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Nutritional and Physicochemical Characteristics of Bread Enriched with Microalgae *Spirulina platensis*

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ABSTRACT
The aim of this study was to increase the nutrient content of bread prepared with white flour, using the valuable metabolites included in *Spirulina platensis*. In this study, conventional breads were added 10% of *Spirulina*. The nutrient composition, protein and lipid content were evaluated and microbiological and sensory analyses were conducted in the breads with microalgal biomass. The addition of microalgal biomass resulted in protein content increase, ranging from 7.40% to 11.63%. While Calcium, Magnesium and Iron contents of bread with *S. platensis* were 721.2, 336.6, 41.12ppm, conventional bread contained 261.7ppm Calcium, 196ppm Magnesium, and 8.72ppm Iron. Enrichment with *Spirulina* had significant influence on the volatile compounds of bread. By using the HS/SPME/GC/MS technique, fourteen volatile compounds were detected in control group and ten compounds were detected in bread with *Spirulina*. The results for the sensory assessment of bread enriched with *Spirulina* were considered satisfactory even if some algae flavor in the samples were perceived. Besides, bread with *Spirulina* stored at room conditions was observed to have a positive effect on the inhibition of mold growth. According to these results, the use of microalgae can enhance nutritional quality of bread without a negative impact on the shelf life of bread.

Keywords: Bread, *Spirulina platensis*, Chemical composition, Protein, Volatiles

I. INTRODUCTION

Humans seeking alternative nutritional sources and tendency to natural and additive-free food have been increasing gradually in developed or developing countries. Therefore, developed countries such as United States of America, Japan, England, Germany, and Norway benefit from the nutritional richness of microalgae [1]. One of the most important algae types cultured today is *Spirulina platensis. Spirulina platensis* is filamentous in nature and it is a microscopic blue-green alga type which is found intensively in alkaline waters, contains 60% to 70% protein, is rich in vitamin B_{12} (193 µg/100 g) and gamma linoleic acid and a source of calcium and iron (1043.62 and 338.76 mg/100 g), contains vitamin E and C, is rich in chlorophyll *a* (1.472%) and phycocyanin (14.18%) pigments, and is used as an essential nutrition support [2-9]. *Spirulina platensis* is digestible because 86% of its cell wall is composed of digestible polysaccharide [10]. The general purpose of *Spirulina* production is to provide protein resource for people and also to benefit from the richness of its biochemical structure. *Spirulina* has a special value among the other algae types. This special case is the lack of cellulose in its cell wall. *Spirulina* is a natural resource rich in GLA, which forms approximately 1% of its dry weight. *Spirulina* contains high amount of iron, which makes it important in anemia disease [11]. *Spirulina* is known with its high biomass productivity in hot and sunny climates. Today, it is produced commercially in many countries such as USA, Taiwan, Thailand, Mexico, Israel and China with a view to providing protein resource to people and benefiting from its biochemical richness as nutrition support. Researches indicate that *Spirulina* was used as nutrition by Aztec Civilization nearly 400 years ago. It is still used as food by Kanembu tribe living in the Chad lake region in Chad Republic. The *Spirulina* which is collected with cloth bags from the lake is dried in the sun within the holes dug in the sand, and then sold as dried bread called “dihe” [2-3]. *Spirulina* contains 50-70% protein, 20% carbohydrate, 5% lipid, 7% minerals and 3 to 6% moisture. Therefore, unlike the proteins obtained

from meat and dairy products, it is a protein resource which is low lipid, low-calorie, and cholesterol-free [8]. It is an energy supplement for elderly people. In Japan, 73% of people aged over 50 eat *Spirulina*. 10 grams of *Spirulina* contains only 36 calories [12].

Nutritional value of the traditional bread types are increased by adding them various foods additives such as walnuts, grapes, and sunflower seeds. Production of bread with different types inevitably brings up the use of a wide range of additives. White bread contains 35-43% moisture, 6-16% proteins, 45-58% carbohydrates, 0.5–1.5% lipids, 0.5-1.5% ash, and 1–1.5% salt; and 100 gram bread has approximately 250-270 calories.

