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QUALITY ASSESSMENT OF CURATIVE AND NUTRITIOUS DATE FRUCTOSE BISCUIT BAR

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ABSTARCT

The demand for enhanced nutrition value biscuits is increasing to curb malnutrition syndromes among children and aged peoples. This study was undertaken to evaluate the effects of adding Indian vetch protein isolate and replacement of sucrose with date fructose on the physicochemical and sensory qualities of date fructose biscuit bars (DFBB). Five treatments of date fructose biscuit bars were prepared with different levels of sucrose replacement with date fructose. Control treatment (T_0) was prepared without date fructose and Indian vetch protein isolate. The results revealed that addition of protein isolate and date fructose in biscuit bars contributed desirable hardness in texture and acceptable crispness. The bars were shelf stable due to low A_w. Dark color was recorded in bars containing 20% date fructose than control. Gross energy values differed significantly among treatments. Mean values ranged from 3.49±0.1 to 4.34±0.2 Kcal/g. In-vitro starch digestibility ranged from 38.90±2.88 to 48.42±1.01mg maltose/g while in-vitro protein digestibility values also showed significant variation (P>0.05) among treatments. Biscuit bars containing 15% Indian vetch protein isolate and 15% sucrose replacement with date fructose were found the best in terms of physical and sensory characteristics. Indian vetch protein isolates (IVPI) and date fructose could be utilized in biscuit bars as a cost effective diet to treat a target populace.

Key words: biscuit bars, protein isolates, fructose, texture, water activity.

INTRODUCTION

Bakery products are an appropriate selection among value added food products for the supplementation of Protein Isolates (PI) from non-conventional plant sources (Bakke and Vickers, 2007). Biscuits prepared from wheat flour alone have not contained good quality protein due to deficiency of certain essential amino acids including lysine that declines the biological value of the products. A legume, Indian vetch (*Lathyrus sativus* L.*)* belongs to the family "*Fabaceae*" which is high in protein (28.70g/100g) and lysine and is a cheapest legume rather least investigated source of protein but it contains toxic factor which can be depleted by the application of various treatments (Rehman *et al.,* 2014). Economic Survey of Pakistan reported that protein deficiency is increasing at alarming rate in Pakistan like other developing countries. Thus, it is imperative that proper nourishment through balanced diets should be a necessary part of the children growth and healthy immune system for disease prevention (GOP, 2013).

Globally, prevalence of diabetes is boosting and in the developing countries, the higher level of increase has been recorded (Grol *et al*., 1997). In 1999, Diabetic Association of Pakistan, had conducted a survey and found that 10% people were suffering from prevalence of diabetes and same rate of impaired glucose tolerance. In world ranking of the diabetes, Pakistan is at 8th position (Samad-Shera, 1999). Dates are good in the diets of diabetic patients, because these contain high contents of invert sugars, which may be better utilized as a source of energy by diabetic patients than sucrose. Date's sugars can be converted into fructose by hydrolysis at low pH. Enzymatic treatments may be applied to extract maximum quantity of fructose from date paste (Rehman and Al-Farsi *et al*., 2005).

Protein energy malnutrition and micro and macro nutrients deficiencies result in poor health and stunted growth, adults face reduced work efficiency and diseases like Kwashiorkor and Marasmus and learning disabilities in the children (Powel, 2007).Considering the aforementioned aspects, this project was designed to explore the effect of adding Indian vetch protein isolate and replacement of sucrose with date fructose on the physicochemical and sensory qualities of date fructose biscuit bars.

MATERIALS AND METHODS

Procurement of raw materials: Wheat flour (maida), vegetable ghee, eggs, baking powder, Indian vetch (*Lathyrus sativus* L) and dates (Iranian dates) were purchased from local market, Faisalabad. Analytical grade chemicals, standards and enzymes (*cellulase, hemicellulase, pectinase, glucoseisomerase*) were purchased from Sigma Aldrich (Seelze, Germany).

Proximate analysis of raw materials: Raw materials and date biscuit bars were analyzed for proximate composition which includes moisture (44-15), crude protein (46-10), crude fat (30-25), crude fiber (32-10) and ash (08-01) according to the standard methods described in AACC (2000).

Removal of β-ODAP from matri: Indian vetch (IV) seeds were detoxified according to the steeping method (Rehman *et al*., 2006).

Extraction of fructose from date pulp: Enzyme assay was applied for the conversion of date pulp into date fructose (El-Sharnouby *et al*., 2009).

