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 T1 (15% protein isolate) and T2 (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%. T1 and T2 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 who 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 *Moringaceae* 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
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 μm) 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.

| Treatments                  | T₀ | T₁ | T₂ | T₃ | T₄ |
|-----------------------------|----|----|----|----|----|
| 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).

pH

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)

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

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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 observed the moisture contents of moringa flowers were 74.18%. The slight difference in observed values 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 T₁ due to higher protein contents (21.35) was selected for further investigation and protein isolate.

Mean values regarding proximate composition of bars are described in Table 3. It can be concluded from the mean Table that in treatment T₁ moisture level was highest 8.33% at the while minimum value was observed in T₀ (6.62%) respectively. The results negotiate that the detected maximum moisture level in T₁ 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).

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 T₁ (2.52%) at 0 day of storage which remained same even after 28 days of storage. In T₀, T₁, T₂ and T₃ 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 T₁, at 0 day of storage and lowest one (6.19) was of T₄ at 28th day of storage. While the pH of T₀ & T₁ 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 had 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).

### Table 2. Proximate composition of moringa flower dried under different conditions.

| Treatments         | Proximate Composition |
|--------------------|-----------------------|
|                    | 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.

| Treatment | Moisture | Protein | Ash | Fat |
|-----------|----------|---------|-----|-----|
| T₀        | 6.62 ± 0.31b | 19.29 ± 0.88d | 1.42 ± 0.21bd | 9.75 ± 0.12bd |
| T₁        | 7.37 ± 0.77ab | 21.55 ± 0.87d | 1.73 ± 0.14a | 12.70 ± 0.22a |
| T₂        | 8.30 ± 0.51a  | 27.33 ± 0.85c  | 1.35 ± 0.12a  | 10.10 ± 0.14b |
| T₃        | 7.40 ± 0.69ab | 35.29 ± 0.96a  | 1.17 ± 0.15bc | 10.09 ± 0.18b |
| T₄        | 6.62 ± 0.56b  | 42.55 ± 0.95c  | 0.92 ± 0.18a  | 10.05 ± 0.14b |