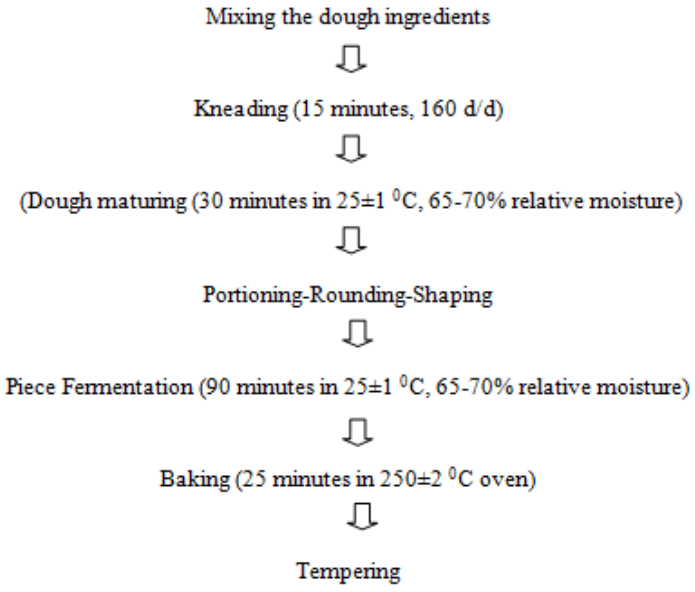
Bread, which is a high carbohydrate food, is habitually traditionally consumed with almost all foods in our country. The aim of this study is to add *Spirulina* to bread, which is the food of people especially having low income, with the purpose of increasing its protein and enriching it in terms of calcium, iron and magnesium. In this way, protein need in society would be fulfilled through bread which is an irreplaceable food consumed a lot. Therefore, the effects of enrichment with *Spirulina* on the quality of bread stored at room temperature (24^0C) and regrigerator storage (4^0C) were investigated in terms of chemical (proximate composition, mineral content and volatile compounds), microbiological (total mold count) and sensorial assessment in the present study.

II. MATERIAL AND METHOD

Microalgae *Spirulina* (*Arthrospira*) *platensis* was used in this study (Ben-Gruionuniv, Israel). The purpose of *Spirulina* addition to bread, which is a carbohydrate source, was to enrich it in terms of nutritional elements. In the study, conventional bread and *Spirulina* additive bread were prepared, and these breads were kept at two different storage conditions (room temperature (24^0C) and refrigerator storage (4 ^{0}C)). In the control group, the bread was made with wheat flour and water, without adding *Spirulina*. Bread with *Spirulina* was made by adding 10% *Spirulina* to the flour. Samples taken from the breads were analyzed in terms of moisture, ash, lipid, protein, and carbohydrate. Besides, microbiological and sensory analyses were performed and their content was evaluated in terms of calcium, iron, and magnesium. Volatile compounds of the control group bread and bread *Spirulina* added determined by using HS/SPME/GC/MS.

1.1. Preparation and Storage of the Breads

Preparation of the bread added 10% *Spirulina* and control group bread were performed according to mechanical dough conditioning method, in the Pilot Oven Unit set in Cukurova University Agricultural Faculty, Food Engineering Department. Kneading process included the use of DIOSNA type kneader with 1 kg flour capacity and ~160 d/d speed (Günsa Machine Ind. Inc.). Bread with *Spirulina* was prepared using 15gr salt, 40gr sugar, 40gr yeast, 900gr flour, 100gr *Spirulina*, and 620ml water. Additive-free bread was prepared using 1kg flour and 550ml water. Both doughs were kneaded for 15 minutes and fermented for 15 minutes. The dough was rested for one hour after 80gr pieces were cut. Temperature of the water to be used in 10% *Spirulina* added bread and control group bread were arranged so that the temperature of the kneaded dough would be 21±1 ^{0}C. The process of bread making was as follows:



At the end of the 15 minute kneading duration, the dough which was left maturing for 30 minutes was shaped by hand and rested for 5 minutes. Then, the dough pieces were placed in the cooking pan appropriately, left for piece fermentation for 90 minutes in the fermentation cabinet having 65-70% relative moisture and 25±1 ^{0}C temperature. The dough which completed its fermentation was baked in 250±2 ^{0}C temperature oven for 25 minutes. Bread with *Spirulina* and control group bread taken out of the pans after 5 minute resting were cooled on wooden grilles until their temperature decreased to room temperature. Shelf life of the bread with 10% *Spirulina* and control group bread were determined under room temperature (24^{0}C) and refrigerator conditions (4^{0}C). For this purpose, random samples were taken from the samples stored at room conditions in 3, 24, 48 and 72 hours intervals, and put in refrigerator bags individually. Besides, random samples were taken from the samples stored in refrigerator in the 96th and 120th hours. Sensory evaluation and microbiological (mold) analyses were performed in these samples taken throughout the storage duration.

