The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology (2018), 42(1), 137-146

ORIGINAL RESEARCH PAPER
Presented at the 8th International Symposium EuroAliment 2017

FOOD DESIGN OF DAIRY DESSERTS WITH ENCAPSULATED CORNELIAN CHERRY, CHOKEBERRY AND BLACKBERRY JUICES

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Received on 19th July 2018
Revised on 20th April 2018

New approach of alginate encapsulation of polyphenolic rich extract from three wild edible forest fruits Cornelian cherry, chokeberry and blackberry was introduced. The aim of the current study was to design new dairy desserts with antioxidant properties by encapsulation and direct incorporation of berries juices The microencapsulation of berries extracts significantly retains ($p < 0.05$) higher amount of polyphenols and antioxidants in dairy desserts not only at the first day, but also after twenty days storage period. The highest antioxidant activity (523 mM TE/100 g (DPPH method) and 510 mM TE/100 g (FRAP method)) demonstrated dairy dessert with encapsulated blackberry juice followed by chokeberry and Cornelian cherry alginate encapsulated juices. The sensory evaluation of obtained dairy desserts with encapsulated berries juices showed satisfactory and overall higher scores for proposed new desserts compared to the direct incorporated fruits juices. Alginate-based encapsulation of polyphenolic extracts from Cornelian cherry, chokeberry and blackberry were evaluated as proper technique for preservation of natural antioxidants in dairy desserts.

Keywords: antioxidant, berries juices, alginate beads, dairy desserts

Introduction

Forest fruits are rich sources of antioxidants. The combination of their health benefits with nutrition potential of fermented lactic acid products revealed new field of value-added dairy products.

Fermented dairy products are important source of different nutrients for humans, to which may be added the biologically active substances affecting beneficial effects on health (Rogeli, 2000; Bhat and Bhat, 2011).

Fruit juices have great application in the creation of different new products, because of their easily and efficiently absorption in gastrointestinal system. Juice obtained from different fruits possesses strong antioxidant properties due to polyphenols and flavonoids, vitamins (C, E and A) and enzymes (Gardener *et al*., 2000; Rodriguez-Roque *et al*., 2015).

The design of dairy products enriched with compounds with well-known biological activity is a promising trend and challenge for science (Jacobsen *et al*., 2008). There is increasing demand for natural antioxidants in food, cosmetics and pharmaceuticals industries which has led to search not only for natural extracts but also for techniques with which final product to keep its nutritional and healthy effect increasing long term storage stability, as well. Encapsulation of fruit juice leads to potential benefits including enhanced stability in the controlled release and bioavailability of bioactive ingredients (Acosta, 2009). Encapsulation has demonstrated to be an alternative technique for the protection of biologically active substances from unfavourable environments (Chaikham *et al*., 2013a,b; Ribeiro *et al*., 2014; Chaikham and Prangthip, 2015). Among numerous carriers for microencapsulation, sodium alginate has attracted great attention, because of its biodegradability, non-toxicity and biocompatibility (Cuji´c´*et al*., 2016). Moreover, encapsulation within Ca-alginate beads is rapid, nontoxic, inexpensive approach and the beads are stable in acidic pH levels.

To the best of our knowledge, literature data for encapsulation of chokeberry, blackberry or Cornelian cherry extracts are limited. Promising results were obtained in our previous research for chokeberry and red beet extracts encapsulated in alginate-inulin gels and their incorporation in dairy desserts (Vlaseva *et al*., 2014).

Therefore, the aim of the present study was to design new dairy desserts with direct incorporated or alginate-encapsulated Cornelian cherry, chokeberry and blackberry juices for better preservation of polyphenols in final product. The evaluation of the changes in total phenols and flavonoids content, as well as antioxidant activity of juices in respect of the type of incorporation and storage period also presented interest in the current research.

Materials and methods

Raw material and ingredients

Raw whole cow milk (3.5 % fat) was purchased from a dairy factory (United Milk Company) in Plovdiv, Bulgaria. Gelatine and sucrose were purchased from a local market in Plovdiv (Bulgaria). A freeze-dried starter culture was used for production of yoghurt by Lactina Ltd. (Bankya, Bulgaria) with the following composition: *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, exceeding 9.5×10^{9} g^{-1} in an amount of 2 %.

The chokeberry and blackberry syrups (Vitanea Ltd, Plovdiv, Bulgaria) with known characteristics were diluted as 10 g is dissolved 100 ml distilled water before incorporation into dairy desserts. Cornelian cherry juice was obtained from compote (250 g Cornelian cherry, 180 g sucrose and 750 ml distilled water) (Ognyanov and Petkova, 2017) after separation of fruits.

