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Formulation and characterization of protein-energy bars prepared by using dates, apricots, cheese and whey protein isolate

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Abstract

Six protein energy-bars (B1-B6) were prepared for Pakistani-athletes using dates, dried apricots, Cheddar-cheese and whey-protein isolate. Bars B1-B3 contained 5 g Cheddar-cheese and 13 g whey-protein isolate while quantity of dates were 74, 68 and 65 g and apricots 8, 14 and 17 g respectively. Bars B4-B6 contained 8g Cheddar-cheese and 12 g whey protein isolate while dates and apricots were same as in bars B1-B3. Bars were sealed in aluminium foil, stored at 20 ± 5 °C for 45 days to evaluate their sensory, compositional, physicochemical, microbial, textural and antioxidant properties. All bars have good sensory attributes except B6 that showed ($p < 0.05$) lower acceptability. The pH, water activity and microbial count decreased ($p < 0.05$) in all the bars. Maximum hardness was noticed in bar B1 and lowest in B6 while highest firmness was recorded in B2 and lowest in B6. The increase ($p < 0.05$) of total phenolic content was noticed in B6 and the highest increase of total flavonoid content was estimated in B2 at day 45, while B5 showed the highest antioxidant-activity on the 30th day afterwards a decline was observed in all the bars. Study revealed that these bars have good sensory attributes, physical characteristics and shelf-stability and could be a healthy snack for athletes.

Keywords: protein energy-bars; dates; apricots; chadder-cheese; whey-protein isolate.

Practical Application: Protein energy bars are minimally processed, good source of functional components and healthy choice for consumers of different age groups, athletes, soldiers, sportsmen and astronauts in order to meet their basic nutritional requirement.

1 Introduction

The trend of natural products has been increased from last few years, retail-stores, specialized and super-markets are special sources to commercialize such products. These products have beneficial and constructive health effects as these are not contained residues of fertilizers and pesticides that are usually present in conventional-food and considered hazardous for human health (Iuliano et al., 2019). In a current busy society, calm sitting down for food is sometimes impossible and consumers are looking for healthy and ready to eat foods. There are almost hundreds of wrapped and potable bars available at gyms, supermarkets and health stores like power, balanced, protein, energy, carbohydrates, breakfast/cereal, brain-boosting and meal replacement bars. These kinds of bars are used as a quick source of energy in sports and breakfast.

To prepare ready to use fruit bars, various fruits are being used to attract the people of different age groups to compensate for their calorie and protein requirements. These bars are mainly composed of cereals, legumes, fruits and nuts with chocolate coating or chips (Munir et al., 2019). Health claims of such natural food bars showed positive effects regarding consumer acceptance due to their potential to act as a functional food. Therefore, stimulates the snack food industry to introduce the healthy snack-bars and encourage the food scientists to understand the value of natural snack-bars that how these products affect the consumer health and their daily food choices (Pinto et al., 2019).

Date palm is the oldest fruit tree of Arab countries and has religious importance for the Muslims due to its health importance and mentioning name at several places in the Quran (Al-Farsi & Lee, 2008). Dates are rich in carbohydrates, dietary fiber, vitamins and flavonoids. Apricot is another delicious fruit, not only contains carbohydrates and minerals but also has a significant quantity of bioactive compounds (Incedayi et al., 2016).

Pakistan is 7th and 10th largest producer of dates and apricot respectively but the export of these fruits is very limited. Almost 40-45% of produce is wasted due to lack of proper supply chain and value addition (Abul-Soad et al., 2015; Kousar et al., 2019). Apricot and dates are very cheap in Pakistan and available as raw material for many food products. Due to taste and aroma, their fruits are highly appreciated in the markets of Pakistan.

Owning the composition and health benefits of cheese, it is being exploited in the production of various health products. During cheese making process the obtained whey is also an important ingredient which is used in a wide range of food products due to its lower cost, good functional and nutritional quality (Alves et al., 2019; Guimarães et al., 2019; Trindade, et al., 2019). Therefore, in current study, cheese and whey protein are

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selected as a source of many essential nutrients such as protein (casein + whey), fat minerals (Ca, P, K, Mg) and vitamins (Kwak et al., 2012; Ahmad et al., 2019a). In Pakistan, the least importance is given to the diet for athletes. The instructors and nutritionist recommend them only whey protein isolate (WPI) for muscle building and they have no other choice. Considering this situation and after reviewing the literature, the present study was planned to develop the protein energy-bars (PE-bars) from dates, dried apricots, ripened Cheddar cheese (CC) and whey protein isolate (WPI) keeping in view the recommended dietary allowance (RDA) guidelines for athletes (carbohydrates and protein 7-12 g and 1.2-2.0 g/kg body weight respectively and fat intake 20-35% of total calories per day) (Jäger et al., 2017). The objective was to newly developed bars could meet the requirements of Pakistani athletes by consuming a serving size of two bars daily.

2 Materials and methods

Dates Aseel (tamer stage), dried apricots (Halman) were procured from a supermarket of Faisalabad, Pakistan. The CC (6 months ripened) was procured from "Fuji Foods" Bhalwal Pakistan while WPI procured from MYPROTEIN, Northwich, England. Most of the chemicals used in the study were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Fisher Scientific (CHEMTREC®, USA).

