STRUČNI RAD / PROFESIONAL PAPER

DEVELOPING THE FORMULATION TO PRODUCE A FERMENTED ALMOND DRINK BY USING DIFFERENT STARTER CULTURES, HYDROCOLLOIDS AND OKARA

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Abstract

This study aimed to investigate the fermentation of almond-based drinks to develop a formulation for an innovative functional product. The almond drink was purchased at the market and fermented by two starter cultures (VEGE 033 LYO and VEGE 053 LYO, IFF Danisco, France). In the preliminary phase of the research, the addition of various hydrocolloids (xanthan gum, guar gum, psyllium, carob seed flour, inulin, starch, and pectin) at concentrations (w/v) 0.5 %, 1 %, and 1.5 % was tested to obtain formulations resulting in acceptable consistency and optimal fermentation time. To improve the experimental drinks' nutritional profile, 1 %, 1.5 %, and 3 % (w/v) addition of rice protein, hemp protein, and okara was also tested. As a result, two formulations containing guar gum and rice protein and the mixture starch/pectin with okara, respectively, were selected for further research, along with the respective control samples. Four fermented formulations were produced and cool stored at 4 °C for 21 days, during which dry matter, ash, acidity, syneresis, water holding capacity, sensory, and microbiological properties were analysed while they were also tested for consumer acceptance. The results showed that adding 1 % okara as a potential dietary fibre reduced syneresis in comparison to all other samples. The formulation with 1 % okara, 1.5 % starch, and pectin received the highest sensory scores and the best acceptance by consumers. Further research is needed to optimize shelf life and nutritional profile of the proposed formulations.

***Keywords**: almond drink, fermentation, vege starter cultures, hydrocolloids, okara*

Introduction

In recent years, an increased trend has been observed among consumers toward a diet based on foods of plant origin such as legumes, cereals, seeds, nuts, fruits, and vegetables. The most common raw materials for the production of plant-based beverages are soy, oats, rice, coconut, and almonds (Sethi et al., 2016). Ethical and health-related aspects, which often lead to the adoption of veganism and vegetarianism, together with the growing ecological awareness and interest in more sustainable food production systems, are considered to be the main reasons why consumers increasingly prefer foods of plant origin. When it comes to plant-based milk alternatives, the most common health problems that drive consumer demand are milk allergy and lactose intolerance, both of which can cause adverse effects in sensitive population groups, including infants, children, and the elderly (Haas et al., 2019; Aydar et al., 2020).

Plant-based beverages are colloidal systems obtained by isolating primarily the fat phase, and sometimes the total dry matter, from plant sources such as soy, oats, coconut, or almonds (Sethi et al., 2016). The isolated fat phase, consisting of triacylglycerols surrounded by phospholipids and proteins, is structurally similar to milk fat (Tzen et al., 1993) and can be dispersed in an appropriate amount of water to form a creamy low-viscosity liquid that mimics cow's milk in sensory characteristics. The aqueous phase of plant-based beverages also contains proteins, minerals, and carbohydrates that contribute to their nutritional value and to a milk-like taste (Queiros et al., 2018).

Because of this composition and functionality, plant-based beverages can be used to improve the nutritional value of various conventional products such as fermented milk, yogurt, ice cream, cheese, chocolate, and butter, or serve as a basis for the development of plant-based alternatives to these products (Yadav et al., 2010).

Fermentation has been recognized as a natural and efficient biotechnological approach for processing numerous foods, and recent research highlights its growing importance in the production of plant-based beverages. It has been shown to contribute to effective proteolysis, increased levels of bioactive compounds, and reduced antinutritional factors, thereby improving the sensory, technological, and functional properties of this product category (Adler et al., 2013). Plant-based fermented beverages are products that mimic the texture and sensory properties of conventional yogurt while maintaining the presence of lactic acid bacteria for extended shelf life. Such products are characterized by a high content of dietary fiber, valuable proteins, and bioactive compounds (Di Cagno et al., 2021).

