PAPER

MULTILEGUME BAR PREPARED FROM EXTRUDED LEGUMES FLOUR TO ADDRESS PROTEIN ENERGY MALNUTRITION

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ABSTRACT

This study was planned to develop and characterize extruded multilegume savory bars as a protein supplementary nutrition. Legumes were extruded to prepare composite flour. Proportions of extruded flour were mixed with whey protein concentrate, honey and palm oil for preparation of protein bar. The product was evaluated for physico-chemical, minerals, calorific value, color, hardness, protein digestibility and sensorial characteristics. *In vitro* protein digestibility was found from 62.04 to74.98% and *in vivo* from 65.30 to 84.01%. Extrusion process and addition of whey protein concentrates significantly affected the nutritional and sensorial parameters of bars.

Keywords: legume protein, extrusion, bar, protein digestibility

1. INTRODUCTION

Malnutrition is an abnormal physiological condition triggered by imbalanced, inadequate or excessive consumption of nutrients (RIZWANA *et al*., 2015) while, proteins energy malnutrition (PEM) is a change of pathological conditions arises due to deficiency of protein calories (ERNEST *et al*., 2013). Developing economies are adversely affected by the malnutrition. Globally, there are more than 150 million children under the age of five years who are malnourished. The majority of these children are residing in just three countries of the South Asia *i.e.* India, Bangladesh and Pakistan where almost 54% of child deaths are linked to this menace (UNICEF, 2016). Unhealthy diet, ecological conditions and general living standard have a strong relation with diseases. According to Global Hunger Index (GHI), Pakistan is at 11th position from 118 countries with respect to malnourished population (22%), stunted growth (45%), wasting (10.5%) and mortality (8.1%) in children under 5 years of age (IFPRI, 2016). Likewise, according to National Nutrition Survey (2011), 58% of the population is facing the food security situation. Due to malnutrition, women and children are facing macro- and micro-nutrient deficiencies. About 31.5% of the children are underweight, 43.7% are stunted and 15.1% are suffering from wasting. Children (39-61%), pregnant women (38-69%) and non-pregnant women (26%-68%) are facing iron, zinc, vitamin A and D deficiencies (GOP-Pakistan, 2011).

PEM is one of the important public health issues in developing countries (VAN DER POLS-VIJLIBRIEF *et al*., 2014). Marasmus, kwashiorkor and marasmic-kwashiorkor are the primary reasons of PEM (ERNEST *et al*., 2013) that is associated with co-morbidities such as anaemia, tuberculosis diarrhea and malaria (le Roux *et al*., 2010) and these causes may lead to death. Several policies have been implemented to overcome the issue of PEM that involves different food based strategies such as dietary modifications, food enrichment and supplementation. School health programs are also initiated in various countries to mitigate this situation (ONIS, 2012).

Protein as a nutrient is considered a dietary component that evokes the widest array of complex scientific, economic and environmental issues, viewed as the most expensive but essential ingredient forming part of a healthy balanced diet (SCHÖNFELDT and HALL, 2012). Edible legumes belong to the family *Leguminosae* entitled as *Fabacae*. These are termed used for grain legumes which are generally grown up for their edible seeds. Legumes also called "a poor man's meat". Legumes are abundantly cultivated in subtropics and tropics areas of the world. They are the good alternative to animal protein for those people who have limited resources (ADEBOWALE and LAWAL, 2004). They possess amounts of amino acids such as leucine, lysine, aspartic acid, arginine and glutamic acid. They are vital sources for food proteins and also give rational essential amino acids when used with grains or other foods (SARWAR *et al*., 2013). It can be used with other food items to enhance the nutrition. They are also an excellent source of micronutrients as contain riboflavin, thiamin, niacin, selenium, folate, and pyridoxine (USDA, Agricultural Research Service 2012). They have good amounts of vitamin A, E and C (RAATZ; The Bean Institute 2010). Application of extrusion process on legumes modifies the physico-chemical parameters for improving functional properties in target applications (OSEN *et al*., 2015). Thermal extrusion has advantages as it helps to hinder anti-nutritional factors such as haemagglutinins, trypsin inhibitors, tannins, phytates which inhibit protein functionality and digestibility (ALONSO *et al*., 2000).

Since, legumes have good sources of protein and micronutrients and can be modified into an extruded product. They can be used to prepare protein rich bars, that can diversified the diet with natural approach to enhance the nutritional requirements and reduce the malnutrition of poor regions by using as a cultural food. The present project was planned to prepare extruded multilegume savory bar in order to mitigate PEM. According to Institute of Medicine the recommended daily allowance of protein for adults older than 18 years is 0.8 g/kg/d and the youngsters (under 18 years of age) required 13-52 g of protein per day (NAP, 2005). The primary objective of this multilegume product was to provide the enough nutrients that fulfill the daily requirement of protein but will not cross the threshold level for children and adults in the absence of meat products to address PEM.

