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RESEARCH ARTICLE

Design of Labneh cheese fortified with alginate-encapsulated pepper (*Capsicum annuum*) extracts

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ABSTRACT

The aim of the present study was to manufacture fresh cheese Labneh enriched with antioxidantive compounds from pepper (*Capsicum annuum*) extracts encapsulated in alginate beads. The carbohydrate content, total phenolic, and carotenoids content, as well as the antioxidant activity of prepared Labneh samples, were evaluated. The microbiological screening on the 1st and 20th day of storage period at 4 ± 1 °C was performed as well. The obtained results for chemical properties illustrated that the addition of alginate-encapsulated pepper (*Capsicum annuum*) extracts in Labneh had a significant effect ($p<0.05$) on the level of total phenols and antioxidant activity in comparison with the control sample. The presence of lactose, galactose and glucose were detected in all samples. It was established that the total viable lactic acid bacterial count (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) in the experimental samples decreased at the end of the storage period. Coliforms, yeasts, and moulds were not detected in the samples with encapsulated pepper extracts. The sensory evaluation demonstrated a higher score for most Labneh samples with encapsulated pepper extracts in comparison with the control sample. A new cheese product was developed and alginate-encapsulated pepper (*Capsicum annuum*) extracts were evaluated as a proper approach for the fortification of natural pigments and antioxidants in Labneh samples.

Keywords: Antioxidants; Encapsulation; Labneh cheese; Pepper

INTRODUCTION

Over the past two decades, there has been substantial interest in the design of a new range of dairy products, which are similar to the existing products but fortified with antioxidants - carotenoids and polyphenol compounds (Ivanova et al., 2018). Creating new foods fortified with biologically active ingredients is an up-to-date task for modern nutritional science and practice. Many of these substances are unstable when incorporated into the food matrix, which requires seeking opportunities to stabilize them before insertion. Encapsulation of biologically active substances is a method that ensures the stability of a substance in a food product. The incorporation of encapsulated biologically active substances in milk and milk products requires an indisputable knowledge of their chemical characteristics and technological properties.

An interesting dairy product in the Middle East is Labneh usually called as cheese spread, which has been traditionally produced in the countries of Lebanon, Jordan, Palestine, Syria, and Egypt (Tamime and Robinson, 2007). It is known and popular as concentrated yogurt in Cyprus, Greece, and Turkey (Senel et al., 2009). Varieties of cheese-making technology have been described by some authors (Abou-Donia et al., 1992a,b; Al-Kadamany et al., 2003; El-Samragy, 1997; Ozer et al., 1999a,b; El-Samragy and Zall, 1998), as well as nutritional and dietary qualities (Tamime and Robinson, 2007).

To expand the product range of existing dairy-based foods, numerous herbs and spices under different forms (i.e. powder, fresh, extract, essential oils, etc.) were added in some dairy products (El-Sayed and Youssef, 2019). Moreover, the influence of the essential oils from medicinal herbs (thyme, marjoram, sage, dill, caraway, cinnamon and *Moringa oleifera* added in Labneh (concentrated yogurt) were investigated (Otaibi and Demerdash, 2008; Zaky et al., 2013; El-Sayed and Youssef, 2019). Fresh herbs as purslane, dill, parsley, cress, coriander, rocket, diplotaxis,

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and mint were successfully used in Labneh production at a concentration up to 8.0 % (Tarakci et al., 2011). Aqueous extracts of oregano, marjoram, sage, and licorice as antimicrobial and taste enhancers were added in Labneh (Al-Turki et al., 2008). Functional Labneh, with different levels of natural antioxidant from water extract of guava leaves, was prepred in order to enhance its antioxidant properties (El-Gazzar et al., 2018). Papaya seeds extract was applied to extend the shelf life of Labneh and to improve its functionality (Mohamed et al., 2016).

Fortification of novel cottage cheese with fresh pepper, together with fresh and dried herbs presented excellent sensory properties, particularly with fresh sweet red pepper (Josipovic et al., 2015). However, to the best of our knowledge, the addition of pepper extract encapsulated in alginate beads into cheese has been not reported. Encapsulation has demonstrated to be an alternative technique for the protection of biologically active substances from unfavourable environments, especially as a form of Ca-alginate beads which are nontoxic, biocompatible and are stable in acidic pH levels (Chaikham et al., 2013; Zlatev et al., 2018). In our previous reports, fruit juices were successfully encapsulated and incorporated in dairy desserts (Vlaseva et al., 2014; Ivanova et al., 2018).

