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Isolation and characterization of *moringa oleifera* l. Flower protein and utilization in functional food bars

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Abstract

Moringa the miracle tree is rich source of protein and its protein content are comparable with meat protein. In the current study protein was isolated from moringa flowers and utilized in preparation of bars. Physicochemical analysis like texture, color, caloric value, water activity, pH and sensory characteristics were determined to evaluate the nutritional and quality attributes of bars. Different concentration of isolated protein was used in the preparedness. Among all treatments T_4 (15% protein isolate) and T_3 (10% protein isolate) were best as they had the highest caloric value i.e. 498.33 and 480.37 kcal and high protein percentage 42.55 and 35.29%. T_4 and T_3 also were good in all textural parameters i.e. hardness 4.68 and 4.85 kg, firmness 3.88 and 3.67 kg, toughness 3.78 and 3.63 kg and work of shear 16.84 and 16.80 kg/mm. The obtained results indicated that the incorporation of moringa protein isolate in bars not only increase their aesthetic value but also increase their nutritional profile. High quality protein isolated from moringa is cheaper and easily grown with little care to provide ample supply of moringa protein to peoples which were in concern of money for provision of proteinaceous food to their families to combat malnutrition.

Keywords: protein isolate; energy bars; malnutrition; texture; sensory characteristics

Practical application: In the current study isolation of protein from moringa flower was done and its applications in bars was carried out to prepare energy rich bars. Moringa flower protein can be used in confectionary and snack industry to prepare candies, bars and different bakery items that will not only create variety in the market but also provide highly nutritious food that will help to expand its use in human food.

1 Introduction

Moringa oleifera L. belongs to the family *Moringa*ceae is extensively cultivated in Africa, Asia, Philippines, North Africa and South Asia (Coppin, 2008). It is significant due to its high health impacting proteins, amino acids and phytochemicals (Vergara-Jimenez et al., 2017). Consumers attention towards plant proteins have proved this as primary source of protein in the diet (Mune et al., 2016). Consumers are becoming more health conscious and tending towards natural products because of no side effects that's why they are being used as functional food constituent. The results of different studies revealed that moringa supplementation can provide protein having essential amino acids, carbohydrates, vitamins and minerals like potassium, iron, calcium and phosphorus. Certain ailments also can be mitigated by using moringa based products (Sengev et al., 2013).

Moringa is also called as miracle tree as it can treat and recover about three hundred diseases (Nwakalor et al., 2014).

Niaziminin (thiocarbamate) is bioactive compound in moringa which has chemo preventive and anti-carcinogenic properties, moreover it can also associate with thyroid hormone treatments. Moringa possess various activities like antihypertensive by having a stabilizing effect on blood pressure, anti-inflammatory by showing positive effect on glandular swelling, antidiabetic as it lowers the blood glucose level, anti-microbial in inhibiting *E. coli* growth i.e. (Vergara-Jimenez et al., 2017).

The isolation of protein from different parts of moringa such as from seeds, leaves and flowers has introduced recently. The differences between isolate and the concentrates of a protein is that in isolate the percentage of protein is 85-90% but in the concentrate the percentage is up to 60%. The protein isolate is preferred and contains all the essential amino acids. Among essential amino acids leucine has the highest amount i.e. 6.65% of the total protein. Phenylalanine, valine, lysine and

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isoleucine are also found in higher amounts than threonine, tryptophan and methionine (Aja et al., 2014)

Protein bars have an important role in our life as these are fast and nourishing baked goods. When the hunger pangs hit between meals, or you have just finished a strenuous workout, bars provide satiety and quality protein, that can combat malnutrition and keep motivated to stay on track to accomplish the weight loss goals. The functionality of moringa protein isolate is in competition with the soy protein isolate. Different methods of protein isolation have been used to isolate protein from moringa and utilize it in functional foods and pharmaceutical products as a protein supplement. Value addition of moringa protein isolate in different products as in protein-energy bars, high energy cookies, soups and beverages can be used to relinquish protein energy malnutrition (Jain et al., 2019). The objective of this work was the extraction of proteins from the moringa flower for preparation and nutritional evaluation of food bars.

2 Materials and methods

2.1 Procurement of Material

Moringa flowers were procured from Multan, Pakistan. Flowers were manually cleaned to remove all types of dirt particles then the flowers were set for drying (sun, shade and hot air oven at 106 °C). Dried flowers were milled (Perton Miller 0.5 μ m) to obtain fine powder of moringa flowers. The obtained powder was packed in sealed polyethylene bags at ambient temperature for further analyses. The obtained powder among three different drying methods with highest protein content was subjected for isolation of protein.