2.1 Preliminary trials for the formulation of PE-bars

Before finalizing the formulation of final bars, thirteen protein energy-bars (PE-bars) (B1-B13) were prepared during preliminary trials using different combinations of dates, dried apricots, cheese and WPI. The formulations of bars were so adjusted that consumption of 2 bars/day can provide 20% and 40% of calories and proteins respectively of RDA for athletes. In B1-B5 and B6-B10 bars, the carbohydrates (55g) were provided by the dates (74 g) and apricot (87.30g) respectively calculated based on carbohydrates in dates (74%) and apricot (63%). In all bars, the quantities of cheese (25% protein) and WPI (88% protein) were adjusted to provide 12.5g of proteins/bars. The quantity of cheese: WPI in B1 (50:0 g), B2 (37.5:3.5 g), B3 (25:7 g), B4 (12.5:10.5 g) and B5 (00:14.20 g) were adjusted to provide 100:0, 75:25, 50:50, 25:75 and 0:100% protein of 12.5g protein/100 bar. The concentration of date: apricot in PE-bars B11, 12 and 13 was 53.5:28.2g, 37.16: 46.65g and 24:62.85g respectively. These quantities were calculated on the ratios of 75:25, 50.50 and 25:75% for dates: apricots based on their contribution to 100% (55 g) of carbohydrates.

In 1st phase of sensory evaluation, 3 bars (B11-B13) out of 13 were selected which were close in sensory attributes while PE-bars B1-B5 and B6-10 were rejected due to high sugary taste and salty or slightly bitter taste respectively. In 2nd phase, taste and texture of PE-bars B11 (5:13 g), B12 (8:12 g) and B13 (10:11 g) were further improved by changing the cheese: WPI concentration as suggested by panellists. After sensory evaluation, the panellist rejected the B13 and suggested to reduce the apricot (<25%) and enhance the concentration of dates >75%) in B11 and B12 respectively.

In Phase 3, the bar B12 was divided into B#1, B#2 and B#3 in which the concentration of cheese and WPI remained same (8: 12 g), while the levels of dates: apricots were 74:8g, 68:14g and 65:17g respectively that provided 90:10%, 85:15% and 80:20% of total carbohydrates (55 g) respectively (Table 1). The bar B11 was also divided into B#4, B#5 and B#6, in which the concentration of cheese: WPI was similar (5:13 g), while the levels of dates: apricots were 72:8 g, 68:12 g and 64:16 g respectively. These quantities provided 90:10%, 85:15% and 80:20% carbohydrates of total 55 g respectively. These six PE-bars (B1-B6) were evaluated for microbiological, antioxidant potential, textural and sensory attributes to find out the best formulation of the bar.

2.2 Preparation of final protein PE-bars

For the formulation of bars, dates and apricots were cleaned, washed, soaked and grinded into the paste. The shredded cheese and WPI were homogeneously mixed according to the formulation (Table 1) in the form of a dough, followed by sheeting and cutting into bars of length 6", width 2", depth 1" and weighing 100 g/bar. These bars were sealed in aluminium foil bags and stored at temperature (20±5°C) for 45 days.

2.3 Compositional analysis

Composition of PE-bars including moisture, protein and lipid contents were determined by following their prescribed methods of AOAC (Association of Analytical Chemist, 2012) and the results were expressed on a dry-weight basis.

2.4 Physicochemical analysis of PE-bars

pH, acidity and water activity (A_w)

The pH of PE-bars determined by using the digital pH meter (Inolab WTW series 720) and acidity was determined by following the titration method of AOAC (Association of Analytical Chemist, 2012). The A_w of PE-bars was determined by using the hygropalm meter (Karl-Fast probe well equipped, A_w-Win, Rotronic model) according to the prescribed method of El-Nimr et al. (2010).

Table 1. Formulations of PE-bars.

Bars No.	Cheese (g)	WPI (g)	Dates (g)	Apricot (g)	Calories
B1	5	13	74	8	240.86
B2	5	13	68	14	237.08
B3	5	13	65	17	232.06
B4	8	12	72	8	271.45
B5	8	12	68	12	270.16
B6	8	12	64	16	272.70

Texture analysis

Texture profile of all PE-bars was evaluated by using the texture analyser (Hardness; TA.XT. plus, stable Microsystem) with a weight of 2 kg load cell through compression test (Zisu & Shah, 2007). Hardness and firmness of samples were measured by applying the force (kg) versus time (sec) with a disk probe (34 mm in diameter) and displacement speed of 56 mm/min. The maximum force that applied is used as an index of hardness and firmness.

Microbial analysis

The total plate count and mold count in PE-bars were determined according to the method of AACC (American Association of Cereal Chemists, 2000). The arithmetic mean was calculated and expressed as a total number of bacteria per gram and total mold count per plate.

Total Phenolic Content (TPC)

The TPC in PE-bars was determined by Folin–Ciocalteu test as described by Igual et al. (2012) with minor modifications. To calibrate the concentration of total phenolics in PE-bars the absorbance of standard Gallic acid solution of 0, 250, 500, 750 and 1000 ppm were also measured at 765 nm. The TPC was calculated (Equation 1) and results expressed in Gallic acid equivalent (mg of Gallic acid equivalent/5 g) of PE-bars.

$$TPC\ (mg\ GAE/g) = \frac{Conc.\ of\ Gallic\ acid\ (mg/mL) \times Vol.\ of\ extract\ in\ mL}{Weight\ of\ bar\ extract\ (g)} \quad (1)$$

Total Flavonoid Content (TFC)

The TFC in PE-bars was determined by following the spectrophotometric method with Aluminum Chloride ($AlCl_3$) (Stankovic et al., 2011). The TFC was calculated (Equation 2) and results expressed in Rutin equivalent (mg of Rutin equivalent/5 g) of PE-bar.

$$TFC\ (mg\ RUE/g) = \frac{Conc.\ of\ Rutin\ (mg/mL) \times vol.\ of\ extract\ in\ mL}{Weight\ of\ bar\ extract\ (g)} \quad (2)$$

Determination of Antioxidant Activity (AA)

The antioxidant activity of PE-bars was measured by DPPH (2, 2-Diphenyl-1-picryle-hydrazyl) test (Baba & Malik, 2015). The DPPH solution was used as a standard to measure the scavenging effect of PE-bars (Equation 3).

$$Scavenging\ effect\ of\ DPPH = \frac{OD\ of\ control\ (DPPH) - OD\ of\ bar\ Sample}{OD\ of\ control} \times 100 \quad (3)$$

3 Sensory analysis

The different sensory attributes (flavor, taste, texture and visual acceptance) were tested based on 9 points hedonic scale that represents the point '1' as least undesirable while point '9' the most-desirable remarks. The serving size of each bite was 3-4 gram that was consistent for each bar. Total of 25 panellists took part and fill their respective proformas. Drinking water offered during evaluation to rinse the mouth before testing the next bar.

