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Effect of Storage on Quality of Spirulina Snack Bars

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***Keywords*— Snack bar, spirulina, storage, quality.**

***Abstract*—*Ready to eat snack and health bars have sturdily occupied space in the global market and are high in demand as nutritional supplements for people on the go and weight watchers especially people consuming calorie-controlled diets. Spirulina is a unicellular micro alga grown in water bodies containing 50-70% protein including all essential amino acids, fatty acids, vitamins and minerals. The soy protein isolate is good source with high bioavability of protein (90%) and it is easily digestible too. Bengal gram is an old pulse with high protein ratio about 80% with several vitamins like vitamin A, C, E, K and B-complex and minerals like phosphorous and magnesium. Peanuts are essential source of protein, lipid and fatty acids and called as poor man's protein due to its low price. It contains essential vitamins and minerals useful for healthy immune system, nerve function, blood coagulation mechanism, bones and teeth formation. In present investigation snack bars prepared with bengal gram, peanut, oats, puffed rice, desiccated coconut, soy isolate, spirulina, cardamom powder and binding syrup were analyzed for organoleptic properties and stored at room temperature after packaging with butter paper and polypropylene pouch for 90 days to record changes in nutritional, functional, organoleptic and microbial quality attributes. The results showed increase in moisture content whereas decrease in ash, fat, protein and fiber content in stored snack bars. The microbial count (Total plate count, yeast and mould) was increased throughout storage period at room temperature from 45th day of storage and sensory score for all the sensory parameters (Colour and appearance, flavour, taste, texture and overall acceptability) was decreased with the advancement in storage period. The snack bars with spirulina could be stored for the period of 90 days in polypropylene pouch at room temperature in good condition.*

I. INTRODUCTION

Energy bar or snack bar is a portable, convenient and proportioned health food with minimally processed, rich in nutrients and excellent in taste which fulfils consumer demand for health foods (Mridula et al., 2011). Snack bars contains mainly cereals and other high energy ingredients having carbohydrate, lipids, proteins and minerals in them, which deliver good sensory and nutritional characteristics to it. (Padmashree et al., 2018).

The low moisture content food items with longer time span of usability and less microbial decay are effectively used for their functional nutrients. Snack bars are commonly reinforced by utilizing different protein, fiber or vitality rich ingredients (Siddique et al., 2018).

The faster developing change in life style leads to rapid increase demand for the processed and convenience foods. Energy bars are one of the nutritionally balanced convenient food which has foothold since after 1980s (Mridula et al., 2011 and Yadav and Bhatnagar, 2015).

Considering increase in participation number of physical activities, snack bars with good range of nutrients with satisfactory amount of proteins, fats, carbohydrates in little packet has been a great source of energy (Yadav and Bhatnagar, 2015).

Protein is important for development and repairing of body cells. The deficiency of protein may lead to different health complications like kwashiorkor, marasmus, impaired mental health, oedema, organ failure, wasting and shrinkage of muscle tissues, and weakness of immune system (Khan et al., 2017). Though protein is considered as an important component of human diet its scarcity is the widest problem in the world. According to global production scenario the ever-increasing world population cannot be lean only on agriculture to nourish them. This leads to identify another protein sources and the best potential of protein source have been found in microbial protein (Usharani et al., 2012). In the last few years, the special properties of microscopic organism have attracted people and scientists from all over the world (Palaniswamy et al., 2018).

Spirulina which is a uni-cellular microphytes contains all essential amino acids with quite amount of methionine, cysteine, and lysine than meat, egg or milk, but comparatively higher than all plant protein. It has a 36% of polyunsaturated fatty acids (γ-linolenic acid, linoleic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid) and 1.5–2.0% of total lipids (Jung et al., 2019). Spirulina have significant effect on reducing serum cholesterol levels in human beings by 4.5% and significantly reduces body weight. It also reduces hepatic damage, inflammatory response, cells degeneration, anaphylactic reaction. It also prevents eye diseases, hypoferric anemia and pernicious anemia due to its vitamin A, iron, and vitamin B12 content spirulina extract induces the tumor necrosis factor in macrophages, suggesting a possible tumor destruction mechanism (Saranraj and Sivasakthi, 2014).

