Innovative Romanian Food Biotechnology Vol. 20, 2021
2021 by Galaţi University Press Received September 2020 /Accepted February 2021

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MICROBIOLOGICAL ANALYSIS OF A NEW NON-LACTIC PROBIOTIC BEVERAGE – CATINOLACT

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Abstract: Sea buckthorn, honey and lactic acid bacteria are known for their many health benefits. Many studies show a significant increase in the number of people with lactose intolerance, allergies to milk proteins, high level of cholesterol, etc. which require special diets. Therefore, we designed a functional product called CatinoLact, which is based on sea buckthorn (puree and juice), two variants of honey (polyflora and linden) and five strains of probiotic lactic acid bacteria (LAB) as starter cultures (*Lactobacillus casei* 431, *Lactobacillus acidophilus, Bifidobacterium* sp., *Lactobacillus plantarum* and *Lactobacillus johnsonii*). All twenty variants of CatinoLact were analyzed from a microbiological point of view: total number of aerobic mesophilic bacteria, the number of yeasts and molds and the number of lactic acid bacteria. The viability of lactic acid bacteria was evaluated every two days, in all variants of CătinoLact bioproduct stored at 4°C for 2 weeks. Although there was a decrease of viable lactic acid bacteria, the CătinoLact bioproduct can be considered as a probiotic food during 7 days of storage, since the viable population remained above the recommended minimum limit of $1x10^6$ CFU/ mL.

Keywords: Sea buckthorn fruits, lactic acid bacteria, honey, bioproduct

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Introduction

There is an increasing interest concerning probiotics from the public, researchers, governmental organizations (FAO/WHO, 2002). Nowadays, the demand for probiotic functional foods is increasing rapidly, as the consumers become more aware about the potential health benefits, due to the fact that probiotics help in maintaining the balance and composition of intestinal flora and protect it from pathogens (Călinoiu *et al*., 2016).

A number of studies have reported that the best matrices to deliver probiotics are fermented milk products, and traditionally, probiotics have been added to yogurt. However, there is an increasing consumer demand for probiotic products in non-dairy foods, mainly sought by vegetarians, vegans, cholesterol controllers and lactose-intolerant individuals (Prado *et al*., 2008; Granato *et al*., 2010; Rivera-Espinoza and Gallardo-Navarro, 2010; Kandylis *et al*., 2016; Panghal *et al*., 2018; Aspri *et al*., 2020; Nazhand *et al*., 2020).

The application of novel technologies aims to better preservation of the bioactive compounds as well as sensory characteristics (Hribar *et al*., 2018). Lactic acid fermentation is considered a valuable technology to enhance shelf life, safety, sensory and nutritional properties of vegetables and fruits (Di Cagno *et al*., 2013). The application of probiotic cultures in different food matrices (dairy and non-dairy based beverages), could represent a great challenge for the viability of probiotics. The success of new probiotic beverages depends on the capability of probiotics to provide enough numbers of viable cells (Shori, 2016; Min *et al*., 2018).

Due to the changes in consumer preference towards natural products with functional properties, in recent years, the use of sea buckthorn berries as a natural food ingredient has been increasing (Bal *et al*., 2011). The transformation of the raw sea buckthorn berry into a sophisticated product requires appropriate harvest, transportation, holding, and storage procedures (Li *et al*., 2002).

Sea buckthorn fruits (*Hippophae rhamnoides* L.) are used for making pies, jams, wines and liquors. The juice or pulp has potential applications as food or beverages (Vinita and Punia, 2018). Along with traditional foods, some new ones, such as condensed juice, mixed juice, sea buckthorn carrot jam, candied fruit, sea buckthorn cheese, sea buckthorn butter, tea and health protection drinks are also being produced (Rajchal, 2009; Chandra *et al.,* 2018).

Sea buckthorn berries contain a large variety of substances which possess a strong biological activity. A supramolecular biocomposite (pectin-lipoprotein-oleosome) structure found in sea buckthorn fruit pulp, a hydrophilic matrix which naturally include both hydrophilic (phenolics) and lipophilic (oils and vitamins) bioactive molecules (Socaciu *et al*., 2009). The fruits are rich in carbohydrates, protein, organic acids, amino acids and vitamins, also contain high contents of carotenoids, tocopherols, omega-3 fatty acids, minerals and polyphenolic acids (Bal *et al.,* 2011; Zeb, 2014;). Sea buckthorn berries could be considered as functional foods due to the medicinal and nutritional properties of their substances (Suryakumar and Gupta, 2011; Christaki, 2012; Krejcarová *et al*., 2015). Active - biologically substances existing in raw fruit, consisting of vitamins, minerals, enzymes, hormones, etc. remain in a big proportion in juices, after cold processing (Varsta and Popa, 2015).

Honey is a functional food with a unique composition (4 - 5% fructooligosaccharides with prebiotic function), with proved antimicrobial effects and bifidogenic effects (Dezmirean *et. al*., 2011; El Sohaimy *et al.,* 2015; Bogdanov, 2016; Luchese, *et al.,* 2017). Romanian linden honey contains a high concentration of glucose and fructose and high content of calcium, magnesium and zinc, contents that can improve the honey usage as a functional food (Dărăban *et al.*, 2016). Significant differences were observed among the 35 honey samples of different floral varieties, from different parts of Romania (especially multi-flower honey), which is strongly correlated with the area's soil composition (Dinca *et al.*, 2015). Compared to the honey from other European countries, the Romanian honey has good market qualities due to its organic character and various botanic sources responsible for the specific flavor and consistency (Isopescu *et al*., 2017).

The aim of the current study was to develop a functional product that uses sea buckthorn fruits (juice and puree) and honey (multi-flowers and linden) as well as its evaluation from a microbiological point of view to assess its stability during refrigeration storage. Therefore, this article offers a potential application of probiotics in non-dairy drinks, being increasingly known and appreciated the benefits of the intake of live probiotic lactic acid bacteria.

