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RESEARCH PAPER

Storage and packaging of roasted quality protein maize (QPM) bar: physical, chemical and microbial studies

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Abstract Microbial safety of cereals products is essential due to its extensive utilization as staple food for humans. Aiming at increased shelf-life of staples, nutritious bar was developed using roasted QPM and assessed for its shelf-life stability in view of different packaging materials (aluminum (AL) and High-density polyethylene (HDPE)) at diverse storage intervals *i.e.* 0, 30, 60, 90, and 120 days, respectively. For this, physical, chemical and microbial analyses were performed. The stored bar was 'liked moderately' till 60 days and 'liked slightly' during the storage period of 90 and 120 days, in both AL and HDPE pouches. The moisture content of the bar was increased by 12.82 per cent and 11.17 per cent in AL and HDPE pouches, correspondingly which affected bar's texture. The value of browning index (OD) was significantly ($p> 0.001$) declined at 30th and 120 days. The free fatty acid content (% oleic acid) was different in HDPE and AL packaged bars; however, this difference was non-significant at 5% level. The microbial load was within the safest limits in the bar stored in HDPE pouches for 120 days and in AL pouches for 90 days, respectively. The bar stored in HDPE pouches was highly acceptable and shelf-stable till four months at refrigerated conditions.

Keywords: Roasted QPM bar • Physical • Chemical composition • Microbial load • Shelf-life

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Introduction

Maize (*Zea mays*), having a massive amount of carbohydrates, fats, proteins, and some important vitamins and minerals, makes it a major cereal for human nutrition worldwide. It is a staple food for more than 200 million people and provides 15 and 20 percent of protein and calories, respectively, of daily diet (Subbulakshmi and Amutha, 2013). The United States, Argentina, Brazil, France, India, Indonesia, Italy, Mexico, and South Africa have a 79 per cent share in the world's maize production. Only 30-40 per cent of total maize produced is used for human consumption, whereas the majority (60-70%) of the produce is utilized as livestock feed domestically (Gwirtz and Garcia-Casal, 2014).

Cereals, including wheat and maize, can be contaminated with mycotoxin-producing fungi and pathogenic bacteria during crop growth, pre-harvesting, post-harvesting, drying, transportation, and storage. Contamination has an adverse effect on cereal yield, its quality, and the nutritional value of the produced flour as well. Simultaneously, mold growth is the most common cause of microbial spoilage and deterioration in the quality of cereal grain and flour during storage. Generally, the moisture content of maize flour ranges from 11 to 14 per cent, but the stipulated limit is 15 per cent. Above this limit, flour is susceptible to microbial attack, which causes food poisoning and off-flavors. Though wheat and maize grains are subjected to vigorous cleaning processes, not all microorganisms and toxins are removed from the final product. Because microorganisms penetrate into the kernel of the grain during crop's growth and likewise, remain in the flour during storage. Hence, the storage conditions after milling and packaging are very important to retain the quality of the flour since these affect the shelf life of the product and safety of the consumer (Victor *et al*., 2013).

The safety of cereals and their products is very important since their consumption frequency is highest among all food groups each day in humans. Bacteria commonly contaminate the exterior part of grains, and there are several aiding factors (pH, moisture, etc.) for it to grow in cereal grains. The pH value of maize is approximately near to neutral, which is considered efficacious for the growth of bacteria. In addition, the rise in the moisture content along with the increase in relative humidity is also responsible for the bacterial growth in cereals. In general, dry cereal grains are not significantly spoiled by bacteria. Their growth is merely possible in processed or cooked cereals. The economic and climatic factors broadly affect the storage of cereals (ICMSF, 2011). The packaging of cereal products remains important since the physico-chemical properties are dependent on the type of packaging. It will also control the microbial growth (Shobha *et al*., 2014).