3.1 Statistical analysis

The results were expressed as means ± standard deviation of triplicate determinations. The data were analysed through analysis of variance (ANOVA) for the comparison of means to evaluate the level of significance at 5%. Tukey's test was used for multiple comparisons between means ($\alpha = 0.05$). Simple linear correlation analysis was used to determine a relationship between the mean values of PE-bars and their storage effect.

4 Results and discussion

4.1 Compositional analysis of PE-bars

The results of the compositional analysis are presented in Table 2. The moisture content of PE-bars is a crucial factor in shelf stability and quality characteristics (taste, texture and visual acceptance) of any product (Pallavi et al., 2015). The moisture content of PE-bars varied significantly ($P < 0.05$) although the difference between B1-B3 and B4-B6 was non-significant that could be due to the similar concentration of cheese in B1-B3 (5 g) and B4-B6 (8 g). The higher concentration of cheese in B4-B6 could be responsible for the increase in moisture content.

Results regarding protein content showed that there was a significant ($P < 0.05$) variation between B1-B3 and B4-B6 due to the similar concentration of CC and WPI in these bars. The addition of WPI and cheese not only enhanced the amount of protein content but also improved the nutritional quality of PE-bars. Veggi et al. (2018) prepared the high-protein diet bars by the addition of soy-protein isolate and whey-protein concentrate and reported the similar results as their product contained 20-23% protein.

The lipid content of B4-B6 was higher as compared to B1-B3 which was again due to higher content of cheese in B4-B6. The WPI is practically free from fat (Haraguchi et al., 2010) therefore, the bar which contains a higher WPI is lower in lipid content.

Table 2. Compositional analysis of PE-bars.

PE-bars	Moisture (%)	Protein (%)	Lipid (%)
B1	13.73 ± 0.07^b	20.53 ± 0.07^b	0.197 ± 0.00^b
B2	13.65 ± 0.10^b	20.60 ± 0.13^b	0.194 ± 0.00^b
B3	13.76 ± 0.12^b	20.48 ± 0.11^b	0.195 ± 0.00^b
B4	22.15 ± 0.09^a	22.26 ± 0.04^a	0.311 ± 0.01^a
B5	22.26 ± 0.05^a	22.38 ± 0.04^a	0.318 ± 0.00^a
B6	22.14 ± 0.12^a	22.38 ± 0.06^a	0.316 ± 0.00^a

Values are means ± standard deviation of 3 replications. Means with different letters within each column represent a significant difference at $P < 0.05$.

4.2 Physicochemical analysis of PE-bars

pH and acidity

The trend regarding pH of PE-bars (Figure 1a) shows storage days and their interaction with bars significantly ($P < 0.05$) affected the pH. There was a decreasing trend in pH of all PE-bars up to 15 days of storage, afterwards, a decrease was noticed only in B2 and B4.

Similar to pH, acidity of PE-bar also varied significantly ($p < 0.05$) due to storage days and their interaction (Figure 1b). There was variation in acidity of all PE-bars except B5 and B6. At day one, the acidity increased in all PE-bars. On 15th day and onward, there was an increasing trend in acidity of all PE-bars except B2 and B3.

The main ingredients of PE-bars were dates, dried apricots, cheese and whey protein isolate which are main contributors of acidity and pH. Highest decline in pH of B1 (0.97%) and increase in acidity of B6 (1.31%) was noticed during storage. It could be due to higher concentration of dates in B1 (74 g of dates and apricots 8 g) and lower in B6 (64 g dates and 16 g apricot). The pH of dried apricots (Halman) is 4.26 and it contained an appreciable amount of ascorbic acid. The acetic acid, citric acid, malic acid and tartaric acid are main the organic acids that produced in dates and apricots thus conferring high acid content to the product (Ghnimi et al., 2018). An increase in invertase activity also produces more reducing sugars are available for the microbial activity which ultimately results in the production of acetate, carbon dioxide and hydrogen ions (H^+) (Mohd-Zaki et al., 2016). Thus content of organic acid increased and sugar concentration decreased that contributes in increased acidity during storage.

Water activity (A_w)

The bar graph of water activity (Figure 1c) shows the significant ($P<0.01$) impact of bars, storage days and their interaction on the A_w. The decreasing trend was observed in A_w during storage. The average higher water activity in B2 could be due to dates (68%) and apricots (14%). Neves (2016) reported that the bars containing 65% carbohydrate had higher A_w due to the higher moisture content and a decreased during storage in which protein powder was added. Moreover, A_w of bars varied as a function of its components and storage period. Silva et al. (2016), reported that the intermediate moisture food usually have the A_w from 0.9 to 0.6 that is lower enough to preserve the product from microbial spoilage and ensured the product stability The factor which shorten the storage life of intermediate moisture food is more often the chemical reaction in which important non-enzymatic browning (Maillard reaction) that occur fastest in the range 0.65-0.75 $A_{w.}$ Thus, bars beyond this range can be stored for an extensive period and allowing their manufacturing on a larger scale (Loveday et al., 2010).