Materials and Methods

Sea buckthorn berries

Fresh sea buckthorn berries (*Hippophae rhamnoides* L.) were collected from two different towns (Murgești, Mărăcineni) in 2018. The sample was collected from a local sea buckthorn population considered as being phenotypically representative for Buzau valley (Romania). Selection was made with regards to productivity, fruit weight, number of thorns, fruit color, etc. The fruit was hand cleaned to remove visible debris, namely dried leaves, branches, and damaged fruit. Cleaned sea buckthorn fruits from the two sources were mixed and portioned into 200 g lots and packed in sterile bags and was frozen at -18°C. Fruit can be frozen to -18°C for long-term storage (1 year) without further loss of ingredients (Rajchal, 2009). Fruits were thawed at room temperature to about 10°C. The puree was obtained by pressing technique without crushing the seeds. Juice was separated with Multipress automatic MP 80 Typ 4290 (BRAUN Entsafter, Germany).

Honey

The organic certified food product that was the subject of this study was honey with the following two assortments: linden and multi-flower. Samples were obtained in 2018 from beekeepers from Buzau branch, members of the Romanian Beekeepers Association.

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Probiotic lactic acid bacteria

A total of five strains of lactic acid bacteria were used in the experimental design. *Lactobacillus casei* 431 (*L. casei*), *Lactobacillus acidophilus* (La5), *Bifidobacterium* sp. (BB12) from Chr. Hansen Company (Denmark), and two belonging to the Microorganisms Collection of the Bioaliment Research Platform (acronym MIUG): *Lactobacillus plantarum* (13GAL) isolated from wheat grains, and *Lactobacillus johnsonii* (La1) isolated from the feces of breast-fed infants. All LAB strains ware stored at -70°C in MRS broth (De Mann, Rogosa and Sharpe – Sigma Aldrich, Germany) supplemented with 20% (v/v) glycerol. The primary culture of which LAB strain was obtained after its cultivation for 12 h, at 37°C, on MRS broth, in order to obtain the mid-logarithmic phase cultures (2-7x10^6 CFU/mL). The starter cultures were spin at 4 800 rpm , at 4°C, for 10 min and were washed twice with sterile 0.85% saline solution in order to prepare the inoculum.

Experimental design

As can see in Table 1, twenty experimental variants were obtained by mixing sea buckthorn berries (50 g puree or juice) with organic honey (5%) and 2% inoculum of which LAB strain (1x10^4 CFU/mL determined by Breed method).

Table 1. Experimental design of CătinoLact product

Sea buckthorn berries	Honey	*Lb. casei* 431	*Lb.acidophilus* La5	*Bifidobacterium* BB12	*Lb. plantarum* 13GAL	*Lb. johnsonii* La1
Puree	Multi-flower	V1	V2	V3	V4	V5
	Linden	V6	V7	V8	V9	V10
Juice	Multi-flower	V11	V12	V13	V14	V15
	Linden	V16	V17	V18	V19	V20

The fermentation was conducted in Erlenmeyer flasks, at 37°C for 48 h because, according to previous tests, the sensory properties of the finished product were the most appreciated after 48 hours of fermentation (data not shown). Moreover, many studies of the dynamics of lactic acid bacteria culture show that the maximum number of cells is recorded between 24 - 48 h (Vodnar, 2010). After fermentation, all samples were stored at 4°C for 14 days.

Microbial analysis

- **Hygiene criteria**

The microbiota of CatinoLact variants was quantified by the indirect counting method after fermentation process and after two weeks of storage in refrigerated conditions (4°C). The samples were homogenized using a Pulsifier equipment (Microgen Bioproduct, London, UK) for five minutes at medium speed. Ten-fold serial dilutions in 0.85% sterile saline solution were plated out following standard methodology. The determination of the total number of aerobic mesophilic bacteria was performed on Plate Count Agar (PCA) (Sigma Aldrich, Germany) by counting the colonies after incubation for 48 h at 37°C. The determination of the total number of yeasts and molds was performed on Malt Extract Agar (MEA) (Sigma Aldrich, Germany) by counting the colonies after incubation at 25°C for 3-5 days. The conventional method was used to determine the probable number of coliforms. The Most Probable Number (MPN) method is a statistical assay consisting of presumptive and confirmed stages. In the presumptive test, serial dilutions of a sample are inoculated into Lactose Broth (LB) (Sigma Aldrich, Germany) for 48 hours at 35°C (3 tube MPN analysis). From each gas positive (fermentation of lactose) tubes a loop of suspension is transferred to a tube of Brilliant Green Lactose Bile (BGLB) (Sigma Aldrich, Germany) broth and incubated for 48 h at 35°C. It scores the number of gas positive tubes from confirmed test then the McCrady statistical table is used to interpret the results with de Man's confidence intervals.

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The number of LAB was determined by cultivation on de Man Rogosa, Sharpe (MRS) agar (Sigma Aldrich, Germany) supplemented with 2% $CaCO_3$ (by double layer method). After 48 h of incubation at 37°C, the LAB colonies appear surrounded by a clear halo. All the experiment was conducted in triplicate for each dilution. The results were expressed as the mean number of the colony forming units per mL (CFU/mL).

- **LAB viability in CatinoLact products**

In order to estimate the shelf life period of CătinoLact bioproduct stored at 4°C, the viability of the LAB in all variants was evaluated every two days, for two weeks, by cultural methods, as described above.

Antioxidant activity

The antioxidant capacity of new bioproduct CatinoLact was determined by using a DPPH (2,2-diphenyl-1-picrylhydrazyl, Fluka Chemie) methanol solution (0.1 M) and quantified using a Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich, USA) calibration curve, under the same conditions, by a spectrophotometric method at 517nm (Yuan *et al*., 2013). The fresh puree and the juice without LAB or honey were analyzed as the control samples. All samples, purees or juices were centrifuged at 5000 rpm for 20 min at 4°C. The supernatant was concentrated to dryness under reduced pressure at 40°C (2-18 Vacuum Concentrator, Christ, UK). Antioxidant activity has been expressed in mg Trolox equivalents per 1 g of dry matter (mg TE/g DM).