2.2 Isolation of protein

Protein isolation was carried out by following the method of Houde et al. (2018) and Wang et al. (1999). Sample was defatted and incorporated in distilled water at ratio of 1:15, after mixing it thoroughly, 2 normal solution of NaOH was introduced in it by drops till pH of the sample become 10. Orbital shaker at speed of 400 rpm was used for mixing for 15 minutes. The obtained sample was put in centrifugation machine after putting them in centrifugation tubes at 6000 rpm speed at 4 °C for half hour. The layers formed after centrifugation were separated from each other i.e. supernatant and residual and kept in different beakers. In the same way obtained 3 supernatants from the residual by repeating the above procedure and mix them all. pH of the obtained supernatants was decreased to 4.5 by addition of 2 normal solution of HCl. The finalized supernatants were centrifuged, and residual remained was isolate of moringa protein. The light pink colored isolate was then stored at -80 °C after adjusting its pH at 7.

2.3 Treatment plan

Functional food bars were developed by using different ratios of moringa isolate keeping all other ingredients (Sugar, white flour, oil and baking powder) according to standard recipe. Treatment plan is given in the Table 1.

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Table 1. Treatment plan for the Moringa flower protein bars.

Treatments	T ₀	T_1	T_2	T ₃	T_4
Moringa flower powder		10%			
Moringa protein Isolate			5%	10%	15%

2.4 Physicochemical analysis of Moringa Bars

Proximate analyses

Proximate analysis (moisture, protein, fat, fiber and ash) of moringa bars were conducted by following the method of AOAC (2016).

Color Measurement:

The color of Moringa protein bars were determined by following the method of Al-Juhaimi et al. (2016) during storage. Minolta Chroma meter was used to determine the color of bars. Colors were expressed in CIE color space (1976) L-a-b, L* stands for lightening, a* stands for Red and Green and b* stands for Yellow.

Texture Analysis

The texture analysis of bars was carried out by the method of Al-Juhaimi et al. (2016). Parameters like hardness (kg), firmness (kg), toughness (kg) and work of shear (kg/mm) were evaluated using a texture analyzer, having a cylindrical probe 8mm in diameter, 36mm in length with speed of 0.5mm/sec, ratio of compression 5% and loading cell of 500N by using texture analyzer (Mod. TA-XT2, stable microsystems, surrey, UK). All tests were carried out at room temperature (20 °C) Using a velocity of 100mm/min. Mean values were determined and compared with that of the literature.

Caloric Value

The caloric values of moringa bars were examined after every seven days by using the Bomb calorimeter following (Heitschmidt, 2015).

Water activity

Water activity of the protein bars were determined at different days interval i.e. 0, 7, 14, 21 and 28 by using a water activity meter (Model Aw-Win, Rotronic, equipped with a Karl-Fast Prob) following the method of Mathlouthi, (2001).

pН

The pH of the bars was determined by following the method of Nadeem et al. (2012). Powdered 5 g sample was taken in flask and 30 mL distilled water was added in it. The sample was mixed thoroughly on orbital shaker at 200 rpm for 10 minutes. Then the pH of the sample was determined by dipping pH meter electrode in the sample.

Sensory Evaluation

Sensory parameters and organoleptic characteristics of bars were evaluated by following the Meilgaard et al. (2007) using 9-point hedonic scale. Sensory panel of the institute was guided to evaluate, flavor, color, mouth feel, texture and overall acceptability.

Statistical analysis

Data was analyzed statistically to check the significance. Two factor factorial design under completely randomized designed was applied to determine the effect of different treatments and storage days (Steel et al., 1997).

3 Results and discussion

The results of proximate analysis of moringa flowers were depicted in Table 2. which revealed that the moisture contents of moringa flowers were in the range of 63.68 to 71.11% which were in corroboration with the previous results of Aremu & Akintola (2014), who obsrved the moisture contents of moringa flowers were 74.18%. The slight difference in observed values was might be due to the change in environmental and varietal differences. The protein, oil, ash and fiber content were in the range of 17.13 to 21.35%, 7.09-8.62, 3.02-4.97 and 2.02-2.61 respectively which were in corroboration with results of Arise et al. (2014), Abbas et al. (2018) and Abdulkadir et al. (2016) whom investigations showed that the following concentrations of protein 25.16%, oil 11.8%, ash 6.01 and fibre 2.1% are present. The differences in different treatments of moringa flower was might be due to different growth conditions, botanical origin and environmental factors. Shade dried T₃ due to higher protein contents (21.35) was selected for further investigation and protein isolation.

Mean values regarding proximate composition of bars are described in Table 3. It can be concluded from the mean Table that in treatment T_2 moisture level was highest 8.33% at the while minimum value was observed in T_0 (6.62%) respectively. The results negotiate that the detected maximum moisture level in T_2 was might be due to contact of sample with air and climate and weather changes (Loveday et al., 2009). Moreover, protein isolate can absorb moisture from atmosphere which may be the cause of increase in moisture content (Loveday et al., 2010).