The objective of the present work was to design a novel Labneh cheese with improved sensory, nutritional and antioxidative properties by incorporation of alginate-encapsulated pepper (*Capsicum annuum* L.) extracts.

MATERIALS AND METHODS

Raw materials

Fresh cow milk was supplied by a dairy factory Bor Chvor Ltd., Parvomay, Bulgaria, with a chemical composition represented in Table 1.

Milk was first separated. Ultrafiltration of skim milk was carried out with the laboratory equipment at the Department of Processes and Apparatus at the University of Food Technologies, Plovdiv, Bulgaria. The system consisted of a removable flat membrane module fitted with a UF10-PAN polyacrylnitrilic ultrafiltration membrane ("Ekofilter" Ltd., Bulgaria) with 10 kDa molecular weight cut-off. The ultrafiltration of cow milk was performed at volume reduction ratio (VRR) - 2, with membrane surface area of 1.250 cm^2, transmembrane pressure 0.5 MPa, temperature 50 ºC and input volumetric flow rate 330 dm^3/h. (Dushkova and Dinkov, 2009). The obtained retentate (concentrate) was standardised and used for the preparation of Labneh cheese with a chemical composition represented in Table 1.

Four pepper varieties (Doux Marconi Geonet, Bulgarian Ratund, Kurtovska kapia, Doux Marconi Geonet x Kurtovska kapia) were grown and kindly donated from Agriculture University- Plovdiv. Pepper fruits were washed, the seeds were removed and the fruit flesh was cut into small pieces. Then samples were homogenized with a laboratory homogenizer Polytron PT45-80 (Kinematica, Switzerland), filtered through nylon cloth and pasteurized. The obtained pepper filtrates were used for the preparation of encapsulated in alginate beads.

Preparation of alginate beads

Sodium alginate (2 g) and 1 g NaCl was suspended in 100 ml pepper extract followed by heating at 50 ºC for 3 min and stirring with a laboratory homogenizer Polytron PT45-80 (Kinematica, Switzerland) - 1600 W, max 250.s^{-1} for 2 min. The procedure was as described in the details (Vlaseva et al., 2014) and finally, alginate suspension was dropped into a cold 2% calcium chloride solution. The resulting alginate beads were washed with distilled water and were used for incorporation into Labneh cheese (Fig 1. A).

Labneh cheese preparation

The retentate was pasteurized at 80 °C for 3 - 5 minutes, then cooled to 44 ± 1 °C and was inoculated with the direct vat set (DVS) culture (YF-3331, Chr. Hansen, Copenhagen, Denmark), with the following composition - *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. The inoculated mixture was incubated at 44 ± 1 ºC up to 3.5 – 4.0 h. Fermented curd was cooled and filtered in cloth bags at 4 ± 2 ºC for 16 - 18 hours to remove the whey from the curd. The obtained fresh cheese Labneh was removed

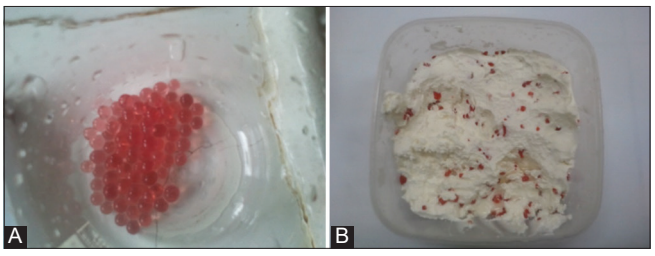


Fig 1. Alginate beads with pepper extracts (A) and Labneh cheese before forming into small balls (B).