Statistical analysis of data

All the data reported in this study represent the averages of triplicate analysis and were reported as mean ± standard deviation. The results were compared statistically using (ANOVA) ($p < 0.05$) was carried out to assess the significant differences between values.

Results and discussion

Microbiological data analysis of CătinoLact product

The microbiological quality of the CătinoLact bioproduct variants was analyzed (figures 1-3). The microbiological limit for yeasts in fruit juices is <1 x 10^3 CFU/mL for unpasteurized fruit juices and <1 x 10^2 CFU/mL for pasteurized fruit juices, and the maximum acceptable level for bacteria is 1 x 10^6 CFU/mL (Mihiretie *et al.*, 2015). The regulations in Romania are in accordance with the European and therefore international ones, even smaller. The microbiological standards for fruit juices are in accordance with EC Regulation 1441/2007 and of the Order of the Minister of Health no. 27/2011 (regarding the approval of the hygienic-sanitary norms for food). According to these documents, the permissible limits for yeasts and molds are 10 CFU/mL, for mesophilic aerobic bacteria are 300 CFU/mL, and for coliforms <10 CFU/mL. The majority of bacteria found in juice or in puree come from the epiphytic microbiota of fruits. The numbers of bacteria change depending on seasonal and climatic variation and are usually Gram negative bacteria belong to the *Pseudomonas* genera or to the family *Enterobacteriaceae* (Braide *et al.*, 2012). We mention that in our study, all the analyzes performed indicated the absence of coliform bacteria.

Usually, the number of mesophilic aerobic bacteria is an indicator of shelf-life span and microbial safety of foods. (Pianetti *et al.*, 2008). The results obtained in this study for mesophilic aerobic bacteria are similar to those obtained by Rahman et al., 2011 in fruit juices (the range of 10^2 to 10^5 CFU/mL with the highest of 2.4 x 10^5 CFU/mL) but the recommendation is to pay special attention to the process of obtaining fresh juices because they generally have a microbiological load higher than packaged juices. In general, the microbiota of puree and juice shows a satisfactory microbiological level for such a functional product obtained by athermic processes. (Rahman *et al.*, 2011). The mechanism of microbial inhibition by berry compounds is considered to be an accumulation of direct and indirect

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actions. Direct actions are primarily considered to be the reactions of the phytochemical compounds with the cell membrane causing inactivation of membrane enzyme complexes. The indirect actions often consist in altering the availability/accessibility of nutrients from environment or in complex mechanisms of modulating gene expression. Such mechanisms result in impairment/disruption of microbial metabolism (Gyawali and Ibrahim, 2012; Lacombe and Wu, 2017).

It was observed that CătinoLact variants show a high level of LAB immediately after fermentation (Figure 1) and the variants: V3, V8, V13, V18 (with sea buckthorn juice and *Bifidobacterium* BB12 as probiotic strain) are noticeable. The variants based on sea buckthorn juice and poly-floral honey were the most suitable environment for the multiplication of lactic acid bacteria, and of all starter cultures, the *Bifidobacterium* BB12 strain developed the fastest reaching values of 6.7 x 10^7 CFU/mL (V13). The lowest number of LAB (8.6 x 10^5 CFU/mL) has been registered at the V10 sample (sea buckthorn puree with linden honey and *Lactobacillus johnsonii* La1). The results show a possible correlation with honey type and with phytochemical compounds in the berries skin. The microbiota of CătinoLact bioproduct made with sea buckthorn juice without epicarp it is more developed than in CătinoLact bioproduct made with sea buckthorn puree. It is known from the literature that in sea buckthorn epicarp are inhibitory compounds which prevent the division and the multiplication of microorganisms like zinc-flavonoids complexes (Wei and Guo, 2014) and carotenolipoprotein complexes (Pintea *et al.*, 2001).

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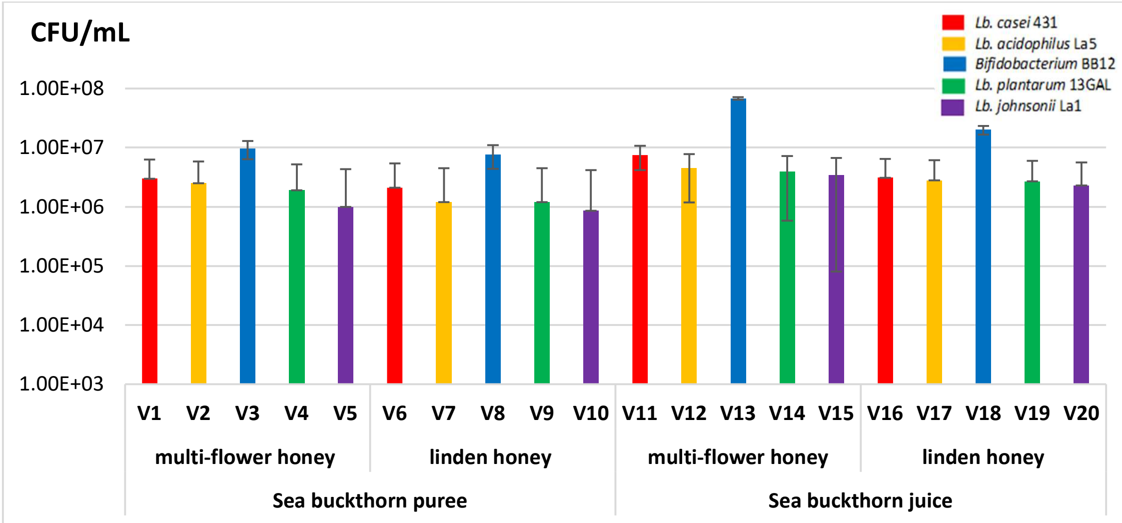

Fig. 1. Quantitative microbiological analysis of LAB in CătinoLact bioproducts (data are the means ± SD)

Braide *et al*., (2012) analyzed the microbiota of fruit juices without the addition of lactic acid bacteria and obtained values of 10^7-10^8 CFU/mL bacteria such as: *Bacillus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Pseudomonas* spp., *Micrococcus* spp. and *Corynebacterium* spp., yeasts and molds like: *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* var. *ellipsoideus*, *Penicillium caseicolum*, *Penicillium notatum*, *Rhizopus stolonifera*.

