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ORIGINAL RESEARCH PAPER

THE EFFECT OF AMARANTH SEEDS ON THE CHEMICAL, MICROBIOLOGICAL AND SENSORY QUALITY OF YOGURT

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Abstract: The objective of this study was to investigate the chemical, microbiological, and sensory characteristics of yogurt enriched with amaranth seeds, stored at a temperature range of 5 ± 1 °C for 21 days. The quantity of amaranth incorporated into the yogurt was 5 % and 10 %. The changes were regularly observed and recorded every 7 days. Statistically significant results ($p \leq 0.05$) show that as storage time increases, *p*H values decrease, and acidity increases. The fat content remains consistent regardless of the amount of amaranth stored. The inclusion of amaranth seeds in yogurt resulted in a statistically significant increase ($p \leq 0.05$) in the protein content of the final product. All yogurt samples demonstrate the absence of Enterobacteriaceae, yeast and molds, Coagulase-positive staphylococci, *Salmonella* spp. and *Listeria monocytogenes* after 21 days of storage, ensuring product safety. Sensory quality was evaluated based on color, taste, aftertaste, texture, and overall acceptability at 1, 7, 14, and 21 days. The results indicate that the sensory quality of yogurt decreases as the concentration of amaranth seeds increases. Except for the control sample (without amaranth seeds), yogurt with 5 % amaranth seeds exhibited superior sensory quality compared to yogurt with 10 % amaranth seeds.

Keywords: *amaranth, functional food, storage, quality, yogurt*

INTRODUCTION

New technological achievements in the food industry cause rapid development and production of minimally processed food, which should be available to every consumer [1]. Today, there is an increasing interest in the consumption of pseudocereal crops such as amaranth and quinoa, which are also called super foods [2, 3]. Increased recognition of the importance of the gut microbiota in maintaining general well-being is driving research into the role of functional food, specifically prebiotics and probiotics. Food products, that offer more health benefits than basic nutrition, are called functional foods. Typical examples of functional foods are prebiotic products (fibers that promote the growth of probiotics) enriched with omega-3 and foods rich in antioxidants [4].
Amaranth is from the *Amaranthaceae* family and is mostly grown in subtropical and tropical regions. Due to its good nutritional composition, this plant is consumed as a vegetable, and its seeds are used as cereals [5]. Amaranth grain mainly consists of about 61.3 - 76.5 % carbohydrates (mostly starch), 13.1 - 21.5 % crude protein, 5.6 - 10.9 % crude fat, 2.7 - 5 % crude fiber and 2.5 - 4.4 % ash [6]. A high concentration of high-quality proteins is 13 - 18 %, more than the three most important basic cereals (rice, wheat and corn) [7].
On the other hand, yogurt is a widely consumed dairy product, which is used globally. It is produced by fermenting fresh or reconstituted milk with lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*). This product is favored by consumers due to its beneficial effects on the intestinal microflora and strengthening the body's immunity [8, 9]. Yogurt is mostly prepared through conventional methods, but recent scientific research has enabled the modification of technologies and processes, especially when yogurt is enriched with other food products and/or nutrients [10, 11]. Today in scientific literature you can find papers on the enrichment of yogurt with various by-products from fruits and vegetables such as blackcurrant pomace [12], pineapple pomace powder (freeze-dried) [13], blackberry pomace [14], olive pomace [15], hydrocolloids [16]. On the other hand, no scientific papers were found regarding the enrichment of yogurt with pseudocereals such as amaranth seeds.
The aim of this paper is to determine the influence of amaranth seeds added in amounts 5 % and 10 % on the chemical, microbiological and sensory characteristics of yogurt for a period of 21 days.

MATERIALS AND METHODS

Materials

Pasteurized cow's milk with the following composition was used to produce yogurt with different amounts of amaranth; lactose 4.30 ± 0.02 %; SNF (solids-not-fat) 11.25 ± 0.31 %, proteins 3.30 ± 0.05 % and acidity 14.00 ± 0.05 °T. Freeze - dried symbiotic starter culture (LBB BY 144-12, LB Bulgaricum LTD) composed of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* was used for yogurt production.