The highest protein amount (42.55%) observed in T_4 . The least amount of protein was found in T_0 (19.29%). The higher amount of protein content was due the addition of higher amount of protein isolate which. Highest amount of ash (1.73%) was observed in $\mathrm{T_{1}}$ and lowest (0.92%) in $\mathrm{T_{4}}.$ The highest amount of ash in T₁ samples was due to the incorporation of moringa flower dried powder while lowest value was due the addition of protein isolate in the recipe. The results are in line with finding of Nadeem et al. (2012) who found that the ash contents of bars prepared from dates supplemented with protein was 2.3%. The maximum fat value was observed in T₁ (12.70%) and least amount of fat was observed in control (T_0) i.e. 9.75%. The rest of the treatments had 10.10,10.09-10.08 & 10.05 $(T_2 T_3 \& T_4)$ fat ranges, respectively. The high amount of fat in T₁ was due to the presence of moringa flower powder which is the source of fat. The results are in corroboration with the findings of Pallavi et al. (2015) and Rao et al. (2016).

Mean values regarding fiber content of bars were depicted in Table 4. From the mean Table it can be observed that maximum fiber content was present in T_1 (2.52%) at 0 day of storage which remained same even after 28 days of storage. In T_0 , T_2 , T_3 and T_4 the amount of fiber was 2.08, 2.06, 2.11 and 2.10 respectively also remained same throughout the storage period. There was no observed effect on the fiber content of bars due to storage period. The similarity was might be due to good storage conditions and handling environment. The results regarding constant level of fiber in bars during storage were same as with the findings of Aramouni & Abu-Ghoush (2011).

Results showed that storage period had a significant affect at the pH of bars. Highest recorded pH (6.74) of T_0 at 0 day of storage and lowest one (6.19) was of T_1 at 28th day of storage. While the pH of $T_4 \& T_3$ were 6.61 & 6.66 at 0 day, which reduced to 6.22 and 6.23 at termination of storage period, respectively. The gradual reduction in pH at different storage days was because the acidity increases, however the change in pH hade not affected on the texture and taste too much. The results of this study were in corroboration with the previous findings of Arise et al. (2014).

Treatments		Proximate Composition						
Treatments	Moisture	Fat%	Protein%	Ash%	Fiber%			
Sun Dried	64.42 ± 0.03	8.52 ± 0.18	19.91 ± 0.08	4.03 ± 0.11	2.18 ± 0.29			
Oven Dried	71.11 ± 0.12	8.62 ± 0.05	17.13 ± 0.13	4.21 ± 0.03	3.15 ± 0.41			
Shade Dried	63.68 ± 0.16	7.09 ± 0.14	21.35 ± 0.11	3.01 ± 0.14	3.13 ± 0.44			

	Table 3. Proximate	composition	of moringa protein bars	s.
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Treatment	Moisture	Protein	Ash	Fat
T ₀	$6.62\pm0.31^{\rm b}$	$19.29\pm0.88^{\text{d}}$	$1.42\pm0.21^{\rm b}$	$9.75\pm0.12^{\rm b}$
T_1	7.37 ± 0.77^{ab}	$21.55\pm0.87^{\rm d}$	$1.73\pm0.14^{\rm a}$	12.70 ± 0.22^{a}
T_2	$8.30\pm0.51^{\rm a}$	$27.33\pm0.85^{\circ}$	$1.35\pm0.12^{\rm b}$	$10.10\pm0.14^{\rm b}$
T_3	$7.40\pm0.69^{\text{ab}}$	$35.29\pm0.96^{\rm b}$	$1.17\pm0.15^{\rm bc}$	$10.09\pm0.18^{\rm b}$
T_4	$6.62\pm0.56^{\rm b}$	$42.55\pm0.95^{\text{a}}$	$0.92\pm0.18^{\circ}$	$10.05\pm0.14^{\rm b}$

 T_0 ; control T_i ; Bars with 10% flour T_i ; Bars with 5% protein Isolate T3; Bars with 10% protein isolate T_i ; sweet bars with 15% protein isolate.