Among the most consumed plant-based beverages on the market are oat, soy, almond, coconut, and rice drinks. Oat beverages are noted for their high nutritional value and significant content of dietary fiber and phytochemicals (Rasane et al., 2015). Soy beverages are a good source of essential monounsaturated and polyunsaturated fatty acids, high-value proteins (Cohen, 2000), and phytosterols (Fukui et al., 2002). Almond-based beverages are globally produced and consumed as one of the three most popular milk alternatives. Almonds have high nutritional value due to the content of proteins, minerals, and vitamins. The main nutrients present in the almond kernel are globulins and albumins (proteins), RRR-α-tocopherol (a highly bioavailable form of vitamin E), monounsaturated and polyunsaturated fatty acids, arginine, and potassium (Berry et al., 1992). The almond skin, on the other hand, is rich in flavones, isoflavones, anthocyanins, procyanidins, and phenolic acids (Frison-Norrie and Sporns, 2002). During the production of almond beverages, most of these nutrients pass into the aqueous phase, which explains why almond drinks are increasingly used as a raw material to produce yogurt analogues in order to develop a functional, plant-based fermented drink with positive effects on human health. Furthermore, almond beverages can be used as an ingredient in the production of a new generation of fermented dairy products (Topcuoglu and Yilmzan-Ersan, 2020). However, one of the greatest challenges in the production of fermented plant-based beverages is achieving a protein gel network similar to that of yogurt. Plant proteins often have different coagulation mechanisms, and because of the naturally lower protein content in plant-based beverages, it is necessary to add auxiliary substances such as stabilizers and emulsifiers. Such technological processes can be costly and may conflict with the principles of sustainable production, which is becoming a key guideline in the modern food industry.

Therefore, the aim of this study was to investigate the fermentation process of an almond-based plant drink to develop a formulation for an innovative functional product with desirable physicochemical and sensory characteristics. To optimize the flavor and nutritional quality of the almond drink, okara (a by-product remaining after soy drink and tofu production) and rice protein were added, while guar gum, starch, and pectin were tested as possible stabilizers. All samples were analyzed for physicochemical, microbiological, rheological, and sensory properties, and product stability was monitored throughout the intended shelf life.

Materials and methods

Materials

To produce the fermented almond drink, an unsweetened almond beverage (Nutrigold, Zagreb) from controlled organic farming was used, and its nutritional value is presented in Table 1.

Table 1. Nutritional value of Nutrigold almond drink (Zagreb)

Tablica 1. Nutritivna vrijednost bademovog napitka Nutrigold (Zagreb)

Nutrient	Value per 100 mL of beverage
Energy	64.7 kJ / 15.5 kcal
Fat	1.35 g
of which saturated fatty acids	0.1 g
Carbohydrates	0.4 g
of which sugars	0.1 g
Fiber	0.2 g
Protein	0.5 g
Salt	0.1 g

In addition to the almond drink, the following ingredients were used for the preparation of formulations: refined white sugar (Viro, Virovitica), okara (PBF, Zagreb), rice protein (Nutrigold, Zagreb), a commercial starch and pectin blend under the trade name GRIND (Danisco, Denmark), and guar gum.

In preliminary experiments, other stabilizers were also tested, including organic psyllium powder (Nutrigold, Zagreb), hemp protein powder (Nutrigold, Zagreb), inulin (Naturmind, Zagreb), carob seed flour (OPG Goravica, Šipan Island), and xanthan gum (Doves Farm Foods Ltd., Berkshire).

For the fermentation of almond beverages, commercially available lyophilized starter cultures produced by IFF Danisco (France) were used:

- VEGE 033 LYO (Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus), and
- VEGE 053 LYO (Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus delbrueckii subsp. lactis, Bifidobacterium lactis HN019™, Lactobacillus acidophilus NCFM®).

Methods

Preliminary formulations

The purpose of the preliminary experiments was to determine the optimal fermentation time and to identify formulations that would yield a product with yogurt-like consistency and satisfactory sensory acceptability for consumers. Sterilized 1 L glass containers were each filled with 300 mL of almond drink. Depending on the test formulation, the following thickening agents were added at concentrations of 0.5 %, 1 %, and 1.5 % (w/v): xanthan gum, psyllium, carob seed flour, inulin, and a starch–pectin blend (GRIND). To enrich the final product nutritionally, rice protein, okara, and hemp protein were added at concentrations of 1 %, 1.5 %, and 3 % (w/v). Sucrose was used as a sweetener at a concentration of 3 % (w/v). All ingredients were homogenized thoroughly using an immersion blender and pasteurized at 73 °C for 15 s with continuous stirring on a hot plate to prevent scorching at the bottom of the vessel. Samples were then cooled to 43 °C and inoculated with the appropriate amount of starter culture (VEGE 053 LYO 0.0129 g per 1 L or VEGE 033 LYO 0.0117 g per 1 L), followed by incubation at 42 °C. Samples were taken at 1–2 h intervals to monitor fermentation progress. The end of fermentation was defined by a pH $\leq$ 4.65.