Maize is administered into a lot of foodstuffs, viz., breakfast cereals, coarse flour, flour, porridge, snacks, arrowroot, and unleavened flatbreads (Shah *et al.,* 2016). Maize is the third most important cereal after wheat and rice and forms a substantial fraction of human food (25%), animal feed (12%), poultry feed (49%), and industrial products such as starch (12%), along with brewery (1%) and seed production (1%), respectively (Anonymous, 2015). It has been observed that products developed from processed flour have increased shelf life and higher starch and protein digestibility than the products formulated from unprocessed flour (Nutan, 2015). Quality protein maize has been upgraded by means of conventional breeding techniques; therefore, it is said to be a bio-fortified food crop (Sumbo and Victor, 2014). The quality of protein is also improved in QPM, which is considered as an alternative food to alleviate protein deficiency in developing countries. Therefore, there was a thought to develop a roasted QPM nutritious bar aimed at studying its physical, chemical, and microbial properties in view of different packaging materials at diverse storage intervals.

Material and methods

Development of quality protein maize-based bars

Descriptive procedure for development of roasted QPM bar has been presented in Figure 1 (a & b). Roasted QPM, cauliflower leaves, carrots and jaggery syrup were blended as 41, 2, 5 and 52 per cent, in that order to develop nutritious bar.

Packaging of roasted QPM bar

Packaging of developed roasted QPM bar was carried out using two types of packaging material *i.e.* aluminum (AL) and high-density polyethylene (HDPE) pouches. Packaged products were stored in refrigerated conditions for four months to ascertain their shelf-life.

Analysis of roasted QPM bar

The fresh and stored bars were analyzed for their physical *i.e.* physical characteristics; chemical *i.e.* moisture, free fatty acids, browning index and microbial growth *i.e.* bacterial and fungal count, continuously for the period of 120 days, at interval of 30 days.

Physical properties

Physical characteristics such as appearance, colour, flavor, taste, texture and overall acceptability of fresh and stored bars were evaluated by a panel of 10 semi trained judges using hedonic scale test (eight-point rating) (Ranganna 2008).

Chemical analysis

Moisture

Moisture analysis was done using the standardized methods (Nagi *et al.,* 2012).

$$\text{Moisture (\%)} = \frac{\text{Wt. of sample before drying (g)} - \text{Wt. of sample after drying (g)}}{\text{Weight of sample taken (g)}}$$

Free fatty acid in terms of oleic acid (% by weight)

Free fatty acids were extracted by following the standard method (Anonymous, 2001). Elaborately, one gram sample of each fresh and stored bar was taken in conical flasks (250 ml), after adding benzene (50 ml) into it. The mixture was kept aside for 30 minutes at room temperature. Accurately 5 ml of extracted aliquot was taken in triplicate in conical flasks followed by the addition of benzene (5 ml), ethanol (10 ml) and two drops of indicator