The variation of the mesophilic aerobic microbiota from the CătinoLact variants can be observed in Figure 2. The highest values are registered in V13 and V18 variants (1 x 10^3 CFU/mL and 7.5 x 10^2 CFU/mL respectively), values that still fall within the maximum level obtained for bacteria by Mihiretie *et al*., (2015) (10^6 CFU/mL), but exceed the standards imposed by national legislation (see the blue line in Figure 2).

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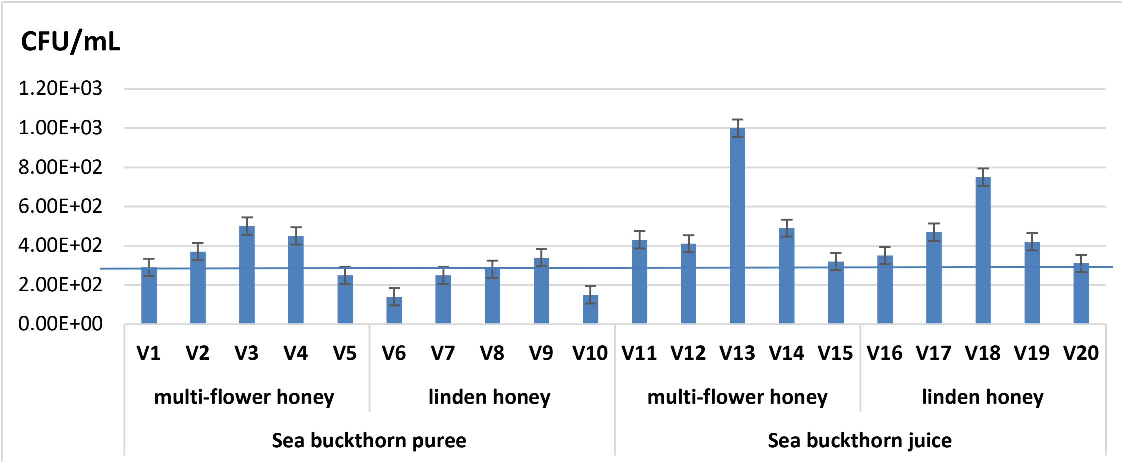

Fig. 2. Quantitative microbiological analysis of aerobic mesophilic bacteria in CătinoLact bioproducts (data are the means ± SD)

As provided by the microbiological standards in force in Romanian legislation (EC Regulation 1441/2007 and of the Order of the Minister of Health no. 27/2011), in case of obtaining unsatisfactory results, measures are required to improve the hygiene of production and selection of raw materials, especially a more rigorous washing of fruits. It can be seen that greatly decreases the total plate count of samples V6 (*L. casei 431*) and V10 (*Lactobacillus johnsonii* La1) at values below 1.5 x 10^2 CFU/mL, which means that these lactic acid bacteria have a strong antimicrobial activity. This antimicrobial activity maybe include the production of hydrogen peroxide, lactic acid, bacteriocin-like molecules or volatile organic compounds (VOCs) (Servin, 2004; Luz *et al*., 2020). The competition for nutrients and adhesion inhibition of pathogens to surface are other mechanisms proposed for this activity (Reid and Burton, 2002). However, the total plate count of CătinoLact bioproducts made with sea buckthorn juice (V11 – V20) without berries skin, it is significantly higher ($p < 0.05$) than in CătinoLact bioproducts made with sea buckthorn puree (V1 – V10), results supported by studies conducted by Criste *et al*., (2020). Many studies have shown experimentally that some compounds in sea buckthorn fruits, some with localization in the pericarp, such as polyphenols, gallic acid, ferulic acid, quercitin, etc. have antimicrobial effect (Cushnie and Lamb, 2006; Borges *et al*., 2013; Wang *et al*., 2018). The variation of the eukaryotic microbiota in CătinoLact products can be interpreted following the graph in Figure 3. Most variants show values around 10^2 CFU/mL of yeasts and moulds. Samples: V1, V3, V6, V8, V11, V13 have values higher than 10^3 CFU/mL. All these results fall within the values obtained by Mihiretie *et al.* (2015) for unpasteurized juices, but are above those imposed by national regulations (EC Regulation 1441/2007 and of the Order of the Minister of Health no. 27/2011). It should be mentioned that among the yeasts, species of the genus *Saccharomyces* are the most common, and the moulds were identified only in the variants with sea buckthorn puree (V1, V3, V6, V8) where they represented below 10 CFU/mL, being species of *Mucor* and *Rhizopus*. We can appreciate that *Bifidobacterium* BB12 and *L. casei* 431 shows a lower antifungal activity.