The efforts were undertaken to develop protein rich snack bar using protein rich ingredients like spirulina, bengal gram, peanut and soy isolate and to study the effect on their quality during storage.

II. METHODOLOGY

2.1 Development of snack bars using spirulina

The spirulina snack bars were prepared by using bengal gram (15%), peanut (15%), soy isolate (4%), desiccated coconut (4%), puffed rice (2%), corn syrup (25%), honey (20%), cardamom (1%) and variable combinations of oats (10-14%) and spirulina (2- 6%). The developed spirulina snack bars wrapped in butter paper and packed in polypropylene pouch were stored at room temperature (90 days) to record changes in nutritional, functional, organoleptic and microbial quality attributes for 15 days of time interval during storage period.

2.2.2 Quality analysis of spirulina snack bars during storage

The moisture, ash, fat, proteins, crude fiber contents, carbohydrates were determined by standard methods suggested by Ranganna (2015) and Thimmaiah (2016).

For determination of total phenolic contents 1g of ground sample was dispersed in 10 ml of 80% ethanol and centrifuged at 1500 rpm for 15 min. After centrifugation, supernatant of sample, supernatant was evaporated to dryness. The dried extract was dissolved in water and different aliquots were taken to made final volume upto 3ml. 0.5ml of FCR and Na2CO3 were added in it and the absorbance was measured at 650nm against a reagent blank. Total phenol content was expressed in mg of GAE/100g (Thimmaiah, 2016).

The antioxidant potential was assessed by using DPPH method. 1g of sample per 100ml ethanol was dispersed thoroughly with the help of vortex mixer. It was allowed to remain still overnight and centrifuged at 3000 rpm for 10min to facilitate separation of sample extract. The 0.2ml of eluted supernatant was taken in a test tube covered with aluminum sheet and 1ml of freshly prepared DPPH solution (80μg/ml ethanol) was carefully added. A control was set up with 0.2ml distilled water as blank and 1ml of DPPH solution was added to it. The sample test tubes were allowed to remain in the dark for 30min and absorbance of the samples and blank sample were measured against ethanol at 517nm (Kumar, 2018). The percentage of inhibition was calculated using the formula (Tailor and Goyal, 2014).

$$\text{Inhibition (\%)} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

2.2.3 Microbial analysis of snack bar

The total plate count and yeast and mould contents were determined by the standard pour plate method (Aneja, 2003).

2.2.4 Sensory analysis

Sensory analysis of prepared snack bars samples were done using 9-point hedonic scale rating (Ranganna, 2015).

III. RESULTS AND DISCUSSIONS

3.1 Nutritional and functional characteristics of stored snack bars

Table 2. Effect of storage period on moisture content (%) of spirulina snack bars

The changes in moisture content of stored snack bars

Raw material	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Crude fibers (%)	Carbohydrates (%)
Bengal gram	10.71 ± 0.49	2.52 ± 0.03	5.19 ± 0.31	22.50 ± 0.35	1.02 ± 0.02	58.06 ± 0.98
Peanut	1.80 ± 0.28	2.87 ± 0.06	49.73± 0.36	24.42 ± 0.40	8.43 ± 0.03	21.23 ± 0.51
Oats	8.02 ± 0.35	1.80 ± 0.05	8.49 ± 0.32	11.72 ± 0.44	9.99 ± 0.04	59.98 ± 0.76
Soy isolate	1.06 ± 0.02	5.33 ± 0.15	0.21 ± 0.06	91.20 ± 0.02	0.02 ± 0.02	2.18 ± 0.16
Spirulina	3.52 ± 0.39	6.74 ± 0.02	9.51 ± 0.56	63.56 ± 0.58	3.18 ± 0.07	13.49 ± 0.42

depicted in Table 2 indicated gradual increase in moisture content of snack bars with advancement of storage period. The moisture content in freshly prepared snack bars was observed in the range between 11.31 and 11.35%. During room temperature storage, moisture content in snack bars was increased to 12.95-13.04% at the end of 90th day of storage. Increase in moisture content during storage might be due to higher vapour transmission rate of PP films (Padmashree et al., 2018 and Ekafitri et al., 2020). The results are in agreement with the results reported by (Mridula et al., 2011, Padmashree et al., 2018, and Kumar et al., 2018). The storage period had non- significant effect on the moisture content of snack bars at 5% level of significance.