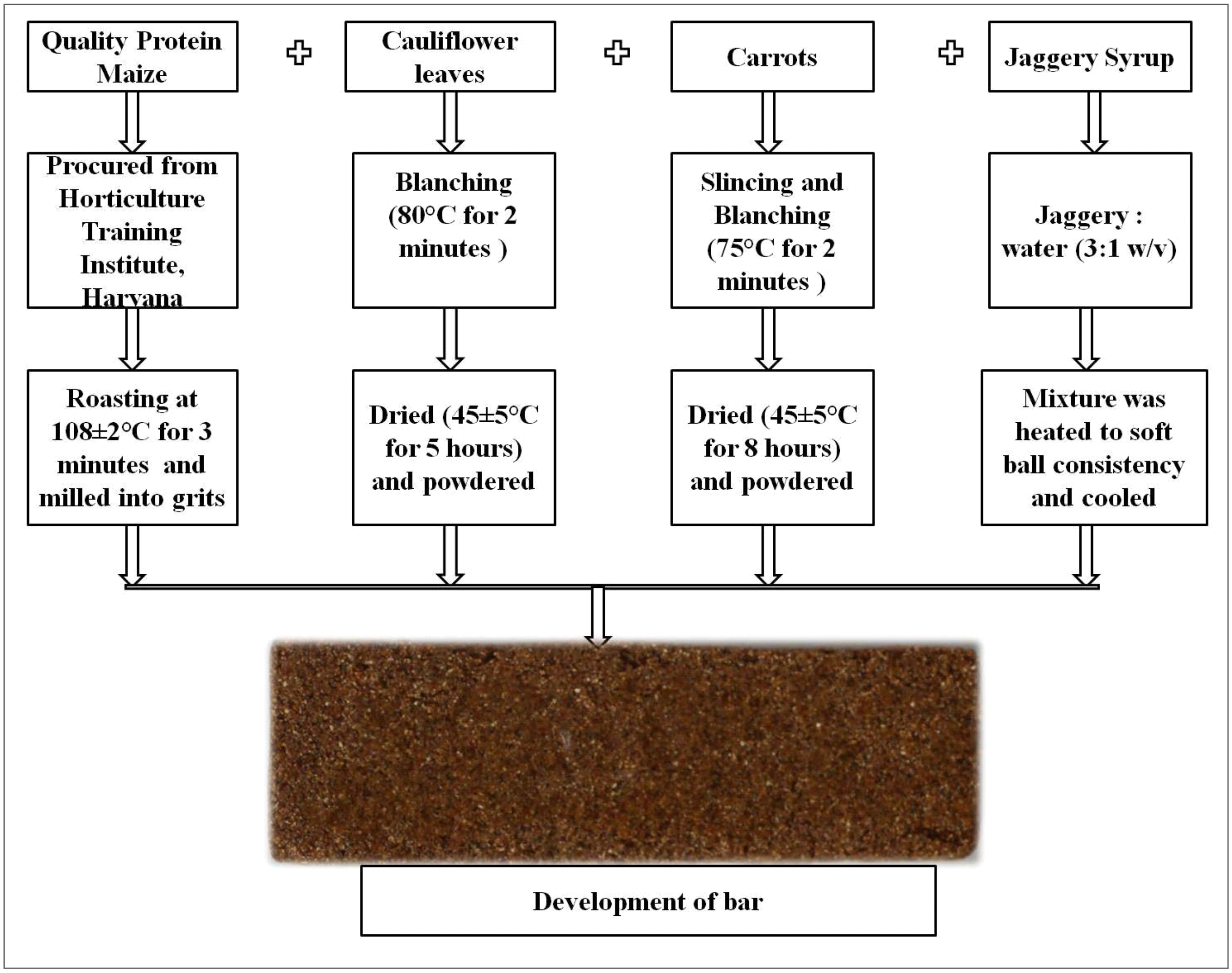

Figure 1 (a). Development of roasted QPM bar

Figure 1 (b). Appearance of roasted QPM bar

phenolphthalein step by step. Afterwards, titration was done using solution of KOH (0.02 N) till a dark pink color appeared.

Calculation

$$\text{FFA (\% oleic acid)} = \frac{28.2 \text{ (Volume of 0.02 N KOH used x Normality of KOH}}{\text{Weight of sample (g)}}$$

Browning index (OD)

Four-gram sample of each fresh and stored bar was taken in conical flasks (250 ml) and after adding distilled water (50 ml), these were kept on mechanical shaker for two hours. Then 5 ml of lead acetate solution (45%) was added followed by shaking for another five minutes and filtration was done using Whatman no. 1 filter paper. The absorbance of cleared filtered extract was read at 420 nm using spectrophotometer (Model LMSP UV 1200) (Padmashree *et al.,* 2013).

Microbial quality

Procedures for media preparation are discussed in Table 1 (Sanders, 2012).

Preparation of dilution blank and media

Distilled water (9 ml) was taken in two different autoclaved test tubes for the preparation of dilution blank. All the weighed ingredients for preparation of media (nutrient agar and glucose yeast extract) except agar agar were taken in volumetric flasks (1000 ml) and dissolved.

Table 1. Preparation of media

Nutrient agar		Glucose yeast extract agar	
Ingredients	Quantity (g)	Ingredients	Quantity (g)
Beef extract	1.5	Yeast extract	2.5
Peptone	2.5	Peptone	2.5
Sodium chloride	2.5	Sodium chloride	5
Agar agar	10	Agar agar	10
Distilled water	500 ml	Distilled water	500 ml

Source: Sanders 2012

Afterwards, agar agar was added and shaken gently. Then, we autoclaved the media and test tubes containing dilution blank for 20 minutes at 15 psi (121°C).

