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Safety Assessment of Organic High-Protein Bars during Storage at Ambient and Refrigerated Temperatures

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Abstract: This study aimed to assess the safety characteristics of organic high-protein bars (HPB) during storage at ambient and refrigerated temperatures based on selected microbiological and chemical indicators. After production, the total number of microorganisms ranged from 3.90–4.26 log CFU/g;. The *Enterobacteriaceae* family was present at 2.81–3.32 log CFU/g, and the count of yeasts and moulds was 2.61–3.99 log CFU/g. No *Salmonella* sp. was found in 25 g of the product. *Bacillus cereus* was present in samples B1 and B2. *Staphylococcus aureus* was presented in samples below the detection limit (<2 log CFU/g). During the storage of products, the number of microorganisms varied. After production and storage, in all samples of HPB, the amount of mycotoxins was below the detection limit. The presence of histamine and tryptamine was not found in the HPB throughout the study period. Regarding TBARS, it can be concluded that the use of prunes and oat flakes (B2 bar composition) in the production of organic bars, and refrigerated storage, reduces the degree of fat oxidation. Among the tested variants, the composition of the B3 bar seemed to be the safest and worth further research, mainly due to the lower frequency of undesirable microorganisms. The protective antioxidative effect of prunes and oat flakes in bars stored at 22 °C indicates the value of the composition of bar B2. The appropriate composition modifications and the use of heat treatment proved to be effective in improving the safety characteristics of HPB. Relying on the results it is possible to store HPB for at least 3 months. Next to standard safety parameters, the unique and effective to increase the safety of HPB is controlling the presence of *B. cereus* and other low water activity (aw) resistant microorganisms.

Keywords: biogenic amines; fat oxidation; food safety; mycotoxin; organic bar; meal replacement

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1. Introduction

Nowadays, one of the major health goals is to achieve and maintain a desirable body weight, and this is accompanied by high awareness of the types of diets used for weight control. Meal replacements are substitutes for a wholesome dish in the form of a snack, bar or powder for making a drink or soup. They should be palatable, portion-controlled, exclude the need to choose and prepare foods, and eliminate food varieties that can spur over-consumption of energy [1,2]. Incorporating meal replacements (1–2 per day) into traditional lifestyle interventions is effective in treating overweight or obese patients [3,4]. Among other food trends, organic food products are considered to have higher nutritional value, compared to conventional ones. As well, organic plant raw materials contain fewer nitrates and pesticide residues [5].

A high-protein bar is an example of meal replacement. Its production should include the use of high-quality raw materials that bring biologically active substances, i.e., the basic nutrients, as well as non-nutritive compounds naturally occurring in the raw material or in

the product subjected to the technological process that affects physiological and metabolic functions of the body [6]. Previous research conducted by Szydłowska et al. [7] concerned the development of bar recipes made from organic plant-origin material (cereal, dried fruits, nuts, chocolate and others), as well as whey protein concentrate (WPC). The protein content was dependent on the product composition. Muesli and coconut bars contained a high amount of proteins in total (at least 20% energy from proteins according to Regulation (EC) No 1924/2006 [8]. All bars were characterized by a high scavenging capacity of DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radicals. However, some organic ingredients were contaminated with *Bacillus cereus*, and these bacteria were also found in muesli and pumpkin bars.

Although these organic bars had low water activity (aw) (0.63–0.74), which has clear advantages concerning controlling the growth of foodborne pathogens, there are nevertheless some major concerns. Desiccation stress is a common event in natural and food industry environments that significantly affects bacterial viability, but they can resist this [9,10].

The study aimed to assess the safety characteristics of organic high-protein during storage at ambient and refrigerated temperatures. Moreover, an attempt was made to define the date of minimum durability and the storage conditions of the bars. Selected microbiological and chemical indicators were evaluated.

2. Materials and Methods

2.1. Preparation of High-Protein Bar

The organic bars were produced under industrial conditions. The ingredients used in the production of the 100 g bar are listed in Table 1. The manufacture of organic bars was as follows. Pumpkin seeds, spelt, oat flakes and coconut shreds when added were roasted and grounded. Prunes and/or dried apricots were mixed with boiling water and allowed to soak for an hour, then the water was discarded, and the fruits were mixed until smooth. Then, water, sunflower oil, honey, and inulin were added and mixed. Dried cherries, freeze-dried raspberries were mixed, and all ingredients were blended. Subsequently, part of the WPC was added, mixed thoroughly with the remaining ingredients and the bar was formed. Next, chocolate (organic chocolate, 70% cacao content, organic cane sugar) was liquefied and the unused amount of WPC was added (proportionally for every 100 g of chocolate to 10 g of WPC). Bars were covered with chocolate glaze, packed in the metalized polyester polyethylene (MP) and sealed. Three different bars were prepared and named B1, B2, and B3. Bar B1 consisted of an equal amount of prune and dried apricots with no addition of oat flakes but coconut shreds. Bar B2 contained only prunes and B3 only dried apricots, and instead of coconut shreds they were made from oat flakes as well.