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

| Treatments | Days | Nutritional analysis |
|------------|------|----------------------|
|            | 0    | 7                    | 14                  | 21                  | 28                  |
| T₀         | 2.08 ± 0.22<sup>a</sup> | 2.08 ± 0.19<sup>b</sup> | 2.06 ± 0.14<sup>c</sup> | 2.05 ± 0.18<sup>d</sup> | 2.08 ± 0.14<sup>abc</sup> |
| T₁         | 2.52 ± 0.13<sup>a</sup> | 2.49 ± 0.12<sup>b</sup> | 2.51 ± 0.16<sup>c</sup> | 2.50 ± 0.14<sup>d</sup> | 2.52 ± 0.15<sup>abc</sup> |
| T₂         | 2.06 ± 0.14<sup>a</sup> | 2.09 ± 0.17<sup>b</sup> | 2.09 ± 0.17<sup>c</sup> | 2.06 ± 0.08<sup>d</sup> | 2.05 ± 0.13<sup>abc</sup> |
| T₃         | 2.11 ± 0.14<sup>a</sup> | 2.07 ± 0.11<sup>b</sup> | 2.08 ± 0.13<sup>c</sup> | 2.09 ± 0.14<sup>d</sup> | 2.07 ± 0.17<sup>abc</sup> |
| T₄         | 2.10 ± 0.14<sup>a</sup> | 2.06 ± 0.12<sup>b</sup> | 2.10 ± 0.22<sup>c</sup> | 2.06 ± 0.19<sup>d</sup> | 2.05 ± 0.16<sup>abc</sup> |
| T₅         | 330.19 ± 9.48<sup>d</sup> | 328.26 ± 9.48<sup>d</sup> | 332.61 ± 14.92<sup>d</sup> | 331.44 ± 16.35<sup>d</sup> | 324.33 ± 15.43<sup>d</sup> |
| T₆         | 331.10 ± 17.4<sup>d</sup> | 342.40 ± 16.23<sup>d</sup> | 333.78 ± 13.39<sup>d</sup> | 342.98 ± 13.57<sup>d</sup> | 329.88 ± 10.74<sup>d</sup> |
| T₇         | 438.18 ± 10.1<sup><small>bc</small></sup> | 436.17 ± 19.67<sup><small>bc</small></sup> | 442.92 ± 15.19<sup><small>bc</small></sup> | 441.89 ± 14.80<sup><small>bc</small></sup> | 431.77 ± 14.10<sup><small>bc</small></sup> |
| T₈         | 480.37 ± 14.1<sup><small>bc</small></sup> | 476.88 ± 14.26<sup><small>bc</small></sup> | 477.14 ± 17.09<sup><small>bc</small></sup> | 481.73 ± 13.66<sup><small>bc</small></sup> | 477.76 ± 9.67<sup><small>bc</small></sup> |
| T₉         | 498.33 ± 19.52<sup>bc</sup> | 502.11 ± 17.95<sup>bc</sup> | 495.11 ± 18.51<sup>bc</sup> | 496.29 ± 18.88<sup>bc</sup> | 501.58 ± 13.25<sup>bc</sup> |
| T₁₀        | 0.36 ± 0.08 | 0.39 ± 0.08 | 0.35 ± 0.08 | 0.33 ± 0.07 | 0.36 ± 0.06 |
| T₁₁        | 0.40 ± 0.06 | 0.40 ± 0.09 | 0.41 ± 0.08 | 0.35 ± 0.04 | 0.41 ± 0.07 |
| T₁₂        | 0.37 ± 0.06 | 0.39 ± 0.07 | 0.37 ± 0.06 | 0.37 ± 0.07 | 0.36 ± 0.06 |
| T₁₃        | 0.37 ± 0.08 | 0.35 ± 0.04 | 0.38 ± 0.07 | 0.35 ± 0.06 | 0.36 ± 0.05 |
| T₁₄        | 0.35 ± 0.07 | 0.37 ± 0.07 | 0.34 ± 0.07 | 0.34 ± 0.04 | 0.36 ± 0.04 |
| T₁₅        | 6.74 ± 0.17<sup><small>bc</small></sup> | 6.61 ± 0.11<sup><small>d</small></sup> | 6.34 ± 0.14<sup><small>cd</small></sup> | 6.40 ± 0.12<sup><small>abcd</small></sup> | 6.35 ± 0.12<sup><small>abcd</small></sup> |
| T₁₆        | 6.56 ± 0.11<sup><small>abcd</small></sup> | 6.45 ± 0.16<sup><small>abcd</small></sup> | 6.48 ± 0.11<sup><small>abcd</small></sup> | 6.26 ± 0.10<sup><small>abcd</small></sup> | 6.19 ± 0.10<sup><small>abcd</small></sup> |
| T₁₇        | 6.68 ± 0.13<sup><small>abcd</small></sup> | 6.49 ± 0.13<sup><small>abcd</small></sup> | 6.38 ± 0.10<sup><small>abcd</small></sup> | 6.28 ± 0.10<sup><small>abcd</small></sup> | 6.21 ± 0.13<sup><small>abcd</small></sup> |
| T₁₈        | 6.66 ± 0.14<sup><small>abcd</small></sup> | 6.49 ± 0.11<sup><small>abcd</small></sup> | 6.39 ± 0.13<sup><small>abcd</small></sup> | 6.26 ± 0.10<sup><small>abcd</small></sup> | 6.23 ± 0.15<sup><small>abcd</small></sup> |
| T₁₉        | 6.61 ± 0.10<sup><small>abcd</small></sup> | 6.45 ± 0.13<sup><small>abcd</small></sup> | 6.34 ± 0.12<sup><small>abcd</small></sup> | 6.26 ± 0.11<sup><small>abcd</small></sup> | 6.22 ± 0.11<sup><small>abcd</small></sup> |

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 T₃ and lowest level was found 7.15 at 0 day of storage in T₁ sample. The observed values showed that maximum value was in T₀ due to presence of higher sugar contents which results in occurrence of millard reaction at higher rate. In T₀ sample, red and green color was 9.41 at 0 day that escalated to 11.47 and 11.57 at 21 and 28 day of study, respectively. The results are in corroborations 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.