Total bacterial count

Pour plate method was performed for the estimation of bacterial count in the fresh and stored bars. One gram of sample was taken with autoclaved spatula and added it in the autoclaved test tubes containing 9 ml of dilution blank (10^{-1}) by mixing thoroughly. Further, the dilutions such as 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were made. For the preparation of plates, one ml of inoculum was added in the each plate followed by the immediate addition of cooled semi solidified nutrient agar media. The inverted plates were kept in incubator for 48 hours at 37°C and total bacterial count (cfu/ g) was recorded.

Total fungal (yeast and mold) count

Similarly, dilutions were prepared till 10^{-4} and one ml of inoculum from broth was added in each petri dish followed by immediate addition of cooled molten glucose yeast extract media. The inverted plates were kept in incubator for 5-7 days at 25°C and total count of yeast and mold growth (cfu/g) was noted.

Calculation

$$\text{CUF/g} = \text{CFU x dilution factor x } \frac{1}{\text{Aliquot taken}}$$

Statistical analysis

The data collected was subjected to Software IBM SPSS Inc. (version 16.0, 2007) to obtain significant ($p \leq 0.05$, 0.01; $p \geq 0.05$, 0.01) difference between different types of packaging material in terms of physical and chemical properties of the stored bar applying chi-square and independent samples t-test, respectively.

Results

Effect of storage on roasted QPM bar

Physical and chemical interaction between ingredients used in bars is observed, which initiates the change in texture and taste of the bars with increasing storage time. The use of an appropriate packaging material along with a suitable storage environment is beneficial to maintain the product's stability, which can enhance the shelf life of nutritious bars (Samuel and Nazni, 2020).

Physical characteristics

Texture plays an important role in acceptance of cereal bars when they are evaluated for physical parameters. The limiting factor of cereal bars was the unbreakable texture developed throughout the storage period, which also affected their shelf-life stability. Level of moisture is also correlated with texture. The taste of the bar was liked slightly by the panel of judges. It might be due to a chemically mixed microenvironment, which leads to a range of physico-chemical reactions (Maity *et al.,* 2016). During our study, physical scores for taste were significantly ($p \leq 0.05$) higher in the cereal bar stored in HDPE (7.80) as compared to the bar stored in AL (7.05) when evaluated on the 30th day of storage (Table 2). During the storage period of 120 days, little difference was observed in color, appearance, and aroma. A non-significant ($p \geq 0.05$) difference was observed in overall acceptability scores of differently packaged bars. The overall scores of HDPE and AL stored bars were 7.66 and 7.24, 7.38 and 7.29, 6.42 and 6.22, and 6.12 and 6.00 after 30, 60, 90, and 120 days, respectively, which showed that the roasted QPM bar was acceptable throughout the storage period. HDPE-stored bars got higher scores as compared to their AL counterparts, but with a non-significant ($p \geq 0.05$) difference between both types of packaging materials.

Chemical analysis

The cereal bars using defatted soy flour indicated that there was no significant effect on free fatty acid during the

Table 2. Effect of storage and packaging on physical properties of bar

Packaging material	Storage periods (days)	Color	Appearance	Aroma	Texture	Taste	Overall acceptability
Fresh	0	7.60±0.26	7.40±0.26	7.30±0.30	7.00±0.42	7.30±0.39	7.32±0.26
AL	30	7.60±0.22	7.20±0.24	7.05±0.33	7.30±0.33	7.05±0.26	7.24±0.24
HDPE		7.90±0.17	7.40±0.22	7.50±0.22	7.70±0.15	7.80±0.13	7.66±0.15
χ^2-value		1.31^{NS}	0.33^{NS}	0.95^{NS}	0.51^{NS}	5.10**	1.31^{NS}
AL	60	7.60±0.16	7.40±0.26	7.45±0.15	7.00±0.25	7.00±0.21	7.29±0.16
HDPE		7.60±0.26	7.20±0.20	7.30±0.30	7.30±0.21	7.50±0.34	7.38±0.23
χ^2-value		0.01^{NS}	0.67^{NS}	0.00^{NS}	0.73^{NS}	2.63^{NS}	0.47^{NS}
AL	90	6.60±0.37	6.10±0.27	6.30±0.39	6.30±0.33	5.80±0.32	6.22±0.30
HDPE		6.70±0.26	6.40±0.16	6.40±0.37	6.40±0.26	6.20±0.44	6.42±0.26
χ^2-value		0.01^{NS}	0.49^{NS}	0.14^{NS}	0.002^{NS}	0.31^{NS}	0.09^{NS}
AL	120	6.40±0.37	5.90±0.34	6.10±0.48	6.00±0.42	5.50±0.40	6.00±0.37
HDPE		6.30±0.30	6.20±0.32	6.40±0.40	6.30±0.30	5.40±0.40	6.12±0.31
χ^2-value		0.22^{NS}	0.26^{NS}	0.22^{NS}	0.15^{NS}	0.01^{NS}	0.07^{NS}