Treatments			Days			- Nutritional analysis
freatments	0	7	14	21	28	 Nutritional analysis
T ₀	$2.08\pm0.22^{\rm b}$	$2.08\pm0.19^{\rm b}$	$2.06\pm0.14^{\rm b}$	$2.05\pm0.18^{\rm b}$	$2.08\pm0.14^{\rm b}$	Fiber
T_1	$2.52\pm0.13^{\rm a}$	$2.49\pm0.12^{\text{a}}$	$2.51\pm0.16^{\rm a}$	$2.50\pm0.14^{\rm a}$	$2.52\pm0.15^{\text{a}}$	(%)
T_2	$2.06\pm0.14^{\rm b}$	$2.09\pm0.17^{\rm b}$	$2.09\pm0.17^{\rm b}$	$2.06\pm0.08^{\rm b}$	$2.05\pm0.13^{\rm b}$	
T_3	$2.11\pm0.14^{\rm b}$	$2.07\pm0.11^{\rm b}$	$2.08\pm0.13^{\rm b}$	$2.09\pm0.14^{\rm b}$	$2.07\pm0.17^{\rm b}$	
T_4	$2.10\pm0.14^{\rm b}$	$2.06\pm0.12^{\rm b}$	$2.10\pm0.22^{\rm b}$	$2.06\pm0.19^{\rm b}$	$2.05\pm0.16^{\rm b}$	
T ₀	$330.19\pm9.48^{\rm d}$	$328.26\pm9.48^{\rm d}$	332.61 ± 14.92^{d}	$331.44\pm16.35^{\rm d}$	324.33 ± 15.43^{d}	Calarianalua
T_1	$331.10\pm17.4^{\rm d}$	$342.40\pm16.23^{\text{d}}$	333.78 ± 13.39^{d}	$342.98 \pm 13.57^{\rm d}$	$329.88\pm10.74^{\rm d}$	Caloric value
T_2	$438.18 \pm 10.17^{\rm bc}$	$436.17 \pm 19.67^{\rm bc}$	$442.92 \pm 15.19^{\rm bc}$	$441.89 \pm 14.80^{\rm bc}$	$431.77 \pm 14.10^{\circ}$	
T_{3}	480.37 ± 14.14^{ab}	476.88 ± 14.26^{abc}	$477.14\pm17.09^{\text{abc}}$	481.73 ± 13.66^{ab}	477.76 ± 9.67^{bc}	
T_4	498.33 ± 19.52^{a}	502.11 ± 17.95^{a}	495.11 ± 18.51^{a}	$496.29\pm18.88^{\text{a}}$	501.58 ± 13.25^{a}	
T ₀	0.36 ± 0.08	0.39 ± 0.08	0.35 ± 0.08	0.33 ± 0.07	0.36 ± 0.06	a _w
T_1	0.40 ± 0.06	0.40 ± 0.09	0.41 ± 0.08	0.35 ± 0.04	0.41 ± 0.07	
T_2	0.37 ± 0.06	0.39 ± 0.07	0.37 ± 0.06	0.37 ± 0.07	0.36 ± 0.06	
T ₃	0.37 ± 0.08	0.35 ± 0.04	0.38 ± 0.07	0.35 ± 0.06	0.36 ± 0.05	
T_4	0.35 ± 0.07	0.37 ± 0.07	0.34 ± 0.07	0.34 ± 0.04	0.36 ± 0.04	
T ₀	$6.74\pm0.17^{\rm a}$	6.61 ± 0.11^{abc}	$6.34\pm0.14^{\rm bcd}$	$6.40\pm0.12^{\rm abcd}$	$6.35\pm0.12^{\text{bcd}}$	pН
T_1	$6.56\pm0.11^{\rm abcd}$	6.45 ± 0.16^{abcd}	$6.48\pm0.11^{\text{abcd}}$	$6.26\pm0.10^{\rm cd}$	$6.19\pm0.10^{\rm d}$	
T_2	$6.68\pm0.13^{\text{ab}}$	$6.49\pm0.13^{\text{abcd}}$	6.38 ± 0.10^{abcd}	$6.28\pm0.10^{\rm cd}$	$6.21\pm0.13^{\rm d}$	
T ₃	6.66 ± 0.14^{ab}	6.49 ± 0.11^{abcd}	6.39 ± 0.13^{abcd}	$6.26\pm0.10^{\rm cd}$	$6.23\pm0.15^{\rm d}$	
T_4	$6.61\pm0.10^{\rm abc}$	6.45 ± 0.13^{abcd}	$6.34\pm0.12^{\rm bcd}$	$6.26\pm0.11^{\text{cd}}$	6.22 ± 0.11^{d}	

Table 4. Effect of storage on nutritional profile of Moringa flower protein bars.

T₀; control T₁; Bars with 10% flour T₂; Bars with 5% protein Isolate T₃; Bars with 10% protein isolate T₄; sweet bars with 15% protein isolate.

The result of present study was also in line with the previous findings of Abraha et al. (2018) who observed the declining pattern of pH in biscuits prepared by fortification of fish fillet protein concentrate. The values of pH declining were similar with the diminution in pH values of energy bars samples found in the present study

The water activity effect on bars can be seen from the Table 4. Results showed that there was a non-significant difference in water activity of bars during storage. Maximum water activity was observed in T_1 , 0.41 & 0.41 at 14th & 28th day followed by minimum level in T_0 (0.33) at 21st day of storage. The water activity remained almost same throughout the storage. The variations in results of study with previous findings of Barrett et al. (2010) was might be due to difference in sugar level and treatments.

From the results it can be concluded that the highest caloric value (498.33 kcal) was seen in T_4 at starting day of storage which persisted almost same at termination of storage which means that storage had no effect on bars calories. However, momentous variation can be observed in various treatments as each treatment had changed protein ratio which results in change the caloric value. The persistence in caloric value with the progression in storage was might be due to good packaging and storage conditions. According to Whybrow (2005) energy content of bars was 400 to 500 kcal so the best treatments were $T_3 \& T_4$ as 480.37 & 498.33 kcal correspondingly having the desirable taste and caloric value in Moringa flower protein bars. The differences

in the study results with the findings (245.47 kcal/100 g) of Lobato et al. (2012) was might be due to variation in treatments.

