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PROBIOTIC VIABILITY AND GLYCEMIC PROPERTIES OF SYNBIOTIC ENERGY BAR PRODUCED FROM GRAINS AND FRUITS

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ABSTRACT
The quest for a creative approach to innovations in food processing to produce revolutionary materials and novel food products that are nutritious, sustainable and functional necessitated this research which explored the probiotic viability of Lactobacillus bulgaricus, glycemic response (glycemic index, glycemic load and glycemic classification of novel synbiotic energy bar formulated from blends of fonio, coconut, tigernut, peanut, sesame seed and snot apple using gum arabic as binder while inulin was incorporated as prebiotic and fat replacer. The synbiotic energy bars were produced using the conventional method while inoculation, fermentation and sheeting were followed by vacuum drying and packaging. It was observed that the energy bars exhibited highest probiotic viability in week one (79-81%), while the least probiotic viability was observed in week eight (7-9%).There were significant differences at (p<0.05) in the probiotic viability among the samples after week zero The study also indicated that the glycemic response values were between 46.06-61.32 and 23.03-32.08 for glycemic index and load respectively which differed significantly (p<0.05) among the samples.

INTRODUCTION
Energy bars contain very little fat, and often only cocoa butter and dark chocolate are used as their primary ingredients. Size and ingredient combinations of energy bars vary, but generally speaking, their main components are whole grains, nuts and seeds, dried fruits, wheat, oil, eggs, and chocolate while some are baked, others can be cold formed or filled and baked (Constantine and Istrati, 2018; Kabuo *et al*., 2017; Silva *et al*., 2014). Fonio is also rich in carbohydrate and has more protein and fibre than rice. The nutritional benefit of fonio outweighs that of other similar cereals (Ballogou *et al*., 2013). Peanuts can be used like other legumes and grains to develop lactose-free, milk-like beverage "peanut milk", which is promoted in Africa as a way to reduce malnutrition among children (Chailangka *et al*., 2023*).*The heart-healthy monounsaturated fats that are essential to the Mediterranean diet can be found in abundance in peanuts. Peanuts are .good sources of vitamin E, niacin, folate, protein and manganese. Coconut also improves insulin secretion and symptoms associated with diabetes and helps protect the body from cancers due to insulin reduction and removal of free radicals that cause premature aging and degenerative disease while promoting heart health, restoration of thyroid function and protecting the body against kidney disease and bladder infection (Nayar, 2016). According to a meta-analysis, eating sesame resulted in modest drops in both systolic and diastolic blood pressure. Studies on sesame oil found lower levels of lipid peroxidation and oxidative stress indicators (FAO, 2002). One of the seeds with the highest oil content is sesame (Elluech *et al*., 2007). It is a staple component in many different cuisines around the world and has a deep, nutty flavor. It can cause allergic responses in some people, just as other seeds and foods.

MATERIALS AND METHOD
Coconut, snot apple, sesame seeds, fonio (acha) and tiger nuts were sourced from the Relief Market, Owerri and were properly identified by a crop scientist at the National Root Crops Research Institute, Umudike,Umuahia-Nigeria. Acacia gum was purchased at ACE Pharmacy, MCC Road, Owerri, Imo

state, Nigeria. Inulin (prebiotic) and *Lactobacillus bulgaricus* (probiotic) were obtained from Pharma Deux, a reputable pharmaceutical company in Beit-Dagan, Israel. All the chemicals used in the preparation of different reagents were analytical grades (AR) and were procured from reputable companies

SAMPLE PREPARATION

Procurement of Raw Materials

Coconut, snot apple, sesame seeds, fonio (acha) and tiger nuts were sourced from the Relief Market, Owerri and were properly identified by a crop scientist at the National Root Crops Research Institute, Umudike,Umuahia-Nigeria. Acacia gum was purchased at ACE Pharmacy, MCC Road, Owerri, Imo state, Nigeria.

Production of coconut flakes

The method described by Okpulor (2015) was modified and adopted for processing of coconut flakes. The coconut shell was removed with knife. The outer brown parts were removed by using a vegetable peeler. The coconut meat was shredded and loaded in a preheated hot air oven (Model No.IK-III, IKON, India) and dried at 65ºC for 2 h. The dry coconut shreds were toasted in a convection oven at 148ºC for 8 min to enhance the nutty flavor and give the coconut shreds a golden brown colour as well as making it flaky.The coconut flakes were stored in a cool dry air tight container for synbiotic energy bar production.