Similarly, the bars B4, B5 and B6 showed a higher decreased in water activity during 45 days of storage. It could be possible

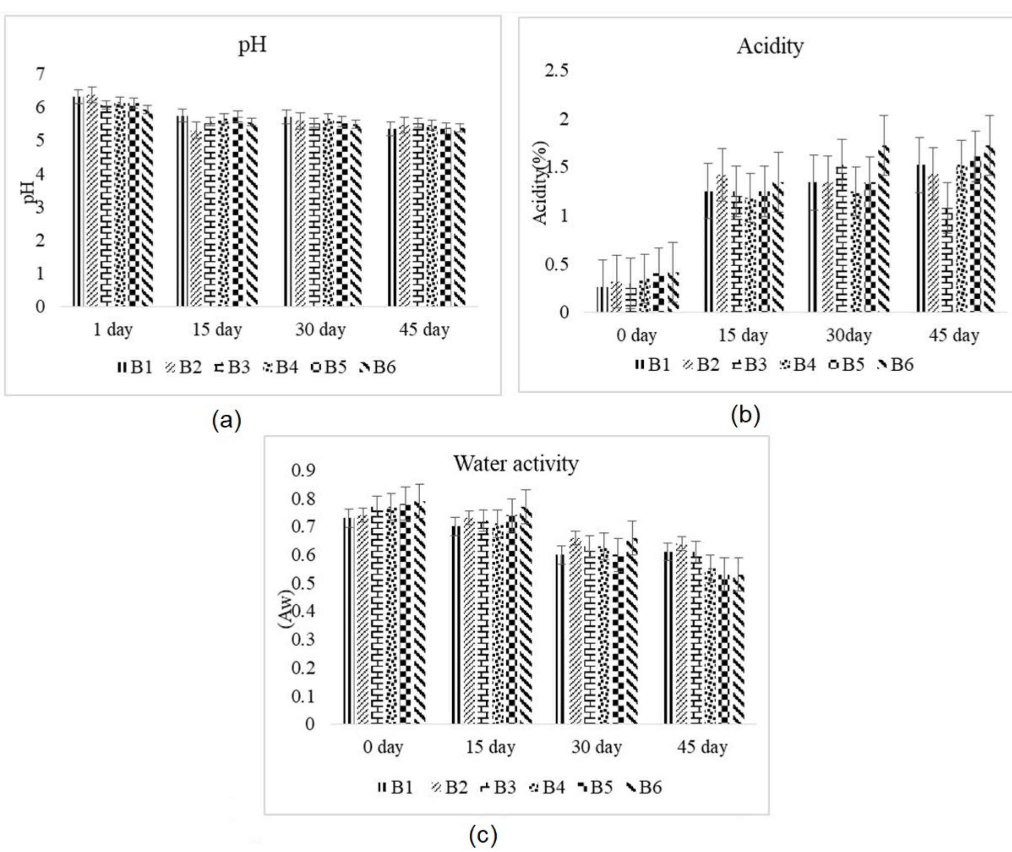

Figure 1. The bar graph showing the effect of storage on pH, acidity, water activity (A_w) of PE-bars.

due to higher concentration of cheese (8%) because casein protein in cheese exhibited the more water-binding property which causes to decrease the A_w of a product. Thus, casein has a significant role to improve the texture and consistency of food (Southward, 2003 ; Ahmad et al., 2019b).

Texture analysis

The changes in the hardness of PE-bars during storage are presented in Figure 2a. The hardness of PE-bars (0.16 kg-0.43 kg at day one) decreased after 15 days in all the bars except B1 in which it increased from 0.28-0.30 kg. Hardness continued to decrease till the 30^{th} day of storage, afterwards an increase was noticed up to 45^{th} day of storage.

The firmness of PE-bars (Figure 2b), revealed that it varied from 0.57-1.05 kg at 1^{st} day of storage. On 15^{th} and 30^{th} days of storage, a progressive decrease in firmness (0.32-0.58 and 0.27-0.39 kg respectively) was noticed in PE-bars. After this, at 45^{th} day of storage, the increase in firmness was observed in B3, B4 and B6, while a further decline was noticed in B1, B2 and B5. Hence, the highest value of firmness was recorded for B2 followed by B3 and B4 that contained 85, 80 and 90% dates respectively. The lowest level of firmness was recorded in B6 that contained a higher concentration of dried apricot (20%).

The hardness of B1, B2 and B3 could be due to high content of WPI (13%), while the comparatively lower hardness in B5 and B6 could be due to higher concentration of apricot and cheese. The WPI contained 90% whey proteins is in the form of dimers. The proteins aggregation and microstructural changes in protein bars usually affected the solubility of proteins. Therefore, this phenomenon has a large effect to induce the hardness in PE-bars immediately after manufacturing and throughout the storage period (Loveday et al., 2010).

The other factor that could be responsible for bar hardening includes the migration of moisture and Maillard reaction that induced the polymerization of protein and caused to plasticize the texture of bar (Rao et al., 2016). However, the results of the present study showed a decrease in hardness in first 30 days, due to the addition of dates and dried apricots, which are the good source of non-reducing sugars glucose and fructose (Tran, 2009). Similarly, the PE-bars that contained more CC also showed less hardness as compared to other PE-bars. Cheddar cheese is a good source of casein that act as an intact protein to reduce the bar hardness during storage (Rao et al., 2016).

Similarly, the firmness of B2 and B3 PE-bars could be due to higher concentration of whey protein, which was responsible for firmness but it results in undesirable texture and rubbery mouthfeel during storage. Therefore, the addition of whey protein in moderate concentration (12% w/w) contribute to improving the firmness of PE-bars (Purwanti et al., 2012). Moreover, the product firmness is significantly affected by the moisture content which is induced by the formation of cross-linking between sugars and proteins while the moisture acts as a plasticizer and reduces the formation of this cross-linkage and cause to decrease the firmness of a product (Rao et al., 2016).