AL: Aluminum; HDPE: High density polyethylene; Values are mean ± standard error; Mean values represented scores (8: liked very much; 7: liked moderately; 6: liked slightly; 5: Neither liked nor disliked); ** Significant at 5%; NS: Non-significant

storage period of 90 days (Yadav and Bhatnagar, 2016). Brown flour-based energy bars enriched with fiber and protein remained stable in terms of chemical characteristics during a storage period of three months (Rawat and Darappa, 2015).

Moisture

Initially, the moisture content in the fresh bar was 14.59 per cent (Table 3). However, it showed a non-significant ($p \geq 0.05$) increase in moisture content during the storage period of 30, 60, and 90 days, correspondingly, in both the packaging materials. A significant ($p \leq 0.05$) increase was observed at the end of the fourth month. Higher moisture content was also observed in the maize bar, with the range between 14.59 and 16.46 per cent on storage till 120 days.

Free fatty acids (% oleic acid)

Free fatty acids are formed in the foods due to fat hydrolysis taking place in them. It may also be escalated by moisture, oxidation in the air, and storage of food at high temperature. The effect of packaging on the free fatty acid (%) (oleic acid) content of the stored bar has been presented in Table 3. During the storage period of 120 days, the value of free fatty acid ranged from 0.48 to 1.18 in bars stored in different types of packaging materials. An increase was observed in free fatty acid composition throughout the study. However, a comparatively higher increase was reported in bars stored in AL pouches than HDPE pouches, but the difference was non-significant at the 5 per cent level of significance.

Browning index

Table 3 summarizes the findings related to the effect of packaging on the browning index (OD) of stored roasted QPM bars. The browning index (OD) was 0.36 in a fresh sample of bar. This value was significantly ($p \leq 0.01$) decreased to 0.26 in the bar stored in the AL bag, whereas it was recorded as 0.24 in the HDPE bag at the 120th day of storage.

Microbial quality

Table 4 depicts the microbial quality of stored QPM bars, assessed for total bacterial and fungal count for 120 days at 30-day intervals. A fresh sample of the bar contained no bacteria. At the end of one month, the count of bacteria was 2 x 105 cfu/g in the AL-packaged bar, which was higher than the bar stored in HDPE pouches (1 x 105 cfu/g). After that, no bacterial growth was reported in bars stored in AL and HDPE pouches on the 90th day. Furthermore, bars stored in AL pouches contained 15 x 105 cfu/g bacteria on the 120th day of their storage (Figure

Table 3. Effect of storage and packaging on chemical properties of bar

Packaging material	Storage periods (days)	Moisture (%)	Free fatty acid (% oleic acid)	Browning index (OD)
-	0	14.59±0.07	0.48±0.04	0.36±0.01
AL	30	14.65±0.83	0.58±0.06	0.34±0.00
HDPE		14.48±0.20	0.46±0.04	0.33±0.00
t-value		0.19 ^{NS}	1.47 ^{NS}	10.69*
AL	60	14.89±0.82	0.59±0.08	0.32±0.00
HDPE		14.72±0.06	0.48±0.12	0.31±0.00
t-value		0.19^{NS}	0.74 ^{NS}	0.46^{NS}
AL	90	15.47±0.12	0.68±0.18	0.29±0.01
HDPE		15.18±0.18	0.63±0.07	0.28±0.01
t-value		1.28^{NS}	0.25 ^{NS}	0.57 ^{NS}
AL	120	16.46±0.09	1.18±0.11	0.26±0.00
HDPE		16.22±0.02	0.97±0.08	0.24±0.00
t-value		2.29***	1.51 ^{NS}	10.25*

