilk;
- BMM: control buttermilk sample enriched with yellow mushroom powder: 34g yellow mushroom for 50g of buttermilk;
- DIM: buttermilk sample enriched with yellow mushroom powder and dill volatile oil: 34g of yellow mushroom powder and 0.5g of dill capsules for 50g of buttermilk;
- CUM: buttermilk sample enriched with yellow mushroom powder and caraway volatile oil: 34g of yellow mushroom powder and 0.5g of caraway capsules for 50g of buttermilk.

2.2. Materials

For the preparation of buttermilk, we used raw milk from a farm in the Sibiu area. The raw milk was pasteurized at a temperature of 85-90˚C for 20 minutes, cooled to 20˚C and lactic culture was added. Volatile oils encapsulated in the sodium alginate and mushroom powder were added to the seed milk. The milk was heated and kept constant at a temperature of 32˚C for 12h and cooled to a temperature of 8˚C. The buttermilk samples were packed in 200g plastic cups and stored in the refrigerator at a temperature between 0-4 °C. During the entire storage period, the glasses were covered with aluminium foil.

The encapsulation of the volatile oil was performed in the laboratory in sodium alginate. 10 ml of 2% alginate solution was prepared. 30µl of volatile oil was added to the solution thus prepared and mixed for homogenization. From this solution, the hardened capsules were obtained with the help of calcium chloride. At the end, they were washed with 30 ml of distilled water to remove excess calcium chloride and left to stand at room temperature for one hour.

2.3. Methods

2.3.1. Sensory Analysis

For the evaluation of the characteristics of the buttermilk samples, the method of scoring with a small number of points and the method of ordering by rank were used. The appreciated sensory characteristics were: color, appearance and consistency, taste and smell [12]. The sensory analysis was performed by 15 tasters, consumers of fermented dairy products, on the first day, on the tenth day and the twentieth day of storage. A questionnaire with a small number of points was used for the consistency analysis as a method of determination. An intensity scale was used for the analysis of taste as follows: 0 - not taken into account, 1 - weak, 2 - moderate, 3 - strong. To assess the smell and the external appearance, the method of arrangement by rank was used, which consists in ordering the samples according to the intensity of the sensory characteristic.

2.3.2. Acidity Determination

The Thörner method was used to determine the acidity of the buttermilk samples [1]. The acidity of the samples was neutralized by titration with 0.1 n sodium hydroxide solution in the presence of phenolphthalein as an indicator. The acidity [°T] of milk and milk products is expressed in degrees Thörner and is calculated by the formula (1):

$$Acidity = \frac{V}{V_1} \cdot 100 \qquad (1)$$

where: V is the volume of 0.1 n sodium hydroxide solution used for titration, in cm^3, and V_1 is the volume of the sample taken for analysis, in cm^3.

2.3.3. Syneresis Determination

The syneresis of the buttermilk samples was determined according to Barkallah's method [6]. 100 g of each sample was placed in a funnel lined with filter paper. After 6 hours of drainage, the resulting volume of whey was measured. The following formula was used to calculate the syneresis index (2):

$$Syneresis = \frac{V_1}{V_2} \cdot 100 \qquad (2)$$

where: V_1 is the volume of whey collected after draining [ml], and V_2 is the volume of the buttermilk sample [ml].

2.3.4. Water Activity Determination

Water activity is the ratio of the vapor pressure of pure water to the vapor pressure of the water above the sample. The Novasina device was used to determine the activity of water in buttermilk samples. The homogeneous sample was introduced into special plastic ampoules, after which the ampoules in turn were introduced into the apparatus, where the activity of the water in the product was determined at 25 degrees Celsius [11].

2.4. Statistical analysis

The results of physicochemical analyzes are expressed by mean±standard deviation (SD) for each sample. Graphical representation and statistical processing were performed using the Minitab 14 program and $p < 0.05$ was considered significant [18].

3. Results and Discussions

3.1. Sensory Analysis

Figure 1 shows the results obtained after analyzing the consistency of the whipped milk samples. Sensory analysis of consistency was performed on the first day, on the tenth day and on the twentieth day of storage.