Bars were stored at refrigerated (4 °C) and ambient temperature (22 °C). The measurements of microbiological quality and water activity (aw) were carried out after production (day 0) and in the 1st, 2nd and 3rd months of storage. The presence of selected biogenic amines and selected mycotoxins were measured on day 0 and after 3 months of storage.

2.2. Microbiological Quality Evaluation

To evaluate the microbiological quality of the organic high-protein bars, the spread plate technique on an appropriate agar medium was performed, determining the total viable count (TVC) following ISO 4833-1:2013 [11] on nutrient agar (LabM, Heywood, UK). The number of rods from the *Enterobacteriaceae* family (ENT) by ISO 21528-2:2017 [12] on MacConkey agar No. 3 (LabM, Heywood, UK), and the number of yeasts and moulds (TYMC), following ISO 21527-1:2008 [13] on YGC agar (Sabouraud Dextrose with chloramphenicol lab Agar, Biomaxima, Lublin, Poland). 10 g of samples B1, B2 or B3 were used for evaluation. The measurements for each bar sample (B1, B2 or B3) were performed in triplicate.

Table 1. Variants of study beer beverages and their composition.

Ingredient (g)	Bar Symbol		
	B1	B2	B3
Whey protein concentrate (WPC)	15.9	16.7	16.3
Pumpkin seeds	14.3	15.0	14.6
Spelt flakes	11.9	12.5	12.2
Prune	7.9	16.7	0.0
Dried apricots	7.9	0.0	16.3
Oat flakes	0.0	8.3	8.1
Coconut shreds	7.9	0.0	0.0
Honey	3.2	3.3	3.3
Sunflower oil	2.4	2.5	2.4
Inulin	2.4	2.5	2.4
Dried cherries	1.6	1.7	1.6
Freeze-dried raspberries	0.8	0.8	0.8
Water	7.9	3.3	5.7
Chocolate	15.9	16.7	16.3
Sum	100	100	100

The presence of pathogenic bacteria was determined using the enrichment culture method with media indicated in the standards, such as XLD agar (Xylose Lysine Deoxycholate Agar, LabM, Heywood, UK) and RAPID'Salmonella agar (Bio-Rad, Hercules, USA) to determine the presence of *Salmonella* spp., following ISO 6579-1:2017 [14]. To determine the presence of *Bacillus cereus* agar PEMBA (LabM, Heywood, UK) was used following ISO 21871:2006 [15]. The presence of *Staphylococcus aureus* was detected using Baird-Parker agar (LabM, Heywood, UK) according to PN-EN ISO 6888-1:2001 [16]. Samples (25 g) of B1, B2 or B3 were used for evaluation. The measurements for each bar sample (B1, B2 and B3) were performed in triplicate.

2.3. Water Activity Evaluation

The measurement of water activity (aw) was performed using an AQUALAB 4TE Series Water Activity Meter (METER Group, Inc., Pullman, WA, USA) according to ISO 21807:2004 [17]. The water activity of the sample was measured with a photoelectric sensor using the dew point detection method. According to the producers' instructions, approx. 5 g of samples B1, B2 or B3 were placed in the test container and then in the measuring chamber of the apparatus. The mean value of three replicates was assumed as the final result of the water activity of the tested samples.

2.4. Mycotoxin Evaluation

Mycotoxin determinations were carried out by an accredited external Hamilton laboratory (https://hamilton.com.pl/en/ (accessed on 21 August 2022)) according to the methodology described in the standards authorized by the Polish Committee for Standardization (PKN), the sum of aflatoxins according to PN-EN 14123:2008 [18], and ochratoxin A according to PN-EN 14132:2010 [19]. The content of zearalenone deoxynivalenol was determined by high-performance liquid chromatography according to the J.S. Hamilton's internal procedures [20,21]. The test was carried out after production and after the 3rd month of storage.

2.5. Biogenic Amines Content

The analysed amines were 2-phenylethyl amine, Cadaverine, Histamine, Putrescine, Spermidine, Spermine, Tryptamine, Tyramine. The content of biogenic amines in organic high-protein bars was determined by an accredited external Hamilton laboratory (https://hamilton.com.pl/en/ (accessed on 21 August 2022)) and immediately after production in industrial conditions, as well as after three months of storage at two temperatures. The presence of biogenic amines was determined using liquid chromatographic analyses.

Biogenic amines were separated using a liquid chromatograph consisting of a quaternary pump, a vacuum degasser, an autosampler, a LC-UV/DAD detector with variable wavelength, and a fluorescence detector. The methodology of the analyses was according to Smělá et al. [22].

2.6. Analysis of Lipid Oxidation TBARS

The determination was performed according to the modified Salih method according to Pikul et al. [23]. The absorbance was measured at a wavelength of 532 nm against a blank containing 5 cm^3 4% perchloric acid and 5 cm^3 TBA reagent.