AL: Aluminum; HDPE: High density polyethylene; Values are mean ± standard error; *Significant at 1%; ***Significant at 10%; NS: Non-significant

Table 4. Effect of storage and packaging on microbial quality of bar (cfu/g)

Packaging material	Storage periods (days)	Total bacteria count (cfu/g)	Total fungal (yeast and mold) count (cfu/g)
Aluminum bags	0	0	0
	30	2 x 10^5	0
	60	0	0
	90	0	0
	120	15 x 10^5	0
HDPE bags	0	0	0
	30	1 x 10^5	0
	60	0	0
	90	0	0
	120	2 x 10^5	0

Bar I: maize; Bar II: maize + green gram; Bar III: maize + chick pea
AL: Aluminum; HDPE: High density polyethylene

2). This growth was the highest when compared to the HDPE stored bar, which showed the presence of 2 x 105 cfu/g bacteria.

Discussion

Storage studies of cereal bars developed using wheat flour and rice flakes revealed that they were acceptable only up to the period of 90 days with respect to all the physical parameters (Yadav and Bhatnagar, 2016). Furthermore, the color, appearance, aroma, texture, taste, and overall acceptability showed no significant difference at the 60th day of the storage period (Verma *et al.,* 2018). Nonetheless, the current study attests that the developed bar was acceptable up to 120 days with no significant ($p \geq 0.01$) difference in its physical characteristics at different storage intervals.

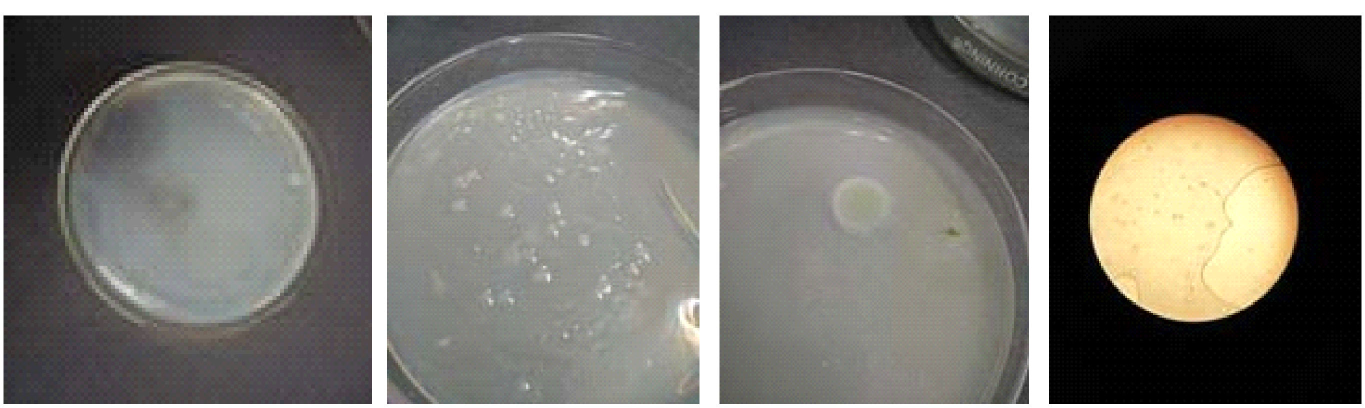

Figure 2. Microbiological assessment of Roasted QPM bar

Rigid texture may limit the shelf life and digestibility of energy– and protein-rich bars. A rigid texture of the stored bar might be developed due to the interactions between thio-disulphide, which leads to aggregation between cross linkages of protein and formation of a network Another reason might be the movement of moisture and development of mainly planned secondary structure and lower surface hydrophobicity of protein particles (Simon *et al.,* 2009). The hardening of protein bars is dependent on Maillard reactions occurring between reducing sugars, and also, an important role of reactive lysine residue is there (Gerard 2002). Padmashree *et al.* (2012) observed the highest overall physical scores (7.9) for bars at the initial storage stage. After that, it was liked moderately for six months. The flaxseed– and oat-based bar was also prepared and found acceptable for twelve months due to the addition of oilseeds. Simultaneously, in another study, a cassava flour-based bar received physical acceptability only till the end of one month (Silva *et al.,* 2013). In our study, the roasted QPM bar was liked very much at the initial stage and it was liked moderately on the 30th day of its storage. Results reported in the above-mentioned investigations are in agreement with Verma *et al.* (2018). In contrast to the present research findings, a millet-based sports bar showed a gradual reduction in appearance (8.0 to 3.7), color (8.2 to 2.3), flavor (8.7 to 1.9), texture (8.7 to 3.9), taste (8.0 to 1.4), and overall scores (38.3 to 13.1) at the 90th day (Sobana, 2017). Furthermore, the overall acceptability scores of the beet root bar were reduced but acceptable for consumption during a storage period of six months (Maity *et al.,* 2016).