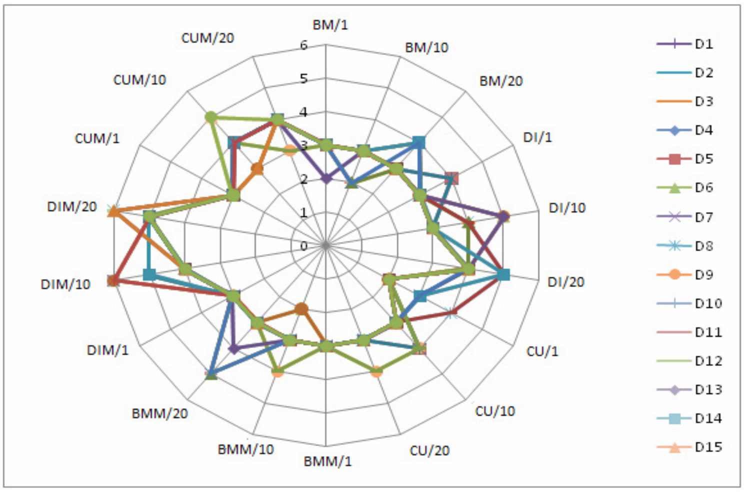

Fig. 1. *Evolution of the consistency of buttermilk samples during the storage period*

From the obtained graph it can be seen that the preferences of the 15 tasters vary differently depending on the product, but also on the tasting period. By calculating a daily average of the product, it is observed that the most appreciated samples in all tasting periods are the sample with dill volatile oil (DI) and the sample with dill volatile oil and mushrooms (DIM).

Figure 2 shows the results obtained from the analysis of the taste of buttermilk samples in the three analysis periods.

Following the graphic representation, a variety of values can be found depending on the preferences of each taster. After calculating an average for each sample during the 20 days, the order of appreciation of the products is as follows: DIM, DI, BMM, CHM, CH, BM.

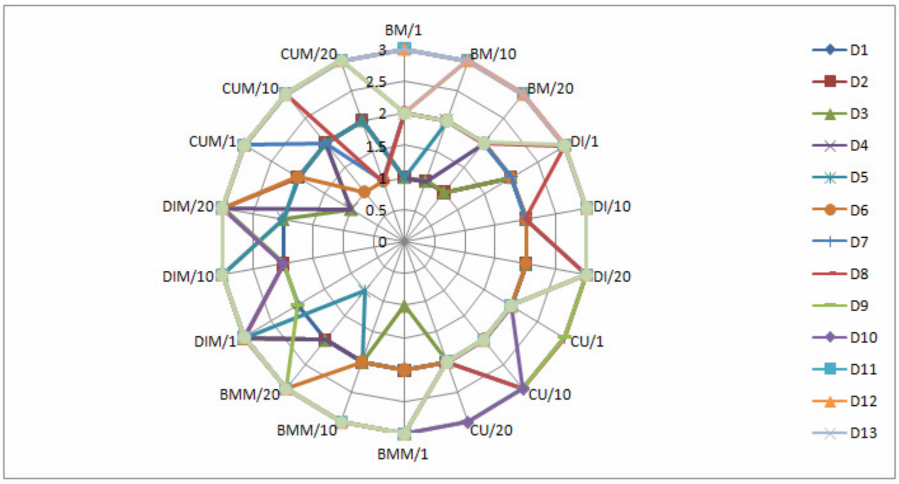

Fig. 2. *Evolution of the taste of buttermilk samples during the storage period*

Figure 3 shows the results obtained from the analysis of the smell of buttermilk samples during the twenty days of storage.