2.7. Presence of Pests

The assessment of the presence of warehouse pests consisted of an inspection of the packaging of B1, B2 and B3 bars immediately after production and during storage, after checking that the packaging is undamaged. Whole pests, fragments of their bodies, droppings, fume and other traces of presence were sought. After the first inspection, the bars were placed in an airtight container. Sticky traps were placed at the place of storage. Observation of pests was carried out after the 1st, 2nd and 3rd months of storage.

2.8. Statistical Analysis

One-way or multiple factor analysis of variance (ANOVA) with a linear model, as well as post hoc Bonferroni test, were applied to the statistical analysis of the data. Statistical significance was recognized when $p < 0.05$. Tests were conducted using Statistica 13 (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. Microbiological Quality Evaluation

The results of the average number of selected groups of microorganisms in the tested variants of high-protein bars are shown in Figure 1.

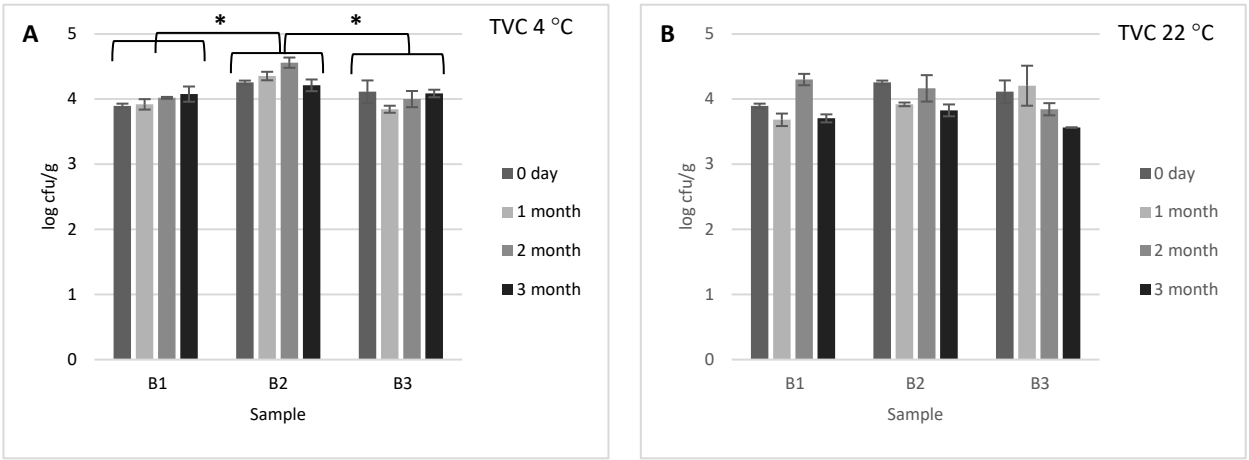

Figure 1. *Cont.*

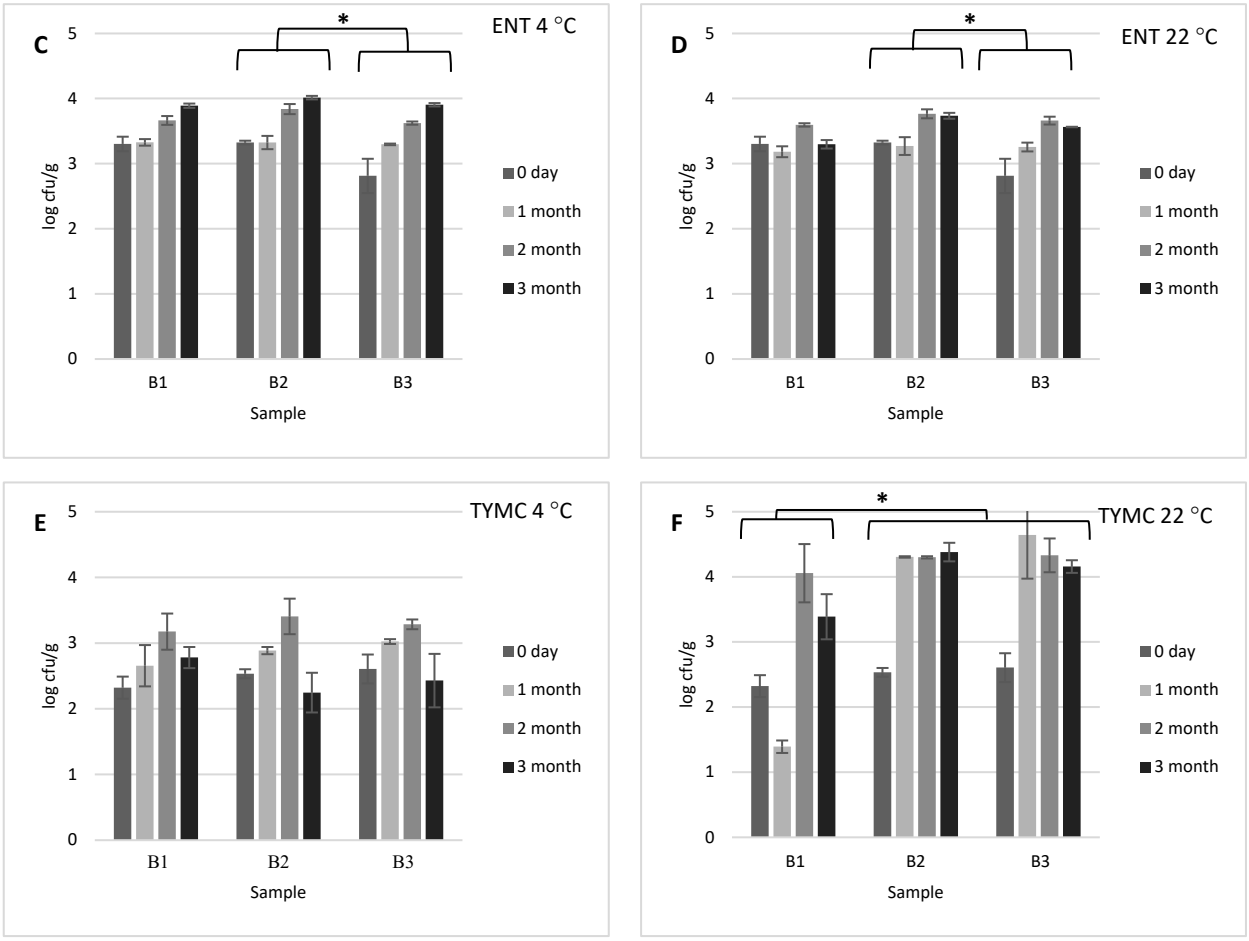

Figure 1. Mean number of selected microorganisms in the organic high-protein bars samples (B1, B2 and B3) stored for 3 months at 4 or 22 °C. (**A**,**B**) Total number of microorganisms (TVC); (**C**,**D**) number of Enterobacteriaceae (ENT); (**E**,**F**) count of yeasts and moulds (TYMC). $n = 3$. Values of the variants of the product (B1, B2, B3) marked with an asterisk differ significantly according to the post hoc Bonferroni test ($p < 0.05$). Bars represent mean standard error.

Immediately after production, the microbiological quality of bars differed, although at a similar level. The total number of microorganisms (TVC) ranged from 3.90–4.26 log CFU/g. The results of the ANOVA analysis revealed that during the storage of products at 4 °C, the TVC did not change significantly ($p > 0.05$). However, the results of the post hoc Bonferroni test indicated that sample B2 contained considerably more TVC versus B1 and B3 bars ($p < 0.05$), whereas at 22 °C, all the values were slightly different (Figure 1A,B).