Production of peanut flakes

Raw peanuts were sorted to remove defective ones and cleaned to remove extraneous materials, soaked in potable water for 6 h to reduce anti nutrients, enhance texture and flavour and steam blanched at 95ºC for 2.5 min to stabilize the oil and prevent rancidity as described by Mayaki *et al.* (2018), dried at 65ºC for 5 h, toasted in a preheated oven at 160ºC for 8-12 min followed by cooling and removal of skins. Thereafter, peanuts were crushed lightly to obtain peanut flakes which were stored for use in production of synbiotic energy bar.

Production of toasted sesame seeds

Sesame seeds were processed by adopting the method described by Chenomics (2002). The sesame seeds were cleaned to remove dirts. The cleaned sesame seeds were soaked for 12 h to reduce the level of oxalate and phytic acid as well as enhance digestibility of the seed. The debittered seeds were drained and dried in a preheated hot air oven (Model No.IK-III, IKON, India) at 45ºC for 5h which was followed by toasting in a preheated electric oven (Model No. Hgd-40d, CHINA) at 160ºC for 5-6 min as described by Nayar and Mehra (2002) to develop the rich nutty flavor and enhance the colour. The toasted sesame seeds were cooled and kept aside for production of synbiotic energy bar.

Production of tiger nut flakes

The tiger nuts were processed using the method described by Bamishaiye and Bamishaiye (2011) with slight modification. Wholesome tiger nuts were selected and cleaned.The cleaned tiger nuts were soaked for 24 h to enhance nutrient availability, improve digestion, flavor and texture.The soaked tigernuts were dried in a cabinet dryer (Model No. BOV-T30C, CHINA) at 55ºC for 3 h. The dried nuts were toasted in a preheated Zanussi electric oven (Model No.ZDF290X, Italy) at 120ºC for 10 min and milled coarsely with an attrition mill (Model No. Hgd-40d, CHINA) to obtain tiger nut flakes.

Production of toasted fonio (acha)

Fonio grains were sorted and cleaned to remove dust and sand particles.The cleaned fonio seeds were steeped in potable water and allowed to sprout for 24 h to enhance nutrient availability, reduce anti-nutrients,improve digestibility and absorption as well as enhance texture and flavour.The sprouted fonio seeds were boiled for 5min to soften the grains, remove bitterness caused by sprouting, and as well improve, texture, flavor and digestibility further before drying in the cabinet dryer (Model No. BOV-T30C, CHINA)at 65ºC for 2 h as described by Ballougou *et al*. (2015) and Jideani (2012). Dried fonio grains were toasted in an electric oven (Model No.Hgd-40d, CHINA) at about 150°C for 8min, cooled at room temperature and then used for energy bar formulation.

Production of dehydrated snot apple extract

The dry snot apple fruits were washed and peeled.The pits were removed while the fruit was crushed and pulped using a tower blender (Model no T12008, Nigeria) for 10 min as described by Maroyi (2017) and the pulp removed, dried in a vacuum oven (Model No.DZF-6090,CHINA) at 40ºC for 6 h at a pressure of 5KPa.The dry snot apple pulp was packaged and used for energy bar formulation.

SAMPLE FORMULATION

SSA : 700 g (Fonio), 100 g(Coconut), 80 g(Peanut), 50 g (Sesame seed), 50 g (tigernut), 20 g(snot appl), 50 g(gum arabic) and 100 g (Inulin).
SSB: 600 g (Fonio), 150 g(Coconut), 100 g(Peanut), 60 g (Sesame seed), 60 g (tigernut), 30 g(snot appl), 50 g(gum arabic) and 100 g (Inulin).
SSC: 500 g (Fonio), 200 g(Coconut), 120 g(Peanut), 70 g (Sesame seed), 70 g (tigernut), 40 g(snot appl), 50 g(gum arabic) and 100 g (Inulin).
SSD: 400 g (Fonio), 250 g(Coconut), 140 g(Peanut), 80 g (Sesame seed), 80 g (tigernut), 50 g(snot appl), 50 g(gum arabic) and 100 g (Inulin).
SSE: 300 g (Fonio), 300 g(Coconut), 160 g(Peanut), 90 g (Sesame seed), 90 g (tigernut), 60 g(snot appl), 50 g(gum arabic) and 100 g (Inulin).
SSF: 0 g (Fonio), 0 g(Coconut), 0 g(Peanut), 0 g (Sesame seed), 0 g (tigernut), 0 g(snot appl), 0 g(gum arabic) and 0 g (Inulin) (**Control sample)**