Table 1: Chemical composition of milk and milk retentate used for the preparation of Labneh cheese

	Moisture, %	Dry matter, %	Protein, %	Fat, %	Lactose, %	Solids, %
Milk	87.56±1.03[a]	12.44±1.20[a]	3.21±0.25[a]	3.60±0.25[a]	4.91±0.08[a]	0.72±0.10[a]
Standardised Retentate	80.84±1.25[b]	19.16±1.24[b]	6.24±0.89[b]	7.00±0.55[b]	5.01±0.01[b]	0.91±0.08[b]

a, b Means with different letters within a column are significantly different ($p<0.05$)

out of the cloth bags then was mixed with 1 % salt (NaCl) and 1 % alginate beads with the pepper extracts (Fig. 1 B). They were formed into small balls, put in glass containers filled with sunflower oil and stored at 4 ± 1 °C for 20 days.

The Labneh cheeses were separated in five batches coded as follows: LC- Labneh cheese control sample; LP1- Labneh cheese with alginate-encapsulated pepper from variety Doux Marconi Geonet; LP2 - Labneh cheese with alginate-encapsulated pepper from variety Bulgarian Ratund; LP3 - Labneh cheese with alginate-encapsulated pepper from variety Kurtovska kapia; LP4 - Labneh cheese with alginate-encapsulated pepper from variety Doux Marconi Geonet mixed with Kurtovska kapia.

Chemical analysis

Milk and retentate fat, protein, lactose content, solids, and moisture were determined using Milk Analyzer MilkoScope Expert™ Automatic 2017 Model.

All cheese samples were analysed during the storage period days on the 1st and 20th day. Titratable acidity (TA) was determined by the Bulgarian National Standard (BNS) 1111-80 and represented as percentages of lactic acid (% LA). The pH was measured by a pH meter (model MS 2000, Mycrosist, Plovdiv, Bulgaria). The moisture of cheese samples was determined by BNS 1109-89. The fat content of cheese samples was determined by the Gerber method (BNS 1671-89). The total protein (TP) content of the cheeses was determined by the Kjeldahl method (BNS EN ISO 8968-1:2014). The solids content of cheese samples was determined according to BNS 6154-74.

Extraction of bioactive substances

Labneh sample (5g) was extracted with 50 ml of 95% ethanol (and with water for sugar analysis) in duplicate using a homogenizing device (T25, IKA Co., Germany) with high-speed shear at 10 000 rpm for 1 min. The sample was filtered and the extract was used for determination of sugars, phenolic content and antioxidant activity.

HPLC-RID analysis of sugars

Chromatographic determination of presenting sugars was performed on an HPLC instrument Elite Chrome Hitachi (Japan), coupled with a refractive index detector (RID) Chromaster 5450 operating at 35 °C. The separation was done with mobile phase distilled H_2O on a column Shodex® Sugar SP0810 (300 mm × 8.0 mm i.d.) with Pb^{2+} and a guard column Shodex SP - G (5 μm, 6 × 50 mm) at 85 °C, with a flow rate 0.5 mL/min (Hadjikinova et al., 2017).

Total Chlorophylls and Carotenoid content

For determination of chlorophyll a, chlorophyll b, total chlorophylls and the total carotenoids, the absorbance of 95% ethanol extracts was measured at three wavelengths 664, 648 and 470 nm. The amount of these pigments was calculated according to Lichtenthaler and Wellburn (1983).

Total phenolic content

The total phenolic content was evaluated using the Folin–Ciocalteu reagent, as the ethanol Labneh extract (0.2 mL) was mixed with 1 mL Folin–Ciocalteu reagent and then 0.8 mL 7.5% Na_2CO_3 was added. The absorption was measured at 765 nm against a blank sample. The results were expressed in mg equivalent of gallic acid (GAE) per g sample (Ivanov et al., 2014).

The total flavonoids content

The total flavonoid content was determined by $Al(NO_3)_3$ reagent. After 40 min the absorbance was measured at 415 nm against a blank. The results were presented as mg equivalents quercetin (QE) per g sample (Ivanov et al., 2014).

The DPPH radical-scavenging ability

The Labneh extract (0.15 mL) was added to 2.85 mL a freshly prepared 0.1 mM methanolic DPPH solution. After 15 min at 37 °C in darkness, the reduction of the absorbance at 517 nm was measured by spectrophotometer in a comparison to the blank sample. The results were expressed in mM Trolox® equivalents (TE) per g (Ivanov et al., 2014).

Microbiological analysis

Total viable lactic acid bacteria count (TVC) (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) was determined according to BNS ISO 7889:2005. Total mould and yeast content were determined according to BNS ISO 6611:2006 and coliform bacterial count according to ISO 4832:2006.