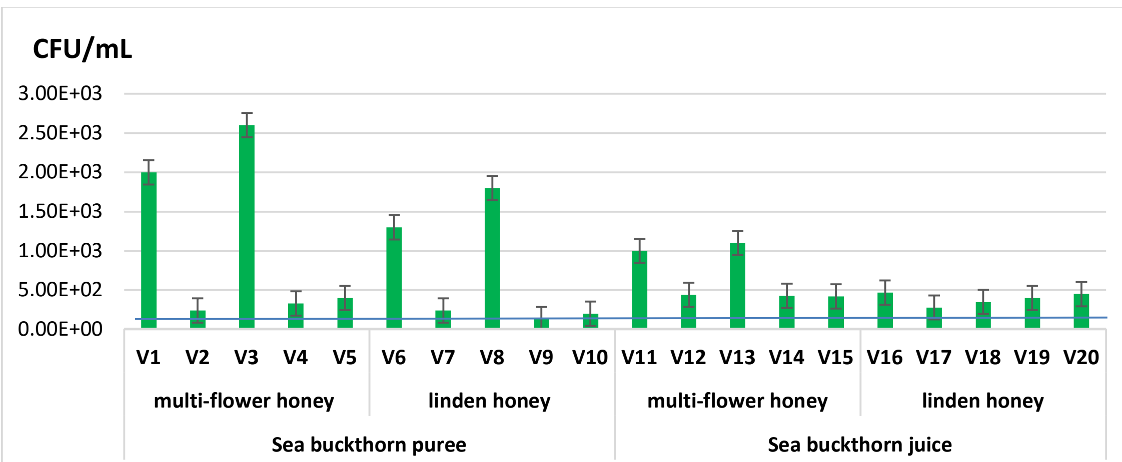

Fig. 3. Quantitative microbiological analysis of yeasts and molds in CătinoLact bioproducts (data are the means ± SD)

The effect of storage on the viability of probiotic bacteria

In the scientific literature, populations of 10^6 - 10^7 CFU/mL in the final product are established as therapeutic quantities of probiotic cultures in processed foods (Talwaker and Kailasapathy, 2004).

The CătinoLact bioproducts were stored refrigerated for 2 weeks at 4°C. Periodically, at a two-day interval, the number of LAB was determined by the indirect cultural method (by

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double-layer cultivation) to assess their viability which gives us valuable information principles to the effect of probiotic drink designed.

From the analysis of figures 4-8, it can be seen that the viability of lactic bacteria is very good in the first 6-8 days of storage. The values of CFU/mL remain in most variants over 10^6. In the second week of refrigeration, CătinoLact bioproduct register a considerable loss of viability of lactic acid bacteria so that they reach values of 10^4 CFU/mL, the loss being two logarithmic units (10^2).

The viable cells count of *L. casei* 431 strain in CătinoLact experimental variants during cold storage is presented in Figure 4. The cell count of *L. casei* 431 remained approximately the same after 6 days of cold storage in all variants (10^6 CFU/mL) respectively 5 x 10^6 CFU/mL in V11 and 2 x 10^6 CFU/mL in V16. This indicated that sea buckthorn juice without compounds with inhibitory effect from sea buckthorn peel, supports the survival in a significant proportion of the *L. casei* 431 strain.

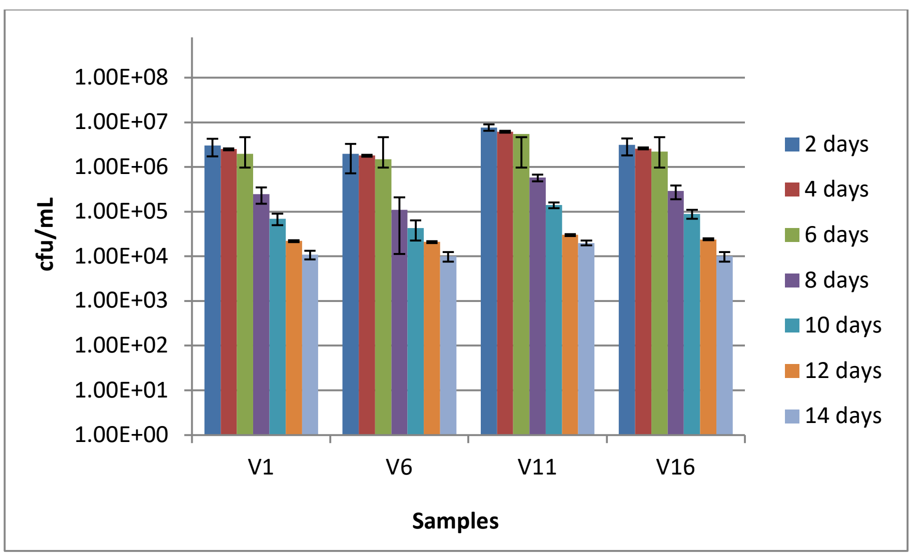

Fig. 4. Viability of *L. casei* 431 in CătinoLact experimental variants during storage at 4°C (data are the means ± SD)

Figure 5 shows the viable count of strain *Lb. acidophilus* La5 in CătinoLact experimental variants during cold storage. The cell count of *Lb. acidophilus* La5 is 2 x 10^6 CFU/mL in V12 after 6 days of cold storage. The similar trend was also observed for the V2 variant (1 x 10^6 CFU/mL).

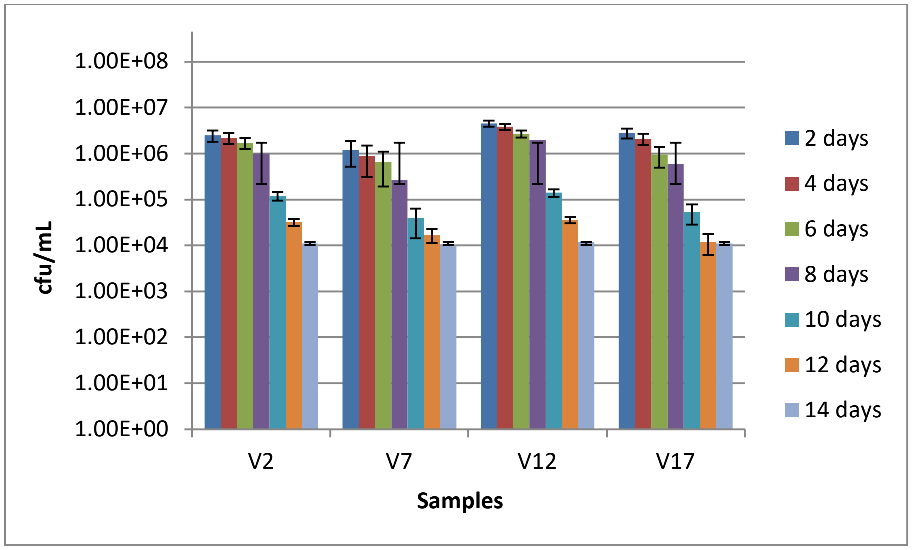

Fig. 5. Viability of *Lb. acidophilus* La5 in CătinoLact experimental variants during storage at 4°C (data are the means ± SD)