The number of Enterobacteriaceae (ENT) family after production was in the range of 2.81–3.32 log CFU/g. Regardless of the product variant and the storage temperature, the number of these microorganisms increased significantly after 3 months of storage ($p < 0.05$) (Figure 1C,D).

The count of yeasts and moulds (TYMC) directly after production was in the range of 2.61–3.99 log CFU/g. After a month of storage at 4 °C, in sample B1 a decrease in TYMC to approx. 1.32 log CFU/g was observed. However, in subsequent cold storage, the number of these microorganisms increased. In the other variants, the TYMC increased, when in the samples stored at 22 °C the increase was more dynamic and higher by about 1 logarithmic order compared to the samples stored at 4 °C (Figure 1E,F). On some bars, aerial mould growth was observed after 3 months of storage.

In the tested variants of high-protein bars, no pathogenic bacteria *Salmonella* sp. was found in 25 g of the product. Pathogenic bacteria of the species *Bacillus cereus* were present in samples B1 and B2 immediately after production and in the following months of storage

at 4 and 22 °C. The presence of *B. cereus* was also found in the B3 high-protein bar variant, with two exceptions (after production and one month of storage at 22 °C) (Table 2).

Table 2. Evaluation of the presence of pathogenic bacteria in the tested products.

Time (Day/Month)	Temperature of Storage (°C)	Pathogen Sample	*B. cereus*			*Salmonella* sp.			*S. aureus*		
			B1	B2	B3	B1	B2	B3	B1	B2	B3
			(Presence)						(log CFU/g)		
0			+	+	–	–	–	–	<2.00	<2.00	<2.00
1	4		+	+	+	–	–	–	3.05	3.06	2.50
	22		+	+	–	–	–	–	3.05	2.36	<2.00
2	4		+	+	+	–	–	–	2.83	3.04	<2.00
	22		+	–	+	–	–	–	<2.00	<2.00	<2.00
3	4		+	+	+	–	–	–	2.80	2.06	<2.00
	22		+	+	+	–	–	–	<2.00	<2.00	<2.00

Explanatory notes: + present; – not present.

Staphylococcus aureus was presented in the samples after the production of the bars below the detection limit, i.e., <2 log CFU/g. After one month of storage at 4 or 22 °C, the S. aureus number was in the range of 2.36–3.07 log CFU/g. In subsequent research periods (2 and 3 months) of storage at 4 °C, S. aureus was detected in samples B1 and B2, but not in the variant B3. All tested variants (B1, B2 and B3) stored for 2 and 3 months at 22 °C contained S. aureus below the detection limit (Table 2).