Encapsulation of the wild berries juices into alginate beads

For the preparation of the alginate beads sodium salt of alginic acid with M-block 61%, G-block 39 % and M/G ratio 1.55 (Sigma Aldrich, France) were used. Sodium alginate (2 g) and 5 g sucrose were suspended in 100 ml juice (Cornelian cherry, chokeberry and blueberry, respectively) followed by heating at 70 ºC for 3 min and stirring with laboratory homogenizer Polytron PT45-80 (Kinematica, Switzerland) - 1600 W, max 250.s^{-1} for 2 min. The obtained suspension was placed in the fridge for 60 min to remove the air bubbles. The alginate beads were prepared as liquid solutions were transferred into a syringe and dropped into cold calcium chloride solution (2 %) with temperature 7 ºC (Vlaseva *et al*., 2014). The resulting alginate beads were washed with distilled water and were used for incorporation in dairy desserts. The encapsulation efficiency of total phenolic content in fruit juices loaded in alginate beads were evaluated as previously described (Isailović *et al*., 2012). In prepared alginate beads the encapsulation efficiency was 85% for chokeberry, 89% for blackberry and 70 % for cornelian cherry.

Preparation of dairy desserts

For the dairy desserts preparation whole cow milk was adjusted to a fat content of 2.0±0.1%, followed by stirring at t = 60÷65 °C and pressure 15-20 MPa, and then heating at 93÷95 °C with 15÷20 min residence time. Then the milk was cooled down to temperature 42÷43 °C. Pasteurised starter culture (2 %) was added to the milk and the sample was incubated for 2-3 h, stirred and cooled down to 20 °C. The stabiliser (gelatine) and sucrose were dissolved in hot water (40 °C) and then cooled to 25 °C). The solution was mixed with juices or alginate beads and added to stirring milk. The products were cooled down. Six dairy desserts, based on addition of fruit juices (three samples) and encapsulated fruit juices (three samples) and one control sample without juice were prepared (Table 1). After the addition of ingredients, the samples were divided into portions of 150 g and stored at 4 °C for 20 days.

Sample preparation of extracts from dairy desserts

The extraction of biologically active substances from dairy desserts was performed as follows: 25 g stirred dairy desserts was extracted with 50 ml 95 % ethanol using homogenizer Polytron PT45-80 (Kinematika, Switzerland) at maximum speed for 5 min. The ethanol extract was separated by vacuum filtration with Buchner funnel under pressure 1.3 kPa. The extraction procedure was repeated twice and the final volume of obtained extracts was collected. Then the dairy desserts extracts were analysed for total phenolic contents, total flavonoids and their antioxidant potential were evaluated by DPPH and FRAP methods.

Table 1. Composition of dairy desserts

Sample	Ingredients				
	cow's milk	**Starter culture**	**Gelatine**	**Sugar**	**Juice**
control	+	+	+	+	no
dairy desserts prepared with juices					
1	+	+	+	+	Cornelian cherry
2	+	+	+	+	blackberry
3	+	+	+	+	chokeberry
dairy desserts prepared with encapsulated in **alginate beads** fruit juices					
4	+	+	+	+	Cornelian cherry
5	+	+	+	+	blackberry
6	+	+	+	+	chokeberry

Total phenolic contents

Total phenolic contents were measured as previously described (Stintzing *et al*., 2005) with slight modifications. Briefly, 1 ml Folin-Ciocalteu reagent diluted five times was mixed with 0.2 ml extract and 0.8 ml 7.5% Na_2CO_3. The reaction was performed for 20 min at room temperature in darkness. Then the absorbance was measured at 765 nm against blank. The results were expressed as mg gallic acid equivalent (GAE) per 100 g product (Ivanov *et al*., 2014).

Determination of total flavonoids content

The total flavonoids content was determinated by $Al(NO_3)_3$ reagent. The absorbance was measured at 415 nm against the control sample. The results were expressed as mg equivalents quercetin (QE) per 100 g product, according to the calibration curve with quercetin as a standard (Ivanov *et al.*, 2014).

Determination of antioxidant activity

The DPPH radical-scavenging ability

Ethanol extracts from dairy desserts (0.15 ml) were mixed with 2.85 ml freshly prepared 0.1 mM solution of DPPH in methanol. The sample was incubated for 15 min at 37 °C in darkness. The reduction of absorbance was measured at 517 nm in comparison to the blank prepared only with methanol and % of inhibition was calculated (Ivanov *et al.* 2014).

