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Shelf life based studies of millet-fortified fruit-based antidiabetic snack bars

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Abstract
Diabetes is a growing concern affecting almost 422 million people worldwide on an annual basis. It is primarily divided into two categories: Type 1 (insulin dependent) and Type 2 (non-insulin dependent). The former one is characterized by absolute insulin deficiency while the latter is marked by relative insulin deficiency. There are several classes of hypoglycemic drugs used to treat this epidemic but besides causing economic burden, these drugs lead to many side effects. Amidst this, moving beyond allopathic medicines and shifting our focus towards functional foods would be a good idea. In this context, snack bars have been considered as a viable option for functional food development. The present research focuses on preparing millet-fortified fruit-based snack bars which also has the goodness of insulin-rich camel milk powder. Millets are easy to digest, gluten-free and contains various antioxidants that are potential enough to treat diabetes. Apple pomace lowers CMPF (3-carboxy-4-methyl-5-propyl-2-furanopropanoic acid) levels, a sensitive marker in human body related to glucose associated disorders thereby promoting diabetes prevention. Last but not the least, camel milk powder is insulin enriched and research reports that its regular consumption can treat diabetes. Therefore, in the current study, antidiabetic snack bars have been developed and its shelf life has been assessed for a period of 3 months. The bars were kept in LDPE (low density polyethylene) zip lock pouches at a temperature of 4 °C (refrigerated temperature) throughout the study. Thiobarbituric acid (TBA), free fatty acid (FFA), peroxide value (PV) and microbial values were recorded in the starting and thereafter, at an interval of 1 month to study the changes in shelf life parameters.

Keywords: Diabetes, hypoglycemic drugs, functional snack bar, shelf life analysis

Introduction
According to latest report by World Health Organisation (WHO), diabetes is a growing concern as its spread is sharply increasing across countries (https://www.who.int/news-room/fact-sheets/detail/diabetes) [1]. Moreover, diabetes is associated with so many other health problems such as cardiovascular diseases, kidney damage, vision loss and organ removal, which makes it even more dreadful (Ellahham *et al*., 2020) [2]. This global health issue is primarily classified into two categories: Type 1 and Type 2. Type 1 diabetes (insulin dependent diabetes), also known as juvenile diabetes, is marked by absolute insulin deficiency which thereby leads to complete elimination of beta cells of islets of Langerhans (Alam *et al*., 2014) [3]. The second kind, type 2 diabetes (non-insulin dependent diabetes), is characterized by relative insulin deficiency and almost 90 -95% patients are affected by this type of diabetes (American Diabetes Association, 2010) [4].
There are several hypoglycemic drugs available in order to treat the disease which can regulate blood glucose levels and linked complexities (Hossain *et al*., 2018) [5]. The most prevalent ones are biguanides and thiazolidinediones (collectively known as "insulin sensitisers"), sulphonylureas and meglitinides (collectively known as "insulin secretagogues"), alpha-glucosidase inhibitors, incretin-based treatments (such as dipeptidyl peptidase-4 inhibitors) and SGLT2 inhibitors (sodium glucose cotransporter 2) (Blahova *et al*., 2021) [6]. In addition to causing economic burden, these drugs can lead to various side effects (Pardeep *et al*., 2019) [7] such as fluid retention, renal dysfunction, renal failures, myocardial infarction and increasing risk of fractures (Hamnvik *et al*., 2019) [8].
Therefore, keeping in view the present circumstances, the paradigm of research has shifted towards finding natural alternatives for diabetes treatment (Alkhatib *et al*., 2017) [9]. One such alternative is the development of "functional foods" (Venkatakrishnan *et al*., 2019) [10] wherein "antidiabetic snack bars" have been explored as a potential option (Gill *et al*., 2017) [11]. The present study focuses on the preparation of millet –fortified (Chandra *et al*., 2016) [12] apple

pomace (Skinner *et al*., 2018) [13] enriched snack bars which also has the goodness of camel milk powder (Alavi *et al*., 2017) [14]. Millets are an excellent source of antioxidants which can contribute immensely towards treating diabetes (Jhan *et al*., 2021) [15]. Apple pomace consumption lowers 3-carboxy-4-methyl-5-propyl-2-furanopropanoic acid (CMPF) (Waldbauer *et al*., 2017) [16], which serves a major role in glucose related disorders. Last but not the least, camel milk powder is rich in insulin, immunoglobulins, lactoferrin, lactoperoxidase and peptidoglycan recognition proteins which play a very crucial role in diabetes treatment and prevention (Agrawal *et al*., 2011) [17].

