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ANTIDIABETIC POTENTIAL OF PROTEIN AND FIBER RICH MILLET BASED SNACK BAR

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Keywords:

Diabetes mellitus, Functional snack bar, Millet mixture, Camel milk powder, Apple pomace powder

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ABSTRACT: Diabetes mellitus has become a global concern with 422 million people affected due to this disease. As the oral hypoglycaemic drugs available in the market have their own set of side effects which can be one of the primary factors towards non-adherence of these medicines among patients, we must explore some natural alternatives in order to deal with this disease in an efficient manner. In such scenario, exploring some intelligent solutions like functional foods can be very helpful as they have the goodness of natural ingredients. The present study focuses on the development of functional food rich in millets, camel milk powder and apple pomace powder. Functional snack bar developed with antioxidant-rich millet mixture (Ragi: Bajra: Kodo: Kutki = 1:1:1:1), insulin-rich camel milk powder and fiber-rich apple pomace powder might have the potential to treat diabetes. Animal studies can be planned in the future in order to validate the product. Moreover, future course of action can comprise of moving into the direction of molecular and cell biology wherein cell line studies can be conducted.

INTRODUCTION: According to World Health Organisation (WHO) Report, 422 million people globally are affected with diabetes and not only this, 1.5 million people die annually because of diabetes being the main reason [1]. Diabetes can be mainly classified into two types: type-1 and type-2 diabetes [2]. Conventionally, oral hypoglycaemic drugs such as biguanides, thiazolidinediones, sulfonylureas, α-glucosidase and α-amylase inhibitors, peptide analogues, incretin mimetics, DPP-IV inhibitors are used to treat this disease [3]. These different kinds of hypoglycaemic drugs have their own set of side effects [4] and sometimes, in almost half of the cases, patients do not properly adhere to the drug therapy [5].

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Therefore, a healthy alternative needs to be developed in order to tackle this health issue [6]. Functional foods can serve as a potential alternative to conventional hypoglycaemic drugs [7]. Research strongly suggests that baked goods such as snack bars can be the ideal vehicle for functional food development [8]. Conventional snack bar recipe incorporated with antioxidant-rich millet mixture [9], insulin-rich camel milk powder [10] and fiber-rich apple pomace [11] leads to the development of such enriched products which can work wonders for the health of diabetes-affected patients (especially type-2 diabetes patients).

MATERIALS AND METHODS: Saipro Agrotech apple pomace powder (200g), Aadvik raw camel milk powder (500g), Aadvik pasteurised camel milk powder (500g), Happilo california almonds (1 kg), True Elements rolled oats (1.2 kg), Puffed rice (1 kg), GirMom Motherly Organic Puffed wheat (200g), Pro Nature Cashew nuts (100g), Originals TM desiccated coconut (250g), SFT sesame white seeds (100g), Urban platter

puffed amaranth (200g), Organic Tattva unpolished peanuts (500g), B&B Organics little millet (1 kg), B&B Organics kodo millet (1 kg), B&B Organics finger millet (1 kg), B&B Organics pearl millet (1 kg), Vedaka jaggery powder (1 kg), ProFoods maltodextrin powder (1 kg) was procured from Amazon. in.

Procedure:

Proximate Analysis of Raw Materials:

Ash Content: Ash content was estimated using standard protocol AOAC (2012). 2g of sample was weighed in a crucible and ignited. The process was continued till the evolving fumes disappeared. The crucible was then placed in a muffle furnace (preheated) at 600*C for 2 hours. Thereafter, the sample was transferred to a desiccator, cooled and weighed. The analysis was conducted in triplicate and results were expressed as percentage of dried sample.

Calculation:

Ash % (w/w) = (Weight of test sample (g) – Weight loss on ashing (g) × 100/ (Weight of test sample (g)

Bulk Density (Untapped and Tapped) and Hausner Ratio: Bulk density and Hausner ratio was calculated according to the procedure outlined by Ho *et al.* (2019) [12]. Untapped bulk density was determined by sifting the sample into a 100 ml measuring cylinder and then weighing. Tapped bulk density was determined by reading the volume after tapping it 100 times. Lastly, Hausner ratio was determined by calculating the ratio of untapped and tapped bulk density.