Sensory analysis

The sensory evaluation of the fresh cheeses was conducted by the Bulgarian National Standard BNS 15612-83 which includes the evaluation criteria flavour, body and texture, consistency, appearance and colour. Each characteristic was evaluated by a 10-points scale. The Labneh samples were evaluated on the 1st day of storage and at the end of the storage period 20th day by 10 experienced panellists.

Statistical analysis

The data were analysed using Microsoft Excel 2010 (ANOVA). Labneh samples were prepared in two separated batches with two replications. The results are presented as mean value ± standard deviation (SD). The significance of the differences between means was evaluated by the LSD method considering $p<0.05$ as significant.

RESULTS AND DISCUSSION

Chemical characteristics of Labneh cheese

The changes in the chemical characteristics of Labneh samples during storage were investigated and represented in Table 2. The changes in active and titratable acidity reflect the way and the intensity of the fermentation process in the cheese samples. An increase in titrable acidity and a decrease in pH values were observed. From the obtained results, it is evident that on the first day of storage in all samples, the titratable acidity was 1.57 - 1.62% lactic acid and pH 4.8. It was found that during the storage period there was a significant tendency to increase the titratable acidity with a corresponding decrease in pH ($p<0.05$), but not between samples ($p>0.05$).

These results were similar to data obtained from other authors (Celik and Bakirci, 2003; Kucukoner and Tarakci, 2004; Tarakci and Kucukoner, 2004; Celik et al., 2006; Desouky et al., 2013; El-Gazzar et al., 2018). There were no significant differences ($p>0.05$) in moisture, fat, total protein content and solids in cheese samples during the storage period. The obtained results are in an agreement with those reported by other authors (Hefnawy et al., 1992; Tamime and Robinson, 2007; Desouky et al., 2013). It was observed that the fortification of Labneh samples with alginate beads containing pepper extracts did not alter the nutritional value of the final Labneh products. Therefore, the suggested approach, for the fortification of Labneh with pepper extracts by encapsulation approach, demonstrates advantages for the preservation of the level of moisture, protein and ash content unchanged in comparison with the control sample.

Besides, the sugar composition of investigated Labneh samples was summarized (Table 3).

The results showed that in the control sample small amount of lactose was observed. Its concentration was ranged from 16 to 11 mg/g. In addition to lactose, monosaccharides such as glucose and galactose in a concentration below 2 and 8 mg/g were detected in other Labneh samples (LP1 - LP4) with encapsulated pepper extracts. The presence of these two monosaccharides together with lactose could be explained with their natural presence in pepper, as well as they are building blocks of lactose and they could be released during enzymatic hydrolysis in process of storage. The content of lactose decreased by more than 50% after 20 days of storage, while the level of monosaccharides increased twice (Table 3). In literature, the lactose content can reach up to 3% only in Labneh prepared camel milk (Desouky et al., 2013). The reported in the current study results for lactose content was compared with data for white brined cheeses and Labneh contained low levels of lactose 0.05 - 1.1 g/100 g (Jaoude et al., 2010). Moreover, the decrease in the content of lactose during the storage

Table 2: Changes in the chemical characteristics of Labneh cheeses during storage