Mean values regarding the instrumental color of bars depicted in Table 5. The utmost color (red and green) value 11.57 was noticed at termination of storage in T_0 and lowest level was found 7.15 at 0 day of storage in T_3 sample. The observed values showed that maximum value was in T_0 due to presence of higher sugar contents which results in occurrence of millard reaction at higher rate. In T_0 samples, red and green color was 9.41 at 0 day that escalated to 11.47 and 11.57 at 21 and 28th day of study, respectively. The results are in corroboration with the findings of Aramouni & Abu-Ghoush (2011) as red and green value was 7.0-8.3 but the changes in color with the storage progression was might be due to difference in protein content and environmental factors.

Highest yellow color (b value) was observed in T₂ (20.34) at the termination day of study while minimum value was observed in T₀ sample which was 13.63 at start day of study. The results described that maximum value was seen in T₂ which was due to high amount of protein present which results in higher degree of millard reaction. In T₁ & T₂, the detected yellow color was 14.45 & 16.52 at initial day of study that increased to 19.62 and 19.30 at 14th day, correspondingly. The variations in results of current study with the findings of Mridula et al. (2013) was might be due to the variations in protein amount present in bars at 0 day but trend of escalation of the yellow color was in line

Treatment -	Days					
ileatiment —	0	7	14	21	28	Colour
T ₀	$43.85\pm0.24^{\rm k}$	$42.88\pm0.11^{\rm m}$	$42.14\pm0.15^{\rm n}$	$41.47\pm0.13^{\circ}$	$41.45\pm0.13^{\circ}$	L* Colour
T_1	$50.03\pm0.14^{\rm c}$	$46.24\pm0.13^{\rm h}$	$44.92\pm0.14^{\rm j}$	$43.59\pm0.16^{\rm k}$	$43.47\pm0.15^{\text{kl}}$	(lightness)
T_2	$50.85\pm0.12^{\rm b}$	$48.42\pm0.16^{\rm d}$	$46.70\pm0.15^{\rm fg}$	45.52 ± 0.17	$45.47\pm0.11^{\rm i}$	
T ₃	$53.29\pm0.14^{\rm a}$	$47.62\pm0.13^{\rm e}$	$46.22\pm0.13^{\rm h}$	$46.42\pm0.13^{\text{gh}}$	$43.13\pm0.14L^{\rm m}$	
T_4	$49.72\pm0.13^{\circ}$	$47.00\pm0.15^{\rm f}$	45.69 ± 0.15^{i}	$44.91\pm0.15^{\rm j}$	$44.68\pm0.18^{\rm j}$	
T _o	$9.41 \pm 0.14^{\circ}$	$9.92\pm0.13^{\circ}$	$10.57 \pm 0.15^{\rm b}$	11.47 ± 0.13^{a}	11.57 ± 0.13^{a}	a* colour
T_1	$7.91\pm0.12^{\rm fg}$	$8.59\pm0.13^{\rm de}$	$9.79\pm0.14^{\rm c}$	$10.67\pm0.13^{\rm b}$	$10.74\pm0.17^{\rm b}$	(red, green
T_2	$7.39\pm0.15^{\rm hi}$	$7.39\pm0.14^{\rm hi}$	$7.69\pm0.13^{\rm fgh}$	$8.55\pm0.14^{\rm de}$	$8.65\pm0.14^{\rm de}$	
T ₃	$7.15\pm0.0.47^{\rm i}$	$7.57\pm0.14^{\rm ghi}$	$7.15\pm0.14^{\rm i}$	$8.72\pm0.14^{\rm de}$	$8.77\pm0.14^{\rm d}$	
T_4	$7.21\pm0.14^{\rm hi}$	7.69 ± 0.13^{gh}	$8.21\pm0.16^{\text{ef}}$	$8.83\pm0.15^{\rm d}$	$8.87\pm0.14^{\rm d}$	
T _o	13.63 ± 0.16^{n}	15.13 ± 0.10^{1}	15.72 ± 0.15^{jk}	$16.69 \pm 0.11^{\text{gh}}$	$16.82\pm0.11^{ m g}$	b* colour,
T ₁	$14.45\pm0.10^{\rm m}$	$15.50\pm0.13^{\rm kl}$	$16.91\pm0.13^{\rm g}$	$17.60\pm0.13^{\rm f}$	$18.25\pm0.13^{\rm e}$	yellow
Τ ₂	$16.52\pm0.11^{\rm gh}$	$17.73\pm0.12^{\rm f}$	$18.78\pm0.16^{\rm cd}$	$19.620\pm0.14^{\rm b}$	$20.34\pm0.16^{\rm a}$	
T ₃	$16.31\pm0.13^{\rm hi}$	$17.45\pm0.10^{\rm f}$	$18.40\pm0.13^{\rm de}$	$19.30\pm0.12^{\rm b}$	$19.63\pm0.12^{\rm b}$	
T_4	15.96 ± 0.14^{ij}	16.88 ± 0.14^{g}	$17.72 \pm 0.15^{\rm f}$	18.78 ± 0.15^{cd}	$18.83 \pm 0.15^{\circ}$	

Table 5. Effect of storage on Colour of Moringa Bars.

T_o; control T_i; Bars with 10% flour T_i; Bars with 5% protein Isolate T₃; Bars with 10% protein isolate T_i; sweet bars with 15% protein isolate.

with Aramouni & Abu-Ghoush (2011) findings as b value for puffed wheat bars was 13.6-19.1 comparable to whole grain sample (Lawless & Heymann, 1998).