Microbiological analysis

The statistical analysis shows that treatment, storage days and their interaction significantly ($P < 0.05$) affected the total plate count and mold count of PE-bars (Figure 3a, b). At day one, the highest plate count was noticed in B6 and lowest value in B1. At 15^{th}, 30 and 45^{th} day, the total plate count was decreased significantly in all PE-bars except B6 that showed a non-significant reduction. For mold count, at day one the mold count of PE-bars varied from 1.32 to 1.63 $\log^{10}$ cfu/2 g. After 15 days, it decreased in all PE-bars. However, the difference between B2 and B4, B5 and B6 were non-significant. From 30^{th} to 45^{th} day of storage, mold count decreased, but there was no significant difference between B2 and B6, B3 and B4.

The small difference in total plate count among all the PE-bars could be due to the difference in the level of major ingredients dates and dried apricots. Dates contained very high sugar content that attributes a hygroscopic behaviour, drawing the water from product and inserting sugar molecules inside thus very less water is available for microbial activity (Kang et al., 2012). The microorganisms are mostly neutrophilic and cannot grow at less than 4.5 pH and 0.8 water activity. The water stress

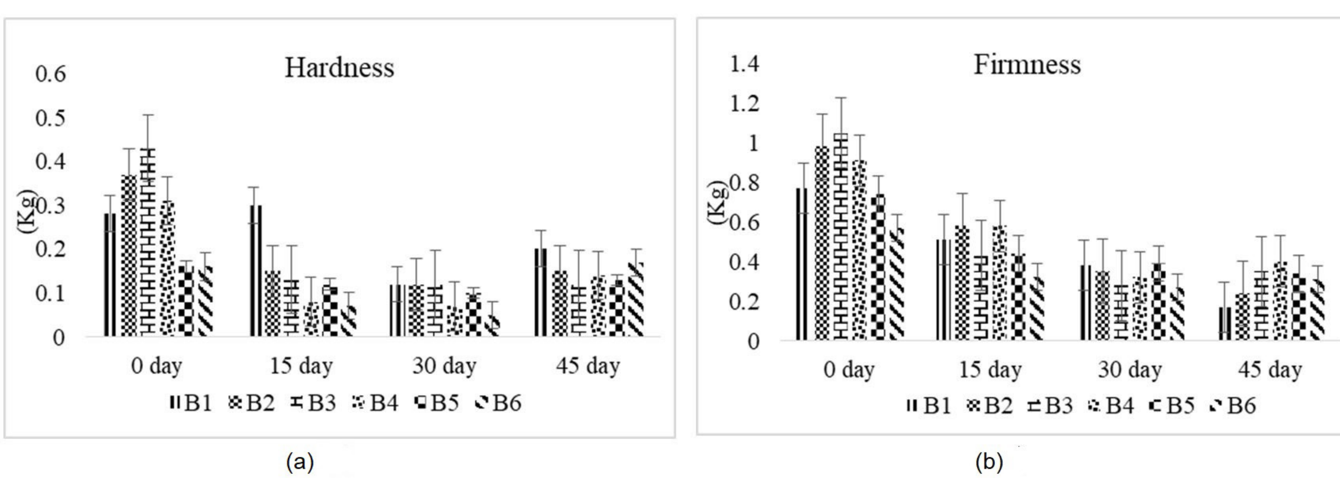

Figure 2. The bar graph showing the effect of storage on hardness and firmness of PE-bars.

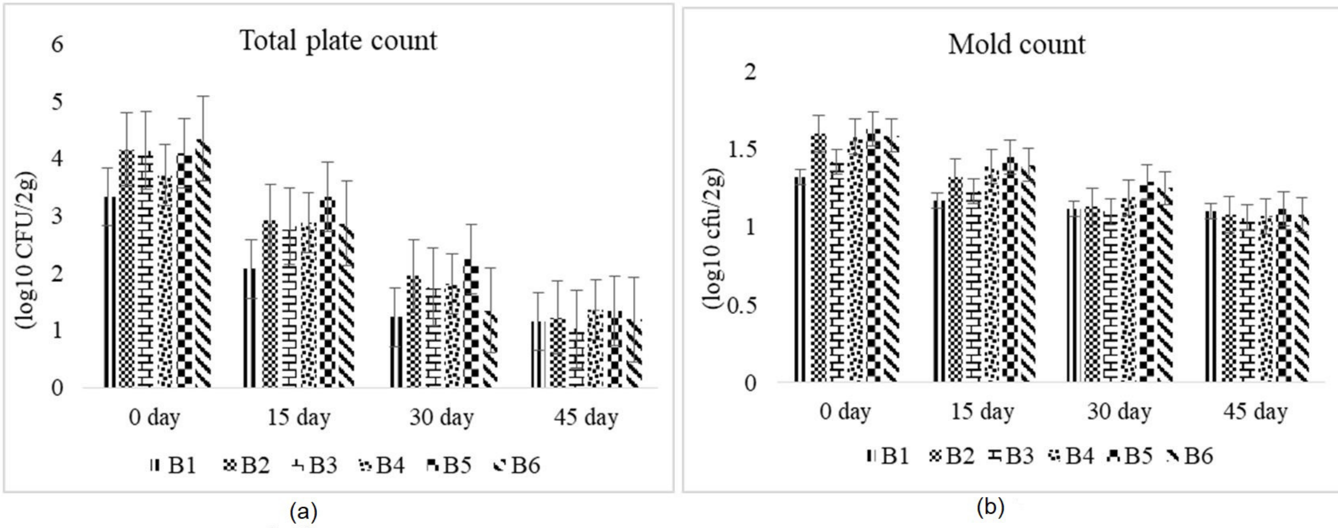

Figure 3. The bar graph showing the effect of storage on total plate count and mold count of PE-bars.