2.2. Chemical Analyses

The analysis performed with a view to determining nutritional components of the bread with *Spirulina* addition and the bread without *Spirulina*, moisture and raw ash were waited in 103 ^{o}C and 550 ^{o}C ovens until the weight was stabilized; protein was performed according to A.O.A.C., 981.10 [13] and lipid was according to the Bligh and Dyer [14] method. Carbohydrates were determined by difference from the total of moisture, lipid, protein and ash contents [15]. The total energy value of the bread formulation was calculated according to Sharoba et al. [15] using the formula as shown in the following equation:

Total energy (kcal/100 g) = [(% available carbohydrates×4) + (% protein×4) + (% lipid×9)]

0.5 g sample was weighed for Ca, Fe and Mg element analysis in *Spirulina* added dough and the dough without *Spirulina* as well as after they were baked and 5 ml nitric acid was added on it. It was then waited under fume cupboard for 20 minutes and the burning procedure was performed in the microwave burning unit (Perkin Elmer, Berghof speedwave NWS-2, Germany). Then, it was diluted 10 times by taking 0.5 mL sample and completing it to 5 mL. For the second dilution, 0.5 mL was taken from this sample and 0.2 mL 12.5% CaCI, 5% 1 mL LaO_3 and 8.3 mL ultra-distilled water was added so that the final volume was completed to 10 mL. These samples were mixed in horizontal shakers and read with Atomic Absorption Spectroscopy (Perkin Elmer A Analyst 400 Atomic Absorption Spectrometer, USA) device.

Extraction of volatile aroma compounds in *Spirulina* added dough and the dough without *Spirulina* as well as after they were baked was performed on +40^{o}C magnetic stirrer for 30 minutes, using Head Space Solid Phase Micro Extraction/Polidimethylsiloxane technique (HS-SPME/PDMS). Volatile aroma compounds adsorbed in SPME needle with extraction phase were desorbed in the injection section of the Gas Chromatography Mass Spectrometer device (GC/MS) (Agilent Technologies Co., Ltd., Palo Alto, USA). Analysis of each sample took 1 hour in the GC/MS device. In the diagnosis of volatile aroma compounds, chromatograms obtained using Wiley, Flavor and Nist libraries were evaluated.

2.3. Microbiological Analysis

In microbiological evaluation, for total mold analysis, samples (10g) was taken control bread and *Spirulina* added bread under aseptic conditions and were mixed with 90 mL of Ringer solution (Merck, 1.15525.0001) (1/4 strength). They were stomached with Stomacher (IUL Instrument, Spain) for 2 minutes. After the decimal dilutions were made, 0.1 mL of appropriate dilution was pipetted onto the surface of the petri plates (Malt Extract Agar, Oxoid) in triplicate. Plates were incubated at 25 °C for 5 days (FDA/BAM, 2001).

2.4. Sensory Evaluation

Sensory evaluation was conducted by six experienced panelists using modified sensory evaluation form which had been described by Özer [16]. The bread samples were assessed in terms of their external characteristics, internal characteristics, odour and flavor properties. Apart from this evaluation table, the panelists were asked whether they would consume the bread they evaluated. Sensory evaluation form used in this study is shown in Table 1.

2.5. Statistical Analysis

Evaluation of the data was done using one-way analysis of variance (ANOVA), and the differences observed between the groups during storage were analyzed using Duncan multiple comparison test. Differences between the control bread and bread with *Spirulina* in terms of food item components were identified using independent groups t-test analysis [17].