Colour Values (L,a,b): The colour values were calculated using Chroma meter CR-400 as per the procedure outlined by Ho *et al.* (2019) [12]. The outcomes were represented in the form of L,a,b where L stands for degree of lightness, a stands for degree of redness and b stands for yellowness traits in various samples.

Water Activity (a_w): Water activity was measured using water activity analyzer as outlined by Ho *et al.* (2019) [12].

Moisture Content: Moisture content was calculated according to the standard AOAC (2012) protocol using hot air oven (RDHO 50 REMI). 2g of sample was weighed in an aluminum dish and dried in the oven at 130*C for 3 hours. Readings were taken in triplicate. The samples were then placed in a desiccator and after 5-10 minutes, these were weighed again.

Calculation:

$$\text{Moisture content} = ((W_1 - W_2) \times 100) / W_1,$$

Where, W_1 = weight of the sample before drying and W_2 = weight of the sample after drying.

Preparation of Functional Snack Bars (Commercial Study): Dry mixture, which comprised of ingredients mentioned in the table below **Table 1**, was prepared. Almonds, rolled oats, puffed rice, cashew nuts, desiccated coconut, sesame seeds, puffed wheat, unpolished peanuts were roasted together on a light flame using a gas stove for approximately 7-8 minutes using a cauldron. Next, the millet mixture (Ragi: Bajra: Kodo: Kutki = 1:1:1:1) was roasted on a light flame for 4-5 minutes. Once roasted, nine different bars with different formulations (see **Table 2**) were prepared using a proper size bakery mold. Conventional mixing was used to mix the ingredients together of each batch. Before transferring the mixture into the mold, butter paper was applied with little amount of oil and placed to cover the mold. Then, the mixture was put into the mold and covered from all sides with the butter paper. Finally, it was placed in the refrigerator for half an hour so that it takes a proper shape.

TABLE 1: DRY MIXTURE COMPOSITION

Dry Ingredients	Percentage (%)
Almonds	16
Rolled oats	28
Puffed rice	14
Cashew	12
Desiccated coconut	6
Sesame seeds	4
Puffed wheat	14
Peanuts	6

TABLE 2: FORMULATION OF 9 FUNCTIONAL SNACK BARS

Trial	Code	Dry mixture (g)	AP* (g)	CM(P)* (g)	CM(UP)* (g)	MM* (g)
1	SC1	100				
2	SC2	61	4	20		15

3	SC3	61	4		20	15
4	SC4	49	6		25	20
5	SC5	49	6		25	20
6	SC6	37	8	30		25
7	SC7	37	8		30	25
8	SC8	32	8	30		30
9	SC9	32	8		30	30

AP* = Apple pomace powder, CM (P)* = Pasteurised camel milk powder, CM (UP)* = Unpasteurised/ raw camel milk powder, MM* = Millet mixture. Note 1: Jaggery powder (15g) and maltodextrin powder (35g) is common to all bar samples. Note 2: The formulations of nine snack bars have been designed through combination of response surface methodology (central composite design) and randomized trials.

FIG. 1: PROTEIN AND FIBER-RICH MILLET BASED SNACK BARS

Proximate Analysis of Functional Snack Bars (Commercial Study):

Ash Content: Ash content was estimated using standard protocol AOAC (2012). 2g of sample was weighed in a crucible and ignited. The process was continued till the evolving fumes disappeared. The crucible was then placed in a muffle furnace (preheated) at 600*C for 2 hours. Thereafter, the sample was transferred to a desiccator, cooled and weighed. The analysis was conducted in triplicate and results were expressed as percentage of dried sample.

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Calculation:

$$\text{Moisture content} = (W_1 - W_2) \times 100) / W_1$$

Where, W_1 = weight of the sample before drying and W_2 = weight of the sample after drying.