3.1.2 Changes in ash content

3.1.1 Changes in moisture content

Table 3. Effect of storage period on ash content (%) of spirulina snack bars

Sample	0 days	15 days	30 days	45 days	60 days	75 days	90 days
S0	1.01 ±0.05	0.92 ±0.04	0.84 ±0.03	0.75 ±0.03	0.69 ±0.02	0.62 ±0.07	0.57 ±0.04
S1	1.38 ±0.02	1.31 ±0.03	1.23 ±0.04	1.14 ±0.04	1.08 ±0.03	1.02 ±0.04	0.97 ±0.03
S2	1.56 ±0.04	1.48 ±0.04	1.40 ±0.05	1.31 ±0.06	1.25 ±0.07	1.18 ±0.05	1.13 ±0.02
S3	1.77 ±0.02	1.70 ±0.02	1.62 ±0.06	1.53 ±0.04	1.47 ±0.04	1.41 ±0.03	1.36 ±0.02
Mean	1.43	1.35	1.27	1.18	1.12	1.06	1.01

The changes in ash content of stored snack bars are presented in Table 4. As regards room temperature storage, there is progressive decrease in ash content of all snack bars throughout the storage period. The highest ash content (1.77%) was recorded in freshly prepared snack bars with 6% spirulina followed by 4% spirulina (1.56%) and 2% spirulina (1.38%). Whereas the lowest ash content (1.01%) was observed in control bars without spirulina. The more ash content in spirulina bars might be due enormous mineral content associated with spirulina (Kumar et al., 2018). The ash content showed declining trend in all snack bars throughout the storage period and recorded 0.57% (Control bars), 0.97% (2% spirulina), 1.13% (4% spirulina) and 1.36% (6% spirulina) ash content at the end of 90 days of room temperature storage. Decrease in ash content during storage might be due to higher degree of deterioration rate associated with PP films (Padmashree et al., 2018 and Siddique et al., 2018). The storage period had significant effect on the ash content of snack bars at 5% level of significance.

3.1.3 Changes in fat content

Table 4. Effect of storage period on fat content (%) of spirulina snack bars

Sample	0 days	15 days	30 days	45 days	60 days	75 days	90 days
S0	9.15 ±0.79	9.07 ±0.87	9.01 ±0.89	8.93 ±0.88	8.85 ±0.70	8.79 ±1.17	8.74 ±0.71
S1	9.17 ±0.93	9.08 ±0.97	9.02 ±1.01	8.94 ±1.06	8.86 ±1.17	8.80 ±0.71	8.76 ±0.94
S2	9.19 ±1.00	9.11 ±0.82	9.05 ±1.04	8.97 ±1.16	8.89 ±1.20	8.83 ±1.01	8.77 ±1.00
S3	9.21 ±1.00	9.14 ±0.81	9.08 ±0.96	9.01 ±0.93	8.92 ±1.09	8.84 ±1.00	8.79 ±1.17
Mean	9.18	9.10	9.04	8.96	8.88	8.82	8.76

The changes in fat content of stored snack bars tabulated in Table 5 indicated gradual decrease in fat content with advancement of storage period. The fat content in freshly prepared was recorded in the range between 9.15-9.21%. During room temperature storage, fat content in the snack bars was decreased to 8.74-8.79% at the end of 90 days of storage. The decline in fat content during storage may be due to degradation products of hydroperoxide which is directly related with moisture content of product (Mridula et al., 2011, Siddique et al., 2018 and Padmashree et al., 2018). The storage period had non-significant effect on the fat content of snack bars at 5% level of significance