Materials and Methods

Materials required

Saipro Agrotech apple pomace powder (200 g), Aadvik raw camel milk powder (500 g), Aadvik pasteurised camel milk powder (500 g), Happilo california almonds (1 kg), True Elements rolled oats (1.2 kg), Puffed rice (1 kg), GirMom Motherly Organic Puffed wheat (200 g), Pro Nature Cashew nuts (100 g), Originals TM desiccated coconut (250 g), SFT sesame white seeds (100 g), Urban platter puffed amaranth (200 g), Organic Tattva unpolished peanuts (500 g), 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), LDPE (low density polyethylene) pouches (pack of 50) were procured from Amazon.in.

Preparation of antidiabetic snack bars

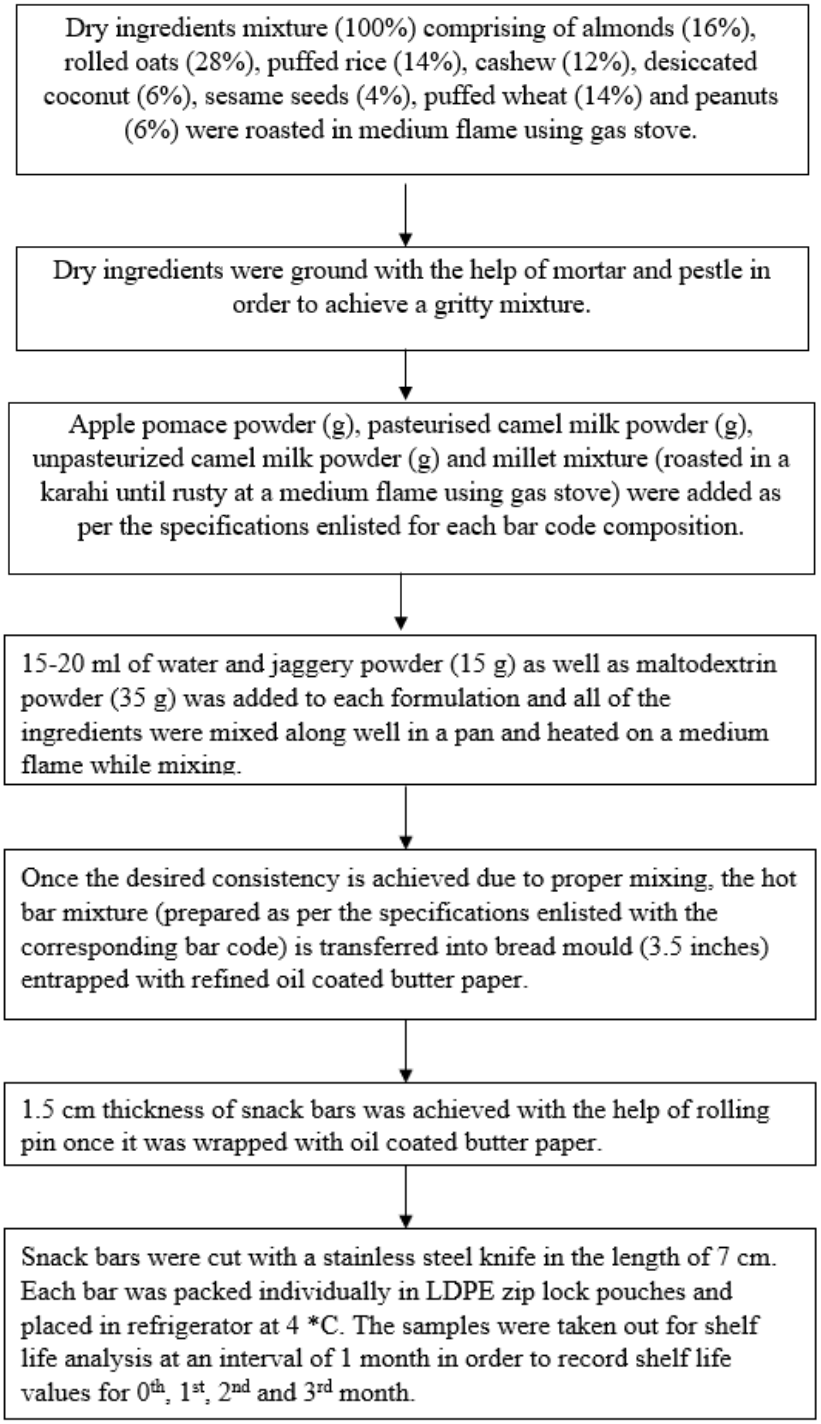

Fig 1: Process flowchart for the development of millet-fortified fruit-based antidiabetic snack bars

Table 1: Formulations of millet-fortified fruit-based antidiabetic snack bars

	Treatments								
Ingredients	**SC1 (Control)**	**SC2**	**SC3**	**SC4**	**SC5**	**SC6**	**SC7**	**SC8**	**SC9**
Dry mixture (g)	100	61	61	49	49	37	37	32	32
Apple pomace powder (g)		4	4	6	6	8	8	8	8
Pasteurised camel milk powder (g)		20		25		30		30	
Unpasteurised camel milk powder (g)			20		25		30		30
Millet mixture (g)		15	15	20	20	25	25	30	30

Note: 1) Dry mixture consists of almonds, rolled oats, puffed rice, cashew, desiccated coconut, sesame seeds, puffed wheat, peanuts
2) Millet mixture consists of Ragi:Bajra:Kodo:Kutki in the ratio of 1:1:1:1

Shelf life analysis

Thiobarbituric acid (TBA) analysis