2. MATERIALS AND METHODS

2.1. Procurement of Materials

Chickpea (*Cicer arietinum* L), mung (*Vigna Radiata*), mash (*Vigna Mungo L*), soybean (*Glycine max*), whey protein concentrates (WPC 80), palm oil and honey were purchased from the markets of Faisalabad, Pakistan.

2.2. Preparation of extruded multilegume savory bars

Thermal extrusion has advantages to destroy the anti-nutritional factors such as haemagglutinins, trypsin inhibitors, tannins, phytates, and helps in production of bioactive peptides and improve protein functionality and digestibility. All legumes were soaked for 15 hrs and dried for thermal extrusion. Legumes were fed into a twin-screw extruder (DNDL 44, Bühler AG, Uzwil, Switzerland) using the method described by Tremaine and Schoenfuss (2014). Optimized extrusion conditions of feed flow rate (60 kg/hr), screw speed (250 rpm), feed moisture content (10%) and barrel exit temperature (160°C) were used for the preparation of extruded powder. Extrudates were pelletized and dried in vacuum oven. Drying continued at 40°C in oven for 26 hrs.

Chickpea flour and other legumes (mash, mung and soybean as composite flour) were mixed in proportions and prepared different treatments as described in table 1. Whey protein concentrates (3%); honey (3%) and palm oil (3%) were added in each sample for better mixing, sensorial and nutritional properties. Purpose of combining proteins from vegan and vegetarian diets is to provide sufficient amounts of some essential amino acids to make complete protein intake. Palm oil has good spreading properties, technically useful and economically beneficial as compared to animal fat as well. After mixing, sheeting was done, and cut into bars of 3.5 centimeter (cm) width, 2 cm height, and 9 cm in length. Each bar of approximately 50 g was packed individually in aluminum foil.

Using AACC 2000 methods, moisture content (method no; 44-15.02), crude protein (method no; 990.03), crude fat (method no; 30-10.01), crude fiber (method no; 962.09), ash (method no; 942.05) and NFE content were determined. Water activity of prepared bars was determined using a previously described AOAC method (AOAC, 2012; method no. 978.18). The color values for each treatment were determined through Color Meter (Color Test II, Neohuaus Neotec, Germany) by following the method described by Hunter (1987). Calorific values of bars were determined through Oxygen Bomb Calorimeter (IKA-WERKE, C2000 Basic, GMBH and CO. Germany) as described by Miller (1959). Hardness of bars was measured according to the method of Piga *et al*. (2005). Results were obtained on the basis of compression force (kg) used to press the bars.

Table 1. Treatment plan.

Treatment	Formulation
T_0	Chickpea flour/composite% as 100/0
T_1	Chickpea flour/composite% as 70/30
T_2	Chickpea flour/composite% as 55/45
T_3	Chickpea flour/composite% as 40/60
T_4	Chickpea flour/composite% as 25/75
T_5	Chickpea flour/composite% as 0/100

Composite flour contains mung, mash and soybean flours.

2.3. *In Vitro* study for protein digestibility

Using the method of Akeson and Stahmann (1964) (with some modifications), *in vitro* protein digestibility was determined. Aliquots of 250 mg of each sample were suspended in 15 mL of 0.1 mol equi/L HCl containing 1.5 mg/mL pepsin (Sigma®, St. Louis, MO, USA) and incubated for 3 hrs at 37°C in a water bath. Hydrolysis from pepsin was stopped after neutralization by adding 7.5 mL of 0.5 mol equi/L of NaOH, then pancreatic digestion started by the addition of 10 mL of 0.2 mol/L phosphate buffer (pH 8.0) containing 10 mg of pancreatin (Sigma®, St. Louis, MO, USA) with 1 mL of 0.005 mol/L sodium azide to hinder microbes growth and incubated at 37°C for 24 hrs. After hydrolysis with pancreatin, 1 mL of 10 g/100 mL of trichloroacetic acid was added and centrifuged at 550×g for 20 min. The supernatant was collected and the total protein content was calculated using Kjeldahl (on the basis of nitrogen content) using AOAC (2012) method.

$$\% \text{ Digestibility} = (Ns - Nb)/Ns \times 100$$

Ns = nitrogen content in the sample, Nb = nitrogen content in the blank.