Extraction of protein isolates: IVPI were prepared by using the method as described by Johnson and Brekke (1983) with certain modifications. 50 g of flour were weighed and 800 mL of alkaline water (pH 9.8) were added to it. This sample was placed on the orbital shaker at room temperature for 40 min and then filtered. Afterward, the sample was subjected to centrifugation at 5000 rpm for 15 min. Due to the presence of protein in the supernatant; precipitation occurred after the adjustment of pH at 4.5 with 1N HCl. Lower layer, which is of high density, serves as the residue. The precipitates formed in supernatant were allowed to settle down. All the proteins were settled at the lower layer, while the upper layer was discarded. The lower layer was collected and centrifuged to get the isolate. The isolate was placed overnight in freeze dryer for drying. Dried isolate was kept in a airtight container.

Preparation of date fructose biscuit bars: In the first step, level of IVPI was optimized through sensory studies (several trials) and then conventional recipe sugar was replaced by quantity of date fructose. Other ingredients remained the same. The DFBB were prepared according to method as described in AACC (2000) with some modifications. The following recipe for "DFBB" was developed after conducting several trials (Table 1).

Physicochemical analysis

Water activity: Water activity in biscuit-bars was determined by the standard water activity meter method with water activity meter (Rotronic Hygro palm *Aw*, Series no. 601089738) (AOAC., 2000).

Texture analysis: Texture analyses of DFBB during storage were determined with the help of texture analyzer (model TA_XT Plus, Stable Microsystems, Surrey, UK) with 5 kg load cell (Rehman and Al-Farsi, 2005).

Color value (Color test): Color value was determined with color meter (Neuhaus Color Test – II, Neotec). It was first calibrated with the standards having lower and upper limits (51 and 170 respectively) (Nadeem *et al*., 2012).

In-vitro starch digestibility (IVSD): The *in-vitro* starch digestibility (IVSD) was assayed by employing porcine pancreatic amylase (Singh *et al.,* 1982). The values of starch digestibility were expressed as milligrams of maltose released per gram of sample in dry weight (Chau and Cheung, 1997).

Preparation of porcine pancreatic amylase solution: Porcine pancreatic amylase (EC 3.2.1.1, 790 units/ mg of protein; catalog No. A6255, Sigma) was used at a concentration of 0.4mg/mL.

Procedure: One unit of amylase liberated 1mg of maltose from starch in 3 min at pH 6.9 at 20ºC. In brief, 50mg biscuit bar sample were incubated with 0.5mL of pancreatic amylase solution (0.4mg/mL) at 20ºC for 2 hrs. Then 2mL 3, 5-dinitrosalicyclic acid reagent were added, and the mixture was boiled for 5 min. After cooling, the absorbance of the filtered solution was measured at 550 nm with maltose used as the standard. The values of starch digestibility were expressed as milligram of maltose released per gram of sample in dry weight.

In-vitro protein digestibility (IVPD): In-vitro protein digestibility was determined according to method described by Saunders *et al.* (1973).

Preparation of pepsin solution: Pepsin enzyme solution (1.5mg/mL) was prepared by using 0.035M solution of HCl with pH near about 2.0 as described in pepsin digestibility method by Mertz *et al.* (1984).

Procedure: In a centrifuge tube, 200 mg of the powdered material was suspended in 35mL of pepsin solution and incubated for 2.0 hrs at 37ºC with gentle shaking. This solution was centrifuged at 12,000 rpm for 15 min at 40ºC and the residue was suspended in 10mL 0.035M HCl and the mixture was re-centrifuged. After removal of the supernatant, the residue was collected and dried overnight at 40ºC, weighed and then analyzed for Nitrogen by micro-Kjeldahl method. The amount of 0.1N H_2SO_4 used was noted and put in the formula to get the total nitrogen of the dried residue. The blank reading was taken without using the enzyme.

$$\% \text{ Nitrogen} = \frac{\text{Vol. of 0.1N } H_2SO_4 \times \text{Vol. of dilution Made} \times 0.0014}{\text{Wt. of sample (g)} \times \text{Vol. of dilution taken (mL)}} \times 100$$