Methods

Production of yogurt with different quantities of amaranth seeds

Production was done at LB Bulgaricum LTD, Production base of Sofia, Bulgaria. Pasteurized cow's milk (84 ± 1 °C, 40 min) in three different batches of 10 L each was used to produce yogurt. After pasteurization, the milk was cooled to 43 °C and bio-herbal honey was added to it. After dissolving the honey in the milk during constant mixing (60 s at 1200 rpm) the starter culture (LBB BY 144-12, LB Bulgaricum LTD) was added for direct inoculation. Previously 5 g/100g and 10 g/100g amaranth seeds were measured in plastic containers. After adding the milk to the containers with amaranth seeds, the contents of the containers were mixed and incubated (Memmert GmbH, Germany) at a constant temperature (43 ± 1 °C). After achieving *p*H 4.6 ± 0.1 (240 min) the yogurt with and without amaranth seeds was stored in a refrigerator (4 °C) for 24 hours. The determination of the chemical, microbiological and sensory quality of the yogurt was determined on 1st, 7th, 14th and 21st day).

Changes during storage of yogurt

The changes in yogurt containing different concentrations of amaranth seeds were evaluated during storage through standard physicochemical analysis methods, including: *p*H value (*p*H meter Testo SE & Co. KGaA, Lenzkirch, Germany), titratable acidity [17] and proteins [18]. Additionally, the following microbiological parameters were analyzed: enumeration of *Lactobacillus* spp. and *Streptococcus* spp. [19], *Enterobacteriaceae* [20], yeast and molds [21], coagulase-positive staphylococci [22], *Salmonella* spp. [23], *Listeria monocytogenes* and *Listeria* spp. [24], enumeration of characteristic microorganisms [25]. Sensory evaluations of the yogurt by 20 panelists were made on a 9-point hedonic scale.

Statistical analysis

Analysis of variance (ANOVA) and least significant differences (Fisher LSD test) at $p \leq 0.05$ were performed using XLSTAT 2019 and Microsoft Office Excel 2016 software.

RESULTS AND DISCUSSION

The strategy of enriching food products with nutritionally rich raw materials helps in improving the lifestyle, consuming a nutritionally balanced diet compared to ordinary food products. Fortification of food products is also considered one of the most effective measures to prevent micronutrient deficiencies in developed countries [26]. Figure 1 shows the *p*H values of yogurt with 0 % (control), 5 % and 10 % amaranth seeds stored at 4 °C for 21 days.

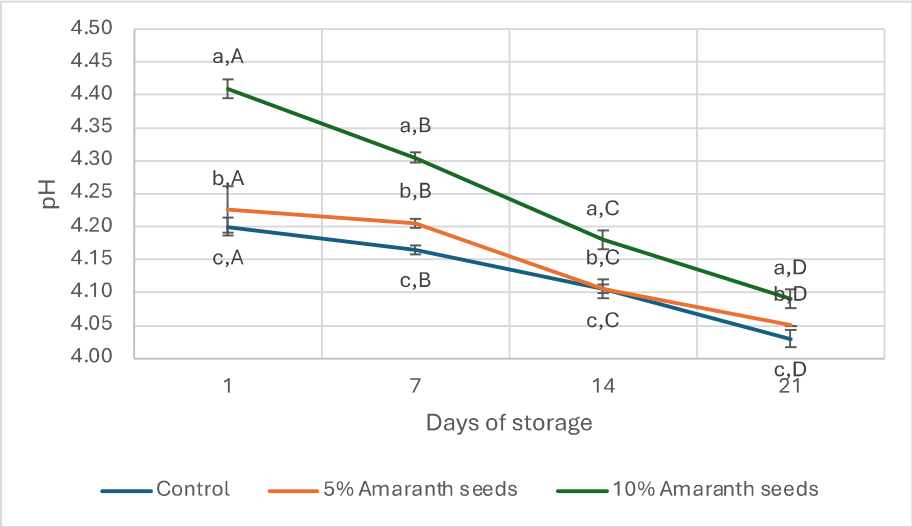

Figure 1. *pH variation during storage of yogurt fortified with different percentages of amaranth*

Note: Small letters indicate statistically significant differences ($p < 0.05$) between the samples with different quantities of amaranth, while capital letters indicate statistically significant differences ($p < 0.05$) between storage days