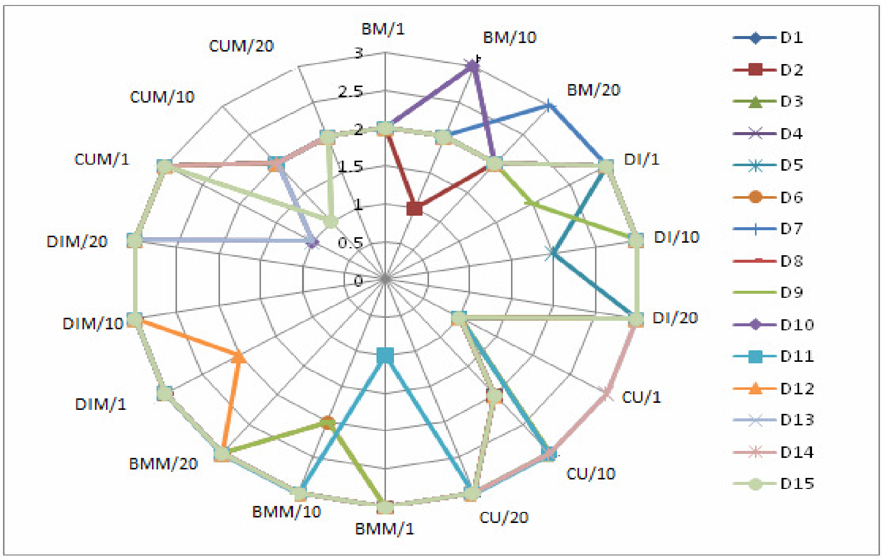

Fig. 3. *Evolution of the smell of buttermilk samples during the storage period*

The order of the samples according to the results obtained after the appreciation of the smell by the tasters is the one found in the figure where it can be stated that the innovative product obtained with the addition of mushrooms and dill capsules is best appreciated in terms of smell, having the highest value followed by the product obtained from whipped milk and dill capsules.

Figure 4 shows the results obtained after analyzing the external appearance of the buttermilk samples. The sensory analysis of the appearance was performed on the first day, on the tenth day and on the twentieth day of storage.

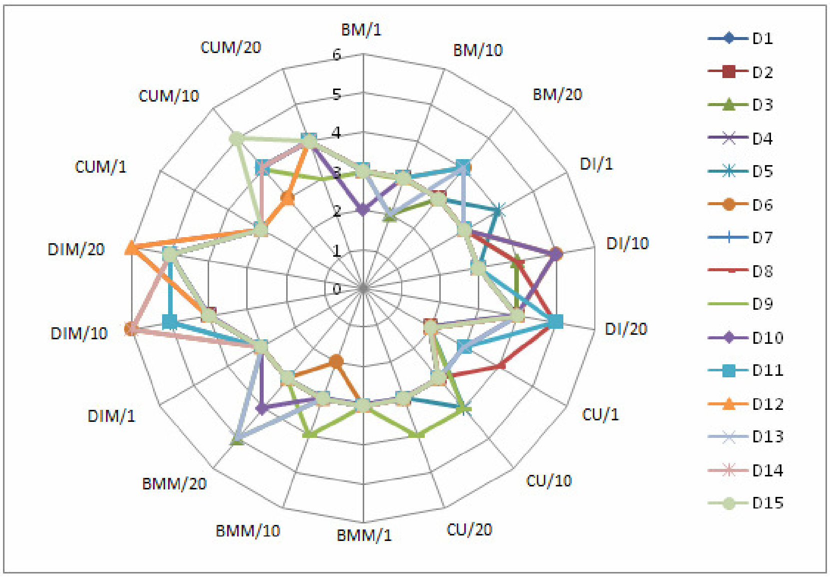

Fig. 4. *Evolution of the appearance of buttermilk samples during the storage period*

The results show that the external appearance presents close results, but also the most appreciated and here remain the samples with the addition of dill capsules compared to those with caraway capsules or the control samples.

3.2. Acidity Determination

To determine the acidity, all six samples were subjected to analysis, and three determinations were made for each sample and the arithmetic mean was calculated. In Figure 5 the acidity results are presented for all six samples on the first day of storage.

As can be seen in Figure 5, the highest acidity is recorded in the buttermilk sample enriched with yellow mushroom powder and caraway volatile oil (CUM) and the lowest acidity is recorded in the control buttermilk sample (BM). A high value of acidity was also recorded in the buttermilk sample enriched with yellow mushroom powder and dill volatile oil (DIM). The mean values for each sample as well as the standard deviations are as follows:

- BM = 130±1.73;
- DI = 132±1;
- CU = 136.67±0.577;
- BMM = 136±1;
- DIM = 137.67±0.577;
- CUM = 143±1.73.

As can be seen in Figure 5, the highest acidity is recorded in the buttermilk sample enriched with yellow mushroom powder and caraway volatile oil (CUM) and the lowest acidity is recorded in the control buttermilk sample (BM). A high value of acidity was also recorded in the buttermilk sample enriched with yellow mushroom powder and dill volatile oil (DIM). The mean values for each sample as well as the standard deviations are as follows:

- BM = 130±1.73;
- DI = 132±1;
- CU = 136.67±0.577;
- BMM = 136±1;
- DIM = 137.67±0.577;
- CUM = 143±1.73.