Where as,

Protein% = Nitrogen % × 6.25

Calorific value of the biscuit-bar (gross energy): Calorific values of the date-biscuit bars were determined by using Oxygen Bomb Calorimeter (IKA-WERKE, C2000 Basic) as described by Krishna and Ranjhan (1981). The amount of heat measured in calories that is released when a substance is completely oxidized in a bomb calorimeter, is called the gross energy of the substance. Finely ground sample of date biscuit bar (0.5g)

was taken in to the metallic decomposition vial. The vial was unscrewed and fastened a cotton thread onto the middle of the ignition wire with a loop before loading the sample. Then the screw cap was tightened. The decomposition vial was guided into the filler head to the open measuring cell cover until was in place. The start button was pushed and the measuring cell cover was closed. The sample within the vial was burnt through electric spark. Heat produced was noted by the software computer and displayed in the form of a graph denoting the temperature against time. The whole procedure was fully automatic. It gave no. of calories per 1 gram of the sample.

Sensory evaluation: Date fructose biscuit bars were subjected to sensory evaluation by panel of judges. The attributes of DFBB were evaluated on 9-points Hedonic Scale (Land and Shepherd, 1988).

Statistical analysis: Each sample was analyzed thrice. Data were compared by using analysis of variance (ANOVA) and the Duncan's multiple range test with a probability $p \leq 0.05$ (Steel *et al*., 1997).

RESULTS AND DISCUSSION

In this study, effects of adding Indian vetch protein isolate and replacement of sucrose with date fructose on the physicochemical and sensory qualities of date fructose biscuit bars (DFBB) were evaluated. Dates sugars were converted into date fructose by applying enzymes assay. The composition of sugars was determined through GC-FID analysis. 83% total sugars were obtained in which 53% were fructose after conversion with isomerase enzyme. Highest amount of fructose followed by glucose (28%) and sucrose (2%) was obtained. In recipe, sucrose was replaced with date fructose sugar in different levels for the preparation of DFBB (Table 1).

The values for proximate composition of vetch flour (detoxified) including moisture content, crude protein, crude fat, crude fiber, ash and NFE were found as 8.07±0.02%, 28.90±0.05%, 1.27±0.06%, 2.04±0.01%, 1.52±0.01%, and 67.26±0.07%, respectively. The values for proximate composition of IVPI i.e. moisture, crude protein, crude fat, crude fiber, ash and NFE were 4.40±0.06, 87.54±0.14, 0.34±0.03, 0.12±0.01, 0.35±0.05 and 11.65±0.05%, respectively (Table 2).

The vetch grains were detoxified to an extent of 93.05% by steeping in double quantity of water (60-70 ^{o}C) for 8 hrs with changing water for 7 times, draining and sun drying (Rehman *et al.*, 2006). Removal of bran portion had further reduced the toxin during milling process. The results are in close agreement with the findings of Rehman *et al.* (2014) who stated that Indian vetch is a rich source of protein and contained 28.38% protein. An appreciable quantity (87.54%) of IVPI was obtained from Indian vetch flour (Table 2). The results are in close agreement with the findings of Onwulata *et al*. (2001). However, at pH 4.5, the percent recovery from *lathyrus clymenum* and *lathyrus annuus* was 60% and the isolates contained 81.07 to 82.4% of protein, respectively (Pastor-Cavada *et al*., 2011).

Dates contained 26.02±0.12% moisture, 2.61±0.05% crude protein, 0.33±0.01% crude fat, 3.48±0.04% crude fiber, 1.44±0.02% ash and 92.14±0.11% NFE. The results of proximate composition of dates are in close agreement and comparable to the findings of research work conducted by the previous researchers. Ismail *et al*. (2006) studied five date varieties and reported that moisture, protein and ash contents in date varieties ranged between 20.25 to 22.14%, 2.3 to 2.7% and 1.83 to 2.36%, respectively.

Date fructose biscuit bars prepared by adding 15% Indian vetch protein isolate and 15% date fructose contained 3.50±0.04% moisture, 15.53±0.15% crude protein, 22.27±0.19% crude fat, 0.67±0.007% crude fiber, 0.94±0.02% ash and 60.59±0.3% NFE while bars prepared from wheat flour (control) and bars containing 15% detoxified Indian vetch flour have moisture, crude protein, crude fat, crude fiber, ash and NFE as 3.25±0.05, 5.45±0.13, 22.08±0.06, 1.31±0.02, 0.60±0.012 and 70.56±0.07 and 3.12±0.14, 7.49±0.21, 22.15±0.07, 1.35±0.03, 1.51±0.04 and 67.86±0.21, respectively (Table 2). It was found that with the addition of Indian vetch protein isolate and date fructose, the nutritional status of biscuit bars was found to improve. The protein level of DFBB was found to enhance to the tune of 10.10% as compared to control. These results are also supported by the findings of other researchers who have worked on composite flours for the production of naan and cookies/biscuits (Farooq *et al*., 2012; Virág *et al*., 2013; Sharma and Gujral, 2014). Similarly, addition of legume flours increased protein, fat, fiber, ash, minerals and vitamins in chocolate bars (Onwuka and Abasrekong, 2006). These findings were on the same lines as observed by Siddique (1989) who stated that quantity of essential amino acids increased by adding 10% gram flour in the chapatties.