The results from Figure 1 show that after the storage of the yogurt with different amount of amaranth seeds there is a decrease in the *p*H value with the extension of the storage time ($p < 0.05$). Yogurt is a popular fermented milk product produced by lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus)*. During production, these bacteria produce lactic acid and thereby reduce the *p*H value of the product itself [27]. Yogurt incubation was terminated when *p*H 4.6 was reached. Twenty-four hours after production it is observed that the *p*H value of the yogurt with 5% and 10 % amaranth seeds decreases the *p*H value more slowly compared to the *p*H value of the yogurt without amaranth seeds. A similar trend of decreasing the *p*H value of yogurt was also observed by Shunekeyeva *et al.* [28] who used amaranth flour to enrich yogurt. One of the reasons for decreasing the *p*H value of yogurt can be the use of residual carbohydrates by living microorganisms and the production of lactic acid. Other reason for decreasing of *p*H value is that the antimicrobial properties of amaranth seeds may account for the slower decline in *p*H observed compared to the control sample. Amaranth seeds are a rich source of bioactive compounds, including saponins, phenolics, and flavonoids, which possess well-documented antimicrobial activity. These bioactive compounds can inhibit the proliferation of pathogenic and spoilage microorganisms, potentially moderating the fermentation process by suppressing the metabolic activity of lactic acid bacteria responsible for lactic acid production and subsequent *p*H reduction [29 – 31].

Amaranth contains polysaccharides and proteins that may interact with lactic acid bacteria during fermentation, potentially accelerating the production of lactic acid. This increase in lactic acid production tends to lower the pH more quickly, resulting in a slightly more acidic yogurt [32]. Amaranth, rich in minerals and bioactive compounds, may also contribute to the buffering capacity of yogurt, which might mitigate some of the *p*H changes over storage time. Shunekeyeva *et al.* [28] reported that yogurt with higher levels of amaranth flour exhibited a slower *p*H decline over time compared to plain yogurt. This

buffering effect can be attributed to the proteins and minerals in amaranth, which interact with the lactic acid produced, thus stabilizing the *p*H to a certain extent. The introduction of amaranth may also impact the dynamics of microbial fermentation. According to Habib *et al.* [33], yogurts with amaranth content saw more robust growth of *Lactobacillus* species, resulting in faster acidification compared to control samples without amaranth. Higher concentrations, however, may slow fermentation, likely due to increased viscosity or other factors affecting microbial activity.

The titration acidity increases statistically significantly ($p < 0.05$) with the addition of amaranth seeds to the yogurt and with the extension of the storage time of the yogurt (Figure 2).

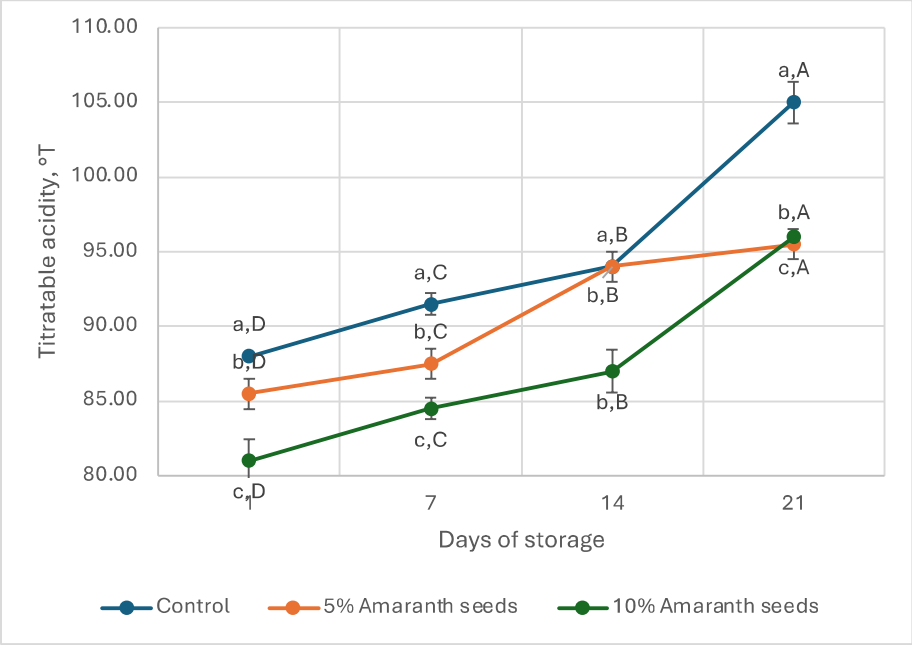

***Figure 2**. Titratable acidity during storage of yogurt fortified with different percentages of amaranth*

Note: Small letters indicate statistically significant differences ($p < 0.05$) between the samples with different quantities of amaranth, while capital letters indicate statistically significant differences ($p < 0.05$) between storage days