***In-vitro* Starch Digestibility (IVSD) of Functional Snack Bars (Commercial Study):** *In-vitro* starch digestibility (IVSD) of snack bar samples was calculated as per the procedure followed by Sharma *et al.* (2019) with minor modifications.100 mg sample was mixed with 20 ml phosphate buffer. The suspension was mixed with 0.1g alpha-amylase. The prepared solution was then kept in water bath at 37*C for 2 hours. Centrifugation was performed at 3000 – 3500 rpm for 15 minutes. 1 ml of supernatant was mixed with 10% aqueous solution of denitrosalicyclic acid (DNS) (w/v) (2 ml). The contents were boiled for 5 minutes in water bath. The final volume was made up to 50 ml with distilled water. The absorbance was read at 540 nm. Standard curve of maltose was prepared and mg of maltose released was calculated corresponding to the absorbance of sample.

Sensory Evaluation of Functional Snack Bars (Commercial Study): 9-point hedonic scale with anchor points, 1(dislike very much) and 9 (like very much) was employed for organoleptic evaluation. A semi-trained panel of 15 students assessed the samples in order to determine the overall acceptability of the developed products. All panelists were between the age of 22 – 27.

The sequence of presentation of the samples was randomized and alpha-numeric codes (SC1-SC9) were used for the samples. The labeled samples were offered to the panelists at room temperature on white disposable plastic plate and distilled water (salt free, taste free) was provided for mouth rinsing. 15 set of readings were then calculated for mean and standard deviation. Finally, the results were depicted in pictorial form using radar graph.

Texture Analysis (Bite Force/ Hardness) of Functional Snack Bars (Commercial Study): Texture (bite force/ hardness) of the bars was measured using texture analyser (stable Microsystems, USA). The sample was placed on HDP/90 heavy duty platform. 30 kg load cell was used and the probe distance from the platform was kept as 25mm. Pre-test speed was 2 mm/sec, post-test speed was 1 mm/sec and test speed was 10 mm/sec. Probe used for the analysis was cutting blade with warner bratzler (HDP/BS). The figure **Fig. 2** below gives a glimpse of the probe used for the texture analysis.

FIG. 2: CUTTING BLADE PROBE USED TO MEASURE THE TEXTURE (BITE FORCE/ HARDNESS) OF BAR SAMPLES (ADAPTED FROM "THE TEXTURE ANALYSIS APPLICATIONS DIRECTORY FOOD PRODUCTS" (STABLE MICROSYSTEMS)

Preparation of Animal Study Bars: Some snack bars have been designed primarily for animal study (as and if it is planned in the future) in order to validate the product for antidiabetic potential. These bars have been designed keeping in view the idea that the antidiabetic potential of the main ingredients (which have been considered in the present study for antidiabetic potential i.e. camel milk powder, millet mixture and apple pomace

powder) can be studied on an individual basis, rather than the synergistic effect. An attempt has been made to avoid masking of the antidiabetic effect of individual ingredients while developing these bars. Secondly, as far as the recipe followed is taken into account, it is very much similar to the one used while preparing commercial study functional snack bars. Kindly see the table (**Table 3**) below for the composition of animal study snack bars.

TABLE 3: FORMULATION OF 9 ANIMAL STUDY BARS

Trial	Code	Puffed rice(g)	Puffed wheat (g)	MM*(g)	AP*(g)	CM(P)*(g)	CM(UP)*(g)
1	SAA	46	46		8		
2	SAB	35	35			30	
3	SAC	35	35				30
4	SAD	35	35	30			
5	SAE	16	16		8	30	
6	SAF	16	16	30	8		30
7	SAG	50	50				

MM* = millet mixture, AP* = Apple pomace powder, CM (P)* = Pasteurised camel milk powder, CM (UP)* = Unpasteurised/ raw camel milk powder. Note 1: Jaggery powder (15g) and maltodextrin powder (35g) and distilled water (15-20 ml) is common to all bar samples.