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 T₁ at 0.41 & 0.41 at 14<sup>th</sup> & 28<sup>th</sup> day followed by minimum level in T₀ (0.33) at 21<sup>st</sup> 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 T₃ 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 T₃ & T₄ 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.

Highest yellow color (b value) was observed in T₂ 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 T₄ 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 28<sup>th</sup> 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 with the diminution in pH values of energy bars samples found in the present study.
Table 5. Effect of storage on Colour of Moringa Bars.

| Treatment | Days | Colour |
|-----------|------|--------|
|           | 0    |        | 7        | 14       | 21       | 28       |
| T₀        |      | 43.85 ± 0.24<sup>a</sup> | 42.88 ± 0.11<sup>m</sup> | 42.14 ± 0.15<sup>n</sup> | 41.47 ± 0.13<sup>n</sup> | 41.45 ± 0.13<sup>n</sup> |
| T₁        |      | 50.03 ± 0.14<sup>b</sup> | 46.24 ± 0.13<sup>b</sup> | 44.92 ± 0.14<sup>c</sup> | 43.59 ± 0.16<sup>d</sup> | 43.47 ± 0.15<sup>i</sup> |
| T₂        |      | 50.85 ± 0.12<sup>b</sup> | 48.42 ± 0.16<sup>d</sup> | 46.70 ± 0.15<sup>e</sup> | 45.52 ± 0.17<sup>j</sup> | 45.47 ± 0.11<sup>m</sup> |
| T₃        |      | 53.29 ± 0.14<sup>b</sup> | 47.62 ± 0.13<sup>b</sup> | 46.22 ± 0.13<sup>b</sup> | 46.42 ± 0.13<sup>b</sup> | 43.13 ± 0.14L<sup>n</sup> |
| T₄        |      | 49.72 ± 0.13<sup>c</sup> | 47.00 ± 0.15<sup>a</sup> | 45.69 ± 0.15<sup>i</sup> | 44.91 ± 0.15<sup>i</sup> | 44.68 ± 0.18<sup>i</sup> |
| T₀        |      | 9.41 ± 0.14<sup>a</sup> | 9.92 ± 0.13<sup>c</sup> | 10.57 ± 0.15<sup>b</sup> | 11.47 ± 0.13<sup>a</sup> | 11.57 ± 0.13<sup>a</sup> |
| T₁        |      | 7.91 ± 0.12<sup>b</sup> | 8.59 ± 0.13<sup>b</sup> | 9.79 ± 0.14<sup>c</sup> | 10.67 ± 0.13<sup>b</sup> | 10.74 ± 0.17<sup>b</sup> |
| T₂        |      | 7.39 ± 0.15<sup>bc</sup> | 7.39 ± 0.14<sup>bc</sup> | 7.69 ± 0.13<sup>gh</sup> | 8.55 ± 0.14<sup>bc</sup> | 8.65 ± 0.14<sup>bc</sup> |
| T₃        |      | 7.15 ± 0.04<sup>gh</sup> | 7.57 ± 0.14<sup>bc</sup> | 7.15 ± 0.14<sup>c</sup> | 8.72 ± 0.14<sup>bc</sup> | 8.77 ± 0.14<sup>d</sup> |
| T₄        |      | 7.21 ± 0.14<sup>bc</sup> | 7.69 ± 0.13<sup>gh</sup> | 8.21 ± 0.16<sup>d</sup> | 8.83 ± 0.15<sup>d</sup> | 8.87 ± 0.14<sup>d</sup> |
| T₀        |      | 13.63 ± 0.16<sup>bc</sup> | 15.13 ± 0.10<sup>n</sup> | 15.72 ± 0.15<sup>gh</sup> | 16.69 ± 0.11<sup>bc</sup> | 16.82 ± 0.11<sup>bc</sup> |
| T₁        |      | 14.45 ± 0.10<sup> bc</sup> | 15.50 ± 0.13<sup>hi</sup> | 16.91 ± 0.13<sup>bc</sup> | 17.60 ± 0.13<sup>bc</sup> | 18.25 ± 0.13<sup>bc</sup> |
| T₂        |      | 16.52 ± 0.11<sup>hi</sup> | 17.73 ± 0.12<sup>bc</sup> | 18.78 ± 0.16<sup>cd</sup> | 19.620 ± 0.14<sup>hi</sup> | 20.34 ± 0.16<sup>bc</sup> |
| T₃        |      | 16.31 ± 0.13<sup>hi</sup> | 17.45 ± 0.10<sup>bc</sup> | 18.40 ± 0.13<sup>bc</sup> | 19.30 ± 0.12<sup>bc</sup> | 19.63 ± 0.12<sup>bc</sup> |
| T₄        |      | 15.96 ± 0.14<sup>hi</sup> | 16.88 ± 0.14<sup>bc</sup> | 17.72 ± 0.15<sup>hi</sup> | 18.78 ± 0.15<sup>cd</sup> | 18.83 ± 0.15<sup>bc</sup> |