The increase in the titration acidity of the yogurt without amaranth seeds is by 19 %, and in the yogurt with 5 % and 10 % amaranth seeds it increases by 11 % and 19 % respectively, during extended storage up to 21 days. The results obtained by Shunekeyeva *et al.* [28] also show an increase in titration acidity with the addition of amaranth flour. The main reason for the increase in titration acidity can be related to the fermentation of yogurt. This process continues during the storage of the yogurt and there is an accumulation of lactic and other organic acids (citric, acetic, formic and acetaldehyde) [34, 35]. In a study by Shleikin *et al.* [36], yogurts enriched with 0, 1, 2 and 3 % amaranth showed an increase in TA in proportion to the amount of amaranth added. The 3 % amaranth yogurt had the highest TA compared to the control, attributed to more substrates for bacterial growth provided by the additional nutrients in amaranth. Amaranth contains

bioavailable carbohydrates and proteins that can stimulate lactic acid bacteria, increasing fermentation efficiency and thus titratable acidity. The buffering properties of amaranth may stabilize the acidity over time, even though higher initial TA levels are observed. Yangilar found that yogurt samples with 3 % amaranth maintained a more stable TA during storage than the control [37]. This effect may be due to proteins and minerals in amaranth, which interact with lactic acid, moderating further increases in acidity as yogurt ages.

The content of protein in yogurt with and without amaranth seeds is shown in Figure 3.

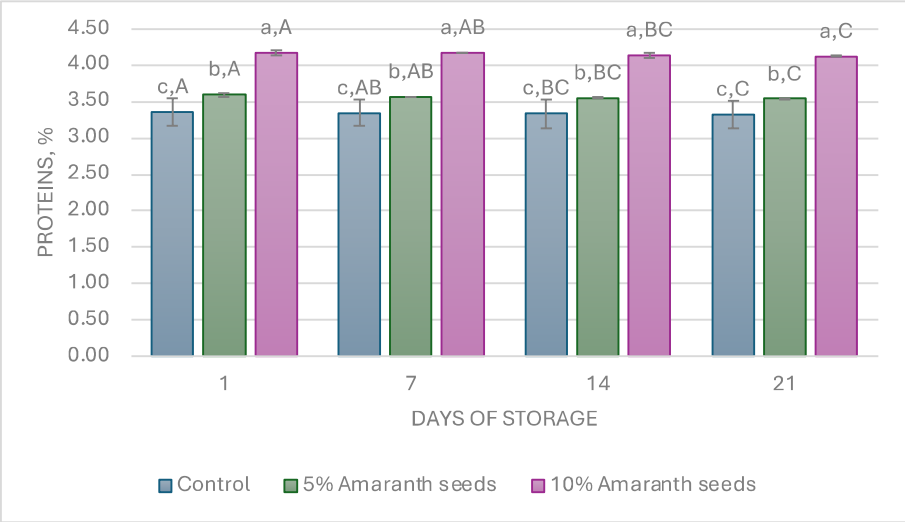

Figure 3. *Total proteins in yogurt fortified with different percentages of amaranth*
Note: Small letters indicate statistically significant differences ($p < 0.05$) between the samples with different quantities of amaranth, while capital letters indicate statistically significant differences ($p < 0.05$) between storage days

Amaranth contains about 13 - 15 % protein, including essential amino acids like lysine, which is typically lower in dairy proteins. Studies show that adding amaranth to yogurt can significantly enhance its protein content [38]. According to Beswa *et al.* [39], yogurts with added amaranth flour exhibited improved protein quality and digestibility scores, especially in samples containing 5 % or more amaranth. The authors noted that amaranth provided additional essential amino acids, enhancing the biological value of yogurt proteins. Higher protein content from amaranth addition also affects yogurt texture, contributing to a thicker and creamier consistency. Zhang *et al.* [40] observed that yogurt samples with amaranth showed better gel structure and viscosity due to the interaction between amaranth proteins and dairy proteins, which created a more cohesive protein network in the yogurt.

Figure 4 shows the change in the content of *L. bulgaricus* and *S. thermophilus* during the storage of yogurt with and without amaranth seeds for 21 days.