Proximate Analysis of Animal Study Bars:

Ash Content: Ash content was estimated using standard protocol AOAC (2012). 2g of sample was weighed in a crucible and ignited. The process was continued till the evolving fumes disappeared. The crucible was then placed in a muffle furnace (preheated) at 600*C for 2 hours. Thereafter, the sample was transferred to a desiccator, cooled and weighed. The analysis was conducted in triplicate and results were expressed as percentage of dried sample.

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Centrifugation was performed at 3000 – 3500 rpm for 15 minutes. 1 ml of supernatant was mixed with 10% aqueous solution of denitrosalicyclic acid (DNS) (w/v) (2 ml). The contents were boiled for 5 minutes in water bath. The final volume was made up to 50 ml with distilled water. The absorbance was read at 540 nm. Standard curve of maltose was prepared and mg of maltose released was calculated corresponding to the absorbance of sample.

RESULTS AND DISCUSSION:

Proximate Analysis of Raw Materials:

Raw material	Average reading ± Standard deviation (%)
AP	1.83 ± 0.29
CM(P)	5.33 ± 0.29
CM(UP)	6.5 ± 0.5
MM	1.5 ± 0.0

Raw material	Untapped bulk density (g/ml)	Tapped bulk density (g/ml)	Hausner ratio*
AP	0.74 ± 0	0.75 ± 0.02	0.98 ± 0.03
CM(P)	0.43 ± 0	0.41 ± 0.05	1.07 ± 0.15
CM(UP)	0.46 ± 0.02	0.46 ± 0.03	0.8 ± 0.05
Puffed rice	0.10 ± 0	0.4 ± 0.52	0.97 ± 0.03
MM	0.85 ± 0.04	0.94 ± 0.06	0.9 ± 0.05
Ragi (finger millet)	0.79 ± 0.08	0.89 ± 0.02	0.89 ± 0.11
Bajra (pearl millet)	0.79 ± 0.03	0.84 ± 0.08	0.95 ± 0.05
Kodo (cow grass)	0.86 ± 0.02	1.03 ± 0.07	0.83 ± 0.08
Kutki (little millet)	0.84 ± 0.01	0.89 ± 0.08	0.95 ± 0.09

Hausner Ratio* is the Ratio of Untapped Bulk Density and Tapped Bulk Density:

Raw material	L	a	b
AP	77.77 ± 0.20	4.19 ± 0.08	23.77 ± 0.16
CM(P)	92.42 ± 0.95	1.52 ± 0.97	8.01 ± 3.43
CM(UP)	92.38 ± 0.29	2.52 ± 0.05	11.43 ± 0.06
Puffed rice	61.65 ± 2.08	0.44 ± 0.08	10.64 ± 0.37
MM	50.64 ± 3.54	2.22 ± 0.34	12.12 ± 0.64
Ragi (finger millet)	24.27 ± 1.06	10.32 ± 1.02	6.84 ± 0.27
Bajra (pearl millet)	24.27 ± 1.06	2.67 ± 0.20	15.65 ± 0.45
Kodo (cow grass)	59.32 ± 1.11	2.67 ± 0.20	15.65 ± 0.45
Kutki (little millet)	53.18 ± 1.75	2.89 ± 0.29	14.99 ± 0.21

L Stands for Lightness, a Stands for Redness, b Stands for Yellowness:

Raw material	Average ± Standard deviation
AP	0.28 ± 0.00
CM(P)	0.26 ± 0.00
CM(UP)	0.33 ± 0.01
Ragi (finger millet)	0.51 ± 0.00
Bajra (pearl millet)	0.55 ± 0.00
Kodo (cow grass)	0.53 ± 0.00
Kutki (little millet)	0.58 ± 0.00
MM	0.56 ± 0.00
Puffed rice	0.58 ± 0.01

Raw material	Average ± Standard deviation (%)
AP	4 ± 0.87
CM(P)	3.5 ± 0.5
CM(UP)	7.5 ± 1
MM	12.3 ± 0.82

Proximate Analysis of Functional Snack Bars (Commercial Study):