TBA analysis was carried out according to method outlined in Padmashree *et al*. (2018) [18]. TBA reagent, TEP (1,1,3,3-tetraethoxypropane) standard and HCl (hydrochloric acid) was prepared. 10 g of snack bar was mixed with 50 ml distilled water using a liquidizer for a time period of 120 seconds. The composition was then relocated into a kjeldahl flask through rinsing with an extra 47.5 ml distilled water. Acidity of 1.5 pH was achieved with the help of 2.5 ml HCl solution. Miniscule quantity of antifoaming agent was added into the flask along with a handful of saddle stones in order to avoid unnecessary jolting. The entire paraphernalia was mustered together and maximum heat possible (as per the Kjeldahl apparatus specifications) was applied to the flasks. Thereafter, 50 ml excerpt was garnered from the distillation unit once the whole heating apparatus functioned for approximately 10 minutes.

The collected decoction was mixed thoroughly and 5ml was pipetted out in a 50 ml glass-stoppered tube, followed by addition of 5 ml TBA reagent. Tubes were locked with the help of stopper, composition was unified by normal swirling until it was placed in a water bath for 35 minutes to achieve boiling. Meanwhile, a distilled water-TBA reagent blank was made ready and given the same treatment as per the bar samples under consideration. Once boiling was done, the flasks were brought to ambient temperature with the help of running water (10 minutes exposure) and a part of decoction was put into the cuvette for recording optical density (in relation to the blank) at 538mμ wavelength using Beckman DU spectrophotometer. The obtained value was multiplied by the factor 7.8 in order to change it into mg malonaldehyde/1000 g snack bar.