2.4. *In Vivo* study for True Protein Digestibility (TPD)

Male Sprague-Dawley rats (350±12 g) of 9 weeks old were procured from Animal House, National Institute of Health, Islamabad, Pakistan and maintained under standard laboratory conditions at 28±2°C with constant light-dark cycle. Rats were fed on standardized chow for an acclimation period of 2 days and then 36 rats were divided into groups of 6 rats. Rats were fed for 10 days in which 2 days were for acclimation period. Rats were weighed on daily basis during study. After 4 days period, spilled food and feces were carefully collected and separated from each rat. The spilled food was dried for 72 hrs in air while collected feces were dried in oven overnight at 100°C, weighed, grinded and analyzed for nitrogen content. Weight of spilled food and uneaten food were minus from the total food supplied to rat to determine the nitrogen intake.
TPD was calculated as:

$$\text{TDP} = \frac{I - (F - F_k)}{I} \times 100$$

I= intake nitrogen, F= fecal nitrogen, and F_k=metabolic or endogenous fecal nitrogen.

2.5. Sensory Evaluation

Attributes like color, texture, folding ability, chewability, taste and overall acceptability of extruded multilegume savory bars were analyzed by a penal of judges using 9- Point Hedonic Scale system as described by Meilgaard et al. (2007). All experiments were conducted in triplicates and average values were considered as mean values. The significance of values was calculated statistically through mean using Analysis of variance (ANOVA) at probability of 0.05.

3. RESUKTS

3.1. Proximate composition of extruded flour

Extruded multilegume composite flours for each treatment were prepared by blending various amounts of mung, mash and soybean with chickpea and then analyzed for moisture, crude protein, crude fat, crude fiber, ash, NFE and mineral content. The mean values regarding proximate composition of composite flour is presented in Table 2.

Table 2. Proximate composition and mineral profile of flour of chickpea/mung, mash and soybean for savory bar development.

Chickpea flour/composite flour (%)	Moisture	Crude Protein[2]	Crude Fat	Crude Fiber[3]	Ash	NFE
T_0	3.98±0.18[1]	29.26±1.32	3.20±0.05	1.02±0.10	3.03±0.06	57.09±2.04
T_1	4.12±0.11	29.89±0.99	3.34±0.03*	1.35±0.13	3.09±0.09	54.17±1.98*
T_2	4.19±0.24	30.28±2.47	3.78±0.05*	1.98±0.10*	3.43±0.05	53.84±2.61*
T_3	4.06±0.11	30.49±3.97	3.86±0.03*	2.87±0.13*	3.51±0.09	50.20±1.39*
T_4	4.29±0.24*	30.58±2.64	4.94±0.05*	3.01±0.10*	4.28±0.05	49.84±2.09*
T_5	4.48±0.35*	31.43±3.29*	5.53±0.09**	3.30±0.08**	4.34±0.10*	47.50±1.87*
Mineral profile (mg/100g)	**Na**	**K**	**Ca**	**Fe**	**Zn**	
T_0	11.51±0.25	477.00±12.45	81.39±19.10	04.70±0.63	02.54±0.09	
T_1	13.65±0.29*	614.50±05.23**	113.85±2.62**	06.62±0.25*	03.01±0.09*	
T_2	14.72±0.21*	682.80±09.19**	129.70±5.69**	07.36±0.14*	03.30±0.03*	
T_3	15.86±0.36*	751.87±07.71**	146.55±2.70**	07.75±0.29*	03.45±0.09*	
T_4	16.87±0.49*	820.93±11.40**	155.40±2.22**	07.92±0.23*	03.75±0.08*	
T_5	18.65±0.39*	925.27±11.35**	188.25±4.50***	08.20±0.20*	03.94±0.09*	

[1]Mean values (on dry basis) ± standard deviation. Different superscripts (*) on values in columns show significance difference ($p< 0.05$) within treatment.

[2]Calculated using N x 6.25 for proteins

[3]Calculated by difference of 100 - (ash + proteins + fat +starch).

The moisture content was ranged from 3.98±0.18 to 4.48±0.35%, crude protein 29.26±1.32 to 31.43±3.29%, crude fat 3.20±0.05 to 5.53±0.09%, crude fiber 1.02±0.10 to 3.30±0.08%, ash 3.03±0.06 to 4.34±0.10% and NFE 47.50±1.87 to 57.09±2.04%. Significant ($p < 0.05$) difference in nutritional composition was observed with the increase of multilegume composite flours in formulations. Maximum Na content was observed in T_5 (18.65±0.39 mg/100g) and minimum (11.51±0.25 mg/100g) in T_0. K content of formulations was ranged from 477.00±12.45 to 925.27±11.35 mg/100g in which maximum content was observed in T_5 and minimum in T_0. Ca content of formulations was ranged from 81.39±19.10 to 188.25±4.50 mg/100g. The highest Ca content was observed in T_5 and the lowest Ca was noted in T_0. Maximum values for Fe were found in T_5 (08.20±0.20 mg/100g) and minimum values in T_0 (04.70±0.63 mg/100g). Zn content was found lowest in T_0 (02.54±0.09 mg/100g) and highest in T_5 (03.94±0.09 mg/100g). Significant ($p<0.05$) difference was found within treatments from control in mineral analysis.