III. RESULTS AND DISCUSSION

In the research, the changes observed chemical composition of the control group bread made using wheat flour without the addition of *Spirulina* and the bread prepared with the addition of 10% *Spirulina* are shown in Table 2.While ash, protein and carbohydrate values of the bread with *Spirulina* were significantly higher than the control group bread ($p<0.05$), no significant differences were observed in terms of moisture and lipid contents ($p>0.05$). Calcium, magnesium and iron contents of *Spirulina* powder, dough, and baked bread were summarized in Table 3. In this case, while the highest calcium, magnesium and iron proportions were detected in *Spirulina*, the lowest values were identified in conventional bread dough. Amounts of calcium, magnesium and iron in conventional bread are much lower than those of the bread with *Spirulina*. As for the amount of iron, approximately 5 times more iron was detected in baked bread with *Spirulina*. Results of the present study have been compared and evaluated with similar studies in the literature. Various studies investigated enrichment with *Spirulina* in pasta, cookies or biscuits [18-23]. The present study revealed that bread with *Spirulina* demonstrated a protein increase in comparison to the control group (Table 2). Similarly, Selmo and Salas-Mellado [24] reported that the addition of *Spirulina* with bread with rice flour on the technological quality and found that increasing the concentration of *Spirulina* from 1% to 4% increased protein content in the proportion of 20%. According to sensory evaluation, it was reported that *Spirulina* addition to rice flour in the proportion of 1% or 4 % made no difference. Fradique et al., [25] indicated that fresh pasta made with commercial durum semolina flour was added *Chlorella vulgaris* and *Spirulina maxima* 0.5, 1.0 and 1.5% (w/w), and it was found, compared to the control group, that there was improvement in quality parameters (optimum cooking time, cooking losses, swelling index and water absorption, physicochemical composition, texture analysis) and enrichment in microalgae. They reported that the groups which were added *S. maxima* displayed an increase in protein content in comparison to the control group.

Ozyurt et al., [18] investigated the pasta produced from semolina with the addition of *Spirulina platensis* at three different levels (5, 10 and 15% w/w) to the effects of *Spirulina* enrichment on some quality parameters of pasta such as cooking quality (weight increase, cooking loss, volume increase), microbiological quality (total mold and yeast count), color (*L*, *a*, *b*) and sensory characteristics were evaluated and compared. They determined that the cooking quality of enriched pasta samples was good for the technological attributes and no threats were detected in terms of microbiological safety. They emphasized that, especially, pasta samples enriched with 10% *Spirulina* had also desirable sensory properties as indicated by the panelists. Zouari et al., [26] studied that the effects of semolina enrichment with blue-green algae *Arthrospira platensis* at three different concentrations (1, 2 and 3 g/100 g of semolina) on the colour, cooking properties, firmness, free radical scavenging activity and sensory characteristics of pasta. It was determined microalgae addition resulted in higher swelling index and lower cooking loss than the control sample. It was indicated that the addition of *A. platensis* significantly increased the protein content of pasta products. The highest protein amounts of 15.3%, and cooking loss 9% were reported for 3g/100g of semolina in the formulation pasta. It was determined that the use of *A. platensis* (2 g/100 g of semolina) can increase the sensory quality and nutraceutical potential as evaluated by free radical scavenging activity of pasta.

Morsy et al., [9] indicated that added *Spirulina* to snack food in the rates of 2.5, 5, 7.5, 10 and 12.5% and determined that in the group which was added 12.5% *Spirulina*, there were decreases in sensory evaluations in many respects. However, overall acceptability was reported to be 59.88. The highest overall acceptability score was found in the *Spirulina* was added at the rate of 2.5% blend group. In the same study, protein content of the product which was not included any protein was 9.43% while the protein contents in the groups which were added *Spirulina* in the rates of 2.5, 5, 7.5 and 10% were found to be 11.38, 13.72, 15.94 and 18.11%, respectively. Microbiological analyses showed that the products which were added *Spirulina* were microbiologically safe. In this study has also found that *Spirulina* addition has positive effects on the inhibition of mold growth in bread stored at room conditions. Rodríguez De Marco et al. [27] investigated that technological and nutritional effect of the incorporation of *Spirulina* in pasta made with bread wheat flour and applied the *Spirulina* biomass in three levels 5, 10 and 20 g /100 g. They found that there was an increase in the protein content of the pasta, but digestibility of the protein decreased. They proposed that this case resulted from the facts that proteins encapsulated starch granules, limited the interaction of starch with amylase enzyme, and thus decreased digestibility of the starch [28]. In another study, added the addition of *Spirulina platensis* rates of 2% and 5% to formulations of cookies made with different types of flour, and investigated the chemical composition, technological, microbiological, sensorial and antioxidant properties. They reported that the nutritional

enrichment showed that the formulation containing composite flour 5% of an increase of the protein, fibre, ash and total phenolic compounds and a high antioxidant capacity [22].