increases with lower water activity and these processes help to prevent the proliferation of microorganisms (Rahman, 2007). The decline in the bacterial count during storage could be due to the gradual decrease in pH. At lower pH, microorganisms expend more energy for the repulsion of excessive protons (H^+) from their cytoplasm to maintain the interior pH but at the same time, they also maintain their haemostasis that is disturbed due to the osmotic pressure stimulated by higher sugar concentration in date product. Both these haemostatic processes assisting in utilizing the maximum energy which initially slows down the growth of microorganisms but when they maintain it for an extended period, it ultimately causes the death of microorganisms as they are unable to supply the required energy to increase the pH and maintain the haemostasis (Abekhti et al., 2013).

There are various factors which influence the mold growth including water activity, relative humidity, temperature, pH and storage time. Molds can easily survive with A_w of 0.80 but no mold growth can occur when A_w is equal or less than 0.62 (Rahman, 2007). The mold growth occurs more frequently at A_w from 0.62 to 0.85 because of nutrition competition due to the absence of a wide range of bacteria. The survival of mold at lower A_w also depends upon the oxygen retention, nutrient availability, solutes concentration, temperature and pH. At lower A_w with high osmotic pressure, the spores and vegetative cells cannot survive. Higher sugar concentration in PE-bars results in the sugar fermentation that produces organic acids, ethyl alcohol, CO_2 and H^+ as by-products that play an important role to inhibit the mold growth in PE-bars (Mohd-Zaki et al., 2016).

Total Phenolic Content (TPC)

The TPC content of these PE-bars during storage are presented in Figure 4a. Results showed that the TPC in all PE-bars increased ($p < 0.01$) with the passage of time during storage. The TPC showed the highest value in B6 (containing 8% cheese and 12% whey) followed by B1. The TPC content of B4 and B5 were similar and the lowest value was noticed in B3. The higher content of TPC in PE-bars could be due to the presence of dates (193.8 mg GAE/100 g) and apricots (6530 mg GAE/100 g) (Al-Farsi & Lee, 2008; Ali et al., 2011). The change in TPC during storage is effected by the storage time and conditions. All PE-bars showed higher polyphenols content and tendency to increase than their initial values. The possible increment of polyphenols due to the reactions between oxidized polyphenols and formation of new antioxidant compounds (Martinez-Flores et al., 2015). The activity of phenylalanine ammonia-lyase in dates also affects and stimulates the biosynthesis of TPC and increase its concentration during storage (Haider et al., 2014). The relatively higher content of TPC in B6 as the addition of cheese that could also be responsible for the enhancement of TPC in PE-bars, which are derived from lactose, oligosaccharides and protein residues such as tyrosine (Yerlikaya et al., 2019). Moreover, through modification in protein structure and catabolism, some bound phenolic contents release from macro-components of cheese that could be the reason to increase the phenolic compounds in PE-bars (Rashidinejad et al., 2015).

Total Flavonoid Content (TFC)

There was a significant ($P < 0.05$) variation in TFC of bars (Figure 4b). The highest increased of TFC in B2 during 30 days could be due to the higher quantity of dates and WPI. The date fruit contained thirteen different types of flavonoids including quercetin and catechin and these compounds have strong interaction with whey protein as it contained more Sulphur containing amino acids (cysteine) that forms new complexes and could be the reason to increase the concentration of TFC during storage (Rahmani et al., 2014). It is worth mentioning that all the PE-bars which contained a higher content of cheese showed the lower value of TFC. In contrary to this, the higher content of dates and apricot showed the higher content of TFC in the PE-bars. Choi et al. (2015) studied the phenolic content of Gouda cheeses supplemented with fruit liquid. They reported that the flavonoid compounds in cheese increased from 300 mg RUE/100 g to 502.8 mg RUE/100 g at 0 week to 15 weeks. The increase in flavonoid compounds was continued