3.2. Proximate composition of multilegume savory bars

The mean values regarding proximate composition of multilegume savory bars are shown in Table 3.

Table 3. Proximate composition of multilegume savory bars.

Savory bar legumes ratio	Moisture	Crude Protein[2]	Crude Fat	Crude Fiber[3]	Ash	NFE	Energy[4] (Calories/100 g)
T_0	3.99±0.21[1]	31.98±0.87	5.03±0.12	0.99±0.26	3.24±0.13	58.03±1.08	418.03±13.04
T_1	4.24±0.09	32.76±0.16	5.63±0.24	1.29±0.31	3.45±0.19	56.23±1.98	436.74±12.09*
T_2	4.35±0.19	33.01±0.34	5.98±0.76	1.92±0.41	3.69±0.23	53.12±2.42*	458.67±10.26*
T_3	4.40±0.20	33.56±0.23	6.73±0.53*	2.76±0.27*	3.93±0.17	51.89±2.32*	526.18±09.87*
T_4	4.43±0.29*	33.93±0.49*	7.09±0.49*	2.86±0.65*	4.56±0.32*	50.63±1.59*	530.17±15.76*
T_5	4.61±0.25*	34.23±0.95*	7.99±1.02*	3.19±0.51*	4.89±0.09*	48.53±0.99**	546.49±19.87**

Mineral profile (mg/100g)	Na	K	Ca	Fe	Zn
T_0	11.65±0.12	479.12±4.32	81.29±9.34	04.65±0.43	02.51±0.04
T_1	13.71±0.18*	618.32±5.76**	114.31±1.93*	06.68±0.18*	02.97±0.12*
T_2	14.78±0.31*	685.47±6.20**	130.82±4.32*	07.71±0.07*	03.23±0.31*
T_3	15.92±0.28*	763.38±8.24**	147.25±3.21*	07.82±0.15*	03.49±0.54*
T_4	16.93±0.17*	824.52±5.91**	156.32±1.99*	07.89±0.09*	03.87±0.42*
T_5	18.69±0.16**	927.36±6.32**	189.17±3.35**	08.40±0.87**	04.02±0.41*

[1]Mean values (on dry basis) ± standard deviation. Different superscripts (*) on values in columns show significance difference ($p < 0.05$) within treatment.

[2]Calculated using N x 6.25 for proteins.

[3]Calculated by difference of 100 - (ash + proteins + fat+ starch).

[4]Caloric values were determined bomb calorimeter.

In all treatments, the moisture content ranged from 3.99±0.21 to 4.61±0.25%, crude protein 31.98±0.87 to 34.23±0.95%, crude fat 5.03±0.12 to 7.99±1.02%, crude fiber 0.99±0.26 to 3.19±0.51%, ash 3.24±0.13 to 4.89±0.09% and NFE 48.53±0.99 to 58.03±1.08%. Significant ($p< 0.05$) difference was found in all treatments in comparison with control for moisture content, crude protein, crude fat, crude fiber, ash and NFE in bars prepared from multilegumes composite flour. Maximum Na content was observed in T_5 (18.69±0.16 mg/100g) and minimum in T_0 (11.65±0.12 mg/100g). K content was ranged from 479.12±4.32 to 927.36±6.32 mg/100g in which maximum content was observed in T_5 and minimum in T_0. Ca content was ranged from 81.29±9.34 to 189.17±3.35 mg/100g. Maximum value for Fe was found in T_5 (08.40±0.87 mg/100g) and minimum in T_0 (04.65±0.43 mg/100g). Zn content was found highest in T_5 (04.02±0.41 mg/100g) and lowest in T_0 (02.51±0.04 mg/100g). Significant ($p<0.05$) difference was found for mineral content in bars within treatments in each column. Maximum calorific value was noticed in T_5(546.49±19.87 calories/100g) while lowest in T_0(418.03±13.04 calories/100g).

3.3. Water activity

Non-significant ($P > 0.05$) difference was found for water activities in bar as all values were recorded around 0.50.