	Storage period	LC	LP1	LP2	LP3	LP4
Titratable acidity, % lactic acid	1	1.62 ± 0.10^{aA}	1.59 ± 0.12^{aA}	1.61 ± 0.10^{aA}	1.57 ± 0.10^{aA}	1.60 ± 0.10^{aA}
	20	1.89 ± 0.12^{aB}	1.85 ± 0.10^{aB}	1.85 ± 0.11^{aB}	1.86 ± 0.12^{aB}	1.85 ± 0.15^{aB}
pH	1	4.78 ± 0.03^{aA}	4.72 ± 0.04^{aA}	4.80 ± 0.04^{aA}	4.77 ± 0.04^{aA}	4.82 ± 0.04^{aA}
	20	4.50 ± 0.06^{aB}	4.52 ± 0.04^{aB}	4.54 ± 0.04^{aB}	4.53 ± 0.04^{aB}	4.54 ± 0.04^{aB}
Moisture, %	1	65.9 ± 0.7^{aA}	66.0 ± 0.7^{aA}	66.1 ± 0.7^{aA}	65.9 ± 0.7^{aA}	65.8 ± 0.4^{aA}
	20	64.9 ± 0.7^{aA}	65.5 ± 0.8^{aA}	65.2 ± 0.7^{aA}	65.1 ± 0.7^{aA}	65.1 ± 0.4^{aA}
Fat, %	1	21.2 ± 0.1^{aA}	21.0 ± 0.2^{aA}	20.8 ± 0.2^{aA}	21.1 ± 0.1^{aA}	21.0 ± 0.1^{aA}
	20	21.6 ± 0.2^{aA}	21.3 ± 0.1^{aA}	21.1 ± 0.1^{aA}	21.3 ± 0.1^{aA}	21.3 ± 0.3^{aA}
Total protein, %	1	19.2 ± 0.1^{aA}	19.0 ± 0.1^{aA}	18.8 ± 0.3^{aA}	19.0 ± 0.2^{aA}	19.1 ± 0.1^{aA}
	20	19.4 ± 0.3^{aA}	19.2 ± 0.3^{aA}	19.1 ± 0.3^{aA}	19.3 ± 0.3^{aA}	19.2 ± 0.2^{aA}
Ash, %	1	1.20 ± 0.05^{aA}	1.21 ± 0.04^{aA}	1.23 ± 0.05^{aA}	1.20 ± 0.04^{aA}	1.22 ± 0.04^{aA}
	20	1.23 ± 0.04^{aA}	1.23 ± 0.04^{aA}	1.25 ± 0.08^{aA}	1.23 ± 0.04^{aA}	1.25 ± 0.06^{aA}

LC-Labneh cheese control sample; LP1-Labneh with encapsulated pepper Doux Marconi Geonet; LP2-Labneh with encapsulated pepper Bulgarian Ratund; LP3-Labneh with encapsulated pepper Kurtovska kapia; LP4-Labneh with encapsulated pepper Doux Marconi Geonet mixed with Kurtovska kapia. a, b -Means with different letters within a row are significantly different ($p<0.05$) A, B-Means with different letters within a column are significantly different ($p<0.05$)

Table 3: Sugar composition of Labneh cheeses during storage

	Storage period, days	LC	LP1	LP2	LP3	LP4
Lactose, mg/g	1	16 ± 1^{aA}	10 ± 1^{bA}	8 ± 2^{bA}	12 ± 1^{cA}	10 ± 0.2^{cA}
	20	11 ± 1^{aB}	4 ± 1^{bB}	4 ± 2^{bB}	8 ± 1^{cB}	5 ± 2^{cB}
Glucose, mg/g	1	N.D.	2 ± 1^{aA}	2 ± 1^{aA}	2 ± 1^{aA}	2 ± 1^{aA}
	20	N.D.	2 ± 1^{aA}	2 ± 1^{aA}	4 ± 1^{aA}	3 ± 1^{aA}
Galactose, mg/g	1	N.D.	2 ± 1^{aA}	2 ± 1^{aA}	4 ± 1^{aA}	3 ± 1^{aA}
	20	6 ± 1^{aA}	3 ± 1^{bA}	4 ± 1^{bA}	8 ± 2^{cB}	6 ± 1^{cB}

N.D.-not detected, LC-Labneh cheese control sample; LP1-Labneh with encapsulated pepper Doux Marconi Geonet; LP2-Labneh with encapsulated pepper Bulgarian Ratund; LP3-Labneh with encapsulated pepper Kurtovska kapia; LP4-Labneh with encapsulated pepper Doux Marconi Geonet mixed with Kurtovska kapia. a, b -Means with different letters within a row are significantly different ($p<0.05$) A, B, C-Means with different letters within a column are significantly different ($p<0.05$)

of Labneh samples could be explained with its expulsion from the cheese during whey draining and its utilization by the starter used in the preparation of cheese.