The maximum Lightness (L value) was 53.29, determined at initial day of study in T_3 , however minimum value was determined in T_0 (41.45) at the termination of storage study. In T_0 the L value was 43.85 at initial day of storage which declined to 41.47 and 41.45 at 21st & 28th day, correspondingly. Moreover, L value of T_1 and T_2 declined to 43.47 and 45.47 at the end of storage study, correspondingly. The results current investigation were in line with the results of Aramouni & Abu-Ghoush (2011) who determined L values for puffed wheat bars were 40.9-50.4. Sugar to protein interaction may be the cause of decrease in the L value of bars with the progression of storage.

The texture profile of bars were depicted in Table 6. It was determined from mean table that maximum hardness was 5.06 kg in T_2 at the termination of storage study and the minimum value was found in T_0 (4.15 kg) at day first of storage. The findings revealed that the hardness level was slightly escalated during storage in all treatments. hardness level in T_0 bars was 4.15 kg at initial day of storage which escalated to 4.32 kg at with the progression in storage at 28th day. The outcomes of the findings showed similarities with previous results of Lobato et al. (2012) who's results showed that hardness level of bars was 2.54 N at preparation time which escalated to 10.00 N after six months of storage study. So, result concluded that escalation in hardness level

was might be due to interactions between protein surfaces and co-solvents. The trend of escalation level was in corroboration with the study of Kim et al. (2009).

Firmness of the bars observed maximum at termination of storage (3.97 kg) in T_4 , but minimum value was found in $T_0 \& T_2$ i.e. 3.30 & 3.31 kg at 7th day of storage. The results of the findings indicated that maximum level of firmness in T_2 was might be due to storage time and conditions. But results of T_4 and T_3 regarding firmness at start of storage period were 3.88 and 3.67 kg which escalated to 3.97 and 3.83 kg at termination of storage period, correspondingly. The variation of results of our study with Kim et al. (2009) were might be due to storage period effect on bars. The escalation of firmness in protein bars were might be due to moisture migration between carbohydrates (i.e. sugars and starches) and the proteins (McMahon et al. 2009).

Similarly, highest toughness level was observed in T_4 (3.97 kg) at end of storage period while lower level of toughness was found in T_0 (3.31 kg) at initial day of storage. The obtained results revealed that maximum level of toughness in T_2 was might be due to storage time and conditions. The inference also revealed that there was a gradual escalation in toughness among all treatments with the progression of storage period. Toughness level in T_0 was 3.31 kg at 0 day of study which escalated to 3.51 and 3.6 kg at 21st & 28th storage day, correspondingly. This increase in toughness was might be due to textural changes in carbohydrates with the advancement in storage period.