3.4. Color of extruded multilegume savory bars

Color reveals the first impression of a food product before consumed. It's the first score of a like and dislike for food commodity. The mean values for color score of extruded multilegume savory bar are shown in Table 4. Highest value (58.67±0.14) of *L* was found in T_2 while the lowest value (50.50±0.13) was noticed in T_0. Maximum color value of *a** was 7.85 in T_2 while the minimum was 5.30 in T_5 that shows coloring trend towards redness. Maximum color value for *b** was 19.4±0.09 in T_5 and lowest was 15.69±0.04 in T_3 that shows coloring trend toward yellowness. T_1, T_2, T_4 and T_5 were significantly ($P<0.05$) different from each other while T_0 and T_3 were non-significantly ($P>0.05$) different in color value of *L**. T_0, T_2, T_3, T_4 and T_5 were significantly ($P<0.05$) different from each other while T_1 was non-significantly ($P>0.05$) different in color value of a*. T_3 and T_5 show significant ($p<0.05$) difference than other treatment in color value of b*.

3.5. Hardness of extruded multilegume savory bars

Hardness is one of the quality attributes, which describe quality of food bars before testing. The mean values of hardness for bars have been listed in Table 4. Maximum force (kg) was noticed on T_5 (8.61±0.76) while minimum on T_0 (5.33±0.34). Highly significant values were observed for T_5, T_4 and T_3 as compared to others.

3.6. *In Vitro* and *in vivo* studies for protein digestibility

Protein digestibility values were calculated for each treatment and results regarding digestibility are shown in Figure 1 for both *in vitro* and *in vivo* studies. *In vitro* digestibility was observed between 62.04 to 74.98% while TPD *in vivo* values ranged from 65.30 to 84.01%. Maximum value for TDP was observed in T_5 (84.01±3.91) and lowest value in T_0 (65.30±3.43). All the treatments were significantly different ($P<0.05$) from control sample in both studies.

Table 4. Mean values of color and hardness (kg) of multilegume savory bar.

Treatments	Color L*	Color a*	Color b*
T_0	[1]50.50±0.13	6.63*	17.8±0.07
T_1	54.81±0.09*	6.33	18.02±0.03
T_2	58.67±0.14*	7.85*	16.03±0.02
T_3	52.33±0.09	6.08*	15.69±0.04*
T_4	55.51±0.09*	5.40*	18.80±0.08
T_5	57.84±0.12*	5.30*	19.4±0.09*

Treatments	Hardness (kg)
T_0	[1]5.33±0.34
T_1	5.89±0.22*
T_2	6.17±0.43*
T_3	7.01±0.52**
T_4	7.99±0.56**
T_5	8.61±0.76***

[1]Mean values of triplicate representations + standard deviation, superscripts (*) show the significant difference ($p<0.05$) in same column.
L* represents the lightness ranging from darkness (0) to lightness (100).
a* represents redness varying from greenness (-a*) to redness (+a*).
b* represents the yellowness varying from blue (-b*) to yellow (+b*).