SAMPLE PRODUCTION

Thirty grams (30 g) of gum arabic powder and hundred grams (100 g) of inulin were dissolved in three hundred and fifty milliliters (350 ml) of treated water at 60ºC. The mix was stirred continuously to ensure complete dissolution of the gum arabic and inulin powder. Measured quantities of processed fonio (acha), coconut flakes, peanut, tigernut, sesame seed and snot apple (see formulation ratio for each sample) were suspended in the arabic gum and inulin solution and mixed by stirring the thick slurry which is the prebiotic mix.The prebiotic energy bar mix was heated in a hot air oven (Model No.IK-III, IKON, India) at 85ºC for 30 min to inactivate enzymes and kill off any remaining organism that may impair or compete with the probiotic.The heated prebiotic mix was allowed to cool to 40ºC.Three grams (3 g) of probiotic powder (*Lactobacillus bulgaricus*) was dissolved in one hundred and fifty milliliters (150 ml) of treated water containing twenty (20 g) grams of gum arabic powder with addition of hundred grams (100 g) of inulin (synbiotic mix) and allowed to stand for 20 min. The cooled prebiotic energy bar mix was inoculated with the reconstituted synbiotic mix and properly mixed using a Hobart mixer (Model No. N50-30, ENGLAND) to ensure proper distribution of the probiotics.The mixed synbiotic energy bar batter was allowed to ferment for 6 h in a sterile proofing cabinet at room temperature. After 6 h, the synbiotic energy bar mix was poured onto a stainless steel table and flattened using a hand sheeter.The sheeted mix was cut ino 90 x 40 x 10 mm with approximate weight of sixty grams (60 g) each and placed on baking trays.They were dried in a vacuum oven (Model No. DZF6090, CHINA) at 45ºC for 5 h at a pressure of 5.5 kPa. Same treatment was given to all the samples for uniformity.

METHODOLOGY

Probiotic viability measurement

In this research work, the probiotic viability of the probiotic cells was determined by total plate count method as described by Sah *et al*. (2015).

Glycemic response

Glycemic response was carried out as described by Foster-Powell *et al*. (2002), using capillary finger sticks and Accu-Answer blood glucose monitoring device from Roche diagnostics, Indianapolis, USA.

Calculation of GI and GL

Geometric calculations were used to determine the incremental area under the blood glucose response curve (iAUC), omitting the region beneath the baseline (FAO/WHO, 2007).

RESULT AND DISCUSSION

RESULT

Table 1: Percentage Cell Viability/Stability for eight weeks

SAMPLES	WK_0	WK_1	WK_2	WK_3	WK_4	WK_5	WK_6	WK_7	WK_8
SSA	$79.0^{a}\pm1.0$	$81.0^{a}\pm1.0$	$77.0^{a}\pm1.0$	$40.0^{a}\pm1.0$	$30.0^{a}\pm10.0$	$28.0^{a}\pm1.0$	$24.0^{a}\pm1.0$	$19.0^{a}\pm1.0$	$9.0^{a}\pm1.0$
SSB	$79.0^{a}\pm1.0$	$80.0^{ab}\pm1.0$	$77.0^{a}\pm1.0$	$40.0^{a}\pm1.0$	$30.0^{a}\pm1.0$	$27.0^{ab}\pm1.0$	$23.3^{ab}\pm0.6$	$19.0^{a}\pm1.0$	$9.0^{a}\pm1.0$
SSC	$78.0^{a}\pm1.0$	$79.0^{b}\pm1.0$	$77.0^{a}\pm1.0$	$39.3^{b}\pm0.6$	$30.0^{a}\pm1.0$	$28.0^{a}\pm1.0$	$23.0^{ab}\pm1.0$	$18.0^{a}\pm1.0$	$7.0^{b}\pm1.0$
SSD	$78.0^{a}\pm1.0$	$79.0^{b}\pm1.0$	$76.0^{a}\pm1.0$	$39.0^{a}\pm1.0$	$28.0^{a}\pm1.0$	$26.0^{bc}\pm1.0$	$22.0^{ab}\pm1.0$	$16.0^{b}\pm1.0$	$7.0^{b}\pm1.0$
SSE	$78.0^{a}\pm1.0$	$79.0^{b}\pm1.0$	$76.0^{a}\pm1.0$	$39.0^{a}\pm1.0$	$27.0^{a}\pm1.0$	$25.0^{c}\pm1.0$	$22.0^{ab}\pm1.0$	$16.0^{b}\pm1.0$	$7.0^{b}\pm1.0$
SSF	$0.0^{b}\pm0.0$	$0.0^{c}\pm0.0$	$0.0^{c}\pm0.0$	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$	$0.0^{d}\pm0.0$	$0.0^{c}\pm0.0$	$0.0^{c}\pm0.0$	$0.0^{c}\pm0.0$
LSD	1.66	1.66	1.66	1.55	7.57	1.66	1.55	1.66	1.66