The viable count of strain *Bifidobacterium* BB12 in CătinoLact experimental variants during cold storage is presented in Figure 6. CătinoLact bioproduct V13 made with sea buckthorn juice, multi-flower honey and having as a starter the *Bifidobacterium* BB12 strain registered a maximum viability, followed by CătinoLact bioproduct V18. The number of lactic bacteria is still high at the end of the 8 days of cold storage (1 x 10^6 CFU/mL) in V13.

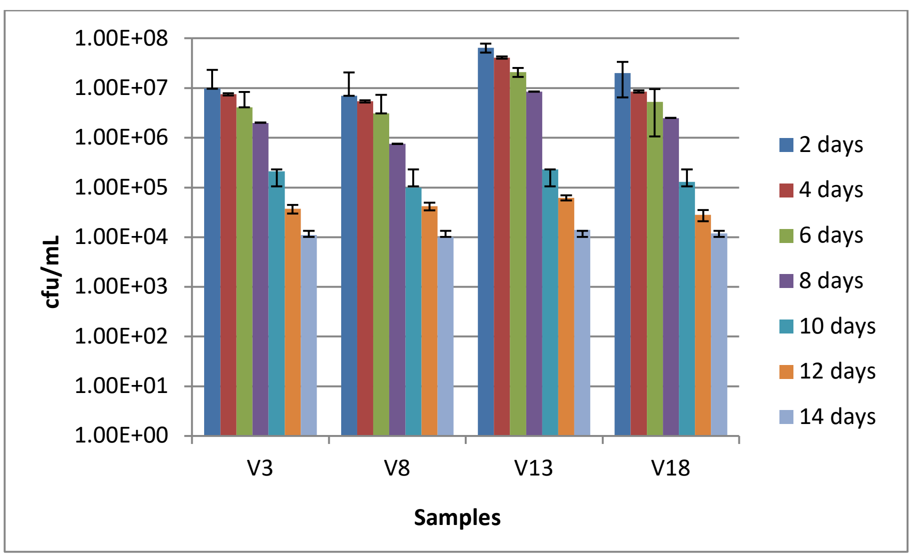

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Fig. 6. Viability of *Bifidobacterium sp.* BB12 in CătinoLact experimental variants during storage at 4°C (data are the means ± SD)

Figure 7 presents a viable count of strain *Lb. plantarum* 13GAL in CătinoLact experimental variants during cold storage. Experimental variants made with sea buckthorn puree V4 and V9 show a decrease in the viability under 10^6 CFU/mL in the first week of cold storage. In contrast, the populations of *Lb. plantarum* 13GAL found in V14 was significantly higher after 6 days of cold storage (1 x 10^6 CFU/mL). This indicated that sea buckthorn puree not supported a good survival of the strain *Lb. plantarum* 13GAL.

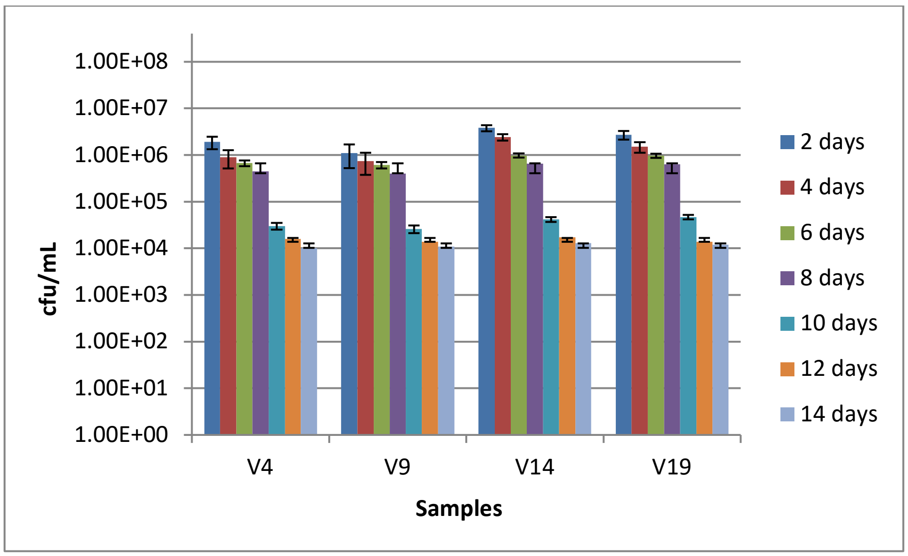

Fig. 7. Viability of *Lb. plantarum* 13GAL in CătinoLact experimental variants during storage at 4°C (data are the means ± SD)

The viable count of strain *Lb johnsoni* La1 in CătinoLact experimental variants during cold storage is presented in Figure 8. Experimental variants made with sea buckthorn puree V5 and V10 show a decrease in the viability under 10^6 CFU/mL in the first week of cold storage.