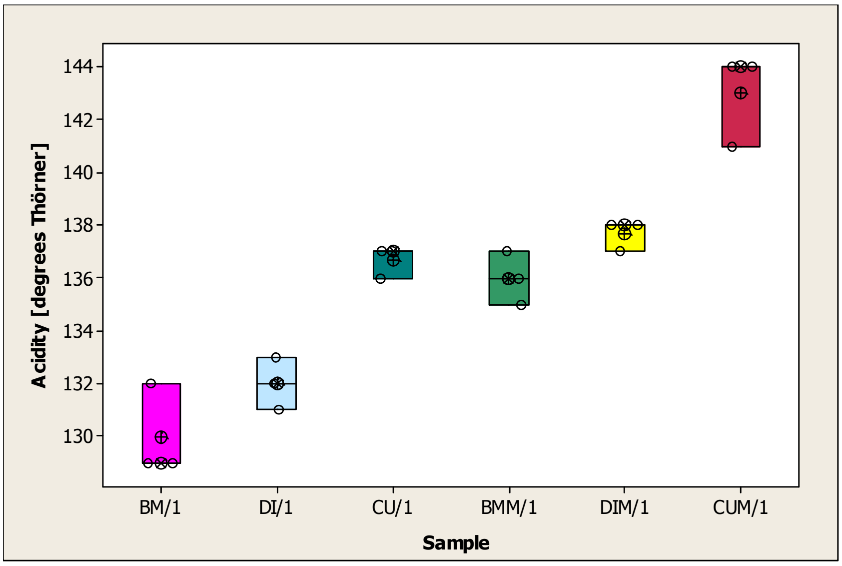

Fig. 5. *Titratable acidity of buttermilk samples on the first day of storage*

As can be seen in Figure 6, the highest acidity is recorded in the buttermilk sample enriched with yellow mushroom powder and caraway volatile oil (CUM) and the lowest acidity is recorded in the control buttermilk sample (BM). The acidity value of buttermilk sample enriched with yellow mushroom powder and caraway volatile oil sample is followed by the acidity of buttermilk sample enriched with yellow mushroom powder and dill volatile oil (DIM). The mean values for each sample as well as the standard deviations are as follows:

- BM = 130±1.73;
- DI = 162±1.15;
- CU = 166.67±0.577;
- BMM = 165.67±0.577;
- DIM = 168±1;
- CUM = 172.33±0.577.

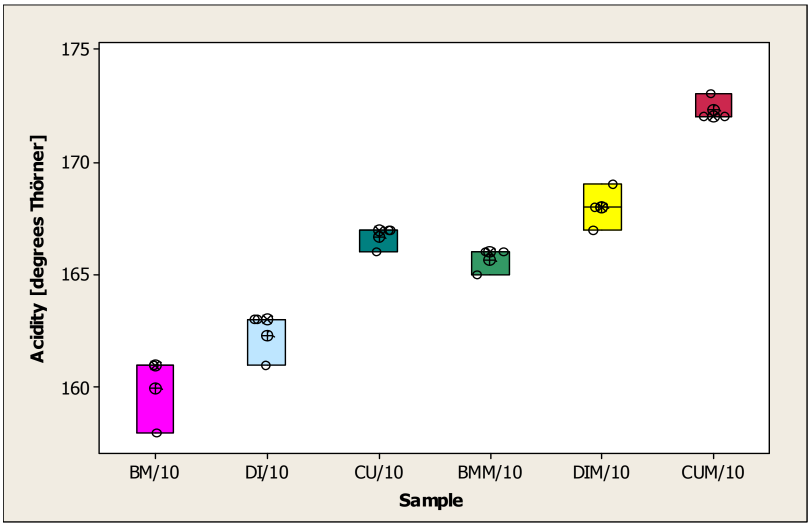

Fig. 6. *Titratable acidity of buttermilk samples on the tenth day of storage*

As can be seen in Figure 7, the highest acidity is recorded in the buttermilk sample enriched with yellow mushroom powder and caraway volatile oil (CUM) and the lowest acidity is recorded in the control buttermilk sample (BM). A high value of acidity was also recorded in the buttermilk sample enriched with caraway volatile oil (CU). The mean values for each sample as well as the standard deviations are as follows:

- BM = 161.67±0.577;
- DI = 163±1;
- CU = 209.33±1.15;
- BMM = 167.67±1.15;
- DIM = 170.33±0.577;
- CUM = 229.33±0.577.