Results are means of triplicate determinations ± standard deviation from the means. Means not having the same superscript are significantly different ($p<0.05$)

Table 2: Average glycemic response of human subjects group fed with formulated synbiotic energy bars.

Samples	0 min	15min	30min	45min	60min	LSD
SSA	$5.68^{c}\pm0.77$	$5.55^{c}\pm0.72$	$5.84^{b}\pm0.74$	$5.87^{b}\pm0.61$	$6.20^{a}\pm0.35$	0.15
SSB	$5.68^{d}\pm0.23$	$5.82^{c}\pm0.26$	$6.25^{a}\pm0.17$	$6.24^{a}\pm0.19$	$5.95^{b}\pm0.25$	0.11
SSC	$5.66^{a}\pm0.19$	$5.48^{b}\pm0.42$	$5.71^{a}\pm0.23$	$5.63^{a}\pm0.66$	$5.62^{a}\pm0.61$	0.13
SSD	$5.89^{a}\pm0.61$	$5.37^{d}\pm0.26$	$5.71^{b}\pm0.17$	$5.57^{c}\pm0.22$	$5.84^{a}\pm0.83$	0.10
SSE	$5.81^{a}\pm0.41$	$5.74^{a}\pm0.46$	$5.76^{a}\pm0.26$	$5.70^{ab}\pm0.41$	$5.59^{b}\pm0.36$	0.12
SSF (Ref food)	$7.06^{a}\pm0.47$	$6.72^{c}\pm0.42$	$6.84^{c}\pm0.25$	$6.87^{c}\pm0.42$	$7.23^{a}\pm0.41$	0.16

Table 3: **Glycemic index/glycemic load and G.I classification of formulated synbiotic energy bars**

Samples	Glycemic index	Glycemic load	G.I Classification
SSA	46.06^{e}	23.03^{e}	Low
SSB	51.93^{d}	25.99^{d}	Low
SSC	54.65^{c}	27.33^{c}	Low
SSD	64.16^{a}	32.08^{a}	Medium
SSE	61.32^{b}	30.66^{b}	Medium
LSD	0.66	0.40	

DISCUSSION

Table 1 Percentage Cell Viability/Stability for eight weeks

In Week 1, Sample SSA had the highest percentage viability value (81%) which was followed by sample SSB (80%) while samples SSC, SSD and SSE maintained viability rate of 79% each. However, no growth was recorded in sample SSF; hence no percentage viability value was recorded. Sample SSA at one week of storage recorded the highest viability value of 81% (Table 1). This report is in agreement with the bacterial growth curve as illustrated by Karimi *et al.*, (2011) and also agrees with the report of Do and Fan (2019), who reported the presence of inulin as having positive impact on the viability of probiotic cells.

In week 2, There was a drop in the number of viable cells in the formulated energy bars with sample SSA having the highest level of cell deaths when compared to its cell growth in week one. The percentage viability value for the samples dropped at all levels though the drop was higher for sample SSA. There was no significant difference ($p<0.05$) in the percentage cell viability value among the formulated energy bar samples. This is also in agreement with Do and Fan (2019) and Silva and Ferrari (2016) where they reported that water activity and temperature variations had considerable influence on the survival of probiotic cells during storage.

From the table, there was a sharp drop in viability /stability of the cells. Samples SSC, SSD and SSE had the highest percentage viability drop (39%) as compared with the zero week viability value (78%) while samples SSA and SSB viability values dropped to 40% compared to week one (81 and 80% respectively) as seen in week 3. No viable growth was recorded for sample SSF. The drop in viability/stability of the probiotics is in agreement with the microbial growth curve as described by Nwanekezi *et al.* (2023).