3.2. Water Activity Evaluation

Figure 2 shows changes in water activity in the samples of organic bars with higher protein content during storage at different temperature conditions. The initial value was in the range of 0.64–0.68, the lowest aw was in sample B3, and the highest was in sample B2. There was no statistical difference between the tested variants (B1, B2 and B3) stored in both studied conditions regarding aw ($p > 0.05$). However, during 3 months of storage, the value significantly increased in all tested organic high-protein bars ($p < 0.05$). At this point at 4 °C, bar B2 had the highest aw d (0.81) and t bar B3 had the lowest aw (0.75) while at 22 °C the water activity of B3 bars was 0.77 versus B2 with 0.74. To sum up, bar B3 most often had the lowest aw.

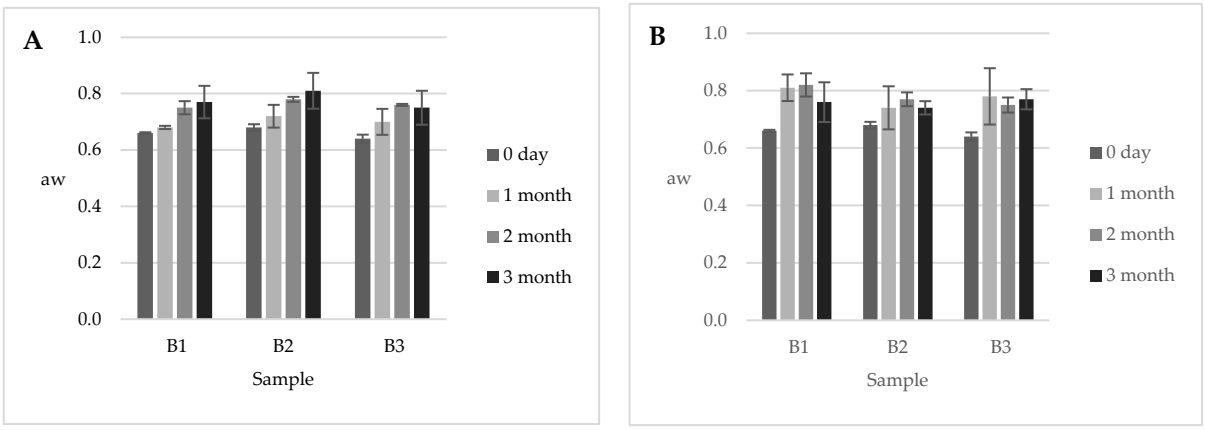

Figure 2. Water activity (aw) of tested products during storage (**A**) at 4 °C and (**B**) at 22 °C; $n = 3$. Bars represent mean standard error.

3.3. Mycotoxin Content

Table 3 presents detailed results of the content of selected mycotoxins measured in tested variants of the organic high-protein bars. Immediately after production and upon 3 months of storage, in all tested samples of high-protein bars, the amount of mycotoxin was below the detection limit of analytical methods.

Table 3. Presence of selected mycotoxins in HPB bars immediately after production (time 0) and after 3 months of storage at 4 °C and 22 °C (n = 3).

Mycotoxin (μg/kg)	Products at Day 0			Products after Storage at 4 °C			Products after Storage at 22 °C		
	B1	**B2**	**B3**	**B1/4**	**B2/4**	**B3/4**	**B1/22**	**B2/22**	**B3/22**
Aflatoxin B1	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Aflatoxin B2	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Aflatoxin G1	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Aflatoxin G2	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Sum of aflatoxin B1, B2, G1, G2	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Ochratoxin A	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
Zearalenone	<10	<10	<10	<10	<10	<10	<10	<10	<10
Deoxynivalenol	<100	<100	<100	<100	<100	<100	<100	<100	<100

3.4. Biogenic Amines Content

The content of biogenic amines in the organic high-protein bars immediately after production in industrial conditions and after a three-month storage period in two temperatures (4 and 22 °C) is shown in Table 4. The presence of biogenic amines, such as histamine and tryptamine, was not found in the study products throughout the research period. There were no statistically significant differences between the three variants compared in a given period ($p > 0.05$). However, changes in the content of biogenic amines under the influence of temperature and storage time were shown. A statistically significant decrease in the content of biogenic amines was observed in the case of putrescine and spermidine in all three tested bar variants ($p < 0.05$).

3.5. Analysis of Lipid Oxidation as TBARS

The lowest value of the TBARS index during 0, 1, and 2 months was found in the B2 bar at 4 °C at 0.38, 0.32 and 0.36 mg MDA/kg of the product, respectively. Among all experimental trials, the greatest changes in fat oxidation were observed in the B1 bar from 0.47 at day 0 to 1.96 mg MDA/kg after 3 months.

3.6. Presence of Pests

After nspection, no damage was found to the packaging in which bars B1, B2 and B3 were placed. The tested products did not contain live pests, parts of their bodies or other traces of their activity. During storage, no traces of the presence of pests were found in the tested products.