Natural pigments, polyphenols, and antioxidant activity

Many types of research dealt with the improvement of biological activity and nutritional properties of Labneh by the incorporation of culinary herbs or medicinal plants (Otaibi and Demerdash, 2008; Zaky et al., 2013; El-Sayed and Youssef, 2019). It is well known that peppers used as flavour enhancer and colorants in cheeses are a rich source of bioactive compounds and antioxidants. The polyphenol content varied in a broad range from 42 mg/100 g fresh weight (FW) to 266 mg/100 g FW. The differences in the flavonoid content are even more significant reaching 60-fold difference - from 1.0 mg/100 g FW to 64.3 mg/100 g FW (Denev et al., 2019). The current research interest in presenting the preservation of bioactive compounds in alginate beads incorporated in Labneh. Therefore, the encapsulation of pepper extract into alginate beads and their addition to Labneh should improve the antioxidant properties of the final product. Moreover, because of natural pigment content in peppers, the Labneh product will attract attention as new products will demonstrate attractive to consumers' appearance. The detailed characterization of natural pigments, total phenols, flavonoids, as well as evaluation of the antioxidant activity of Labneh cheese during storage was done (Table 4). The content of total chlorophylls and carotenoids was detected only in Labneh with encapsulated pepper extract. In the control sample, only carotenoids were found at 33.25 μg/100 g. The highest total chlorophylls content was observed in Labneh LP3, followed by LP4, while the highest carotenoids were detected in LP4 - 482.33 μg/100 g. Regarding the total phenolic content, the Labneh made with encapsulated pepper extract was the higher content of phenolic compounds, compared with the control.

In general, Folin–Ciocalteu reagent can detect also protein in sample which explains the high level of phenol content in control sample. The highest content of phenolic compounds was detected in Labneh fortified with alginate bead containing pepper extract from the Bulgarian variety Kurtovska kapia (34.24 mg GAE/100 g). A slight decrease in the level of natural pigments, total phenols, and total flavonoids was observed in Labneh samples during storage.

The reported values for total phenolic content in Labneh with encapsulated pepper extract were compared with data reported for fresh peppers (Denev et al., 2019). Moreover, the reported in the current research data for total phenolic content in Labneh with encapsulated pepper extracts was near or higher than the total phenolics of Labneh made with different concentrations of aqueous extract of Guava leaves (El-Gazzar et al., 2018). In the current research, the Labneh sample demonstrated the highest pigment content-carotenoids and chlorophyll, as well. Naturally carotenoids are a typical component in full-fat dairy products. This fact explains the presence of detected total carotenoids in control sample. All these compounds brought about radical scavenging activity of this Labneh (Table 4). It was found that total carotenoids, total phenols, and total flavonoids content highly correlated with antioxidant activity (evaluated by DPPH assay) of Labneh samples, as correlation coefficients r^2= 0,8450, r^2=0,8572 and $r^{2=}$ 0,9045, respectively.

Microbiological characteristics

Microbiological analysis of "hygienic" microorganisms in Labneh cheese is necessary to control the microbiological

Table 4: Total chlorophylls, carotenoids, phenolic content and antioxidant activity in Labneh cheeses

	Storage period, day	LC	LP1	LP2	LP3	LP4
Chlorophyll a, μg/100 g	1	N.D.	50.55 ± 1.21^{aA}	71.87 ± 2.21^{bA}	285.95 ± 3.20^{cA}	60.82 ± 1.21^{dA}
	20	N.D.	48.66 ± 2.00^{aA}	70.67 ± 1.50^{bA}	282.65 ± 2.22^{cA}	58.51 ± 1.21^{dA}
Chlorophyll b, μg/100 g	1	N.D.	76.89 ± 1.20^{aA}	128.22 ± 1.12^{bA}	684.00 ± 3.21^{cA}	130.33 ± 3.15^{dA}
	20	N.D.	74.84 ± 1.15^{aA}	122.44 ± 1.15^{bB}	677.64 ± 3.22^{cA}	126.35 ± 2.210^{dA}
Total Chlorophylls, μg/100 g	1	N.D.	127.44 ± 1.21^{aA}	200.09 ± 2.00^{bA}	969.99 ± 3.96^{cA}	191.15 ± 2.52^{dA}
	20	N.D.	123.49 ± 1.20^{aB}	193.11 ± 1.50^{bB}	960.29 ± 2.22^{cB}	184.86 ± 2.12^{dB}
Total carotenoids, μg/100 g	1	38.25 ± 2.21^{a}	41.00 ± 2.23^{aA}	98.00 ± 1.61^{cA}	576.02 ± 1.61^{cA}	499.23 ± 3.61^{dA}
	20	33.25 ± 1.21^{a}	39.17 ± 1.18^{bA}	94.52 ± 1.00^{cB}	570.02 ± 2.12^{dB}	482.33 ± 3.61^{eB}
Total phenols, mg GAE/100 g	1	10.55 ± 0.21^{a}	12.56 ± 0.21^{bA}	14.52 ± 0.10^{cA}	36.25 ± 0.12^{dA}	19.25 ± 0.25^{eA}
	20	9.53 ± 0.21^{a}	10.39 ± 0.26^{bB}	13.16 ± 0.32^{cB}	34.24 ± 0.15^{dB}	17.12 ± 0.12^{eB}
Total flavonoids, mg QE/100 g	1	N.D.	0.01	0.03 ± 0.01^{aA}	0.05 ± 0.01^{aA}	0.02 ± 0.01^{aA}
	20	N.D.	N.D.	0.02 ± 0.01^{aA}	0.04 ± 0.01^{aA}	0.01 ± 0.01^{aA}
DPPH, mM TE/100 g	1	13.21±0.15a	18.63 ± 0.12^{bA}	22.95 ± 0.50^{cA}	28.95 ± 1.21^{dA}	25.95 ± 0.25^{eA}
	20	12.46± 0.21a	15.63 ± 0.21^{bB}	20.95 ± 0.21^{cB}	$26,04\pm0.21^{dB}$	21.63 ± 0.21^{eB}