Turnet	Days					
Treatment –	0	7	14	21	28	Texture
T ₀	$4.15\pm0.15^{\rm i}$	$4.19\pm0.12^{\rm i}$	$4.23\pm0.13^{\rm hi}$	$4.28\pm0.11^{\rm hi}$	$4.32\pm0.10^{\rm ghi}$	Hardness
T_1	$4.39\pm0.10^{\rm fi}$	$4.45\pm0.14^{\rm e-i}$	$4.47\pm0.10^{\rm d\text{-}i}$	$4.52\pm0.12^{\text{c-i}}$	$4.59\pm0.12^{\rm b-h}$	(Kg)
T ₂	$4.97\pm0.14^{\rm ab}$	4.98 ± 0.15^{ab}	$5.01 \pm 0.12^{\text{a}}$	$5.01\pm0.13^{\rm a}$	$5.06\pm0.14^{\rm a}$	
T ₃	$4.85\pm0.13^{\rm a\cdot d}$	$4.87\pm0.11^{\rm abc}$	$4.88\pm0.11^{\rm abc}$	4.93 ± 0.16^{ab}	$4.96\pm0.16^{\rm ab}$	
T_4	$4.68\pm0.11^{\text{a-g}}$	$4.68\pm0.13^{\text{a-g}}$	$4.72\pm0.13^{\rm a-f}$	$4.76\pm0.12^{\rm a-f}$	$4.82\pm0.12^{\text{a-e}}$	
T ₀	$3.36\pm0.09^{\text{gh}}$	$3.30\pm0.12^{\rm h}$	$3.43\pm0.14^{\rm e-h}$	$3.51\pm0.14^{\rm c\text{-}h}$	$3.63\pm0.13^{\rm a-h}$	Firmness
T_1	$3.49\pm0.10^{\rm c\text{-}h}$	$3.40\pm0.12^{\rm fgh}$	$3.51 \pm 0.12^{\text{c-h}}$	$3.67\pm0.10^{\rm a-h}$	$3.62\pm0.12^{\rm a-h}$	(Kg)
T_2	$3.40\pm0.11^{\rm fgh}$	$3.31\pm0.13^{\rm h}$	$3.46\pm0.15^{\rm d-h}$	$3.51\pm0.11^{\rm b-h}$	$3.61\pm0.10^{\text{a-h}}$	
T ₃	$3.67\pm0.10^{\rm a\cdot h}$	$3.60\pm0.12^{\rm a\text{-}h}$	$3.71\pm0.14^{\text{a-g}}$	$3.76\pm0.12^{\rm a-f}$	$3.83\pm0.15^{\rm a-d}$	
T_4	$3.88\pm0.13^{\rm abc}$	$3.80\pm0.13^{\text{a-e}}$	$3.90\pm0.14^{\rm ab}$	$3.94\pm0.16^{\rm a}$	$3.97\pm0.16^{\rm a}$	
T ₀	$3.31\pm0.11^{\rm f}$	$3.32\pm0.13^{\rm f}$	$3.43\pm0.13^{\rm def}$	$3.52\pm0.11^{\rm b-f}$	$3.60\pm0.12^{\rm a-f}$	Toughness
T_1	$3.51\pm0.12^{\rm b-f}$	$3.59\pm0.14^{\rm a-f}$	$3.61\pm0.10^{\rm a-f}$	$3.67\pm0.13^{\rm a-f}$	$3.71\pm0.11^{\text{a-e}}$	(Kg)
T ₂	$3.40\pm0.13^{\rm ef}$	$3.43\pm0.13^{\rm def}$	$3.48\pm0.15^{\rm c-f}$	$3.50\pm0.17^{\rm c-f}$	$3.61\pm0.10^{\rm a-f}$	
T ₃	$3.63\pm0.14^{\rm a\text{-}f}$	$3.64\pm0.16^{\rm a\cdot f}$	$3.73 \pm 0.12^{a-e}$	$3.77\pm0.12^{\text{a-e}}$	$3.82\pm0.13^{\text{abc}}$	
T_4	$3.78\pm0.12^{\text{a-e}}$	$3.81 \pm 0.11^{\text{a-d}}$	$3.90\pm0.10^{\rm ab}$	$3.94\pm0.11^{\rm a}$	$3.97\pm0.14^{\rm a}$	
T ₀	16.74 ± 0.13	16.74 ± 0.19	16.74 ± 0.14	16.75 ± 0.17	16.75 ± 0.13	Work of
T_1	16.80 ± 0.17	16.80 ± 0.17	16.81 ± 0.13	16.81 ± 0.11	16.81 ± 0.12	shear
T ₂	16.70 ± 0.22	16.71 ± 0.12	16.71 ± 0.18	16.70 ± 0.16	16.70 ± 0.15	(Kg/mm)
T ₃	16.80 ± 0.17	16.81 ± 0.15	16.77 ± 0.13	16.76 ± 0.17	16.80 ± 0.17	
T_4	16.84 ± 0.17	16.85 ± 0.20	16.84 ± 0.14	16.84 ± 0.16	16.85 ± 0.14	

Table 6. Effect of storage on texture of Moringa Bars.

T₀; control T₁; Bars with 10% flour T₂; Bars with 5% protein Isolate T₃; Bars with 10% protein isolate T₄; sweet bars with 15% protein isolate.

The mean Table 6 depicted the results of work of shear in kg/mm. It was obvious from the results that highest work of shear was seen in T₄ (16.85kg/mm) on 28th storage day while minimum value was seen in T_{2} (16.70 kg/mm) during the whole study period. From the results it was revealed that minimum work of shear was in T₂ was might be due to storage time and conditions. The obtained inferences also revealed that there was insignificant escalation in work of shear of bars in all variants. Work of shear in T_o was 16.74 kg/mm at the initiation of storage which had nonsignificant changes at 21st & 28th day of storage 16.75 and 16.75 kg/mm, correspondingly. Likewise, the work of shear in T₁ and T₂ was 16.80 and 16.70 kg/mm at initial day which remains constant till the end of storage period. Temperature and time had effect on the change in work of shear as reported by Banach et al. (2016) and Rawat & Darappa, (2015). The variation of result was might be due to effect of time and ingredients of bar on work of shear.

Results regarding sensory color are expressed in Figure No. 01. From the figure it can be concluded that maximum values for color were observed in T_0 and T_3 and lowest one was in T_4 . Slight decline was seen in the T_0 and T_3 color with the progression of storage may be due to lower storage temperature but the increase in T_4 was might be due to more protein contents, these results are in corroboration with the previous findings of Banach et al. (2014) as they found color changes with the advancement in storage. From the Figure No. 02. it can be concluded that the best flavor accepted by the panelist was of T_0 and T_3 at 0 day of storage which showed slight decline in flavor till 28th day of storage. The rejected flavor was of T_1 and T_4 which also declined till 28th day of storage. The difference in the results with the previous findings of Srebernich et al. (2016) was might be due to different treatments and preparatory conditions. These results were in corroboration with the previous findings of Banach et al. (2014) who supported the idea that flavor would be adversely affected by the physicochemical changes in the food matrix during storage.