Ferric reducing antioxidant power (FRAP) assay

The FRAP reagent was freshly prepared by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6- tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM $FeCl_3.6H_2O$ in d. H_2O. The reaction was started by mixing 3.0 ml FRAP reagent with 0.1 ml of investigated extract. The reaction time was 10 min at 37 °C in darkness and the absorbance was measured at 593 nm against blank prepared with 95 % ethanol (Ivanov *et al.* 2014).

Antioxidant activity evaluated by DPPH and FRAP assays was expressed as mM Trolox equivalents (TE) per 100 g dairy desserts.

Sensory evaluation

Dairy desserts were evaluated for sensory characteristics according to BNS 15 612-83. The evaluation criteria included the following indicators: flavour, aroma, colour, body (texture) and appearance. A 10-point rating scale was used for sensory evaluation.

Statistical Data Analysis

Statistical analysis of the results was performed using the program Microsoft Excel 2010 (ANOVA). Multiple comparisons were made by the LSD method. The results were presented as mean value ± standard deviation (n = 3) and statistical significance was set at $p < 0.05$.

Results and discussion

The content of biologically active substances and antioxidant activity of dairy desserts with wild berries were summarized in Table 2 and Table 3. In general, the total phenolic content on the first day of storage ranged from 0.5 to 14.53 mg GAE/ 100 g dairy dessert. The lowest level except control was found in desserts prepared with Cornelian cherry juice – 4.12 mg GAE/100 g sample (sample 1).

Table 2. Phenolic compounds and antioxidant potential of dairy desserts after 1st day storage at 4 ºC

Sample	Total phenolic content, mg GAE/100 g	Total flavonoids, mg QE/ 100 g	DPPH, mM TE/100 g	FRAP, mM TE/100 g
control	n.d.	n.d	56.22±2.15^{a}	45.23±2.00^{a}
1	4.12±0.52^{b}	0.76±0.15^{a}	136.55±1.15^{b}	112.52±2.01^{b}
2	7.81±0.44^{c}	0.56±0.16^{a}	385.36±2.23^{c}	320.21±3.16^{c}
3	6.54±0.22^{d}	0.96±0.23^{b}	320.23±2.50^{d}	310.32±2.75^{d}
4	5.02±0.25^{e}	0.34±0.15^{a}	357.72±2.36^{e}	353.25±2.95^{e}
5	14.53±0.25^{g}	0.81±0.15^{b}	523.22±2.00^{g}	510.33±3.52^{g}
6	7.53±0.23^{f}	1.16±0.31^{b}	433.60±1.19^{f}	496.21±2.62^{f}

n.d – not detected; a÷g Means with different letters within a column are significantly different ($p < 0.05$)

The highest content of phenolic compounds was detected in encapsulated with alginate samples; especially desserts prepared with alginate beads with blackberry juice (sample 5) – 14.53 mg GAE/100 g sample. The highest level of total flavonoids was found in desserts prepared with encapsulated chokeberry juice (sample 6). The antioxidant activities evaluated by DPPH and FRAP methods showed similar trend. The highest value for antioxidant potential was found in desserts prepared with blackberry juice encapsulated in alginate beads (523.22 and 510.33 mM TE/100 g dairy dessert). Therefore, alginate capsules preserve the

bioactivity of wild berries extracts. This approach for enrichment of dairy desserts with phenolic compounds presented promising results for production of functional dairy products with antioxidant activity.

The dairy desserts were analyzed after the 1st and 20 th days storage with respect to the contents of total phenolic, total flavonoids content and antioxidant activity was also evaluated. The changes of polyphenols in samples were assessed during storage period in terms of gallic acid equivalents (Table 3). A significant decrease in polyphenol content was observed in all six samples with increase of time of storage. After 20 days at 4º C the total phenolic content in desserts with encapsulated juices in beads was approximately two times lower than the samples after 1st day storage. Significantly decrease in total phenolic content and antioxidant activity ($p < 0.05$) was observed in desserts prepared with direct incorporation of berry juices (samples 1 to sample 3). The flavonoids content significantly decreased ($p < 0.05$) as in dairy desserts with Cornelian cherry juice (sample 1) was not detected at all. The highest total phenolic content and antioxidant activity were found in desserts with encapsulated blackberry juices (sample 5). The antioxidant activity of desserts with encapsulated juices was approximately two to three times higher than these prepared by juices (Table 3).