Singh et al., [23] studied that biscuits were prepared from sorghum, guar gum and whole wheat flour with the addition of *Spirulina platensis* powder to produce high fibre and high protein biscuit. It was determined the biscuit purchase from the market was the increased protein 5.60%, while this rate of increased 13.70% in the biscuit enriched with *S. platensis* and the rate of fibre increased to 4.40% from 2.20%. They indicated that in the study that a negative effect is observed in the sensory and textural structure in case the rate of *S. platensis* is over 7%. In this study, the panelists indicated in sensory evaulation suggested that *Spirulina* addition should be less, which displays similarity with the study mentioned above. Similarly, in this study, protein content in bread without *Spirulina* was found as 7.40% while it was found as 11.63% in the group which was added 10% *Spirulina.* Protein enrichment with *Spirulina* addition was a parallel finding with studies conducted by Morsy et al., [9], Zouari et al., [26], Fradique et al., [25], Selmo and Salas-Mellado [24], and Bolanho et al., [22]. Rodríguez De Marco et al., [27] also reported that *Spirulina* addition in pasta increased protein content, but decreased digestibility of the protein.Volatile compounds of the control group bread and bread *Spirulina* added determined by using HS/SPME/GC/MS are shown in Table 4. Before they were baked, conventional dough and dough with *Spirulina* were found to have 13 volatile compounds. While the fundamental compounds of control group dough were 11-hexadecen-1ol,acetate (70.94%), 9-octadecenoic acid (11.05%), those of dough with *Spirulina* were 5-nonadecen-1-ol (49.25%), 9-octadecenoic acid (14.43%) and ethanol (13.27%). After they were baked, 11-hexadecen-1ol,acetate was found to decrease to 0.18% and 5-nonadecen-1-ol to 22.12%. 14 compounds were identified in the control group bread after they were baked. The highest proportions belonged to Z-11-tetradecen-1-ol trifluoroacetate (17.05%) and 1,3-benzenediamine (14.84%). Differently, 10 volatile compounds were identified in bread with *Spirulina* addition after it was baked. The highest proportions were determined as 5-nonadecen-1-ol (22.12%), 1,3-benzenediamine (18.26%) and 2H-benzimidazol-2-one (14.43%) from ketones. These findings show that baking procedure and *Spirulina* addition have significant effects on fundamental volatile compounds; and aroma structure of the bread changed with *Spirulina* addition.

The results of microbiological analysis of samples taken from control bread and bread with 10% *Spirulina* stored at refrigerator conditions did not mold growth show no during the storage. Although bread with *Spirulina* stored at room conditions had no mold growth, total mold count in the control group was found as 2.74±0.06 logcfu/g (Figure 1). These values were found to be higher than the 1.0×10^2cfu/g limit for bread identified in Turkish Food Codex Microbiological Criteria Communique. This finding suggests that *Spirulina* addition has positive effects on the inhibition of mold growth in bread stored at room conditions.Sensory evaluation was conducted by six experienced panelists for the control group bread and *Spirulina* added bread. Findings of the sensory analysis were shown in Table 5. In this sudy, the panelists reported that crust colour and crumb colour of *Spirulina* added bread were different, but base width, colour homogeneity, pore structure, and softness scores were similar. Besides, while the panelists evaluated the smell, aroma and taste differences of 10% *Spirulina* addition as consumable, they suggested that *Spirulina* addition in less proportions would ensure acceptability of these changes in bread by larger mass of people. While shelf life of both groups stored at room conditions was determined as 72 hours, it was 120 hours for the groups stored at refrigerator; no differences were detected between the groups. The data obtained from sensory evaluations showed that *Spirulina* addition had no negative effects on shelf life of the breads.