Table 4. Presence of selected biogenic amines in HPB bars immediately after production (time 0) and after 3 months of storage at 4 °C and 22 °C.

Biogenic Amines (mg/kg)	Products at Day 0			Products after Storage at 4 °C			Products after Storage at 22 °C		
	B1	**B2**	**B3**	**B1/4**	**B2/4**	**B3/4**	**B1/22**	**B2/22**	**B3/22**
2-phenylethyl amine	1.24 ± 0.13 aA	1.31 ± 0.1 aA	1.14 ± 0.12 aA	n.d.	2.95 ± 0.65 aA	1.66 ± 0.37 aA	1.24 ± 0.27 aA	1.41 ± 0.31 aA	1.35 ± 0.30 aA
Cadaverine	2.09 ± 0.24 aA	2.68 ± 0.21 aA	2.05 ± 0.27 aA	n.d.	2.47 ± 0.35 aA	2.99 ± 0.42 aA	3.30 ± 0.46 aA	3.94 ± 0.55 aA	3.09 ± 0.31 aA
Histamine	<1.00	<1.00	<1.00	n.d.	<1.00	<1.00	<1.00	<1.00	<1.00
Putrescine	11.8 ± 0.31 aA	12.2 ± 0.27 aA	12.7 ± 0.27 aA	n.d.	14.9 ± 3.9 aA	13.8 ± 3.60 aA	8.63 ± 2.24 aB	7.38 ± 1.92 aB	8.08 ± 2.10 aB
Spermidine	7.61± 0.21 aA	9.49 ± 0.29 aA	7.67 ± 0.25 aA	n.d.	7.25 ± 1.02 aA	6.8 ± 0.96 aA	4.03 ± 0.56 aB	5.86 ± 0.82 aB	5.19 ± 0.73 aB
Spermine	3.71 ± 0.15 aA	4.53 ± 0.17 aA	3.67 ± 0.26 aA	n.d.	3.84 ± 0.85 aA	3.44 ± 0.76 aA	1.74 ± 0.38 aA	2.77 ± 0.61 aA	2.35 ± 0.52 aA
Tryptamine	<5.00	<5.00	<5.00	n.d.	<5.00	<5.00	<5.00	<5.00	<5.00
Tyramine	2.47 ± 0.35 aA	2.55 ± 0.31 aA	2.29 ± 0.32 aA	n.d.	2.24 ± 0.18 aA	2.16 ± 0.17 aA	2.17 ± 0,17 aA	1.66 ± 0.13 aA	2.01 ± 0.16 aA

Explanatory notes: n.d.-no data. Data are expressed as mean ± SD. Means in the same row within the same column followed by different lowercase letters represent significant differences ($p < 0.05$). Means in the same row between different columns followed by different capital letters represent significant differences ($p < 0.05$).

4. Discussion

Consumer interest in food quality and safety is considered to be one of the main reasons for the growing demand for organically grown food, which consumers perceive as healthier and safer [24]. Previous research proved that it is possible to apply organic raw materials to develop the product intended for physically active people (high protein content) with high nutritional value and acceptable, high sensory quality [7]. However, a disturbing presence of *B. cereus* has been observed during storage. Organic products seem to be susceptible to microbial contamination compared to conventional products on the same level [25]. Therefore, broader research was needed to evaluate the safety of the product and to set possible storage times. For this reason, the presented study was planned to investigate more deeply the safety characteristic of high-protein bars made from organic raw materials.

The composition of high-protein bars determined by Szydłowska et al. [7] was modified to combat a few safety dilemmas revealed during the experiment and for wider use of raw materials native to Poland. The reformulation consisted of removing coconut flour and replacing it with oat flakes. Dried dates were replaced with prunes and/or apricots. Cane sugar was replaced with honey. Due to the microbiological contamination of raw materials, as in pumpkin seeds among others, the production process was modified. The change concerned the use of heat treatment, i.e., roasting of pumpkin seeds, oat and spelt flakes as well as soaking prunes and/or apricots in hot water to reduce the number of microorganisms in the finished product.

Insects present in food can have both direct and indirect effects on human health. The direct effect is the contamination of food with arthropod fragments as well as related contaminants e.g., allergenic, carcinogenic or microorganisms. The most significant indirect effect is that their presence can change the storage microenvironment, making durable products suitable for the rapid growth of fungi and other microorganisms present in the environment [26]. The tested products during storage were not infested with pests and it can be concluded that all food safety characteristics were connected with the natural microbial content and its activity or chemical changes during storage.