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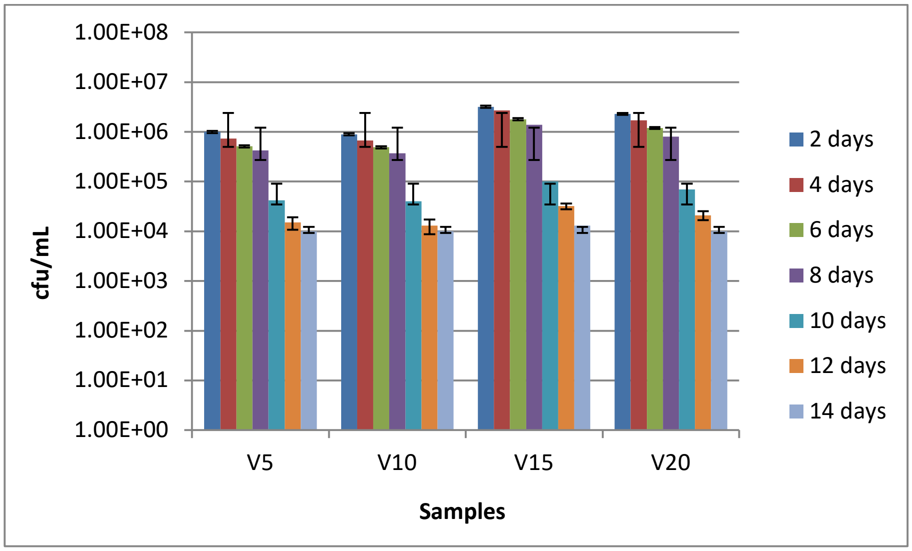

Fig. 8. Viability of *Lb. johnsoni La1* in CătinoLac experimental variants during storage at 4°C (data are the means ± SD)

A higher population of *Lb. plantarum* 13GAL was found in V15 and V 20 after 6 days of cold storage (1 x 10^6 CFU/mL).

As can be seen, the viability of the bacteria was increased in samples made with sea buckthorn juice and multi-flower honey than the samples made with sea buckthorn puree and linden honey. It was possible to preserve a probiotic population around 1 x 10^6 CFU/mL only 7 days of storage at 4°C indicating that CătinoLact bioproduct could be considered a functional product along with its shelf life. Functional shelf life was found to be 8 days only for the V13 variant.

Numerous studies have reported large losses in viability during storage of functional beverages like probiotic fermented drink made of a mixed extract of soy and rice byproducts with added waxy corn starch (Costa *et al*, 2017), cashew apple juice (Pereira *et al*., 2011) or cabbage juice (Yoon *et al*., 2006).

The evolution of the number of microorganisms was analyzed for each sample after incubation and during at the end of storage period. The changes in microbial load (total number of aerobic bacteria and number of yeasts and molds) during the storage period at 4°C was significant ($p < 0.05$) and safe for consumption (Figures 9 and 10). Literature reports the

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bactericide or bacteriostatic effect of raw and processed honey (Chauhan *et al*., 2010). Regarding mesophilic aerobic bacteria, it can be observed (in Figure 9) that after 14 days of refrigeration, all variants register a numerical decrease below the limit of 300 CFU/mL (see blue line), with one exception, variant V4 (sea buckthorn puree, poly-flower honey and *Lb. plantarum* 13GAL where the values decrease significantly (by 22% compared to the initial values shown in Figure 2) and reach 3.5 x 10^2 CFU/mL. We can say that the presence of LAB greatly limits the multiplication and viability of the mesophilic aerobic microbiota. The best antimicrobial activity is manifested by the *Bifidobacterium* BB12 strain in variant V13 because it reduces by 80% (see comparative with Figure 2) the mesophilic aerobic microbiota of the CătinoLact product during storage at 4°C. Lactic acid bacteria produce a variety of metabolic products that are capable of interfering with the growth of other microorganisms. Lactic acid bacteria are important and modern "tool" of biopreservation with advantage as they are considered as GRAS (Zinoviadou *et al*., 2016). These bacterial end products have been applied to food systems to prevent the growth of certain undesirable bacteria (Vandenbergh, 1993).

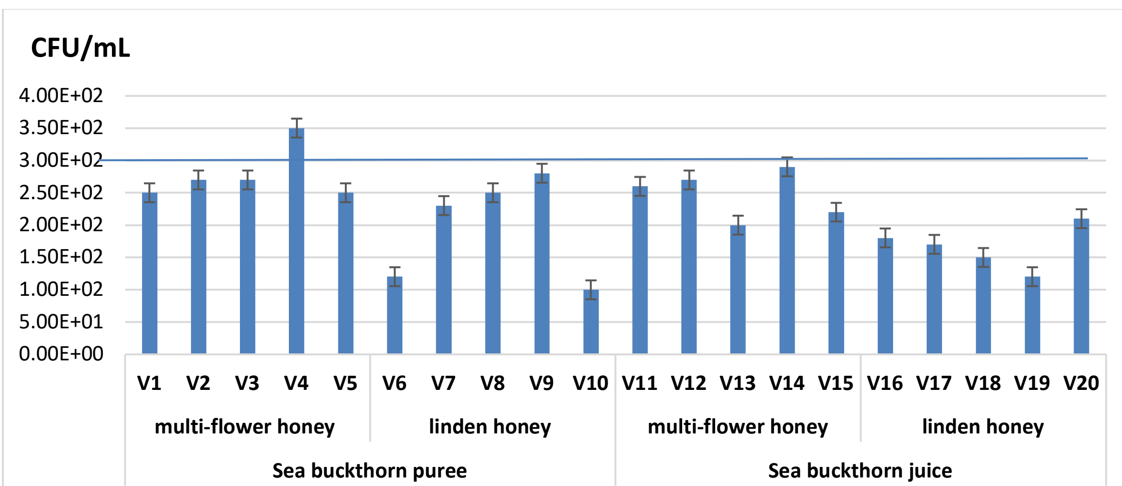

Fig. 9. Quantification of mesophilic aerobic bacteria in CătinoLact variants after 14 days of refrigeration (data are the means ± SD)

The data shown in Figure 10 show that the number of yeasts and molds decreases during refrigeration, on average with a logarithmic unit compared to the initial values shown in Figure 3. The steepest reductions were recorded in the case of samples V1, V3, V6, V8 ($p < 0.05$). The highest values (in variants V12 and V15) are 3.2 x 10^2 CFU/mL (see the Figure 10).