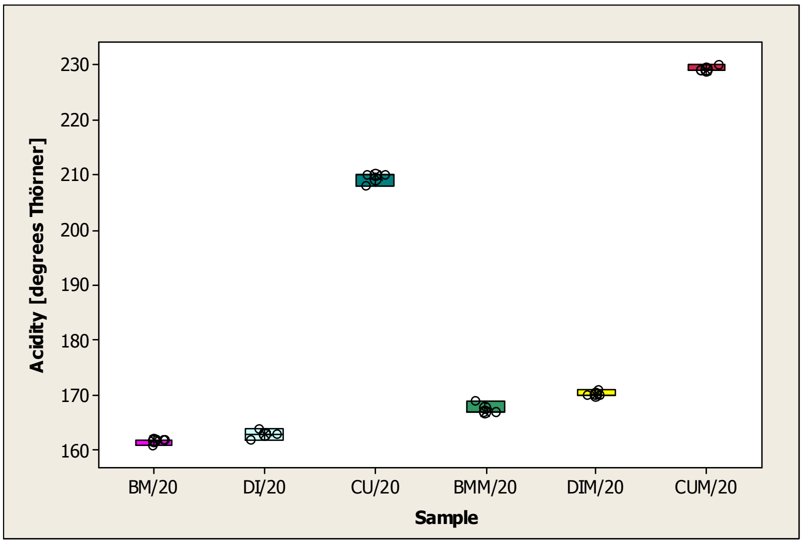

Fig. 7. *Titratable acidity of buttermilk samples on the twentieth day of storage*

3.3. Syneresis Determination

To determine the syneresis, all six samples were subjected to analysis and three determinations were made for each sample and the arithmetic mean was calculated. In Figure 8 the syneresis results are presented for all six samples on the first day of storage.

In Figure 8 is represented the syneresis for all sample on day 1 of storage. The highest value of syneresis is recorded in the buttermilk sample enriched with yellow mushroom powder and caraway volatile oil (CUM) and the lowest acidity is recorded in the control buttermilk sample (BM). The syneresis value of the buttermilk sample enriched with yellow mushroom powder and caraway volatile oil sample is followed by the syneresis of the buttermilk sample enriched with yellow mushroom powder and dill volatile oil (DIM). The mean values for each sample as well as the standard deviations are as follows:

- BM = 0.972±0.2;
- DI = 1.44±0.173;
- CU = 2.967±0.153;
- BMM = 1.8533±0.00577;
- DIM = 12.893±0.0115;
- CUM = 14.42±0.0173.

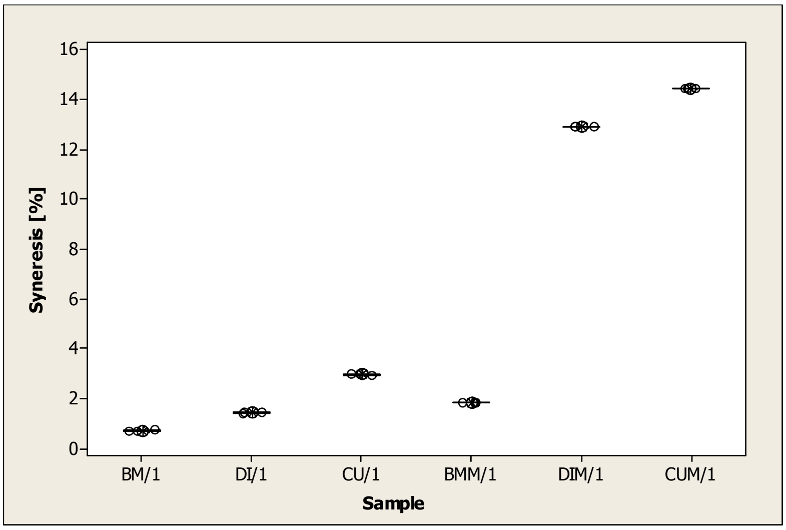

Fig. 8. *Syneresis of buttermilk samples on the first day of storage*

As can be seen in Figure 9, the highest syneresis is recorded in the buttermilk sample enriched with yellow mushroom powder and caraway volatile oil (CUM) and the lowest syneresis is recorded in the control buttermilk sample (BM). A high value of syneresis was also recorded in the buttermilk sample enriched with yellow mushroom powder and dill volatile oil (DIM). The mean values for each sample as well as the standard deviations are as follows:

- BM = 11.81±0.01;
- DI = 16.82±0.0173;
- CU = 13.347±0.00577;
- BMM = 17.31±0.01;
- DIM = 28.507±0.0115;
- CUM = 24.13±0.0173.