Free fatty acid (FFA) value determination

FFA value was determined as per the procedure outlined in AOAC (1995) [19] (https://www.dairyknowledge.in/sites/default/files/11.11.pdf). 7.05 g of oil sample from bar sample (extracted using soxhlet apparatus) was measured and transferred into a 250 ml flask. Thereafter, 50 ml alcohol (counterpoised using 2 ml phenolpthalein solution and 0.1N NaOH in order to produce persisting faded pink colour) was poured into the flask. The constituents were now titrated against 0.25N NaOH with energetic flask oscillations so that persevering pink colour develops which lasts for atleast more than 60 seconds. The obtained titre value is synonymous to FFA%, represented as % oleic acid.

Peroxide value (PV) determination

Peroxide value was determined as per method outlined by Bansal *et al*. (2022) [20]. Acetic acid-chloroform solution (3:2, v/v), saturated potassium iodide solution and sodium thiosulfate standard solutions were prepared. Acetic acid-chloroform solution was prepared by simply mixing 3 volumes of acetic acid with 2 volumes of chloroform. Saturated KI solution was prepared by diffusing surplus KI in freshly bubbling water. Extra solid must persist while preparing saturated KI solution. In order to check the validity of the solution prepared, it was examined on a daily basis by adding 0.5-30 ml acetic acid-chloroform solution (3:2, v/v). Finally, 2 droplets of 1% starch solution were poured into the solution. Thirdly, sodium thiosulfate standard solutions of 0.1 M and 0.01 M were prepared.

5 g of oil sample extracted from bar sample (using soxhlet apparatus fat extraction method) was weighed and poured into 250 ml glass – corked Erlenmeyer flask. 30 ml of acetic acid-chloroform solution (3:2, v/v) was added into the flask and whirled properly in order to achieve homogenization. Thereafter, 0.5 ml of saturated KI solution was added with the help of Mohr pipet and allowed to stand for atleast 1 minute (interspersed with frequent swirling). Lastly, 30 ml of water was added into the flask. Now, titration was conducted wherein 0.1 M sodium thiosulfate solution functioned as a titrant and the prepared solution in the flask served as the analyte. End point to be recorded is disappearance of yellow colour. Please make sure that the flask is subjected to full-swing shaking while titration is being carried out. 0.5 ml 1% starch solution was added in next step and titration was still continued in order to release all the iodine from chloroform layer and disappearance of blue colour is achieved. If less than 0.5 ml 0.1 M sodium thiosulfate solution is consumed, kindly carry out the titration again using 0.01 M sodium thiosulfate solution. Prepare blank accordingly and subtract it from the main sample. Peroxide value ($meqO_2$/kg oil) was calculated using the formula (S*M*1000/g test sample), wherein S stands for volume of sodium thiosulfate (ml, blank rectified) and M stands for molarity of sodium thiosulfate solution.

Microbial analysis

Total plate count (TPC) was determined as per the method outlined by Abedelmaksoud *et al*. (2018) [21] total microbial count was detected as per the procedure referred by Abalaka *et al*. (2022) [22], coliform count was assayed according to the procedure mentioned in Singh *et al*. (2022) [23].

Results and Discussion

Table 1: TBA (mg malonaldehyde/kg sample) analysis

Parameter	TBA (mg malonaldehyde/kg sample) (mean ± standard deviation)			
Months	0th month	1st month	2nd month	3rd month
Bar codes				
SC1	2.15 ± 0.02	7.45 ± 0.12	10.98 ± 0.22	14.04 ± 0.18
SC2	1.04 ± 0.15	6.03 ± 0.44	8.99 ± 0.17	14.02 ± 0.11
SC3	2.33 ± 0.20	7.00 ± 0.38	10.02 ± 0.45	13.26 ± 0.24
SC4	2.10 ± 0.59	6.99 ± 0.30	9.05 ± 0.80	13.89 ± 0.39
SC5	2.00 ± 0.18	6.05 ± 0.24	10.00 ± 0.78	14.06 ± 0.05
SC6	1.98 ± 0.84	7.09 ± 0.56	10.02 ± 0.62	13.09 ± 0.04
SC7	2.03 ± 0.24	7.02 ± 0.43	8.43 ± 0.47	13.77 ± 0.12
SC8	2.09 ± 0.07	6.74 ± 0.57	9.68 ± 0.38	14.01 ± 0.32
SC9	2.04 ± 0.03	7.08 ± 0.42	10.04 ± 0.87	14.07 ± 0.08

Note: Values are (mean ± standard deviation) of three independent readings.