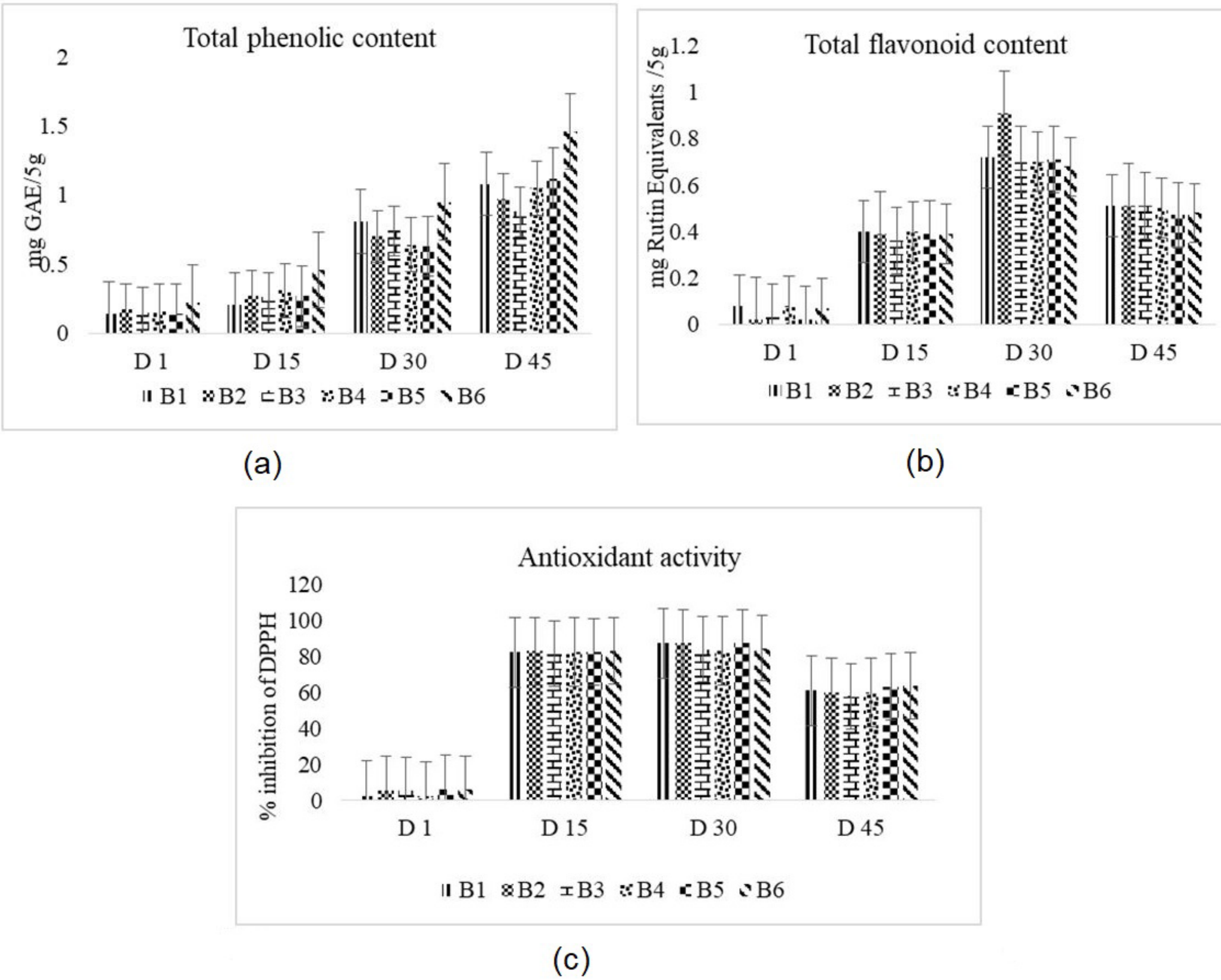

Figure 4. The bar graph showing the effect of storage on total phenolic, flavonoid contents and antioxidant activity of PE-bars.

until the 9th week of storage, afterwards a reduction in TFC was observed. They concluded from their study that these flavonoid contents may be the indicator of protein breakdown in ripened cheese and responsible for the increase of the antioxidant activity. The addition of WPI showed the higher content of TFC as compared to cheese, which shows the higher proteolytic activity of WPI in PE-bars.

Antioxidant Activity (AA)

It is apparent from Figure 4c that lowest antioxidant activity was at day one of storage. Afterword's, an abrupt increase in antioxidant activity was noticed is all the PE-bars significantly ($P<0.05$) at 15th day of storage. AA elevation was continued up to 30th day of storage but the elevation level was not that much as noticed during the first 15th days. At 45th day of storage, significant ($p < 0.01$) reduction in AA was observed in all the bars.

The composition of fruits and fruit-based products is very diverse and their AA depends on the type and amount of bioactive compounds present in them. A strong association between polyphenols and AA which support the present finding (Castro-Lopez et al., 2016). The reduction in AA of bars after 30 days of storage is according to the findings of Zheng & Lu (2011) who reported the variation in AA during storage of strawberries and pineapple juice. In present work, TPC increased up to 45 days and TFC increased up to 30th days during storage, similarly, AA also increased with the passage of time up to 30th days then decline was started. However, some other compounds may also contribute to AA of PE-bars including enzymatic antioxidants (peroxidase, catalase and superoxide dismutase) that are present in considerable amount in dates and known to provoke health benefits. Moreover, dates and apricots contained a significant amount of vitamins and minerals that either directly scavenges the free radicals or activate internal antioxidants (phytochemicals) by stimulating the antioxidant enzymes and providing the vitamins or minerals as cofactors for their optimal activity (Fatima et al., 2018). Dried apricots are a valuable source of bioactive compounds other than phenolic and flavonoids including anthocyanins (3.08 ± 0.40 mg CGE/100 g) and β-carotene (0.56 ± 0.03 mg/100 g) that could also be responsible for the increase of AA (Ali et al., 2011).

Sensory evaluation of PE-bars

The graphs of sensory evaluation (Figure 5a-d) shows the significant variation in bars regarding flavor ($p<0.01$), taste and visual acceptance ($p < 0.05$), while storage days only effected ($p < 0.01$) the visual acceptance. The interactive effect remained non-significant ($p > 0.05$) for flavor and taste while significant for texture ($p < 0.01$) and visual acceptance ($p < 0.05$).

The non-significant difference in flavor and taste of PE-bars (B1-B5) could due to the minor difference in concentration of dates and dried apricot which could not be judged by the panellist, which are the rich source of organic acids and volatile flavouring compounds (Ali et al., 2018). Xi et al. (2016) reported that the sucrose, γ-decalactone, β-Ionone and citrate are key flavouring compounds that contribute to the consumer acceptance for apricots. While the alcohols are identified the main volatiles compounds in dates that are responsible for the flavor in which Methyl-propionate and Ethyl-acetate are dominant ester compounds. Therefore, it is difficult to conclude that which specific compounds are responsible for flavor of PE-bars.

Klee (2010), reported that the organic acids and sugars in fruits are primary factors that responsible for the taste while volatile organic components contribute to the aroma. The PE-bars B1 showed relatively higher scores for a taste that contained a higher concentration of dates (74 g) than the others. The reason for low acceptability for PE-bars B5 and B6 could be the high concentration of cheddar cheese. As in Pakistan, the people are not very much familiar with the cheese flavour due to its consumption by a special segment of the population.