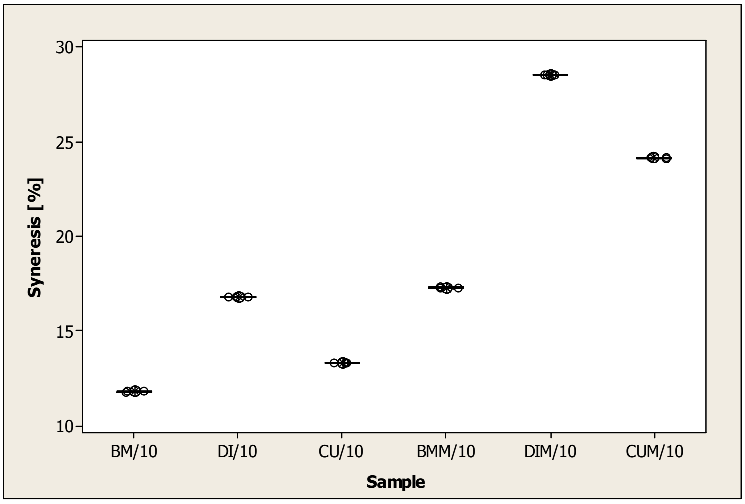

Fig. 9. *Syneresis of buttermilk samples on the tenth day of storage*

In Figure 10 is represented the syneresis for all sample on day 20 of storage. The highest value of syneresis is recorded in the buttermilk sample enriched with yellow mushroom powder and dill volatile oil (DIM) and the lowest acidity is recorded in the control buttermilk sample (BM). The syneresis value of the buttermilk sample enriched with yellow mushroom powder and dill volatile oil sample is followed by the syneresis of the buttermilk sample enriched with yellow mushroom powder and caraway volatile oil (CUM). The mean values for each sample as well as the standard deviations are as follows:

- BM = 12.303±0.00577;
- DI = 14.027±0.00577;
- CU = 17.54±0.0173;
- BMM = 25.067±0.00577;
- DIM = 32.613±0.0115;
- CUM = 30.487±0.00577.

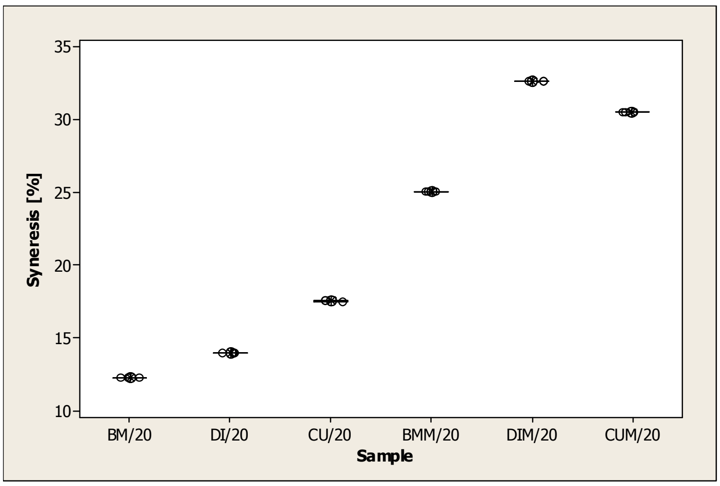

Fig. 10. *Syneresis of buttermilk samples on the twentieth day of storage*

3.4. Water Activity Determination

To determine the water activity, all six samples were subjected to analysis and three determinations were made for each sample and the arithmetic mean was calculated. In Figure 11 the water activity results are presented for all six samples on the first day of storage.

As can be seen in Figure 11, the highest water activity is recorded in the buttermilk sample enriched with dill volatile oil (DI) and the lowest water activity is recorded in the buttermilk sample enriched with yellow mushroom powder and caraway volatile oil (CUM). A high value of water activity was also recorded in the buttermilk sample enriched with yellow mushroom powder (BMM). The mean values for each sample as well as the standard deviations are as follows:

- BM = 0.96767±0.000577;
- DI = 0.97267±0.000577;
- CU = 0.96667±0.000577;
- BMM = 0.96767±0.000577;
- DIM = 0.96467±0.000577;
- CUM = 0.96267±0.000577.