Table 3. Phenolic compounds and antioxidant potential of dairy desserts after 20th day storage at 4 ºC

Sample	Total phenolic content, mg GAE/100 g	Total flavonoids, mg QE/ 100 g	DPPH, mM TE/100 g	FRAP, mM TE/100 g
control	n.d	n.d	15.56±0.36^{a}	2.30±0.43^{a}
1	2.03±0.21^{a}	n.d	20.82±1.23^{b}	20.3±1.56^{b}
2	1.92±0.11^{a}	0.26±0.06^{a}	19.28±2.08^{b}	16.2±1.63^{c}
3	1.29±0.16^{b}	0.45±0.11^{b}	30.39±3.15^{c}	14.8±1.56^{c}
4	2.43±0.15^{c}	0.35±0.08^{b}	33.33±2.35^{d}	21.6±1.85^{b}
5	7.39±0.15^{e}	0.55±0.12^{b}	47.16±2.02^{e}	56.7±1.56^{e}
6	4.26±0.18^{d}	0.86±0.15^{c}	48.47±2.65^{e}	37.0±2.31^{d}

a÷e Means with different letters within a column are significantly different ($p < 0.05$)

Therefore, encapsulation of wild berry extracts in alginate beads and their incorporation in dairy desserts presented a promising method for stabilization of total phenols in obtained products. In comparison with dairy desserts on the first day the antioxidant activity decreased more than 10 times. The prepared dairy desserts preserved their health potential effect up to 20th day of storage.

In addition, some authors reported in soy-based beverages FRAP values ranged from 8.54 to 16.27 μmol/g dry matter in aqueous-organic extracts and between 12.79 to 18.98 μmol/g dry matters in residues. In UHT milk, samples exhibited an average FRAP value of 3.10 μmol/g dry matter (Durazzo *et al.*, 2015). De Almeida Callou *et al.* (2010) and Durazzo *et al*. (2015) have studied different brands of soy-

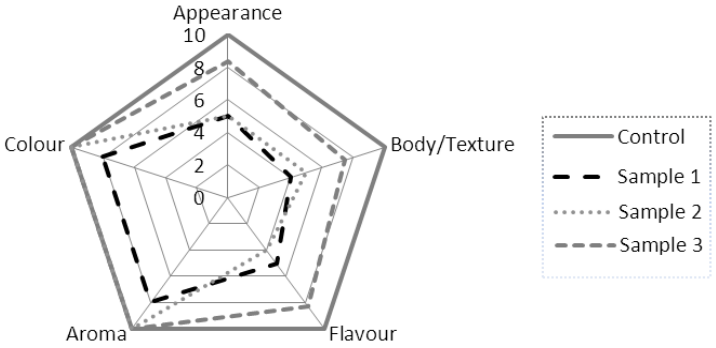
based beverages (including products from soy protein isolate and soy milk, mixed with fruit juice) and their results showed that antioxidant activity varied significantly ($p < 0.05$) among products. Our study added additional information about stability of polyphenols during storage. As it was shown that not only the storage at room temperature (Durazzo *et al*., 2015), but also at refrigeration temperature caused a significant decrease ($p < 0.05$) of antioxidant capacity and soluble phenolic compounds. The authors reported that the loss of phenolic compounds had occurred at a slower rate when they were in encapsulated form.

The lower levels of polyphenols contained in alginate beads after 20th day storage could be explained with influence of pH and small encapsulation capacities of water soluble components of wild berries. Weakly encapsulation capacities such as polyphenols from chokeberry could be attributed to the fact that they can easily diffuse out into the alginate gelling solution.