3.1.4 Changes in protein content

Table 5. Effect of storage period on protein content (%) of spirulina snack bars

Sample	0 days	15 days	30 days	45 days	60 days	75 days	90 days
S0	12.72±0.83	12.50±0.96	12.33±0.95	12.18±0.87	12.03±0.89	11.88±0.81	11.71±0.70
S1	13.75±0.77	13.53±0.99	13.36±0.82	13.21±0.75	13.06±0.73	12.91±0.73	12.75±0.61
S2	14.78±0.96	14.57±1.01	14.43±0.81	14.28±0.76	14.14±0.68	13.98±0.61	13.81±0.66
S3	15.83±0.88	15.61±1.03	15.44±0.88	15.29±0.79	15.14±0.79	15.00±0.63	14.82±0.54
Mean	14.27	14.05	13.89	13.74	13.59	13.44	13.27

The Table 6 showed gradual decrease in protein content of snack bars throughout the storage period. The highest protein content (15.83%) was recorded in freshly prepared snack bars with 6% spirulina followed by 4% spirulina (14.78%) and 2% spirulina (13.75%). Whereas the lowest protein content (12.72%) was observed in control bars without spirulina. During room temperature storage, protein content in snack bars showed declining trend and found to be decreased to 14.82% (6% spirulina), 13.81% (4% spirulina), 12.75% (2% spirulina) and 11.71% (control) at the end of 90th day of storage. Decrease in protein content during storage might be due the lipolytic and proteolytic activities related to higher moisture content leading to loss in protein content (Butt et al., 2003 and Siddique et al., 2018). The similar decrease in protein content during storage was reported by (Siddque et al., 2018). The storage period had significant effect on the protein content of snack bars at 5% level of significance.

3.1.5 Changes in fiber content

Table 6. Effect of storage period on fiber content (%) of spirulina snack bars

Sample	0 days	15 days	30 days	45 days	60 days	75 days	90 days
S0	3.06 ±0.04	3.02 ±0.04	2.97 ±0.06	2.94 ±0.03	2.92 ±0.05	2.91 ±0.05	2.90 ±0.04
S1	2.94 ±0.03	2.88 ±0.03	2.83 ±0.04	2.80 ±0.06	2.78 ±0.04	2.77 ±0.06	2.76 ±0.03
S2	2.80 ±0.01	2.75 ±0.04	2.70 ±0.03	2.67 ±0.02	2.65 ±0.05	2.64 ±0.04	2.63 ±0.04
S3	2.67 ±0.02	2.62 ±0.05	2.58 ±0.01	2.55 ±0.04	2.53 ±0.06	2.52 ±0.03	2.51 ±0.03
Mean	2.87	2.82	2.77	2.74	2.72	2.71	2.70

Changes in fiber content of stored snack bars depicted in Table 7 indicated significant effect of storage period on fiber content of snack bars. The fiber content in the freshly prepared snack bars was found to be in the range between 2.67 and 3.06%. During room temperature storage, fiber content in snack bars was decreased to 2.51 and 2.90% at the end of 90th day of storage. The decrease in fiber content during storage might be due to to higher degree of deterioration rate associated with PP films (Padmashree et al., 2018). The findings are in close conformity with the results reported by (Siddique et al., 2018). The storage period had significant effect on the fiber content of snack bars at 5% level of significance.

3.1.6 Changes in total phenolic content

Table 7. Effect of storage period on total phenolic content (mg GAE/g) of spirulina snack bars

Sample	0 days	15 days	30 days	45 days	60 days	75 days	90 days
S0	1.07 ±0.07	0.94 ±0.06	0.81 ±0.04	0.71 ±0.03	0.50 ±0.05	0.34 ±0.05	0.16 ±0.02
S1	3.02 ±0.08	2.75 ±0.04	2.50 ±0.06	2.36 ±0.04	1.97 ±0.03	1.53 ±0.09	1.09 ±0.05
S2	5.12 ±0.05	4.82 ±0.03	4.67 ±0.05	4.49 ±0.06	4.05 ±0.04	3.60 ±0.08	3.19 ±0.04
S3	7.20 ±0.05	6.88 ±0.04	6.62 ±0.04	6.47 ±0.05	5.98 ±0.07	5.57 ±0.09	5.03 ±0.06
Mean	4.10	3.85	3.65	3.51	3.13	2.76	2.37