N.D.-not detected, LC-Labneh cheese control sample; LP1-Labneh with encapsulated pepper Doux Marconi Geonet; LP2-Labneh with encapsulated pepper Bulgarian Ratund; LP3-Labneh with encapsulated pepper Kurtovska kapia; LP4-Labneh with encapsulated pepper Doux Marconi Geonet mixed with Kurtovska kapia. a, b -Means with different letters within a row are significantly different ($p<0.05$) A, B-Means with different letters within a column are significantly different ($p<0.05$)

quality and to ensure the safety of the product. Table 5 represents the results for the presence of coliforms, moulds, and yeasts during the storage period. The absence of coliforms is a leading indicator of quality and high hygiene observed during the production and storage of all Labneh samples.

These results were in agreement with the reports of Tamime and Crawford (1984). A minimum concentration (<10 cfu/g) of moulds and yeasts in the Labneh control sample is found at the end of the storage period. It is period is probably due to the absence of a pepper antioxidant inhibitor effect. Similar results have been reported by Mutlag and Hassan (2008) and Manso et al. (2013). No moulds and yeasts were detected at the end of the study period in Labneh cheese with alginate-encapsulated pepper extracts. This inhibitory effect can be explained by the antimicrobial properties of different pepper extracts (Hassan et al., 2001; Schelz et al., 2006; Mutlag and Hassan, 2008; Manso et al., 2013), by the antimicrobial activity of the organic acids produced by the lactic acid bacteria (Fraga et al., 2005) and by the direct inhibition of moulds by *Lactobacillus* ssp. (Lavermicocca et al., 2000; Ström et al., 2002; Valerio et al., 2004; Coloretti et al., 2007; Dalié et al., 2010; Delavenne et al., 2012; Ahmadova et al., 2013; El-Mabrok et al., 2013).

Table 5: Presence of coliforms, moulds, and yeasts in Labneh cheeses during storage

	Storage period	LC	LP1	LP2	LP3	LP4
Yeast and moulds, cfu/g	1	N.D.	N.D.	N.D.	N.D.	N.D.
	20	N.D.	N.D.	N.D.	N.D.	N.D.
Coliforms, cfu/g	1	N.D.	N.D.	N.D.	N.D.	N.D.
	20	< 10	N.D.	N.D.	N.D.	N.D.

N.D.-not detected, LC-Labneh cheese control sample; LP1-Labneh with encapsulated pepper Doux Marconi Geonet; LP2-Labneh with encapsulated pepper Bulgarian Ratund; LP3-Labneh with encapsulated pepper Kurtovska kapia; LP4-Labneh with encapsulated pepper Doux Marconi Geonet mixed with Kurtovska kapia.