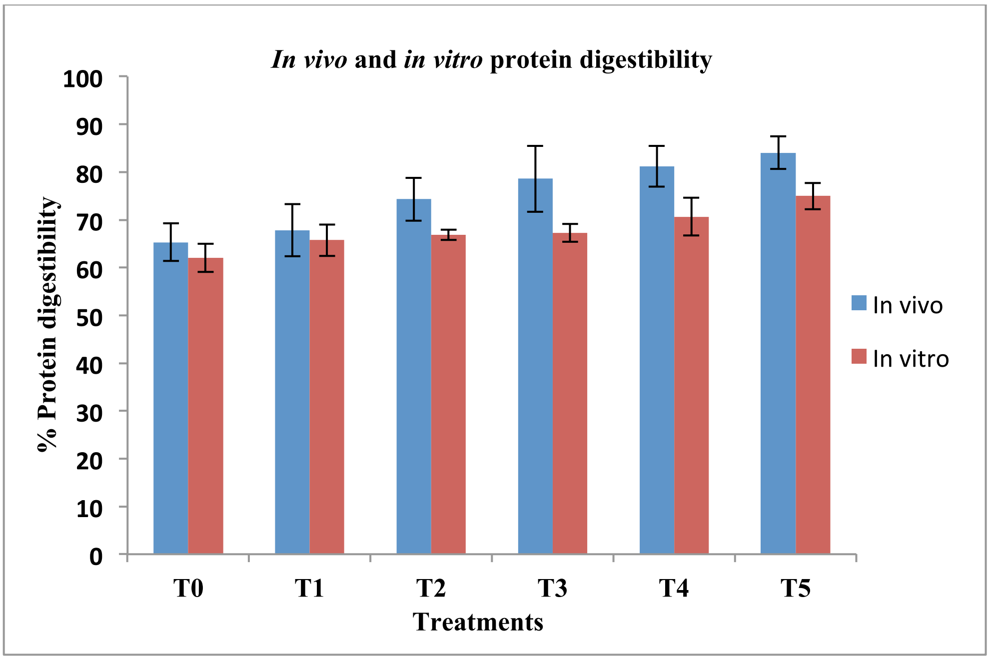
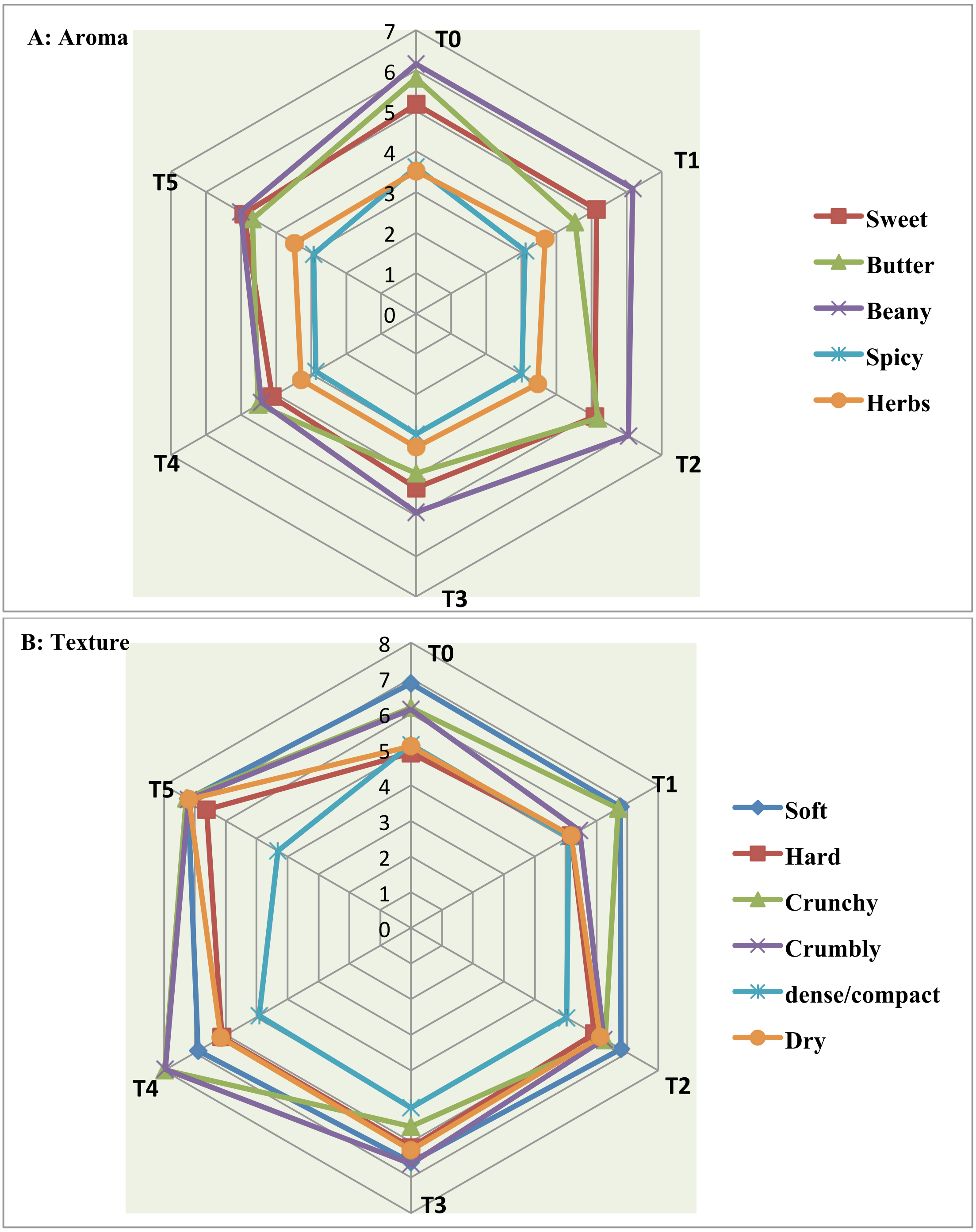


Figure 1. The *in vivo* and *in vitro* protein digestibility values (%) of different treatments. *In vivo* protein digestibility was evaluated using rats model and *in vitro* through pepsin and pancreatin.

3.7. Sensory evaluation of extruded multilegume savory bars

Values regarding aroma, texture and flavor analysis are shown Figure 2.