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.

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 T₀, however minimum value was determined in T₀ (41.45) at the termination of storage study. In T₀ the L value was 43.85 at initial day of storage which declined to 41.47 and 41.45 at 21<sup>st</sup> & 28<sup>th</sup> day, correspondingly. Moreover, L value of T₁ and T₂ 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 T₂ at the termination of storage study and the minimum value was found in T₀ (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 T₀ bars was 4.15 kg at initial day of storage which escalated to 4.32 kg at with the progression in storage at 28<sup>th</sup> 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 T₀ but minimum value was found in T₀ & T₁ i.e. 3.30 & 3.31 kg at 7<sup>th</sup> day of storage. The results of the findings indicated that maximum level of firmness in T₀ was might be due to storage time and conditions. But results of T₁ and T₂ 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 T₁ (3.97 kg) at end of storage period while lower level of toughness was found in T₀ (3.31 kg) at initial day of storage. The obtained results revealed that maximum level of toughness in T₁ 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 T₀ was 3.31 kg at 0 day of study which escalated to 3.51 and 3.6 kg at 21<sup>st</sup> & 28<sup>th</sup> storage day, correspondingly. This increase in toughness was might be due to textural changes in carbohydrates with the advancement in storage period.
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Table 6. Effect of storage on texture of Moringa Bars.