Lower moisture content (8.2 to 9.3%) was reported in bars till the 60t60th day that were prepared using butter instead of water (Siddique *et al.,* 2018). Aigster *et al.* (2011) also found that a moisture content of 15.1 per cent produces a harder texture of bar during storage. Conversely, the fresh bar had a harder texture as compared to stored samples in our study. The increments in moisture percentage were higher in AL pouches than HDPE pouches. Hence, it can be scrutinized that higher moisture content affects the texture of bars.

A chemical change occurred in the form of free fatty acid production during the storage period of the developed product under present investigation. Fungal spoilage in cereals was measured, formed due to the activity of mold lipase on lipid glycerides and in the dearth of fungi. An increase in storage time and temperature, moisture percentage, and attack by fungi leads to free fatty acid augmentation during storage of grains (ICMSF, 1998). A gradual increase in free fatty acid content of QPM-based convenience food was also reported, and it is stated that the values may vary from 0 to 4.55 till six months of storage (Triwedi, 2008). Similarly, the free fatty acid content of polyethylene stored QPM-based products revealed 0.14 and 0.18 per cent free fatty acid at the initial stage, which was increased to 0.84 and 0.97 per cent on 180 days of storage (Kaur *et al.,* 2018).

As the moisture content increased, bars oxidized as those came in contact with air, and their color changed from dark to light. The value of the browning index (OD) ranged from 0.24 to 0.36, which was comparable to the range (0.15 to 0.44) reported by Padmashree *et al.* (2013). Rafiya (2014) found a non-significant increase (0.14 to 0.15 OD) at the initial stage of storage of the wholesome nutrient bar, but after 30 days, the browning index significantly increased to 0.28 OD till the end of the third month.

An increase in bacterial growth could be due to the presence of high moisture content in the bars. Garg *et al.* (2010) reported that the normal and safe range for total bacteria count was 1,000,000 cfu. It may be concluded that the roasted QPM bar was acceptable for 90 days in AL pouches. On the other hand, HDPE-stored bars were safe to consume up to 120 days. The growth of yeast and mold was not reported during the entire storage period. Total yeast and fungal count were increased from 3.4 to 4.6 and 3.45 to 3.62 in energy-rich bars after the storage period of three months (Mridula *et al.,* 2013). There was no microbial growth reported until the storage period of 45 days in a millet-based bar during a study (Sobana, 2017). It was also reported that after the period of 60 days, the aerobic bacterial growth was there, and the yeast, mold, and other bacterial growth was not observed at the end of 90 days. The shelf life of the cereal bar was stable for three months in polypropylene packaging material under ambient conditions (15 to 34^{o}C). However, the bar remained safe to consume even for the period of six months in different packaging materials such as metalized polyester films and paper-aluminum foil polyethylene laminates (Padmashree *et al.,* 2012).

Conclusions

Conclusively, the findings regarding the shelf life of the roasted QPM bar revealed that the bar was acceptable till the end of the storage period in terms of its physical characteristics. The HDPE stored bar obtained higher physical scores as compared to its AL counterpart. Comparatively, a higher but non-significant increase in free fatty acid content was observed in the AL-packaged bar than in the bar in the HDPE pouch. Microbial analysis showed that the bar contained safe limits of bacteria count till 120 days in HDPE pouches; therefore, it was considered safe for human consumption. No growth of yeast and mold was reported in all the bar samples during the entire storage period. Thus, the present investigation affirmed that the developed roasted QPM bar is recommended for human consumption as fresh and even after its storage for up to 120 days in HDPE pouches.

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