Fermentation was stopped by rapid cooling of the samples in cold water, after which they were stored in a refrigerator at +4 °C. All preliminary samples were subjected to sensory evaluation by a trained panel. Once the most acceptable formulations of fermented almond drink were identified, they were reproduced and analysed by physicochemical methods to characterize the final products, as described in next Section.

Production and characterization of fermented almond drinks with the addition of GRIND/guar gum and okara/rice protein

For this phase of the study, selected formulations of fermented almond beverages were prepared as shown in Table 2.

Table 2. Formulations of fermented almond drinks

Tablica 2. Recepture fermentiranih bademovih napitaka

Sample	Formulation
K1-33	Control sample 1 – Fermented almond drink without additives, inoculated with starter culture VEGE 033
K2-53	Control sample 2 – Fermented almond drink without additives, inoculated with starter culture VEGE 053
A	Fermented almond drink with 0.5 % (w/v) guar gum, 3 % (w/v) sucrose, and 1 % (w/v) rice protein, inoculated with VEGE 053
B	Fermented almond drink with 1.5 % (w/v) GRIND (starch–pectin mixture), 3 % (w/v) sucrose, and 1 % (w/v) okara, inoculated with VEGE 033

Samples were produced as described in Section *Preliminary formulations*. During fermentation, the pH was measured every 1–2 hours to determine the endpoint, and fermentation was stopped by rapid cooling. After cooling, the samples were stored at +4 °C for 21 days, with analyses performed at 7-day intervals.

For all samples, acidity was measured using a Multi 340i pH-meter (WTW, Wellheim, Germany). Syneresis (S, %) and water-holding capacity (WHC, %) were determined by centrifuging 20 g of sample at 5000 rpm and 4 °C for 10 min, following the method of Feng et al. (2018) as described by Barukčić et al. (2022).

To evaluate the microbiological stability of the produced samples, plate inoculation methods were used to determine the presence of yeasts and

moulds, lactic acid bacteria, Enterobacteriaceae, and the total viable bacterial count, according to Božanić et al. (2010).
Sensory evaluation was carried out by a trained panel using a weighted-score method. The evaluated attributes were appearance, colour, taste, odour, and consistency. Each property was rated on a 1-to-5 scale, and average scores were multiplied by their significance factors (Fv) to obtain weighted points. The maximum total score attainable per sample was 20.
In addition, consumer acceptance testing was performed with untrained potential consumers belonging to the target population for the product. The Peryam hedonic scale was used, offering nine response options. This method is simple to apply and provides extensive possibilities for statistical interpretation of results (Tratnik and Božanić, 2012).
All obtained results were processed using Microsoft Excel 2007 and expressed as mean values. One-way analysis of variance (ANOVA) was applied to analyse consumer acceptance data.

Results and Discussion

Acidity, Syneresis and Water-Holding Capacity

The percentage of lactose conversion to lactic acid is one of the most important technological parameters in the production of fermented milk beverages. Accordingly, in the production of their analogues this parameter is also important because it affects the incubation time and product storage. The acidity of fermented beverages was determined by measuring pH values during 21 days of storage of samples in a refrigerator at 4 °C, as shown in Figure 1.
After the 7th day of fermentation, a slight increase in pH values was noticeable in all samples, except for the control sample K1-33, which showed the opposite trend. During the 14th day of storage, a slight decrease in pH values could be observed, which can be attributed to subsequent acidification caused by the activity of the starter cultures VEGE-033 and VEGE-053 during sample storage. Subsequent acidification is considered an undesirable process in fermented beverages because it shortens shelf life and can cause some defects including excessive acidity and syneresis (Deshwal et al., 2021). The pH values of the control samples during 21 days of storage ranged between 4.96 and 5.11, which can be attributed to the fact that a pH of 4.6 was not achieved during fermentation of the control samples, most likely due to an insufficient carbon source required by microorganisms for further growth. The pH values of samples A and B during the 21 days of storage were almost identical, with a mean value of 4.55, and they did not show significant variations; they remained stable throughout the entire storage period. Sample A showed slightly lower pH values ranging from 4.3 to 4.5, which is in accordance with the results of Bernat et al. (2015), where pH values of around 4.6 were reported for a probiotic almond beverage. In sample B a slight increase in pH values was visible, and