| Treatment | 0 | 7  | 14  | 21  | 28  | Texture |
|-----------|---|----|-----|-----|-----|---------|
| T_0       | 4.15 ± 0.15^a | 4.19 ± 0.12^a | 4.23 ± 0.13^a | 4.28 ± 0.11^a | 4.32 ± 0.10^a | Hardness |
| T_1       | 4.39 ± 0.10^a | 4.45 ± 0.14^a | 4.47 ± 0.10^a | 4.52 ± 0.12^a | 4.59 ± 0.12^a | (Kg)  |
| T_2       | 4.97 ± 0.14^b | 4.98 ± 0.15^b | 5.01 ± 0.12^b | 5.01 ± 0.13^b | 5.06 ± 0.14^b |        |
| T_3       | 4.85 ± 0.13^c | 4.87 ± 0.11^bc | 4.88 ± 0.11^bc | 4.93 ± 0.16^bc | 4.96 ± 0.16^bc |        |
| T_4       | 4.68 ± 0.11^d | 4.68 ± 0.13^d | 4.72 ± 0.13^d | 4.76 ± 0.12^d | 4.82 ± 0.12^d |        |
| T_5       | 3.36 ± 0.09^e | 3.30 ± 0.12^e | 3.43 ± 0.14^e | 3.51 ± 0.14^e | 3.63 ± 0.13^e | Firmness |
| T_6       | 3.49 ± 0.10^h | 3.40 ± 0.12^h | 3.51 ± 0.12^h | 3.67 ± 0.10^h | 3.62 ± 0.12^h | (Kg)  |
| T_7       | 3.40 ± 0.11^h | 3.31 ± 0.13^h | 3.46 ± 0.15^h | 3.51 ± 0.11^h | 3.61 ± 0.10^h |        |
| T_8       | 3.67 ± 0.10^h | 3.60 ± 0.12^h | 3.71 ± 0.14^h | 3.76 ± 0.12^h | 3.83 ± 0.15^h |        |
| T_9       | 3.88 ± 0.13^h | 3.80 ± 0.13^h | 3.90 ± 0.14^h | 3.94 ± 0.16^h | 3.97 ± 0.16^h |        |
| T_10      | 3.31 ± 0.11^e | 3.32 ± 0.13^e | 3.43 ± 0.13^e | 3.52 ± 0.11^e | 3.60 ± 0.12^e | Toughness |
| T_11      | 3.51 ± 0.12^e | 3.59 ± 0.14^e | 3.61 ± 0.10^e | 3.67 ± 0.13^e | 3.71 ± 0.11^e | (Kg)  |
| T_12      | 3.40 ± 0.13^e | 3.43 ± 0.13^e | 3.48 ± 0.15^e | 3.50 ± 0.17^e | 3.61 ± 0.10^e |        |
| T_13      | 3.63 ± 0.14^e | 3.64 ± 0.16^e | 3.73 ± 0.12^e | 3.77 ± 0.12^e | 3.82 ± 0.13^e |        |
| T_14      | 3.78 ± 0.12^e | 3.81 ± 0.11^e | 3.90 ± 0.10^e | 3.94 ± 0.11^e | 3.97 ± 0.14^e |        |
| T_15      | 16.74 ± 0.13 | 16.74 ± 0.19 | 16.74 ± 0.14 | 16.75 ± 0.17 | 16.75 ± 0.13 | Work of |
| T_16      | 16.80 ± 0.17 | 16.80 ± 0.17 | 16.81 ± 0.13 | 16.81 ± 0.11 | 16.81 ± 0.12 | shear |
| T_17      | 16.70 ± 0.22 | 16.71 ± 0.12 | 16.71 ± 0.18 | 16.70 ± 0.16 | 16.70 ± 0.15 | (Kg/mm) |
| T_18      | 16.80 ± 0.17 | 16.81 ± 0.15 | 16.77 ± 0.13 | 16.76 ± 0.17 | 16.80 ± 0.17 |        |
| T_19      | 16.84 ± 0.17 | 16.85 ± 0.20 | 16.84 ± 0.14 | 16.84 ± 0.16 | 16.85 ± 0.14 |        |

T_i: control; T_j: Bars with 10% flour; T_k: Bars with 5% protein Isolate T3; Bars with 10% protein isolate T_j: 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 T_1 (16.85kg/mm) on 28th storage day while minimum value was seen in T_0 (16.70 kg/mm) during the whole study period. From the results it was revealed that minimum work of shear was in T_3 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_3 was 16.74 kg/mm at the initiation of storage which showed slight decline in flavor till 28th day of storage.

From the Figure No. 02, it can be concluded that the best flavor accepted by the panelist was of T_4 and T_5 at 0 day of storage which showed slight decline in flavor till 28th day of storage. The rejected flavor was of T_2 and T_3 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_3 and T_4 which was slightly affected with the progression of storage. The most rejected treatment by the panelist was T_8 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_3 and T_4 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_1 and T_1 not acceptable by the sensory panelist.

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_9 and T_10 and lowest one was in T_11. Slight decline was seen in the T_0 and T_11 color with the progression of storage may be due to lower storage temperature but the increase in T_11 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.
because there developed a grainy texture and sticky mouthfeel in the T\textsubscript{4} and hardening in T\textsubscript{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\textsubscript{0} and those having intermediate protein contents as in T\textsubscript{3} were accepted more than those of the T\textsubscript{1}, T\textsubscript{2} and T\textsubscript{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).
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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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