IV. CONCLUSION

Bread is a carbohydrate-based food which is consumed widely by the majority of societies. In this study has found that when bread dough is added *Spirulina platensis*, a microalgae type rich in nutritional value, it enriched the content of the bread in terms of protein, calcium, magnesium, and iron. No mold growth was detected in samples taken from the control group and 10% *Spirulina* added bread group stored at refrigerator conditions. In addition to this, no mold growth was detected in *Spirulina* added bread which was stored at room conditions and rejected in the 72nd hour, while total mold count was found 2.74±0.06 logcfu/g in control group samples. Consequently, the results of this study indicate that 10% *Spirulina* addition did not have any negative effects on shelf life of the breads and it improved the nutritional value.

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Table 1.Sensory Evaluation Form

	Score	**3 hours**	**24 hours**	**48 hours**	**72 hours**	**96 hours**	**120 hours**
External Characteristics							
Proportion of base width / height	0-10						
Volume	0-15						
Shell Colour	0-5						
Shell structure	0-5						
Internal Characteristics							
Colour homogeneity	0-5						
Pore structure	0-10						
Softness	0-10						
Elasticity	0-10						
ODOUR							
Odour	0-10						
FLAVOR							
Aroma	0-10						
Taste	0-10						
Would you consume this bread?	Yes No						

Table 2. Chemical composition of bread enriched with *S. platensis* (10%)

	Control	10% *Spirulina* added bread
Moisture (%)	32.71 ± 1.02^{a}	34.03 ± 1.35^{a}
Ash (%)	1.53 ± 0.05^{b}	1.89 ± 0.05^{a}
Protein (%)	7.40 ± 0.34^{b}	11.63 ± 0.06^{a}
Lipid (%)	1.91 ± 0.54^{a}	1.36 ± 0.16^{a}
Carbohydrate (by difference)	56.45	51.09
Total Energy (kcal)	272.59	256.32

***Different letters in the same line (a,b) indicate statistically significant differences ($p < 0.05$).**

Table 3. Calcium, magnesium and iron contents of *Spirulina*, dough, and breads (ppm)

	Calcium	Magnesium	Iron

***Spirulina* Powder**	8140 ± 16^{e}	924.2 ± 4^{a}	297.8 ± 1.6^{a}
Conventional Dough	248.4 ± 6^{d}	168.5 ± 4^{e}	8.17 ± 0.4^{d}
Dough with *Spirulina*	546.6 ± 3^{b}	278.6 ± 2^{c}	33.8 ± 0.1^{c}
Baked Bread	261.7 ± 8^{c}	196 ± 1^{d}	8.72 ± 0.5^{d}
Baked Bread with *Spirulina*	721.2 ± 4^{a}	336.6 ± 3^{b}	41.12 ± 0.7^{b}

***Different letters in the same column (a-e) indicate statistically significant differences (p< 0.05).**

Table 4. Volatile compounds of Conventional bread and Bread with *Spirulina* (%)

	Dough with *Spirulina*	Conventional Dough	Baked Bread	Bread with *Spirulina*
Ethanol	13,27	2,34	0,45	9,45
1,3-benzenediamine	7,55	4,25	14,34	18,26
2H-benzimidazol-2-one	4,11	-	9,18	14,43
Beta-ionone	1,33	-	-	1,1
Phenol	0,97	1,06	8,45	9,18
Oxalicacid	1,88	-	-	-
1-hexadecanamine	4,76	-	-	-
9-octadecenoic acid	14,43	11,05	15	7,75
10-heneicosene	2,82	2,61	8,53	-
Z-11-tetradecen-1-ol trifluoroacetate	2,22	1,39	17,05	-
1,19-eicosadiene	3,78	2,78	13,73	-
cyclododecanemethanol	1,03	0,51	1,69	-
11-hexadecen-1-ol,acetate	-	70,94	0,18	-
Dodecane	-	0,41	6,45	-
18-nonadecenoic acid	-	0,78	2,79	-
2,3-dihydroxypropyl elaidate	-	1,78	-	-
13-docosen-1-ol	-	-	0,79	-
1,16-hexadecanediol	-	-	0,74	-
2-propenoic acid	-	-	-	6,48
Hexadecane	-	-	-	0,83
Undecanal	-	-	-	3,38
5-nonadecen-1-ol	49,25	0	0	22,12
	94,13	97,56	98,92	83,53

-: Not detected

Table 5. Sensory Evaluation Results of Control and *Spirulina* added bread throughout storage G1: Control Group, G2: 10 %*Spirulina* added bread