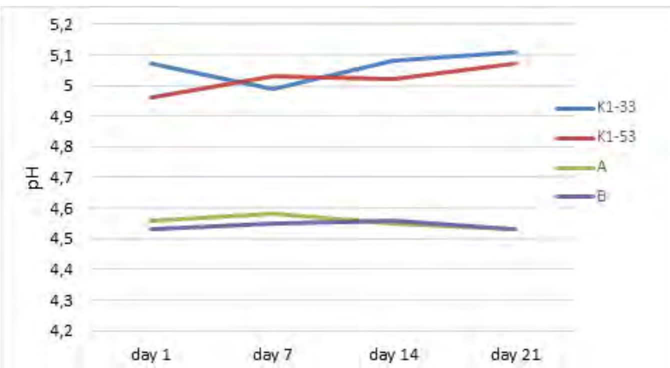

Figure 1. *Acidity (pH) of the produced fermented almond drink samples (K1-33, K1-53, A, B) during 21 days of cold storage*

Slika 1. *Kiselost (pH) proizvedenih fermentiranih bademovih napitaka (K1-33, K1-53, A, B) tijekom 21 dan hladnog skladištenja*

with further storage the pH remained in the range 4.5 to 4.6. Similar results were obtained by Mudgil et al. (2017), who produced yogurts with the addition of partially hydrolysed guar gum.

Syneresis and water-holding capacity were determined during 21 days of cold storage of samples, and the results are shown in Figure 2.
Acid formed during fermentation causes a decrease in pH, which leads to changes in protein charge and consequently to protein coagulation. If the coagula have low structural stability, this leads to increased syneresis, during which liquid (whey in the case of dairy products) separates and is expelled from the gel (Garcia-Perez et al., 2005). From the consumer's point of view, the occurrence of syneresis impairs product quality; therefore, syneresis is one of the important indicators of the quality of fermented dairy products and their analogues that must be considered during production. Stabilizers such as starch, pectin and guar gum are therefore used to stabilize the structure and viscosity of fermented beverages. During storage of the samples produced in this study, a significant increase in syneresis could be observed in all samples (Figure 2a). Control samples K1-33 and K1-53 showed high percentages of syneresis immediately after fermentation and during the storage period. Sample A showed a somewhat lower percentage of syneresis on the first day after fermentation; however, during further storage it showed a considerably higher percentage of syneresis compared with sample B (Figure 2a). Similar results were obtained by Zhao et al. (2021), where the addition of rice protein to a fermented soy beverage also resulted in pronounced syneresis. Compared with sample A, sample B, which contained the starch-pectin mixture, showed a lower percentage of syneresis (Figure 2a), which is consistent with the results obtained in the study conducted by Grasso et al. (2020) on a fermented coconut beverage where starch and pectin were also used as thickening and gelling agents.

In accordance with the syneresis results (Figure 2a), the water-holding capacity (WHC) decreased during storage (Figure 2b). The WHC values ranged between 1.36 % (K1-33) and 100 % (A). Sample B initially had the highest WHC (42.75 %) and this trend was maintained throughout the entire storage period. The control samples had a low ability to bind water, which is most likely the result of the lack of stabilizers or other ingredients that could bind water and contribute to a stable consistency. Garcia and Perez (2005) concluded that yogurt with the highest proportion of added fibres has the greatest water-binding capacity. Accordingly, sample B (45.55 %), with the addition of okara as a rich source of dietary fibre, had the highest water-binding capacity among all samples.

Microbiological and Sensory Quality of the Produced Fermented Beverages

The number of microorganisms was determined by counting the grown colonies of fermented almond drink inoculated on appropriate nutrient media, and the presence of yeasts and moulds, the total number of bacteria, lactic acid bacteria, and Enterobacteriaceae was determined. The total number of bacteria ranged from 1.79 to 5.7 log CFU mL^{-1}, the number of Enterobacteriaceae from 0.69 to 4.35 log CFU mL^{-1}, while the values for the number of yeasts and moulds in fermented almond drinks ranged from 0 to 3.66 log values (Table 1). Yeasts and moulds are characterized by a good ability to survive in an acidic medium and at low temperatures and therefore can be the cause of spoilage in yogurt and other fermented beverages (Božanić et al., 2010). However, according to the obtained results (Table 1), it is evident that the values of these types of microorganisms decreased or were not detected at all during the storage period, which may indicate unfavourable conditions in fermented almond drinks such as low concentrations of utilizable sugars, fats, and proteins that would be necessary for the metabolic activity of yeasts and moulds. On the other hand, somewhat higher initial values of yeasts, moulds, and Enterobacteriaceae and of the total number of aerobic