Functional snack bar	Average ± Standard deviation (%)
SC1	1.67 ± 0.29
SC2	2.83 ± 0.29
SC3	2.33 ± 0.29
SC4	2.5 ± 0
SC5	2.67 ± 0.29
SC6	2.33 ± 0.29
SC7	2.67 ± 0.29
SC8	3 ± 0.5
SC9	2.67 ± 0.29

Functional snack bar	L	a	b
SC1	42.81 ± 2.87	4.5 ±0.40	14.65 ± 1.20
SC2	42.76 ± 1.91	5.09 ± 0.38	15.34 ± 1.08
SC3	45.96 ± 4.01	5.55 ± 0.95	17.16 ± 2.20
SC4	42.7 ± 3.15	6.19 ± 0.67	14.95 ± 2.08
SC5	44.22 ± 1.79	5.93 ± 0.65	17.20 ± 0.19
SC6	41.97 ± 4.14	6.72 ± 0.33	14.77 ± 0.82

SC7	47.30 ± 2.97	5.52 ± 0.80	17.03 ± 1.62
SC8	43.07 ± 2.25	5.10 ± 0.66	14.67 ± 1.75
SC9	48.06 ± 8.57	3.90 ± 1.16	12.71 ± 1.00

L stands for lightness, a stands for redness, b stands for yellowness.

Functional snack bar	Average ± Standard deviation
SC1	0.74 ± 0.00
SC2	0.61 ± 0.00
SC3	0.72 ± 0.01
SC4	0.63 ± 0.01
SC5	0.68 ± 0.01
SC6	0.66 ± 0.00
SC7	0.70 ± 0.01
SC8	0.66 ± 0.02
SC9	0.72 ± 0.00

Functional snack bar	Average ± Standard deviation (%)
SC1	10 ± 1
SC2	10.67 ± 0.76
SC3	11.17 ± 1.44
SC4	10.17 ± 0.58
SC5	9 ± 1.5
SC6	9.33 ± 0.29
SC7	9.83 ± 0.76
SC8	8.83 ± 1.04
SC9	10.17 ± 0.58

Functional snack bar	IVSD (mg maltose/ g of sample)
SC1	139.95
SC2	179.87
SC3	172.04
SC4	187.83
SC5	183.09
SC6	196.33
SC7	191.61
SC8	188.14
SC9	183.25

Functional snack bar	Colour	Taste	Flavour	Texture	Overall acceptability (OAA)
SC1	7.5 ± 1.13	6.4 ± 1.59	6.5 ± 1.77	6.3 ± 1.54	6.5 ± 1.28
SC2	7.5 ± 1.13	5.6 ± 1.80	5.9 ± 1.71	6.9 ± 1.41	6.0 ± 1.45
SC3	7.5 ± 0.83	6.5 ± 1.64	6.6 ± 1.75	6.6 ± 1.45	6.8 ± 1.70
SC4	7.2 ± 1.01	6.6 ± 1.64	6.4 ± 1.49	6.5 ± 1.45	6.3 ± 1.44
SC5	7.1 ± 0.92	6.3 ± 1.50	6.3 ± 1.40	7.2 ± 1.55	6.6 ± 1.40
SC6	7.0 ± 1.20	6.3 ± 1.28	6.3 ± 1.21	7.0 ± 1.18	6.6 ± 0.99
SC7	7.3 ± 1.29	6.9 ± 1.27	6.7 ± 1.41	7.0 ± 1.73	6.8 ± 1.16
SC8	7.2 ± 1.30	5.8 ± 1.47	6.1 ± 1.29	5.6 ± 1.45	5.9 ± 1.54
SC9	7.3 ± 1.05	6.6 ± 1.20	6.6 ± 1.06	6.2 ± 1.47	6.6 ± 1.55