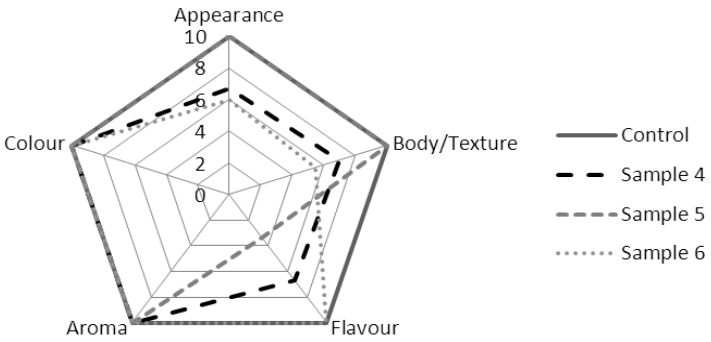
According to Cuji´c´*et al.* (2016) the release of phenolic compounds from alginate particles in water was better than in acidic environment. In our case pH values of dairy desserts prolonged the polyphenolic degradation and release of active compounds from alginate beads and even after 20 days small amount of polyphenols was detected. Leick *et al.* (2011) demonstrated that anthocyanins at pH 3 were positively charged and alginate negatively charged due to the presence of carboxylic anions. Possible explanation was that some of anthocyanins and polyphenols polymerize, and interaction made polyphenols release difficult and some part of them remained trapped in the particles. Higher levels of total phenolic content in dairy desserts prepared with alginate beads could be explained also with interaction of polyphenol compounds with uronic acids residues of alginate and steric caused by capture of polyphenols in cross-linked calcium alginate (Cuji´c´*et al.*, 2016).

The results obtained with regard to the sensory profile of dairy desserts were an assessment that characterized the acceptability of the product by consumers and experts.

The obtained results showed that the encapsulated juice in alginate beads samples were characterized by a higher sensory score compared to the directly added juice variants on the 1st day of storage (Figure 1 a and b). This trend persisted throughout the shelf life of dairy desserts. Especially clear tendency was observed in the appearance and body (texture) indicators (Figure 2). Probably the low pH of the juice changed the body (texture) of the final product more sensibly. Desserts contained alginate encapsulated chokeberry and blackberry juices had the highest total score that were closer to the control sample (Figure 1b and Figure 2b). At the end of the study period (Figure 2), all indicators were degraded, but established trends remained. Desserts with incorporated juices into alginate beads had higher sensory scores compared to the desserts obtained after direct juice incorporation (Figure 2). It was advisable to enrich the dairy desserts with antioxidants in the form of encapsulated juices.

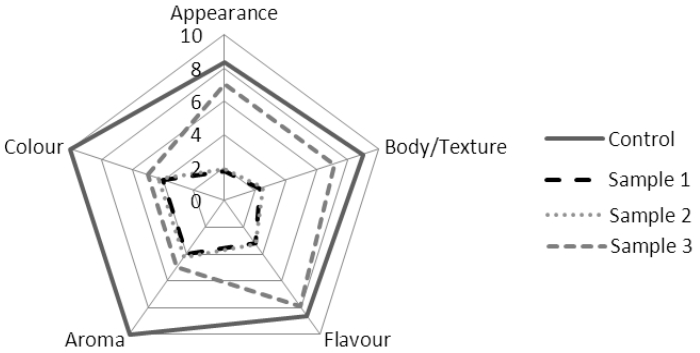


a)

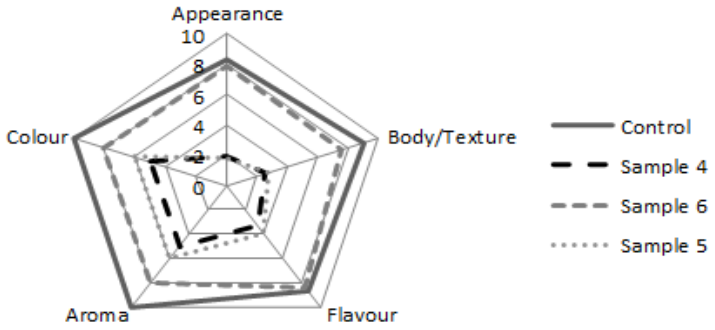


b)

Figure 1. Sensory evaluation at 1st day of storage of dairy desserts enriched with biologically active fruit juice (*a*) and encapsulated in alginate beads fruit juices (*b*).



a)



b)

Figure 2. Sensory evaluation at 20th day of storage of dairy desserts enriched with biologically active fruit juice (a) and encapsulated fruit juices (b).

Conclusions

The current study demonstrated food design of dairy desserts enriched in antioxidants from encapsulated wild fruits extracts. The obtained alginate-berries beads and pure berries juices were used for preparation of dairy desserts enriched in polyphenols. Alginate-based encapsulation of polyphenolic extracts from Cornelian cherry, chokeberry and blackberry were evaluated as proper technique for preservation of natural antioxidant in dairy desserts. Compared to the direct incorporated fruits juices encapsulated ones demonstrated higher antioxidant potential and sensory profiles. Since the incorporation of berries-derived active ingredients into the dairy desserts have caused minimal changes in the sensory evaluation of the final product it could be widely applied and have the potential application as nutraceuticals in dairy industry.

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