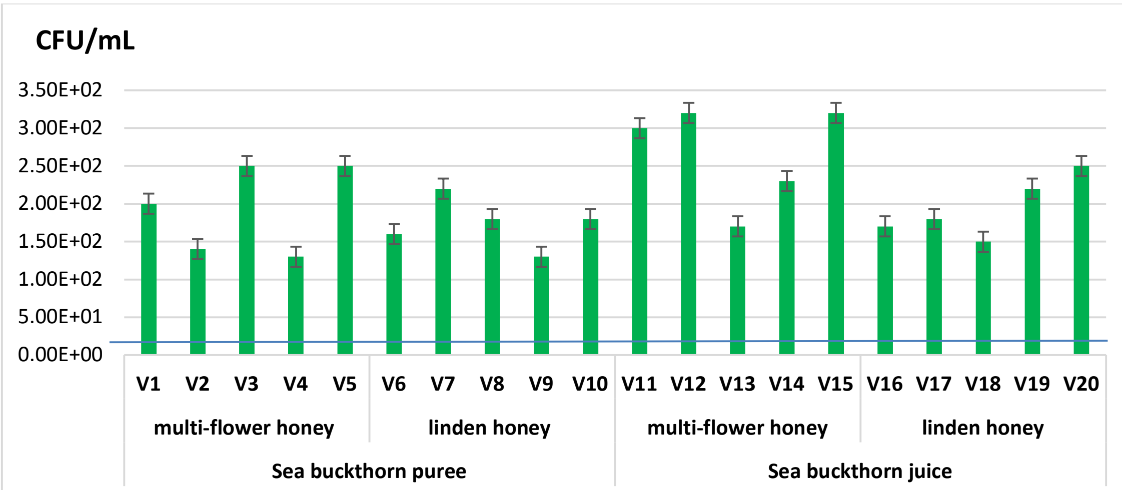

Fig. 10. Quantification of yeasts and molds in CătinoLact variants after 14 days of refrigeration (data are the means ± SD)

So, the use of probiotic starter cultures for the fermentation of sea buckthorn fruit juices is a very good method of bioconservation but it also brings additional benefits to these drinks. Several studies have shown that nonviable probiotics can also have beneficial effects, and this approach can be explained by the production of secondary metabolites (such as B vitamins, bioactive peptides, exopolysaccharides, bacteriocins, and organic acids) during fermentation, mainly by LAB (Vinderola, 2008). Non-viable lactobacilli are equally adherent to intestinal mucus (Ouwehand *et al*., 2000).

Antioxidant activity

Table 2 presents the antioxidant activity obtained using the DPPH method (expressed as mg TE/g DM) for all 20 variants of CatinoLact compared to the control samples of puree and juice (without the addition of honey and lactic acid bacteria). All the evaluated extracts

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Received September 2020 /Accepted February 2021

showed antioxidant potential attributed to hydrophilic compounds: polyphenols, chlorophyll, vitamin C and lipophilic compounds including carotenoids, tocopherols and vitamin E (Sne *et al.*, 2013).

Table 2. Antioxidant activity of CatinoLact variants, evaluated using DPPH method, expressed as mg TE/g DM (mean±SD)

Control	Honey	***Lb. casei* 431**	***Lb.acidophilus* La5**	***Bifidobacterium* BB12**	***Lb. plantarum* 13GAL**	***Lb. johnsonii* La1**
Puree 40.15±0.25	Multi-flower	45.86±0.18	43.40±0.23	47.38±0.30	42.35±0.14	44.67±0.18
	Linden	44.19±0.22	41.89±0.28	45.66±0.31	40.78±0.08	42.88±0.27
Juice 32.62±0.28	Multi-flower	42.05±0.15	37.88±0.30	40.22±0.26	39.56±0.35	38.74±0.18
	Linden	40.74±0.24	37.44±0.21	39.88±0.12	36.79±0.38	36.95±0.34

The results obtained are similar to those published by Criste *et al.*, (2020) which compared four varieties of Romanian sea buckthorn (between 36.61 and 42.25 mg TE/g) and larger compared to those obtained by Sne *et al.*, (2013), from green sea buckthorn berries (23.60 mg TE/DM). The highest activity, was found for V3 variant of Catinolact (from sea buckthorn puree, poly-flower honey and *Bifidobacterium* BB12 strain) – 47.38±0.30 mg TE/gDM. The V1-V10 variants, based on sea buckthorn puree, showed the higher antiradical activity, probably due to the fact that in the puree were found compounds from the whole fruit, both endocarp and pericarp but also from seeds. However, the variants obtained with *Lb. casei* 431 and *Bifidobacterium* BB12 as starter cultures, showed the strongest antioxidant activity, values that correlate with the best viability of the respective strains in the product.

Conclusions

CătinoLact could be served as a functional beverage for vegans, vegetarians and consumers who have allergies to dairy foods. Sea buckthorn juice, without skin-specific inhibitory compounds, together with polyfloral honey were the most suitable substrate for obtaining the CatinoLact functional drink. Of all the probiotic strains of lactic acid bacteria used in the design of the product, the *Lb. casei* 431 and *Bifidobacterium* BB12 strains were noted. *Lb acidophilus* and *Lb. johnsonii* have proven the best antimicrobial activity against the associated microbiota. The CătinoLact bioproduct showed its probiotic effect lasted only 7 days during which a number greater than 1 x 10^6 CFU/mL of live probiotic lactic acid bacteria is found in the refrigerated product. Further studies should evaluate supplementation with components that could improve the viability of probiotic cultures, in order to maintain the probiotic effect for a longer time.

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