In the present study, the results suggested that color value increased and hardness of DFBB decreased with the replacement of sucrose with date fructose and addition of IVPI in the biscuit bars. Decrease in color value indicated dark color that might be due to the browning reaction (Maillard reaction) between added protein isolate and date fructose (Asikin *et al*., 2014). Also, it caused serious effects on food properties such as the color, texture, flavor and nutritional values of bakery products (Zhou *et al*., 2013). Similarly, decrease in hardness in biscuit bars might be occurred due to more moisture retention capacity of date fructose used in date fructose biscuit bars formulation (Table 3).

Water content changes the texture from soft to hard and brittle texture as the moisture content decreased. However, knowledge of water activity, or the ratio of vapor pressures, is necessary to control shelf stability. The water activity values of all biscuit bars were found in safe range reflecting good shelf stability (Table 3). Controlling water activity in confections like candies, toffees with low moisture content render them stable for a very long time (Ergun *et al*., 2010). In the present investigations, water activity of biscuit bars ranged from 0.235 to 0.335 which were found lower than previous findings.

In cereal products, the biological exploitation of starch and protein is chiefly relianced on their digestibility. In-vitro protein digestibility is reported to very much narrate to true digestibility and is normally applied as a convenient and rapid alternative. In this study, in-vitro protein digestibility values of two biscuit bars were 70.91±1.14 and 88.29±1.69% in treatments T_0 and T_1, respectively, which were affected significantly due to the incorporation of Indian vetch protein isolate (Table 3). These results are considerably higher as compared to those reported by Fadlallah *et al*. (2010), where they have supplemented the sorghum flour with chickpea flour. High IVPD values indicate that the more protein may be available for the body's nourishment as compared to low content (Adam *et al*., 2013).

In the present investigations, it was found that starch digestibility improved/decreased with the addition of vetch protein isolate (Table 3). These results were found in line with findings of previous researchers. In-vitro starch digestibility was higher in banana fruit bars that might be due to increased level of rapidly digestible starch in banana flour (Utrilla-Coello *et al*., 2010). Moreover, date fructose and vetch protein isolate are rich in readily digestible starch and protein that might be involved in increased in-vitro starch and protein digestibilities. (Yadav and Kheterpaul, 1994). Gross energy values differed significantly in treatments. Mean values ranged from 3.49±0.1 to 4.34±0.2 Kcal/g. The calorific value reduced as a result of substitution of sucrose with date fructose.

In the present study, all the DBB containing IVPI had achieved good sensory score for color, texture, taste, flavor, mouth feel and overall acceptability.

Results pertaining to sensory characteristics are presented in the Table 4. Analysis of variance showed non-significant ($P>0.05$) difference in color score of biscuit bars. The highest color score was achieved by T_2 followed by T_1. Highly significant ($P<0.01$) differences were recorded in the Taste score of biscuit bars. The results revealed that maximum taste score was obtained by T_2. Analysis of variance for flavor score of biscuit bars indicated highly significant differences ($P<0.01$) among treatments. Maximum flavor score was achieved by treatment T_3 followed by T_2. Texture score of biscuit bars indicated highly significant ($P<0.01$) variation among treatments. Texture score of T_3 is the highest followed by T_2. Significant ($P<0.05$) variations exist in mouth feel score of biscuit bars among treatments. Overall acceptability of biscuit bars varied from 33.41±0.85 to 38.05±0.34. The highest overall acceptability score was achieved by T_3 and followed by T_2.

The results suggested that addition of IVPI had improved the sensory characteristics of DFBB. These results are agreed with the findings of previous researchers (Grankivist and Biel, 2001). They stated that aroma and flavor had high impact on consumer liking, followed by taste and appearance of cereal bars. Moreover, preference was also associated with sweetness, filling flavor, chew and crunchy texture (Bower and Whitten, 2000). However, food products with darker color are less preferred because of their unappealing color as in case of bars containing black bean obtained less score for color characteristic as compared to bars with red bean (Maurer *et al.*, 2005). In this study, IVPI had not significantly disturbed the color of the DFBB and had been utmost liked by the judges.