The changes in total phenolic content of stored bars tabulated in Table 8 indicated significant effect of storage period on total phenol content of snack bars. The total phenolic content of freshly prepared snack bars was found to be increased with increase in spirulina level and recorded as 1.07mg GAE/g, 3.02mg GAE/g, 5.12mg GAE/g and 7.20mg GAE/g for snack bars containing 0%, 2%, 4% and 6%, spirulina respectively. Noticeable decrease in total phenolic content was seen in stored snack bars and recorded as 0.16mg GAE/g, 1.09mg GAE/g, 3.19mg GAE/g and 5.03mg GAE/g in snack bars containing 0%, 2%, 4% and 6%, spirulina respectively at the end of 90th day of storage. The higher phenolic content in spirulina variants is associated with more phenolic content of spirulina powder (Kumar et al., 2018). Carvalho and Silva (2018) and Siddique et al. (2018) reported decrease in total phenolic compounds in stored bars throughout the storage period. The storage period had significant effect on the total phenolic content of snack bars at 5% level of significance.

3.1.7 Changes in antioxidant activity

Table 8. Effect of storage period on antioxidant activity (%) of spirulina snack bars

Sample	0 days	15 days	30 days	45 days	60 days	75 days	90 days
S0	9.33 ±0.72	8.05 ±0.51	7.12 ±0.14	6.32 ±0.22	4.83 ±0.42	3.66 ±0.31	2.33 ±0.19
S1	20.16±0.44	18.15±0.43	16.10±0.23	14.04±0.38	11.83±0.21	8.85 ±0.49	5.99 ±0.50
S2	33.33±0.82	31.24±0.33	29.30±0.22	27.37±0.55	24.91±0.43	22.14±0.24	20.00±0.32
S3	46.61±0.98	44.12±0.21	42.18±0.23	40.09±0.55	37.92±0.32	35.02±0.75	32.81±0.28
Mean	27.36	25.39	23.68	21.96	19.87	17.42	15.28

The antioxidant potential of stored snack bars was assessed by % DPPH inhibition as free radical scavenging activity and findings are recorded in Table 9. The antioxidant potential of freshly prepared snack bars was found to be increased with increase in spirulina level and recorded as 9.33%, 20.16%, 33.33%, and 46.61% for snack bars containing 0%, 2%, 4% and 6%, spirulina respectively. The antioxidant activity showed declining trend in all snack bars throughout the storage period and recorded 2.33% (Control bars), 5.99% (2% spirulina), 20.00% (4% spirulina) and 32.81% (6% spirulina) at the end of 90 days of room temperature storage. The better the antioxidant activity is associated with high amount of phenolics (Kumar et al., 2018). Carvalho and Silva (2018) and Siddique et al. (2018) reported similar decrease in antioxidant potential of stored bars throughout the storage period. The storage period had significant effect on the antioxidant potential of snack bars at 5% level of significance.

3.1.8 Changes in hardness of stored snack bars

Table 9. Effect of storage period on hardness (g.sec) of spirulina snack bars

Sample	0 days	15 days	30 days	45 days	60 days	75 days	90 days
S0	3630.33 ±1.52	3656.67 ±1.52	3668.00 ±1.00	3688.33 ±2.08	3714.33 ±1.52	3723.00 ±2.00	3729.00 ±1.00
S1	3628.33 ±1.52	3652.00 ±2.0	3666.67 ±2.51	3686.67 ±1.52	3710.33 ±1.52	3716.00 ±2.00	3723.00 ±2.00
S2	3625.00 ±2.0	3648.67 ±1.52	3665.33 ±3.51	3684.67 ±2.08	3706.33 ±2.08	3714.33 ±1.52	3720.00 ±1.00
S3	3621.67 ±2.51	3642.67 ±2.51	3662.67 ±1.52	3681.33 ±2.30	3703.00 ±2.00	3712.00 ±1.00	3718.67 ±1.52
Mean	3626.33	3650	3665.67	3685.25	3708.50	3716.33	3722.67

The changes in hardness of stored snack bars tabulated in Table 10 indicated gradual increase in hardness with advancement of storage period. The hardness of freshly prepared snack bars was recorded in the range between 3621.67-3630.33g.sec. During room temperature storage, hardness of snack bars was increased to 3718-3729g.sec at the end of 90 days of storage. Increase in hardness during storage might be due to thiol–disulphide interchange reactions during storage which lead to protein cross linking aggregation and network formation (Silva et al., 2013). The hard texture development in protein rich bar may also be due to the migration of moisture as well as formation of most ordered secondary structure and lower surface hydrophobicity of protein particles (Padmashree et al., 2012). The storage period had non-significant effect on the hardness of snack bars at 5% level of significance.