Bread Characteristics	Groups												
	Room Condition						Refrigerator storage						
	3	24	48	72	96	120	3	24	48	72	96	120	
Volume (0-15 Points)	14.17 ±0.41^{c}	13.17 ±0.41^{b}	13.00 ±0.00^{b}	10.00 ±0.00^{a}			14.00 ±0.00^{f}	13.33 ±0.52^{c}	13.00 ±0.00^{d}	11.00 ±0.00^{c}	10.00 ±0.00^{b}	9.17 ±0.41^{a}	G1
	15.00 ±0.00^{c}	13.17 ±0.41^{b}	13.17 ±0.41^{b}	10.00 ±0.00^{a}			15.00 ±0.00^{c}	13.67 ±0.52^{d}	13.00 ±0.00^{c}	11.17 ±0.75^{b}	10.00 ±0.00^{a}	9.50 ±0.55^{a}	G2
Base Width /Height Ratio (0-10 Points)	9.00 ±0.00^{d}	7.50 ±0.55^{c}	6.83 ±0.41^{b}	5.67 ±0.52^{a}			9.00 ±0.00^{d}	7.67 ±0.52^{c}	7.50 ±0.55^{c}	6.83 ±0.41^{b}	6.33 ±0.52^{a}	6.00 ±0.00^{a}	G1
	9.00 ±0.00^{d}	7.83 ±0.41^{c}	7.17 ±0.41^{b}	5.83 ±0.41^{a}			9.00 ±0.00^{d}	8.00 ±0.00^{c}	7.00 ±0.00^{b}	7.00 ±0.00^{b}	7.00 ±0.00^{b}	6.00 ±0.00^{a}	G2
Colour (0-5 Points)	5.00 ±0.00^{c}	5.00 ±0.00^{c}	4.17 ±0.41^{b}	2.67 ±0.52^{a}			5.00 ±0.00^{d}	5.00 ±0.00^{d}	4.17 ±0.41^{c}	3.50 ±0.55^{b}	3.33 ±0.52^{b}	2.33 ±0.52^{a}	G1
	4.00 ±0.00^{b}	4.00 ±0.00^{b}	3.83 ±0.41^{b}	2.67 ±0.52^{a}			4.00 ±0.00^{d}	4.00 ±0.00^{d}	4.00 ±0.00^{d}	3.50 ±0.55^{c}	3.00 ±0.00^{b}	2.33 ±0.52^{a}	G2
Shell Structure (0-5 Points)	5.00 ±0.00^{c}	5.00 ±0.00^{c}	3.67 ±0.52^{b}	2.50 ±0.55^{a}			5.00 ±0.00^{d}	5.00 ±0.00^{d}	4.50 ±0.55^{c}	3.00 ±0.00^{b}	2.83 ±0.41^{ab}	2.50 ±0.55^{a}	G1
	3.17 ±0.41^{a}	3.00 ±0.00^{a}	3.00 ±0.00^{a}	2.84 ±0.41^{a}			3.17 ±0.41^{b}	3.00 ±0.00^{ab}	3.00 ±0.00^{ab}	3.00 ±0.00^{ab}	3.00 ±0.00^{ab}	2.83 ±0.41^{b}	G2
Colour Homogeneity (0-5 Points)	5.00 ±0.00^{c}	5.00 ±0.00^{c}	3.83 ±0.41^{b}	2.50 ±0.55^{a}			5.00 ±0.00^{d}	5.00 ±0.00^{d}	4.33 ±0.52^{c}	3.17 ±0.41^{b}	3.00 ±0.00^{b}	2.33 ±0.52^{a}	G1
	5.00 ±0.00^{c}	3.83 ±0.41^{b}	3.50 ±0.55^{b}	2.33 ±0.52^{a}			5.00 ±0.00^{c}	4.33 ±0.52^{d}	3.67 ±0.52^{c}	3.00 ±0.00^{b}	2.83 ±0.41^{b}	2.00 ±0.00^{a}	G2
Pore Structure (0-10 Points)	9.00 ±0.00^{c}	9.00 ±0.00^{c}	7.50 ±0.55^{b}	4.33 ±0.52^{a}			9.00 ±0.00^{c}	9.00 ±0.00^{c}	7.83 ±0.41^{d}	6.83 ±0.41^{c}	6.00 ±0.00^{b}	4.17 ±0.98^{a}	G1