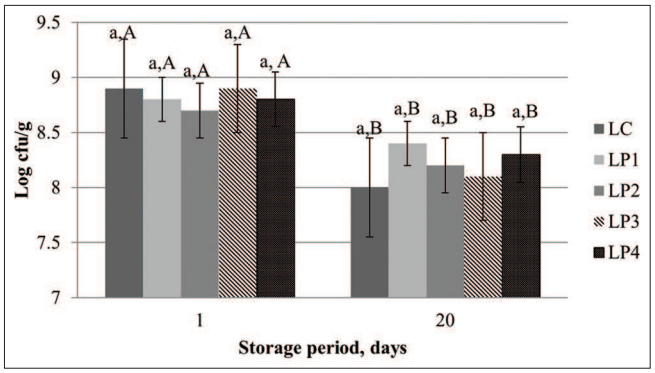


Fig 2. The total viable lactic acid bacterial count. a, b Means with different letters within a row are significantly different (p<0.05); A, B Means with different letters within a row are significantly different (p<0.05)

Total viable lactic acid count (TVC), representing the survival of the starter microflora, is represented in Fig. 2.

a,b Means with different letters within a row are significantly different (p<0.05); A, B Means with different letters within a row are significantly different (p<0.05)

The obtained results show that at the beginning (1st day) of the storage period (at 4 ± 1 °C), TVC values in the samples with added pepper extracts are similar to these of the control sample (p>0.05). This tendency persisted in the end of the storage period (20th days) when no significant difference was found (p>0.05). All analysed Labneh cheese samples showed a decrease in lactic microflora at the end of the storage period (p<0.05). These data are in agreement with Sharal et al. (1996), Hamann and Marth (1984), Otaibi and Demerdash (2008) and Thabet et al. (2014).

Sensory analysis

The acceptance of the final product is appreciated by the sensory profile (Fig. 3).

The obtained data showed that the samples of fresh Labneh cheese were with a very good sensory profile at the beginning of the storage period but at the end of the storage period, all the samples with encapsulated pepper extracts demonstrated better sensory profile than the control samples, which proved the high acceptance of the products enriched with biologically active compounds. Moreover, the addition of alginate beads with pepper improved the color, appearance, and flavour of the Labneh products, especially at the end of storage period (Fig. 3). Therefore, the results from sensory evaluation demonstrated that samples LP3 and LP4 contained not only the highest level of bioactive compounds with

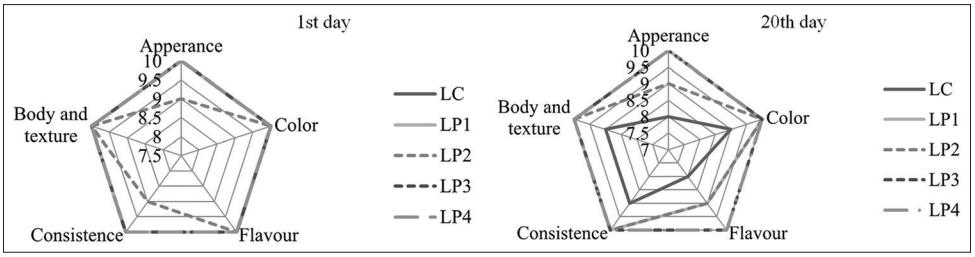


Fig 3. Sensory profile of Labneh cheese during period

antioxidant action but also were accepted with the highest sensory evaluation.

CONCLUSION

This study revealed that the addition of different pepper extracts encapsulated in alginate beads in Labneh cheese did not affect the chemical characteristic of the final products during the storage period. The total lactic acid bacterial count was similar in all the samples. The obtained products were characterized by better sensory profiles at the end of the storage period. From analysed samples Labneh, this with encapsulated pepper Kurtovska kapia showed the highest content of bioactive substances and the highest radical-scavenging activity. The nutritional and healthy effects of designed Labneh cheese were improved by enrichment with antioxidants and bioactive substances encapsulated in alginate beads.

Authors' contribution

Mihaela Ivanova and Tatyana Balabanova designed the experiment, performed most of the experiments, analysed the data and wrote the paper; Nadezhda Petkova conducted experiments for sugar determination, as well as natural pigments, polyphenols, and antioxidant activity, performed data interpretation, wrote part of the manuscript and edited final version; Nikolay Panayotov grew and donated the pepper varieties for the alginate beads encapsulation.

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