3.2 Organoleptic characteristics of stored snack bars

3.2.1 Colour and appearance

The colour and appearance score of fresh bar was found in the range between 7.1-7.5. Throughout the storage period there is no marked difference in colour and appearance score of snack bars. The highest colour and appearance score (7.4) was observed with control snack bars followed by 4% spirulina bars (7.3) after 90th day of storage. Whereas, 6% spirulina snack bars showed lowest colour and appearance score (6.9) at the end of 90th day of storage. The storage period had significant effect on the colour and appearance score of snack bars at 5% level of significance.

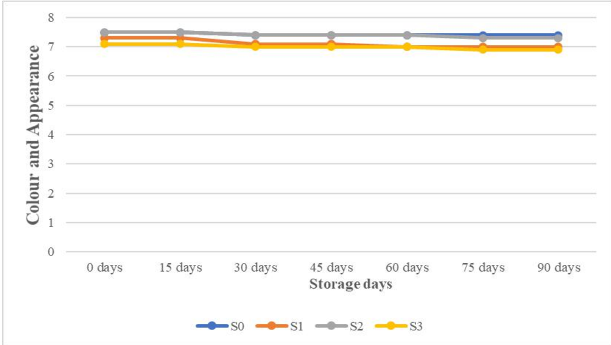

Fig. 1 Changes in colour and appearance of stored spirulina snack bars

3.2.2 Flavour

The flavour score of fresh snack bars was found in the range between 6.9 and 7.4. Throughout the storage period there is no marked difference in flavour score of snack bars. The highest flavour score (7.0) was observed control snack bars followed by bars with 4% spirulina (6.9) after 90th day of storage. Whereas, snack bars with 2% and 6% spirulina exhibited lowest score (6.4) and (6.0) at the end of 90th day of storage. The storage period had significant effect on the flavour score of snack bars at 5% level of significance.

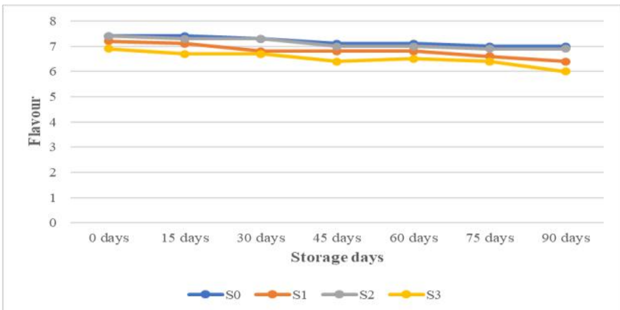

Fig. 2 Changes in flavour of stored spirulina snack bars

3.2.3 Taste

The data tabulated in Table 13 reflects on the effect of storage on average taste score of developed snack bars. The taste score of fresh snack bars was found in the range between 6.9 and 7.5. The snack bars with 4% spirulina and without spirulina were observed to be with higher taste score (7.1) as compared to 2% and 6% spirulina snack bars which depicted lower scores (6.5 and 6.3) at the end of the 90 days of storage. The storage period had non-significant effect on the taste score of snack bars at 5% level of significance.

