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Isolation of probiotics and nutritional evaluation of fermented lactose-free foods as a potential treatment for diarrhoea

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ABSTRACT

Probiotics are live bacteria that can be found in fermented local foods and confer health benefits on the host when administered in adequate amounts. However, there is little awareness of local fermented, lactose-free foods as good and cheap sources of probiotics that could be employed in the dietary management of diarrhoea instead of the expensive antibiotic drugs. The local food materials were fermented and prepared into their respective meals using traditional methods. The probiotic population, nutrient quality, and energy value of the fermented foods, and the prepared meals were investigated. Probiotics were isolated and enumerated from the meals using standard pour-plating techniques, while the proximate composition of the samples was evaluated using standard methods. The used food samples include; Sorghum 'ogí' (SP), Soya flour, 'Garri', and formulated meals from Sorghum 'ogí' (SP) and Soya four as 90% 'ogí'+10% soya (TS), 80% 'ogí'+20% soya (PS), and 70% 'ogí'+30% soya (ST). The probiotic (LAB) population identified in the meals includes *Bifidobacterium lactis* at concentration ranges of ($3.41x10^2$–$7.77x10^6$ CFU/g), *Lactobacillus acidophilus* ($1.48x10^3$–$3.69x10^6$ CFU/g) and *Lactobacillus bulgaricus* ($1.01x10^2$–$7.39x10^4$ CFU/g). *Bifidobacterium lactis* had the highest population ($7.77×10^6$ CFU/g) in PS, while *Lactobacillus acidophilus* ($3.69×10^6$ CFU/g) and *Lactobacillus bulgaricus* ($7.39×10^4$ CFU/g) were highest in ST. However, only the PS meal met the RDA standards for both *Bifidobacterium lactis* ($\geq10^6$ CFU/g) and *Lactobacillus bulgaricus* (10^3 CFU/g) implying a good source of probiotics, and exhibited good nutrient quality; (69.48%) moisture, (7.10%) protein, (3.34%) ash, (6.70%) crude fat, (2.76%) fibre and (10.60%) carbohydrates. Thus, the formulated PS meal with an adequate concentration of probiotics of acclaimed health benefits possesses the potential for a therapeutic diet that could help in ameliorating the effect of gastrointestinal disorder and diarrhoea. Also, the good nutrient quality and energy value of the meal indicates its capability for faster recuperation after a health disorder like diarrhoea.

Introduction

Mostly, a condition known as diarrhoea or gastroenteritis in infants and young adults is often brought about by eating food or drinking water that has been contaminated by pathogens. It is T conventionally usually treated with expensive antibiotic drugs (Jay et al., 2005; DuPont, 2014; Bruzzese et al., 2018). Some of these drugs have

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dangerous, and sometimes fatal side effects, which should never even be administered to children below five (5) years of age (Bevan et al., 2008; Baù et al., 2020). Moreover, some anti-diarrhoea and anti-emetic drugs have no practical benefits for children with acute or persistent diarrhoea as they do not prevent dehydration or improve nutritional status, which should be the main objective of a treatment (Bevan et al., 2008; Florez et al., 2020). However, eating nutritive food that is rich in probiotics can have therapeutic effect, but this knowledge is majorly obscured to the general public and limited in scientific backings.

Probiotics are live microorganisms that are intended to have health benefits. The concept behind probiotics was introduced in the early 20th century, when Nobel laureate Elie Metchnikoff, known as the "father of probiotics" proposed that consuming beneficial microorganisms could improve people's health. Researchers continued to investigate this idea, and the term "probiotic" – meaning "for life" eventually came into use (Richard, 2017).

These organisms contribute to the nutritional status of the host, regulation of intestinal angiogenesis, and the development of immunity in infants and older children, as well as to the protection from a diarrhoea-causing pathogen (Johnson and Versalovic, 2012). Pathogens which include *Salmonella choleraesuis* CECT4155, CECT409 and CECT443, *Escherichia coli* CECT439 and *E. coli* O157:H7 serovar CECT4076, *Staphylococus aureus* CECT4013 and CECT976, and *Listeria monocytogenes* Scott A, the spoilage strain *Clostridium tyrobutyricum* CECT4011 that attack primarily the GI tract were previously examined. It was discovered that probiotics provided prevention and relief against these pathogenic bacteria and the associated symptoms (Olivares et al., 2006). The two main groups of interest and the most frequently used probiotics are *Lactobacillus* and *Bifidobacterium* (Bergogne, 2017). There were studies conducted to determine the efficacy of the administration of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* as probiotics on various subjects in the prevention of antibiotic-associated diarrhoea (AAD) and *Clostridium-difficile*-associated diarrhoea (CDAD) in comparison with the conventional antibiotic drugs. The results of these studies have shown that probiotics were effective in the prevention of AAD (Gotz et al., 1979; Buts et al., 1986; McFarland, 2006; Surawicz et al., 2013**)**.