Regarding microbiological quality, the changes implemented in the bar's production did not considerably lower the TVC in the reformulated bars. However, in comparison with the pumpkin bars used in the Szydłowska et al. [7] study (TVC approx. 4.6 log CFU/g), the determined value in the present study was lower (TVC approx. 4.06 log CFU/g) and justifies the introduction of thermal processes during the manufacture of the bars. It is worth noting that products manufactured under industrial conditions usually contain a higher number of microorganisms compared to laboratory-scale production, due to the more diverse microbial environment and a greater number of people involved in the production. The proof of that is the higher number of Enterobacteriaceae family in tested products (about 4 log CFU/g) (Figure 1) whereas in the bars obtained from laboratory production the count was around 2 log CFU/g [7]. Other researchers found varied data regarding the TVC number in similar products i.e., in bars made from oat and corn flakes with brown rice and dried fruits detected the TVC on the level of about 2.08 log CFU/g [27]. Bhakha et al. [28] counted aerobes on the level of 8.9 log CFU/g in the pulse-based snack bars with cereals and dried fruits addition.

Fungi cause major spoilage of foods and feedstuffs. Certain environmental factors can induce the production of secondary metabolites i.e., mycotoxins. Contamination of food and feed by mycotoxins is considered one of the most serious food safety problems in the world because these fungal metabolites can be teratogenic, mutagenic, carcinogenic and immunosuppressive, and can cause serious damage to animal and human health [29,30]. In the study of Szydłowska et al. [7], just after production, the TYMC was below the detection threshold, but during one month of cold storage (4 °C), substantial growth was observed to average 5.5 log CFU/g, whereas in the modified product the TYMC was approximately

about 3 log CFU/g during the whole 3 months cold storage period. A similar count of the TYMC was identified in the study of Bhakha et al. [28], where pulse-based snack bars contained yeasts and moulds at around 5.1 log CFU/g during storage at room temperature but 4.3 log CFU/g stored at 4 °C. The research of Munshi et al. [27] revealed approx. 0.78 log CFU/g in cereals-based bars, whereas in popped pearl millet bars during storage at ambient temperature the TYMC was below the detection limit [31]. Nevertheless, after analysing the presence of yeasts and moulds it can be stated that applied changes positively influenced this safety characteristic mainly because selected mycotoxins were not detected (Table 3), which means that moulds did not find the proper condition for toxin production.

Regarding food pathogen occurrence, the tested high-protein bars were free from Salmonella spp. but Staphylococcus aureus and *B. cereus* could be possible hazards in this kind of product. Although the number of S. aureus did not exceed 3 log CFU/g, and the possibility of toxin production was low, additional preventive measures should be implemented to protect the product more efficiently. The more serious risk arises from the occurrence of *B. cereus*. Under EU food law, the limits are set only for dried infant formulas and dried dietary foods for special medical purposes intended for infants below six months of age, and allow the presence of *B. cereus* up to 500 CFU/g [32]. Most cases of food-borne outbreaks caused by the *B. cereus* group have been associated with concentrations above 5 log CFU/g. Nevertheless, cases of both emetic and diarrheal illness have been reported involving lower levels of *B. cereus*. At postharvest, the main management option for controlling *B. cereus* group strains in the food chain is to maintain the foods refrigerated at ≤ 7 °C. Other efficient control measures include heat treatment, high hydrostatic pressure, pulsed light, irradiation and chemical sanitisers. Because some of these may fail to inactivate spores, effective methods should be established, for example, a combination of the above-mentioned procedures [33]. For example, Hamanaka et al. [34] found infrared radiation (IR) effective in the inactivation of Bacillus subtilis spores, and the combination of IR heating with UV irradiation markedly accelerated the killing efficiency of microorganisms on fresh figs [35]. The results of Singh et al. [31] suggest that proper packaging material may improve the microbial stability of snack bars. In this research, the product packaged in metalized polyester polyethylene (MP) contained about 1 log CFU/g less the TVC than wrapped in high-density polyethylene (HDPE) foil.

The iroasting step of spelt flakes was not effective enough to inactivate all *B. cereus* cells or spores. The presence of *B. cereus* was found in all tested variants. It is worth noting that the B3 bar was the least contaminated with pathogenic spores, so perhaps its composition played a role in the dynamics of pathogen growth.

Water activity (aw) as an indicator of water availability allows prediction of the course of biological processes, especially the growth and development of microorganisms. A higher aw value indicates a better condition for microbial growth. Generally, the minimum aw at which microorganisms can grow is 0.60, but these numbers are variable. The minimum aw of most bacteria is approximately 0.87. Survival of only a few cells of some foodborne pathogens may be sufficient to cause disease. In a desiccated state, metabolism is greatly reduced, i.e., growth does not occur, but vegetative cells and spores may remain viable for several months or even years [10]. Measured values were approx. 0.65 at the beginning of the storage and increased to approx. 0.79 after 3 months of storage (Figure 2). Even though aw was in the range considered safe, the growth of some pathogens (Table 2) suggests that further action is needed to protect the product fully for example the addition of ingredients that have strong antimicrobial activity. Another possibility is the application selected thermal processes.