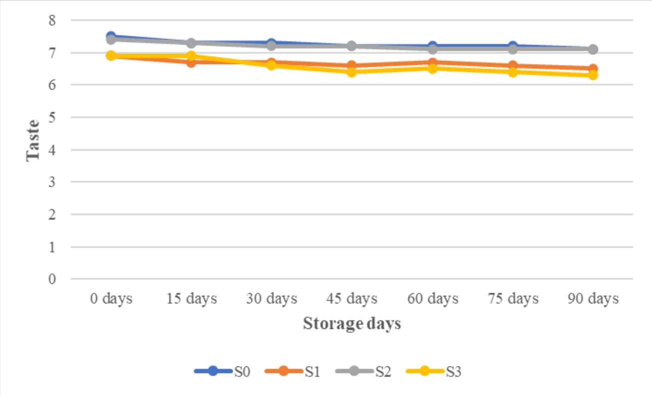

Fig. 3 Changes in taste of stored spirulina snack bars

3.2.4 Texture

The Table 14 represents changes in texture score of snack bars during storage. The texture score of fresh snack bars was recorded as 7.1-7.5. At the end of 90 days of storage, snack bars with 0% and 4% spirulina recorded higher texture score (7.1) followed by bars with 2% spirulina (7.0). The lowest texture score (6.7) was observed with 6% spirulina snack bars. The storage period had significant effect on the texture score of snack bars at 5% level of significance.

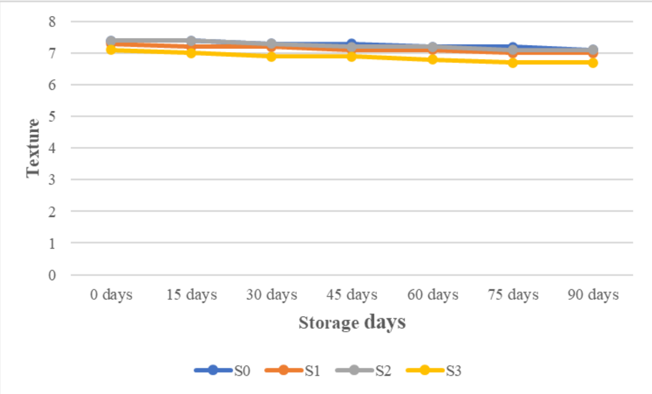

Fig. 4 Changes in texture of stored spirulina snack bars

3.2.5 Overall acceptability

The data tabulated in Table 15 represents effect of storage period on overall acceptability of snack bars. The overall acceptability score of fresh snack bars was recorded as 7.0, 7.3, 7.5 and 7.6 respectively. It was found to be decreasing throughout the storage period in all snack bar samples. The highest overall acceptability (7.4) was recorded with snack bars containing 4% spirulina followed by control bars (7.3) at the end of 90 days of storage. Whereas the lowest overall acceptability was observed with snack bars containing 6% spirulina (6.8). The storage period had significant effect on overall acceptability of snack bars at 5% level of significance. The snack bars with 4% spirulina justified its suitability on the basis of highest organoleptic score for colour and appearance, taste, flavour and texture and overall acceptability throughout the storage period of 90 days.

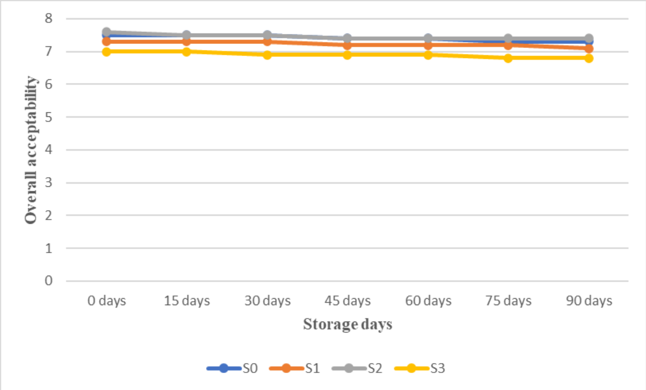

Fig. 5 Changes in texture of stored spirulina snack bars

IV. CONCLUSION

In the study, protein rich snacks bars were developed by replacing oats with spirulina by 2%, 4% and 6% and effect of room temperature storage on nutritional, functional, sensorial and microbial quality was assessed for 90 days. The ash, fat, protein, crude fibers, carbohydrates, total phenolic content and DPPH free radical scavenging activity of snack bar was decreasing whereas moisture content was increasing throughout the storage period of 90 days. Organoleptic characteristic viz., colour and appearance, flavour, taste, texture, and overall acceptability were decreasing during storage of snack bars.The total plate count and yeast and mould counts were not detected up to 30 days of storage, thereafter increasing trend was observed from 45th day to 90th day of storage at room temperature. The protein rich snack bars could be stored for the period of 90 days in polypropylene pouch at room temperature.

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