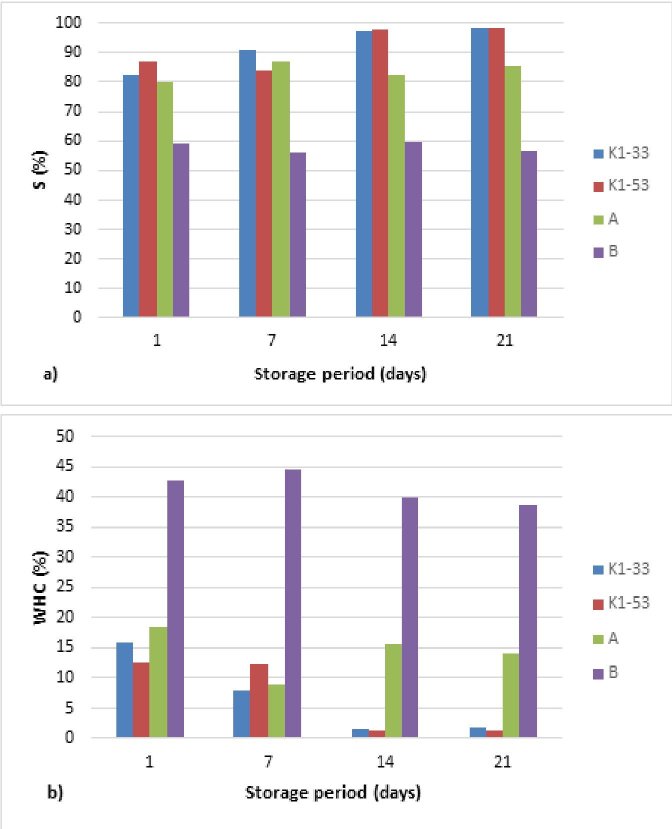

Figure 2. a) *Syneresis (S, %) and* ***b)*** *water-holding capacity (WHC, %) of the produced fermented almond drink samples (K1-33, K1-53, A, B) during 21 days of cold storage*

Slika 2. a) *Sinereza (S, %) i* ***b)*** *kapacitet zadržavanja vode (WHC, %) proizvedenih fermentiranih bademovih napitaka (K1-33, K1-53, A, B) tijekom 21 dan hladnog skladištenja*

mesophilic bacteria (Table 1) can be associated with an inadequate pasteurization regime carried out at the minimum temperature and holding time (72 °C / 15 s). During 21 days of sample storage, a decrease in the survival of lactic acid bacteria was observed in almost all samples, which may be the result of a lack of sugar, the main carbon source for the further growth of microorganisms. Comparing the 1st and 21st day of cold storage of samples, the survival of lactic acid bacteria averaged 70–80 %. The best survival of lactic acid bacteria was shown by sample B with the addition of 1 % okara, 3 % sucrose, and 1.5 % starch–pectin mixture, which can be attributed to okara as a rich source of dietary fibre, i.e., prebiotics that serve as food for microorganisms, while sample K1-33, without additives, had the lowest survival of lactic acid bacteria (Table 1). Sensory analysis was carried out by five panellists who evaluated the appearance, colour, consistency, smell, and taste of the produced fermented almond drinks. The results of sensory evaluation of fermented drink samples for the 1st and 21st day of cold storage are shown in Figure 3, where it is evident that the highest-rated samples were A and B, which were enriched with sources of protein, fibre, and stabilizers. The panellists gave similar ratings for smell to all samples, but the control samples generally received lower scores.

During cold storage, the consistency ratings of the products were generally maximal for samples that contained additives, while the samples without additives showed the presence of lumps, which is why they received lower consistency ratings compared with the samples with additives. The panellists concluded that the samples with additives (A and B) had better consistency, with sample B, enriched with okara and the starch–pectin mixture, being the best rated. Among the most important parameters is taste, since it is the decisive factor in consumers' decisions whether to buy a product. Because the results of microbiological analysis

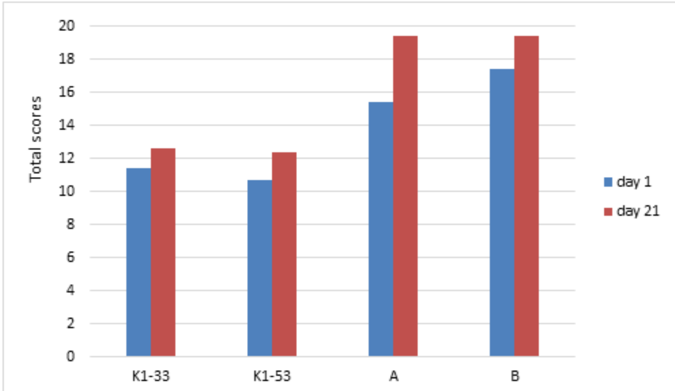

Figure 3. *Total pondered scores of sensory evaluation of samples (K1-33 , K1-53, E, F) on the 1st and 21st day of cold storage*