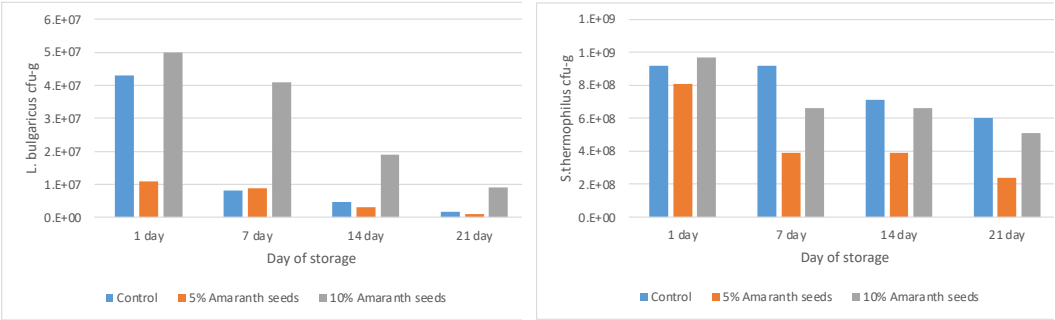

Figure 4. *L. bulgaricus and S. thermophilus during the storage of yogurt*

From the results presented, it can be observed that the content of *L. bulgaricus* and *S. thermophilus* decreases with the extension of the storage time. The reduction of *L. bulgaricus* is 96, 90 and 82 %, respectively, for yogurt with 0 %, 5 % and 10 % amaranth seeds. The reduction of *S. thermophilus* with the extension of the storage time is by 35, 70 and 47 %, respectively, for the yogurt with 0 %, 5 % and 10 % amaranth seeds. Enterobacteria, yeast and molds, coagulase-positive staphylococci, *Salmonella* and *Listeria monocytogenes* were not detected in all yogurt samples after 21 days of storage. Table 1 shows the results of the sensory analysis done on the 1st, 7th, 14th and 21st day.

Table 1. *Sensory analysis of yogurt fortified with different percentages of amaranth*

Parameter	**1 day**	**7 day**	**14 day**	**21 day**
Color				
Control	8.4	7.6	6.8	6.6
5 % Amaranth seeds	8.4	7.0	6.4	4.6
10 % Amaranth seeds	7.4	6.0	5.8	4.4
Taste				
Control	8.2	8,2	7.6	7.0
5 % Amaranth seeds	7.01	6.0	4.8	3.4
10 % Amaranth seeds	6.8	5,8	3.6	3.0
After taste				
Control	8.6	8.2	8.0	7.2
5 % Amaranth seeds	6.8	6.6	4.8	3.4
10 % Amaranth seeds	6.4	6.0	3.6	2.8
Texture				
Control	7.6	7.4	7.2	6.8
5 % Amaranth seeds	7.6	7.6	6.6	4,2
10 % Amaranth seeds	7.0	5.8	5.4	4.2
Overall acceptance				
Control	8.2	8.0	7.8	7.4
5 % Amaranth seeds	7.2	6.6	5.8	3.6
10 % Amaranth seeds	6.6	5.6	4.4	3.4

The results of the sensory analysis show that yogurt containing 5 % amaranth seeds consistently received higher ratings across all evaluated parameters when compared to yogurt with 10 % amaranth seeds. This suggests that a lower concentration of amaranth seeds may be more favorable for maintaining the desired sensory qualities of yogurt. Additionally, as indicated in Table 1, there is a noticeable decline in sensory quality for all yogurt samples as the storage time is extended. This trend underscores the importance of considering both the seed concentration and storage duration in the formulation and shelf-life assessment of yogurt products.

CONCLUSIONS

The addition of amaranth seed (5 and 10 %, respectively) to yogurt significantly changes the chemical, microbiological and sensory quality of the yogurt. The *p*H value of yogurt and total protein increased and the titration acidity decreased with increasing amount of amaranth seed. On the other hand, the addition of 5 % and 10 % amaranth seeds does not affect the total fat content. By monitoring the changes up to 21 days, it was determined that the *p*H value and total proteins decrease and the titration acidity increases with the extension of the storage time. Sensory characteristics during prolonged storage of yogurt with 5 and 10 % amaranth seeds deteriorate. The content of *L. Bulgaricus* and *S. thermophilus* decreased with the extension of storage time.

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