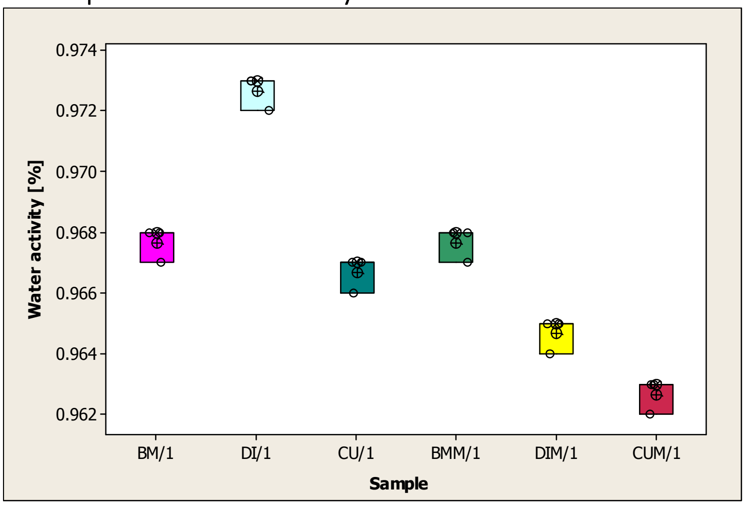

Fig. 11. *Water activity for buttermilk samples on the first day of storage*

In Figure 12 is represented the water activity for all sample on day 10 of storage. The highest value of water activity is recorded in the buttermilk sample enriched with dill volatile oil (DI) and the lowest water activity is recorded in the buttermilk sample enriched with yellow mushroom powder and caraway volatile oil (CUM). The water activity value of the buttermilk sample enriched with a dill volatile oil sample is followed by the water activity of the buttermilk sample enriched with yellow mushroom powder (BMM). The mean values for each sample as well as the standard deviations are as follows:

- BM = 0.94567±0.000577;
- DI = 0.94633±0.000577;
- CU = 0.94367±0.000577;
- BMM = 0.94633±0.000577;
- DIM = 0.94267±0.000577;
- CUM = 0.94167±0.000577.

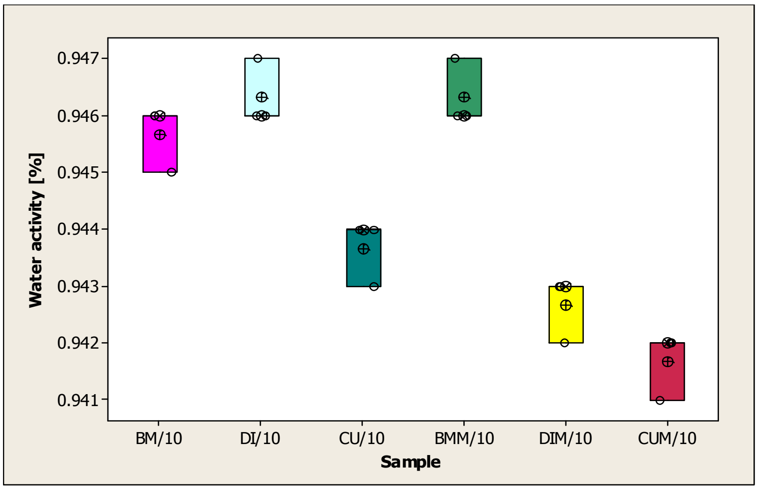

Fig. 12. *Water activity for buttermilk samples on the tenth day of storage*

As can be seen in Figure 13, the highest water activity is recorded in the control buttermilk (BM) and the lowest water activity is recorded in the buttermilk sample enriched with yellow mushroom powder and dill volatile oil (DIM). A high value of water activity was also recorded in the buttermilk sample enriched with yellow mushroom powder and caraway volatile oil (CUM). The mean values for each sample as well as the standard deviations are as follows:

- BM = 0.94433±0.000577;
- DI = 0.94167±0.000577;
- CU = 0.94033±0.000577;
- BMM = 0.93867±0.000577;
- DIM = 0.93767±0.000577;
- CUM = 0.94167±0.000577.