The panellists commented that B6 exhibited savory and salty taste, which could be possible due to the higher concentration of dried apricots and cheddar cheese that had higher acidity as well. Regarding texture, the panellists like the PE-bars B1-B4 due to its firm texture and a higher concentration of WPI. It was noted that the addition of whey proteins contributed to improves the textural properties of bars due to its water-holding and gelling capacity (Loveday et al., 2010). Loose texture and low firmness of PE-bars B5 and B6 could be due to the higher concentration of dried apricots and cheese and low concentration of WPI.

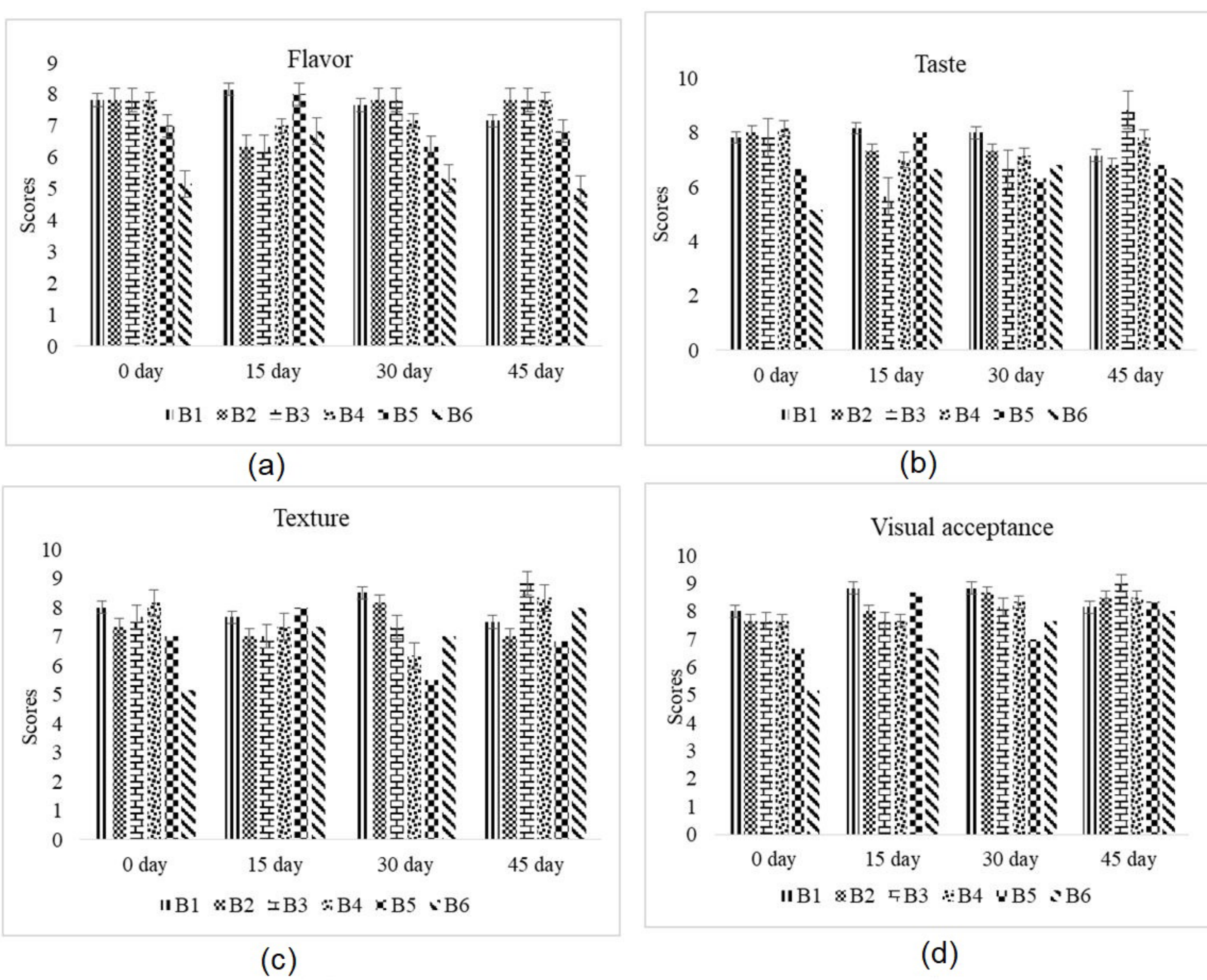

Figure 5. The bar graph showing the effect of storage on flavor, taste, texture and visual acceptance of PE-bars.

Thehigher level of moisture can be the other reason for reduced firmness in these bars.

The similar visual acceptance and overall acceptability of bar B6 could be possible due to its low texture quality and flavor. The sensory analysis of PE-bars B1-B5 was good in flavor, taste, texture, visual acceptance and overall acceptability. Therefore, extra work is required to improve the quality of B6 as compared to the other PE-bars.

5 Conclusion

All PE-bars (B1-B6) were presented the good sensory attributes except B6 that showed lower acceptability. Addition of dates and apricots increased the bioactive components while the addition of cheese and WPI increased the protein content of PE-bars that helps to improve the nutritional quality and textural properties of PE-bars. The water activity (A_w) and microbial count decreased during storage that presented the long and good shelf stability. In conclusion, our findings demonstrated that the PE-bars can be used as a meal replacement bar for older people, soldiers, in fitness clubs and for school-going children. Protein-energy malnutrition (PEM) is a common health problem in Pakistan. Therefore, these PE-bars can be modified keeping in view the RDA of children to combat the PEM.

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