Biogenic amines (BAs) are naturally occurring ingredients in food products and influence the sensory properties of foodstuffs. In low concentrations, they do not pose a threat to health, and their increased content may be the result of the activity of endogenous enzymes contained in raw materials used for food production or microbial decarboxylation of amino acids taking place [36]. The toxic effects of BAs depend on individual sensitivity, lifestyle and diet, including consumption of ethanol, as well as on the kind of biogenic amine and

its concentration in food. BAs such as histamine, tyramine, cadaverine, putrescine and spermidine or spermine are involved in several pathogenic syndromes, representing a risk for consumer health [36–38]. The study bars with increased protein content we analysed to assess the potential presence of biogenic amines as a significant threat to human health. The content of biogenic amines in the study bars did not indicate high levels of these compounds. In food, the presence of biogenic amines such as histamine and tyramine is a public health concern, since these are the most notorious foodborne toxins [38]. The toxic concentration of tyramine, a biogenic amine also found in fruit, especially prunes, is 100–800 mg/kg. The values in the study bars did not exceed this range. In the case of histamine, the maximum concentration of this compound in food products should not exceed 200 mg/kg of the product [39]. In the investigated bars, this amount did not exceed 1 mg/kg of the product. It should be noted that very low amounts of histamine and tyramine, considered to be one of the most toxic biogenic amines, were found in the tested products. Despite the toxic influence of biogenic amines on food quality and human health, there is no specific regulation regarding BA content in food, except for the European Food Safety Authority, which developed a qualitative risk assessment concerning biogenic amine presence in fermented foods and concentrations that can induce adverse effects in consumers [40]. In out study of non-fermented, organic high-protein bars, no high concentration of biogenic amines was found that would pose a risk of food poisoning.

Fat oxidation is one of the significant problems in the production and storage of food products, as oxidative rancidity causes quality deterioration, loss of nutritional value, and the formation of toxic compounds. Factors that accelerate these changes include oxygen, light, and high temperatures. Based on the results of the research, it was found that the applied storage conditions of the bars under refrigerated conditions (4 °C) had an inhibitory effect on lipid oxidative changes, as evidenced by the results of the TBARS index (Table 5). The TBARS values of bars stored at 4 °C were lower than the rancidity period (1–2 mg MDA/kg sample) for food products and proved their good physicochemical quality [41]. The use of prune and oat flakes in B2 bars had a significant ($p < 0.05$) effect on the inhibition of oxidative changes in fat. The protective effect of prunes and oat flakes was particularly evident in bars stored at 22 °C. The antioxidant properties of prunes and oat flakes were also confirmed in their research by Vinson et al. [42], and Ryan et al. [43]. According to the values of TBARS, it can be concluded that the use of prunes and oat flakes (B2 bar composition) in the production of organic bars and refrigerated storage reduces the degree of fat oxidation.

Table 5. The value of the bar TBARS index (mg MDA/kg).

Time and Temperature of Storage (Month/°C)	Bar Symbol		
	B1	B2	B3
0	0.47 ± 0.03 aB	0.38 ± 0.03 aA	0.48 ± 0.02 abB
1/4	0.59 ± 0.03 abC	0.32 ± 0.02 aA	0.47 ± 0.02 aB
2/4	0.53 ± 0.06 abB	0.36 ± 0.03 aA	0.45 ± 0.07 aAB
3/4	0.63 ± 0.05 bAB	0.75 ± 0.06 bB	0.61 ± 0.06 bA
1/22	0.90 ± 0.03 cB	0.54 ± 0.05 abA	1.01 ± 0.05 dB
2/22	0.81 ± 0.03 cB	0.45 ± 0.02 abA	0.74 ± 0.04 cB
3/22	1.96 ± 0.17 dA	1.33 ± 0.41 cA	1.57 ± 0.11 eA

Explanatory notes: data are expressed as mean ± SD. Means in the same row within the same column followed by different lowercase letters represent significant differences ($p < 0.05$). Means in the same row between different columns followed by different capital letters represent significant differences ($p < 0.05$).

5. Conclusions

The nutritional quality and safety of organic products should be evaluated simultaneously to deliver scientific evidence of the quality of an organic product. The results of this research provide unique knowledge about a product that modern consumers and food producers are interested in.

Manufactured in industrial conditions, the organic high-protein bars were of good but not the best microbiological quality, with only slight heat treatment as preservation. Nevertheless, based on the presented results it is possible to store the organic high-protein bar for at least 3 months.

There is the possibility of further modifications to achieve microbial stability at an ambient storage temperature; for example, selecting ingredients that contain fewer microorganisms and/or are rich in antimicrobial compounds. In addition, choosing proper packaging materials and/or storage temperature may improve the safety characteristics of organic high-protein bars.

It can be concluded that the composition of the organic high-protein bars and their microbiological and physicochemical stability indicate the safety of this innovative product. Among the tested variants, the composition of the B3 bar seemed to be the safest and worth further research, mainly due to the lower frequency of undesirable microorganisms. On the other hand, the protective antioxidative effect of prunes and oat flakes in bars stored at 22 °C indicates the value of the composition of bar B2.

Next to standard safety parameters, increasing the safety of HPB involves controlling the presence of *B. cereus* and other low-aw-resistant microorganisms.

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