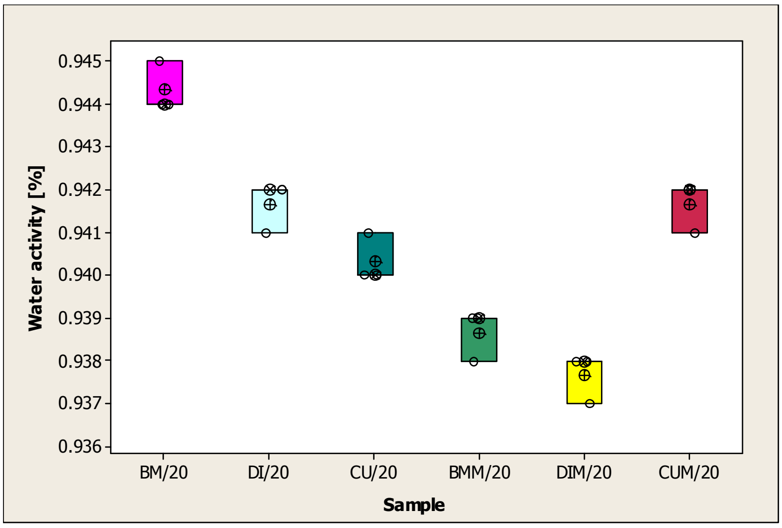

Fig. 13. *Water activity for buttermilk samples on the twentieth day of storage*

4. Discussions

Following the sensory analysis, buttermilk enriched with mushroom powder obtained better results compared to buttermilk in which we did not add this powder in case of all four characteristics analyzed. In the case of buttermilk samples enriched with volatile oils, the sensory characteristics analyzed were highlighted during storage, the highest grades being obtained on the tenth and twentieth days. In the case of plain buttermilk and buttermilk samples with the addition of mushroom powder, the highest grades were obtained on the first day of storage for all four characteristics analyzed. The buttermilk sample with the addition of mushroom powder and encapsulated dill volatile oil obtained the highest ratings for consistency, taste, smell and appearance. The lowest grades were obtained in the case of plain buttermilk for the same characteristics.

The highest acidity values were obtained on the twentieth day of storage and the lowest on the first day of storage for all six samples of buttermilk. The sample of buttermilk with added mushroom powder and caraway volatile oil recorded the highest value of acidity during the storage period. High values of acidity were also recorded in the buttermilk sample with the addition of encapsulated caraway volatile oil. The lowest values of acidity were recorded in the plain buttermilk sample throughout the storage period.

In the case of the syneresis, its lowest values were obtained on the first day of storage, and the highest on the twentieth day of storage. The buttermilk sample with the addition of mushroom powder and dill volatile oil obtained the highest values of syneresis, especially on days ten and twenty of storage. The buttermilk sample with the addition of mushroom powder and caraway volatile oil recorded high values of the syneresis, especially on day one of storage. The lowest values of the syneresis for the entire storage period were recorded for the plain buttermilk sample.

During the twenty days of storage, the water activity decreases in the case of all six samples of buttermilk. The highest values of water activity were recorded in the buttermilk sample enriched with encapsulated dill volatile oil and in the plain buttermilk sample. The lowest values of water activity were recorded in the samples of buttermilk with the addition of mushroom powder and encapsulated dill and caraway volatile oil.

5. Conclusions

Fermented dairy products are a wide range of foods that have a high nutritional value due mainly to the nutrients they contain. Buttermilk is part of fermented dairy products and is a product consumed and appreciated in Romania due to its sensory and nutritional qualities.

In Romanian forests, we find a wide range of edible mushrooms, and their consumption in the fresh or dry states has increased a lot in recent years due to the health benefits it brings to consumers.

Chanterelles (*Cantharellus cibarius*) are edible mushrooms that are high in iron, copper, vitamin D and vitamin B, all of which help strengthen the immune system and maintain the cardiovascular and connective system.

Dill (*Anethum graveolens*) contains numerous compounds and chemical derivatives that offer their antioxidant and disease prevention capacity. Dill is also known for its antimicrobial properties. Caraway (*Carum carvi*) is rich in minerals and vitamins, being used especially to treat digestive diseases and to strengthen the immune system.

Dill and caraway are excellent sources of volatile oil. Being a product very sensitive to environmental factors, we decided to encapsulate it in the sodium alginate and form capsules that were added to the dairy product. The addition of mushroom powder in buttermilk positively influenced its sensory characteristics, and the results obtained from physicochemical analyzes were superior to plain buttermilk. The addition of volatile oil of dill and caraway has positively influenced both the sensory and physical-chemical characteristics of buttermilk.

We can conclude that by adding mushroom powder and volatile oils we obtained a dairy product enriched with bioactive compounds.

Acknowledgements

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