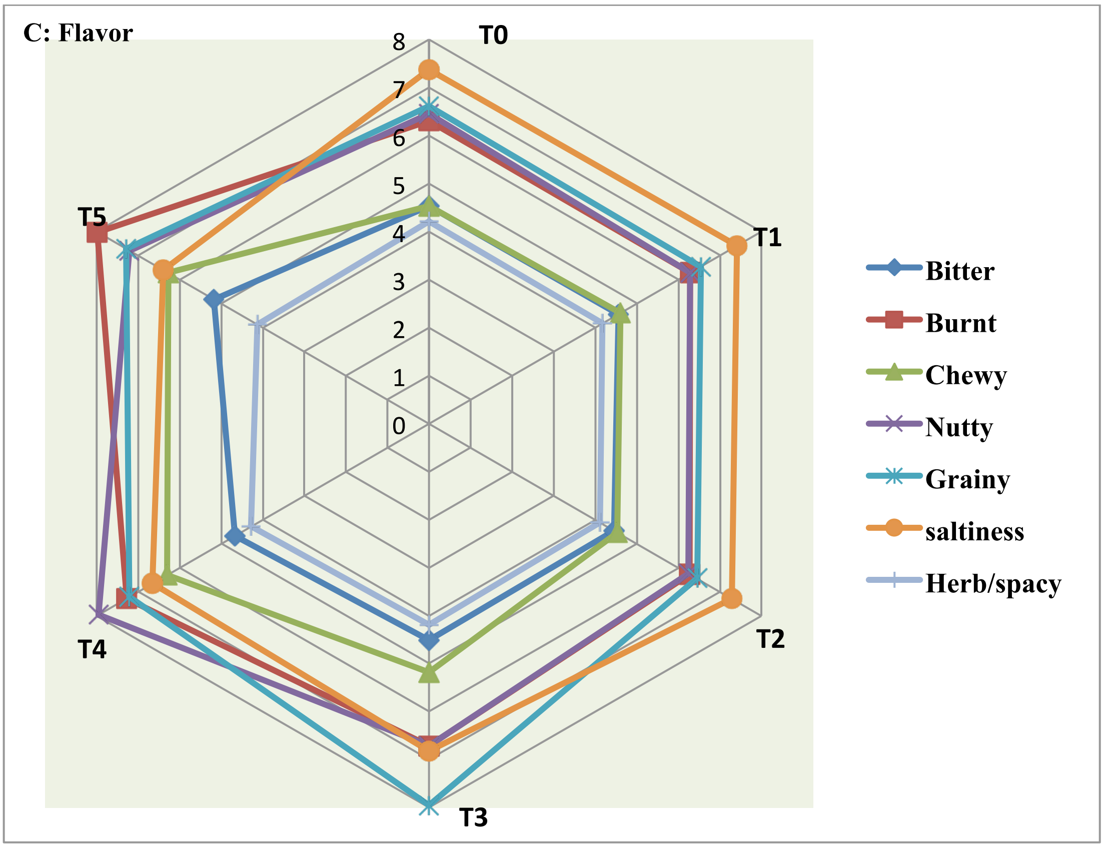


Figure 2. Mean values were obtained through 3 repetitions by 19 panelists each. (A= aroma, B= texture, C= flavor) Score range from 1 = dislike extremely to 9 = like extremely evaluated after 60 days of storage interval.

Texture observation of bar in T_3, T_4 and T_5 showed significant sweet, butter and bean aroma. Texture observation of extruded multilegume savory bars showed that all the treatments had rough texture; T_2 showed significant dryness in its texture, T_3, T_4 and T_5 showed crunchy and crumble texture. Bars made from T4 showed compact and dense texture whereas bars from T_5 were observed significantly soft in texture. Flavor analysis showed that T_3 has significant flavor of chewy, nutty and grainy, whereas flavor of bitter, burnt and saltish was found in T_5. Similarly, T_4 showed significant saltish flavor.

4. DISCUSSION

Legumes are good source of proteins and transformation of these into bars helps to mitigate the threatening situation of PEM. Addition of whey protein concentrate, honey and palm oil into bar was also added to improve nutritional and sensorial characteristics. Production of protein rich bars lower down PEM but also provide various minerals and vitamins to consumer in sufficient quantity to address various body functions. As legumes proportions were increased in treatments, values of protein, ash and energy increased. So, it is clear from results that addition of legumes in composite proportions helps to increase the nutritional status of bars. Nutritional value of proteins depends on the availability, digestibility and quantity of essential amino acids present in it. Extrusion helps in the inactivation of anti-nutritional factors and improves nutritional values. So, preparation of bar through extrusion treatment is one of the best method to conserve protein constituents for mitigation of PEM.