It can be seen in the Figure No. 03 that sensorial texture of high protein bars best accepted by sensory analysts was of T_0 and T_3 which was slightly affected with the progression of storage. The most rejected treatment by the panelist was T_4 which showed that sugar to protein interaction at lower temperature would affect the texture and hardened the bars. The results are linked with the previous findings of Mezzenga (2007) who investigated that physical and chemical interaction among the ingredients in snack bars can occur over time and begin to affect the texture of product.

From the Figure No.04 it can be divulged that the sensory attribute of mouthfeel was best in T_0 and T_3 at 0 day of storage which reduced slightly till 28th day of storage and this pattern was also observed in rest of the treatments but the results of mouthfeel in T_2 and T_4 not acceptable by the sensory panelist

because there developed a grainy texture and sticky mouthfeel in the T_4 and hardening in T_2 . The results are linked with the previous findings of Lu & Zhou (2019) who supported the idea of undesirable flavor and sticky mouthfeel.

Results of sensory evaluation about the overall acceptability are provided in the Figure No. 05. From the figure it can be concluded

that the bars of T_0 and those having intermediate protein contents as in T_3 were accepted more than those of the T_1 , T_2 and T_4 . There a gradual reduction in overall acceptability of protein bars was seen with the advancement in storage days. This shit in overall acceptability with the progression of storage time was might be due to physicochemical changes in the bars during storage which affected all sensory parameters to some extent.

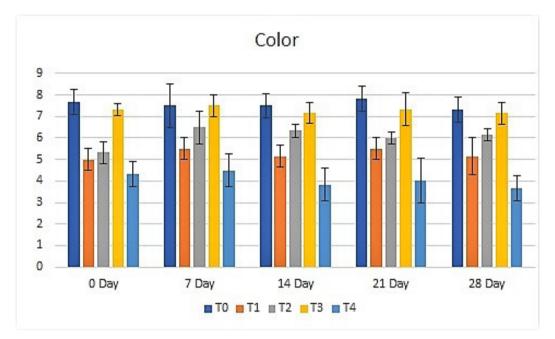


Figure 1. Effect of storage on Color (Sensory Evaluation) of Moringa Bars 127x76mm (96 x 96 DPI).

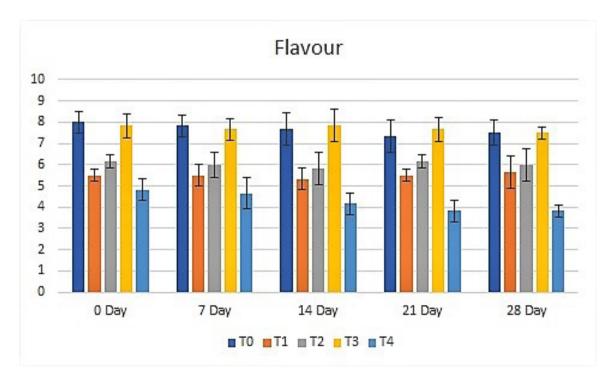


Figure 2. Effect of storage on Flavor (Sensory Evaluation) of Moringa Bars 127x76mm (96 x 96 DPI).

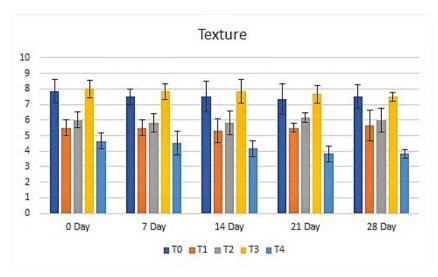


Figure 3. Effect of storage on Texture (Sensory Evaluation) of Moringa Bars 127x76mm (96 x 96 DPI).

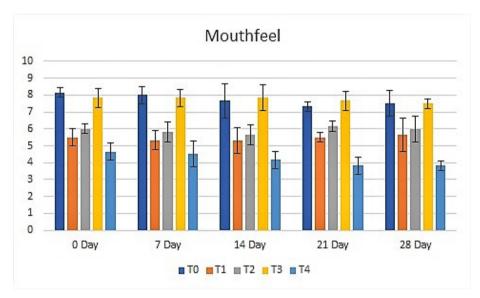


Figure 4. Effect of storage on Mouthfeel (Sensory Evaluation) of Moringa Bars 127x76mm (96 x 96 DPI).

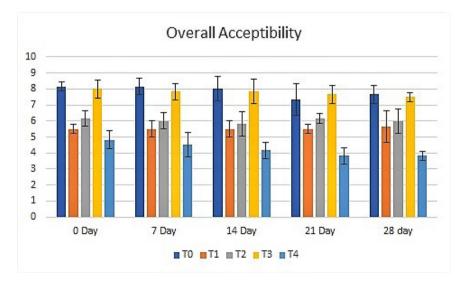


Figure 5. Effect of storage on overall acceptability (Sensory Evaluation) of Moringa Bars 127x76mm (96 x 96 DPI)

4 Conclusion

The moringa is affordable and people who cannot afford meat can overcome their energy requirements by using moringa products. The results of current studies revealed that snack should be enriched with protein isolates extracted from moringa which not only increase protein content of snacks but also help to combat malnourishment which is focus of modern world.

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