There were further drops in viability /stability of the probiotic cells from 40% (in week 3) to 30% (in week 4) for samples SSA, SSB, and SSC. The percentage cell viability values of energy bar samples SSD and SSE dropped to 28% and 27% respectively at week 4 (see Table 1). No probiotic cell viability was observed in sample SSF (control sample). While In week 5, the viability/stability of probiotic cells in synbiotic energy bar samples SSA, SSB and SSC dropped from 30% that was recorded for them in week 4 to 28%, 27% and 28% respectively (table 1), while those of samples SSD and SSE dropped from 28% and 27% respectively to 26% and 25%. No cell growth/viability was observed for sample SSF (control).

There were further drops in percentage viability /stability of the probiotic cells from 28%, 27%, 28%, 26% and 25% in week for samples SSA, SSB, SSC, SSD and SSE respectively to 24%, 23%, 23%, 22% and 22% in week six. No viability was observed for sample SSF. While in the 7th week, there were further drops in percentage viability/stability of the probiotic cells to 19% in samples SSA and SSB, while the percentage viability/stability of the probiotic cells in SSC, SSD and SSE dropped to 18%, 16% and 16% respectively. There was no significant difference ($p<0.05$) in the percentage viability/stability values of samples SSA, SSB and SSC.The percentage viability/stability values of samples SSA, SSB and SSC differed significantly from those of samples SSD and SSE at 5% level. The percentage viability/stability of probiotic cells in the synbiotic energy bars at eight weeks of storage dropped to 9% for samples SSA and SSB, 7% for samples SSC, SSD and SSE while none was recorded for sample SSF (control sample). There was no significant difference ($p<0.05$)

in the percentage viability/stability values of the cells in samples SSA and SSB as they respectively had 9% value.

Table 2 and 3 Glycemic response, index/glycemic load and G.I classification of formulated synbiotic energy bars

The Tables represents the glycemic response, index, glycemic load and glycemic classification of the test group fed with formulated energy bar samples. The glycemic index is a number used to gauge how much a particular food may raise blood sugar levels (Jenkins *et al.*, 2001). As a tool, it is often used to promote better blood sugar management. It can be inferred from the Table that the glycemic index of the diet ranged from (46.06-61.32) in sample SSA and SSD respectively. The low glycemic index recorded in the samples may be as a result of the different components (inulin, gum arabic, fonio, tigernut, sesame seed and coconut which are excellent ources of fibre) used in the formulation of the synbiotic energy bar. Moreso, fonio which is a major component of the formulated energy bar under study has been discovered to have a low glycemic index of 66 which is moderate.

The glycemic load of a given food sample is a number that estimates how much a particular food will raise a person`s blood sugar (Augustin *et al.*, 2015). Unlike the glycemic index, glycemic load gives more clarity using the GI and the amount of total carbohydrate per serving of a specific food to estimate how quickly a food material causes blood sugar to rise and how much blood sugar will rise after eating (Augustin, 2015). The glycemic load ranged from (23.03 -32.08) in samples SSA and SSD respectively. The values obtained were in agreement with RDA (Recommended Dietary Allowance) range of a daily dietary intake of glycemic load in food samples (Metegor *et al.*, 2007) thus making the glycemic index invariably low in sample SSA, SSB and SSC while SSD and SSE had medium glycemic index classifications.However, there was significant difference in the glycemic indices among the samples at ($p<0.05$).Also the samples exhibited significant differences in glycemic load at ($p<0.05$).

CONCLUSION

Inulin which is one of the most abundant prebiotics has proved to be effective for growth of probiotics (*lactobacillus spp*) for production of synbiotics and a good fat replacer when combined with gum arabic in food formulations. Also gum arabic which proved a good binder for this purpose is relatively cheap and available. However, it was observed that temperature variation during storage had negative influence on the viability/stability of the probiotic cells.Synbiotic food products can be formulated by combining prebiotics with specific probiotics with the ingredients during formulation.

RECOMMENDATION

It is also recommended that research on optimization of the probiotic cells using different techniques be carried out while efforts to commercialize synbiotic energy bar should be encouraged in order to create variety of products that will meet the expectations of health conscious consumers, increasing the utilization of some under-utilized cereals, legumes, pulses/nuts and fruits since they form the bulk of the ingredients used in energy bar manufacture. It is also advised that in-vivo research on the synbiotic energy bar be done to determine the probiotic survivability in-vivo.

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