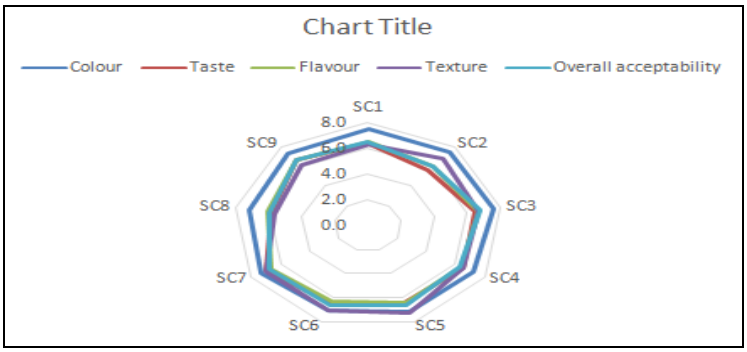

FIG. 3: PICTORIAL DEPICTION OF SENSORY ANALYSIS OF SNACK BARS

Functional snack bar (commercial study)	Hardness/ Bite force (g)
SC1	2043.8
SC2	1140
SC3	5437.2
SC4	8182.9
SC5	7280.44
SC6	8566.7
SC7	14791.8
SC8	2113.7
SC9	1050.6

Proximate Analysis of Animal Study Bars:

Animal study bar	Average ± Standard deviation
SAA	3.0 ± 0.50
SAB	3.33 ± 0.29
SAC	3.83 ± 0.58
SAD	2.50 ± 0.50
SAE	2.67 ± 0.29
SAF	2.67 ± 0.29
SAG	3.0 ± 0.0

Animal study bar	L	a	b
SAA	36.79 ± 0.02	4.59 ± 0.98	12.15 ± 0.19
SAB	45.97 ± 3.30	4.16 ± 0.70	14.49 ± 2.14
SAC	44.18 ± 6.87	4.2 ± 1.66	15.43 ± 0.63
SAD	41.59 ± 3.06	3.84 ± 0.31	13.54 ± 2.34
SAE	44.45 ± 0.79	4.68 ± 0.16	15.68 ± 0.53
SAF	41.32 ± 3.15	5.36 ± 0.64	15.79 ± 0.51
SAG	42.06 ± 2.05	4.0 ± 0.9	13.06 ± 0.45

L stands for lightness, a stands for redness, b stands for yellowness.

Animal study bar	Average ± Standard deviation
SAA	11.17 ± 0.29
SAB	13.33 ± 1.26
SAC	10.67 ± 0.58
SAD	11.17 ± 0.29
SAE	10.67 ± 1.26
SAF	9.33 ± 1.15
SAG	9.38 ± 0.58

Animal study bar	IVSD (mg maltose/ g of sample)
SAA	191.76
SAB	188.87
SAC	180.78
SAD	179.67
SAE	186.35
SAF	180.61
SAG	206.51

CONCLUSION: Protein and fibre-rich millet based snack bars can be a very organic and natural way to treat insulin-resistant (type-1) diabetes as they have the goodness of insulin, antioxidants such as polyphenols. Millet mixture of Ragi (finger millet), Bajra (pearl millet, kodo (cow grass) and kutki (little millet) can contribute immensely towards enriching the snack bar for diabetes treatment. The serious state of affairs of non-adherence of diabetic patients towards oral hypoglycaemic drugs has opened avenues for the growth of natural alternatives like functional foods. These developed snack bars (SC1-SC9) have been developed with the trust to treat type-1 diabetes as camel milk powder is rich enough in insulin which can pass through the human blood unmutilated. Also, research provides enough evidence to support the fact that camel milk can treat diabetes and the

best example for this is the Raica community. Secondly, millets and apple pomace are a storehouse of antioxidants which can treat diabetes. Moreover, the utilization of apple pomace for new product development provides an opportunity to divert resources back into the mainstream thus paving way for circular bio-economy.

Future Direction: The developed antidiabetic snack bars can be subjected to cell biology and molecular study wherein the snack bar solutions can be prepared alongwith a negative and a positive control solution (metformin or glibenclamide treated cells) in 96 well plates and then, MTT and glucose consumption assays can be conducted for antidiabetic studies.

If cell study provides good validation for the anti-diabetic trait of the product, then animal studies can be omitted since ethical issues are involved in the procedure.

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