ABDEL-GAWAD *et al*. (2016) prepared composite flours using legumes and wheat and observed protein content between 11.76 to 19.05%, fat content between 1.36 to 3.25%, crude fiber between 0.59 to 1.55% and ash content between 0.63 to 2.40%. All values are slightly different from current study that is due to use of whey protein concentrate and different legume species, as composition varies within species. JAHREIS *et al*. (2015) prepared legume flour and found Ca content (47-221 mg/100g), K content (1030 to 1760 mg/100g), Fe content (4.3 to 7.7 mg/100g) and Zn content (2.5 to 4.1 mg/100g); these observations are slightly different from present study. This might be due to addition of different legume species.

NADEEM *et al*. (2012) prepared date bars and showed moisture content (15.56 to 18.70%), protein content (7.41 to 14.96%), crude fat (5.55 to 8.37%), crude fibre (3.58 to 3.88%) and ash content (2.30 to 2.91%). As multilegume increases in the bar protein, crude fibre and ash content also increases and improves minerals profile such as Na, Ca, K, Fe, Zn and essential amino acids without disturbing the sensorial parameters (Bower and Whitten 2000). Added proteins are functioned to keep the ingredients of food bars intact, maximize the strength, set the structure and contribute to water holding capacity. Whey protein possesses viscosity, water holding properties and gel strength that contribute to bar firmness (ORTIZ *et al*., 2008). Fat content not only provide caloric values but also increases the palatability of bars. Additionally, fat possess to act as a binder with sweeteners in agglutination of the ingredient present in bar that helps to impart compactness and firmness to texture of food bars (ESCOBAR *et al*., 1996). Color is the first impression of a food product. It's the first score of a like and dislike food commodity. SREBERNICH *et al*. (2016) prepared cereal bar with different formulations and found L^* value (52.78 to 62.70), a^* values (8.39 to 11.93) and b^* values (23.53 to 27.90), slight difference was found in our findings in accordance to current findings; this might be due to different composition of bars. RAJABI (2017) prepared high protein extruded bars and found hardness values between 6.08 to 9.33 kg; these values are comparable to current findings. Increase in harness was observed with the change in composite flour (%) that is might be due to the cumulative effect of different flours.

ALMEIDA *et al*. (2015) prepared whey protein supplements and found their protein digestibility between 88.4 to 91.7% *in vitro* and these values are not comparable to current findings because in this study vegetable protein sources were used that have more anti-nutritional factors and can form more complex protein structure and may hinder protein digestibility (BUTTS *et al*., 2012). *In vivo* true protein digestibility increased as the extruded multilegumes proportions increased in bars. ERBERSDOBLER *et al*. (2017) stated that digestibility of protein is high around 89-96% in weaning foods based on beans and rice and these values have contradictions to current findings and this might be due to use of different legumes and processing conditions. NOSWORTHY *et al*. (2018) stated that extrusion of beans/legumes ameliorate the protein digestion as compared to other processing conditions. Changes in extrusion variables also affect the protein digestibility. With the increase in extrusion temperature, the protein digestibility values are increased as inactivation of protease enzymes occur rapidly as temperature increases. Increase in the shear forces increases the protein digestibility, this might be due to denaturation of protein increase with increasing screw speed (Bhattacharya and Hanna, 1985). In this study screw speed was 250 rpm and barrel exit temperature was 160°C, these conditions might be the reason of increase in protein digestibility of protein bars.

Extruded multilegume savory bar first time prepared, which has sensorial and nutritional qualities. Both nutritional and sensorial qualities of puffed cornmeal were enhanced when blended with milk protein isolates (ONWULATA, 2010), same is happened in current study. BANACH *et al*. (2016) prepared protein bar by using extruded milk protein concentrates that were more cohesive, softer and less textural changes were observed than bars prepared with the spray drying method. Hence, extrusion process modifies the physico-chemical parameters of ingredients not only the structural-function relationships of proteins and same findings are observed in the current work.

5. CONCLUSIONS

Compositional and sensorial characteristics of multilegume savory bars were assessed to consider the supplementation of protein from plant source (legumes) as alternate of meat protein. Proximate analyses showed that proportion of different legumes have significantly increased the nutritional values of savory bar. Significant increase in protein and minerals content were observed in bars and they have better protein digestibility in *vivo* and *vitro* studies conducted on rats. Protein from plant source can be an economical approach to produce these bars to mitigate PEM. So, these bars can be considered as well balanced fast food from nutritional point of view to handle the problems associated with PEM.

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Paper Received March 18, 2019 Accepted October 1, 2019