Slika 3. *Ukupni ponderirani bodovi senzorske ocjene uzoraka (K1-33, K1-53, E, F) 1. i 21. dan hladnog skladištenja*

showed borderline values of Enterobacteriaceae on the 1st day of cold storage, the panellists evaluated the taste attribute only on the 1st and 21st day of cold storage. The highest taste score was given to sample B, while the lowest scores were given to the control samples without additives. The panellists commented that the low scores of fermented almond drinks without additives were due to the bland taste of the beverages. Considering all previous results, the highest overall rating on both the 1st and 21st day of storage was obtained by beverage sample B with the addition of 3 % sucrose, 1 % okara, and 1.5 % starch–pectin mixture (Figure 3). Consumer acceptability determination was carried out by testing 47 participants of various ages (20–60 years) who evaluated all four samples of fermented almond drink. The composition and distribution of ratings using the hedonic scale for fermented almond drinks of different formulations are shown in Table 3. The consumer ratings were summarized, and the mean value (x), standard deviation (s), coefficient of variability (Cv), and percentage of desirability were calculated and are also presented in Table 3.

Table 3 shows the results for the desirability of the evaluated samples of fermented almond drinks, where it is evident that the mean value of the samples ranges from 4.40 (sample K1-33) to 5.66 (sample B), and the calculated percentage of desirability ranges from 46.81 % (sample K1-33) to 70.21 % (sample B). The best ratings and desirability percentage were obtained by the fermented almond drink with the addition of sucrose, starch–pectin mixture, and okara (sample B). Sample A received a significantly lower rating than sample B (x = 4.34), which can be attributed to the different product formulation. The control samples received significantly lower ratings (approximately 4.40) than samples A and B, which can be attributed to the fact that no sweeteners or stabilizers were added to the samples. The least desirable product was sample K1-53 (P = 44.68 %), which is a fermented almond drink with VEGE culture 053 without any additives. The overall acceptability of the tested products is low, since all samples have a mean value lower than 7.5. The main shortcomings reported by respondents were a lack of sweetness or acidity. The low acceptability of yogurt analogues can be attributed to the discrepancy between consumer expectations that are primarily based on experience with dairy products, and the actual sensory perception of fermented plant-based drinks, which is consistent with various studies on the acceptability of plant-based fermented beverages.

Table 3. Evaluation of the acceptability of fermented almond drinks by consumers using the hedonic scale

Tablica 3. Prihvatljivost fermetiranih bademovih napitaka među potrošačima primjenom hedonističke skale

Score	Sample			
	K1-33	K1-53	E	F
9	2	3	2	5
8	2	0	0	7
7	4	8	7	9
6	2	5	5	6
5	12	5	9	6
4	8	5	5	3
3	9	12	5	5
2	6	7	13	3
1	2	2	1	3
∑*	47	47	47	47
x*	4.40	4.47	4.34	5.66
s*	1.98	2.19	2.10	2.39
Cv*	45.08	48.91	48.35	42.21
desirability	46.81 %	44.68 %	48.94 %	70.21 %

Conclusion

The results of this research show that among all tested formulations, the highest water-holding capacity was achieved in the sample with the addition of 1 % okara, 1 % starch–pectin mixture, and 3 % sucrose (45.55 %), while the lowest was in the fermented almond drink without additives (1.36 %). Syneresis was highest in the control samples (98.50 %), while it was lowest in the sample with the addition of 1 % okara, 1 % starch–pectin mixture, and 3 % sucrose. The best survival of lactic acid bacteria was observed in the sample with the addition of 1 % okara, 1 % starch–pectin mixture, and 3 % sucrose, which can be attributed to okara as a source of dietary fibre. According to sensory analysis, the highest overall score was obtained by the samples with the addition of 1 % okara, 1 % starch–pectin mixture, and 3 % sucrose, as well as by the samples with the addition of 1 % rice protein, 0.5 % guar gum, and 3 % sucrose. Overall, considering the results of all conducted analyses, the fermented almond drink enriched with okara, sucrose, and starch and pectin as stabilizers was well accepted by consumers and shows the greatest potential for further optimization of the final product formulation.

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