Table 2: FFA (% oleic acid) analysis

Parameter	FFA (% oleic acid) (mean ± standard deviation)			
Months	0th month	1st month	2nd month	3rd month
Bar codes				
SC1	0.40 ± 0.10	0.89 ± 0.22	1.03 ± 0.88	1.24 ± 0.39
SC2	0.35 ± 0.22	0.85 ± 0.16	1.08 ± 0.09	1.22 ± 0.23
SC3	0.28 ± 0.32	0.78 ± 0.24	1.00 ± 0.05	1.20 ± 0.17
SC4	0.22 ± 0.56	0.86 ± 0.19	1.06 ± 0.97	1.13 ± 0.28
SC5	0.14 ± 0.20	0.73 ± 0.11	0.98 ± 0.10	1.18 ± 0.30
SC6	0.50 ± 0.86	0.80 ± 0.10	0.86 ± 0.08	1.10 ± 0.24
SC7	0.29 ± 0.77	0.74 ± 0.15	0.99 ± 0.34	1.29 ± 0.37
SC8	0.48 ± 0.65	0.88 ± 0.12	1.02 ± 0.24	1.15 ± 0.20
SC9	0.33 ± 0.42	0.76 ± 0.14	1.00 ± 0.17	1.23 ± 0.34

Note: Values are (mean ± standard deviation) of three independent readings

Table 3: PV (meq O_2/ kg oil sample) analysis

Parameter	PV (meq O_2/ kg oil sample) (mean ± standard deviation)			
Months	0th month	1st month	2nd month	3rd month
Bar codes				
SC1	2.7 ± 0.05	2.9 ± 0.04	3.2 ± 0.02	3.7 ± 0.04
SC2	2.5 ± 0.02	2.8 ± 0.03	3.3 ± 0.04	3.6 ± 0.02
SC3	2.6 ± 0.03	2.9 ± 0.02	3.1 ± 0.03	3.5 ± 0.04
SC4	2.4 ± 0.01	3.0 ± 0.04	3.2 ± 0.01	3.7 ± 0.05
SC5	2.3 ± 0.04	2.8 ± 0.02	3.3 ± 0.05	3.8 ± 0.07
SC6	2.7 ± 0.03	2.7 ± 0.04	3.4 ± 0.02	3.6 ± 0.03
SC7	2.5 ± 0.02	2.9 ± 0.08	3.3 ± 0.03	3.5 ± 0.04
SC8	2.3 ± 0.01	3.0 ± 0.03	3.2 ± 0.04	3.8 ± 0.02
SC9	2.4 ± 0.05	2.7 ± 0.05	3.1 ± 0.02	3.8 ± 0.04

Note: Values are (mean ± standard deviation) of three independent readings.

Table 4: Microbial analysis

Parameters	TPC (cfu/g sample)	TMC (cfu/g sample)	Coliform count (cfu/ g sample)
Time period	0th – 3rd month	0th – 3rd month	0th – 3rd month
SC1	ND	ND	ND
SC2	ND	ND	ND
SC3	ND	ND	ND
SC4	ND	ND	ND
SC5	ND	ND	ND
SC6	ND	ND	ND
SC7	ND	ND	ND
SC8	ND	ND	ND
SC9	ND	ND	ND

Note: ND stands for "not detected".

Conclusion

The present study reverberates the idea that developed snack bars are fit for consumption for a period of 3 months (90 days), when kept at 4 °C in LDPE pouches. Shelf life analysis parameters, namely Thiobarbituric acid (TBA), free fatty acid (FFA), peroxide value (PV) and microbial counts, all hint in the direction that snack bar can be safely consumed within 3 months.

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