Table-1. Sucrose replacement with date fructose in DFBB.

Ingredients (g)	T0 (0%),	T1 (5%),	T2 (10%),	T3 (15%)	T4 (20%)
Flour	500	-	-	-	-
Sugars (Sucrose:Fructose)	250 (250:0)	250 (237.5:12.5)	250 (225:25)	250 (212.5:37.5)	250 (200:50)
Vegetable Ghee	300	-	-	-	-
Eggs	100	-	-	-	-
Baking Powder	12	-	-	-	-
IVPI (g per 500g flour)	75	-	-	-	-

Table 2. Proximate composition of raw materials and DFBB (%).

Raw Material	Moisture	Crude protein	Crude fat	Crude fiber	Ash	NFE
Indian Vetch(IV)	8.15±0.05	28.45±0.07	1.38±0.02	2.24±0.04	1.61±0.01	66.32±0.08
Dates	26.02±0.12	2.61±0.05	0.33±0.01	3.48±0.04	1.44±0.02	92.14±0.11
IVPI	4.40±0.06	87.54±0.14	0.34±0.03	0.12±0.01	0.35±0.05	11.65±0.05
DFBB	3.5±0.04	15.54±0.15	22.27±0.19	0.67±0.007	0.94±0.015	60.58±0.03
Wheat flour (control)	3.25±0.05	5.45±0.13	22.08±0.06	1.31±0.02	0.60±0.012	70.56±0.07
Wheat flour+15% IV	3.12±0.14	7.49±0.21	22.15±0.07	1.35±0.03	1.51±0.04	67.86±0.21

Table 3. Comparison of means of physical properties, in-vitro protein digestibility (%), in-vitro starch digestibility (mg maltose/g) and calorific value (Kcal/g) of DFBB.

Parameter	T_0	T_1	T_2	T_3	T_4
Color	172.0±2.1A	168.0±3.5A	153.00±3.6B	149.0±3.0BC	142.0±2.8C
Hardness	2139.2±15.1A	2087.6±14.5A	1897.2±49.9B	1521.8±26.6C	1369.2±26.3D
Fractureability	72.7±0.9A	71.3±1.5AB	71.6±1.7AB	68.4±1.4B	62.4±1.2C
A_w	0.235±0.003B	0.240±0.006B	0.33±0.006A	0.33±0.01A	0.335±0.003A
IVSD	48.42±1.01A	-	-	38.90±2.88B	-
IVPD	70.91±1.14B	-	-	88.29±1.69A	-
Calorific value	4.34±0.2A	-	-	3.49±0.1B	-

Means sharing similar letters in a row are statistically non-significant (P>0.05)

T_0= Wheat flour; T_1= with 5% date fructose+15% IVPI; T_2= with 10% date fructose+15% IVPI; T_3= with 15% date fructose+15% IVPI; T_4= with 20% date fructose+15% IVPI

Table 4. Comparison of means of sensory properties of DFBB.

Treatment	Color	Taste	Flavor	Texture	Mouth feel	Overall acceptability
T_0	7.45±0.08A	6.75±0.15A	6.89±0.14D	7.49±0.19C	6.80±0.13B	35.38±0.65C
T_1	7.51±0.20A	6.81±0.23A	7.67±0.21C	8.29±0.14AB	6.70±0.16B	36.98±0.86AB
T_2	7.54±0.18A	6.84±0.06A	7.91±0.21A	8.50±0.10A	6.60±0.09BC	37.39±0.99A
T_3	7.39±0.11A	6.84±0.06A	7.95±0.11A	8.52±0.17A	7.35±0.14A	38.05±0.34A
T_4	7.26±0.12A	5.59±0.07B	7.21±0.08C	6.05±0.08D	6.30±0.14A	32.41±0.85D

Means sharing similar letters in a column are statistically non-significant (P>0.05)

Conclusion: The results revealed that all treatments containing IVPI and different concentrations of date fructose were found acceptable in sensory attributes. However, date fructose biscuit bars supplemented with 15% protein isolate and 15% sucrose replacement with date fructose were found the best in terms of physicochemical and sensory characteristics.

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