	9.00 ±0.00^{c}	8.17 ±0.75^{b}	7.67 ±0.52^{b}	4.33 ±0.52^{a}			9.00 ±0.00^{d}	8.83 ±0.41^{d}	8.67 ±0.52^{d}	6.50 ±0.55^{c}	5.83 ±0.41^{b}	3.83 ±0.75^{a}	G2
Elasticity (0-10 Points)	9.00 ±0.00^{c}	7.33 ±0.52^{b}	7.00 ±0.00^{b}	3.33 ±0.52^{a}			8.50 ±0.55	7.33 ±0.52	7.00 ±0.00	6.00 ±0.00	5.00 ±0.00	3.83 ±0.75	G1
	9.33 ±1.03^{d}	7.33 ±0.52^{c}	5.17 ±0.41^{b}	3.67 ±0.52^{a}			9.33 ±1.03^{d}	7.17 ±0.41^{c}	5.67 ±0.52^{b}	5.50 ±0.55^{b}	5.00 ±0.63^{b}	3.17 ±0.41^{a}	G2
Softness (0-10 Points)	8.00 ±0.00^{d}	7.00 ±0.00^{c}	6.33 ±0.52^{b}	4.00 ±0.00^{a}			8.00 ±0.00^{c}	7.00 ±0.00^{d}	6.83 ±0.41^{d}	5.83 ±0.41^{c}	4.67 ±0.52^{b}	3.83 ±1.17^{a}	G1
	8.00 ±0.00^{d}	6.33 ±0.52^{c}	5.17 ±0.41^{b}	4.00 ±0.00^{a}			8.00 ±0.00^{d}	6.17 ±0.41^{c}	5.33 ±0.82^{b}	4.50 ±0.55^{a}	4.33 ±0.82^{a}	3.83 ±0.41^{a}	G2
Smell (0-10 Points)	10.00 ±0.00^{c}	9.50 ±0.55^{bc}	9.33 ±0.52^{b}	6.33 ±0.52^{a}			10.00 ±0.00^{c}	9.83 ±0.41^{bc}	9.67 ±0.52^{bc}	9.50 ±0.84^{bc}	9.00 ±1.26^{b}	6.66 ±0.52^{a}	G1
	7.17 ±0.41^{c}	7.00 ±0.00^{bc}	6.50 ±0.55^{ab}	6.33 ±0.52^{a}			7.33 ±0.52^{bc}	7.67 ±0.52^{c}	7.17 ±0.41^{bc}	6.83 ±0.41^{b}	6.17 ±0.41^{a}	6.00 ±0.00^{a}	G2
Aroma (0-10 Points)	10.00 ±0.00^{d}	9.00 ±0.00^{c}	8.50 ±0.55^{b}	3.50 ±0.55^{a}			10.00 ±0.00^{c}	9.83 ±0.41^{c}	9.50 ±0.55^{c}	9.17 ±0.98^{bc}	8.50 ±0.55^{b}	5.83 ±1.47^{a}	G1
	5.33 ±0.82^{b}	5.00 ±0.00^{b}	4.00 ±0.00^{a}	3.50 ±0.55^{a}			5.00 ±0.00^{c}	5.00 ±0.00^{c}	5.00 ±0.00^{c}	5.00 ±0.00^{c}	4.50 ±0.55^{b}	4.00 ±0.00^{a}	G2
Taste (0-10 Points)	10.00 ±0.00^{c}	8.67 ±0.52^{b}	8.17 ±0.75^{b}	3.33 ±0.52^{a}			10.00 ±0.00^{c}	9.00 ±0.00^{d}	9.00 ±0.00^{d}	7.83 ±0.41^{c}	6.50 ±0.84^{b}	4.33 ±0.52^{a}	G1
	8.00 ±0.00^{c}	7.33 ±0.52^{c}	5.50 ±0.55^{b}	3.83 ±0.98^{a}			8.00 ±0.00^{d}	8.00 ±0.00^{d}	7.00 ±0.00^{c}	5.83 ±0.41^{b}	4.33 ±0.52^{a}	4.00 ±0.00^{a}	G2

*Different letters in the same line (a-f) indicate statistically significant differences ($p < 0.05$).