Additionally, in the last few years, much interest has accumulated for probiotic bacteria due to their potential benefits in the field of normal intestinal microflora, stimulation of immune systems, relief in diarrhoea, and other GI afflictions (Borges et al., 2006). Especially, the stimulation of immune systems by the activities of probiotics is of tremendous importance for natural body defence against infectious diseases such as in the current situation of Coronavirus (Covid-19) pandemic. There are several mechanisms through which probiotics aid the body in fighting off pathogens ranging from fighting for adhesion on the tract itself to displaying some sort of antibacterial properties (Bouzaine et al., 2005). The gastrointestinal (GI) tract is home to thousands of microorganisms, among which beneficial species are referred to as probiotics which include *Bifidobacterium* and *Lactobacillus*. Many of the microorganisms in probiotic products are the same as or similar to microorganisms that naturally live in human bodies (WHO, 2013).

Food products sold as probiotics include yoghurt (a fermented dairy product). Nevertheless, it has been recommended that a nutritious diet that does not cause diarrhoea to worsen should be a kind of lactose-free-diet (PDCU, 2016). Additionally, most of the non-dairy processed fermented foods such as pickles, meat, and wine (Wang et al., 2019) are expensive and out of reach of the general populace. This class of people also has little or no knowledge about the presence of probiotic and the embedded anti-diarrhoea potential in some local foods that are cheap and available in their locality. Thus, proper documentation of research findings on locally available lacto-free food sources that could meet the standard concentration of anti-diarrheoa probiotics and also of good nutritive value is necessary. It will take a lot of time to improve the knowledge of the people about the use of local foods as a simple dietary therapy in treating diarrhoea. Concentration is one major and basic parameter used in the evaluation of the health effect of probiotics. Therefore, the aim of this study is to determine the probiotic potential of some locally fermented lacto-free foods with respect to the recommended allowance claim of health benefits.

Materials and methods

Materials

Raw materials used for this study include cassava (*Manihot esculenta*), sorghum (*Sorghum bicolor*) grains, and soya bean (*Glycine max*) seeds; all were purchased from "Oja-Oba" market in Ado-Ekiti, Ekiti State, Nigeria. All used reagents were of analytical grade.

Methodology

Processing of the fermented food materials

The processing of all the fermented food materials and their preparation into meals were carried out at the Microbiology Department of Afe Babalola University, Ado-Ekiti using local methods.

Processing of 'garri'

Cassava tubers were peeled and washed with cleaned water, then cut into medium sizes for grinding. After grinding, they were packed in a sack bag, drilled on a jack for 3 days for ultimate dewatering and to allow fermentation to take place. Then, the dewatered mass was sieved and fried; cooled at room temperature, and packaged in polyethylene bag prior to usage. The described method of Airaodion et al. (2019) was adopted for garri processing.

Processing of Sorghum 'ogi'

Sorghum grains were sorted, thoroughly washed, and soaked for 3 days for fermentation to take place. The fermented grains were washed and wet milled; potable water was added and sieved with fine muslin cloth; then allowed to sediment for 8 hours and decanted. The sediment ('ogi') was tightly retained in the muslin cloth for 6 h for dewatering before drying in an air-draughtoven (60 °C), then cooled and pulverised as Sorghum 'ogi' flour/powder. The method of Akingbala et al. (1978) as adopted by Osundahunsi and Aworh (2002) was used with the modification of using Sorghum in place of maize.

Processing of soya bean flour

The method described by Igbua et al. (2019) with certain modifications was employed in the processing of soya bean flour. The soya bean seeds were sorted and washed with clean water, then soaked for 72 hours while the water was changed at intervals of 12 hours. After 72 hours, the fermented seeds were dehulled and thoroughly washed to remove the coat and lessen the fermentation odour. The dehulled seeds were boiled over a gas cooker for 30 min and washed again with clean water, drained and sun-dried; then, locally roasted and milled to obtain soya flour with the aid of Hammermill.

Preparation of meals from the process food materials

Preparation of sorghum ('ogi') meal

The sorghum 'ogi' powder was made into a thin gruel by rehydration; boiling 'ogi' liquor as water was poured into the gruel and cooked with continuous stirring until a thick gel was obtained as the meal. The method described by Igbua et al. (2019) was used; with the modification of thinner gruel. The decanted water from the processed raw 'ogi' was used as 'ogi' liquor.

Preparation of soy-pap (soy-'ogi') meal

The same procedure used in the preparation of Sorghum 'ogi' meal was also adopted in preparing meals with the formulated 'soy-ogi' samples. The sorghum 'ogi' flour was formulated with soya bean flour at ratios of 90% sorghum 'ogi'+10% soya flour; 80% sorghum 'ogi' flour+20% soya flour; 70% sorghum 'ogi'+30% soya flour; rehydrated with little water (at room temperature) to form thin gruel in each case. Boiling 'ogi' liquor was then added to form gelatinised gruel and cooked with constant stirring until the wanted thickness for a ready-to-eat-meal was obtained.

Preparation of 'Garri' meal

Processed 'garri' was simply soaked in table water (at room temperature) and left for few minutes until soft. Part of the water-soak was decanted and used as 'garri' liquor.

Isolation of probiotics

Two strains of lactic acid bacteria (LAB); *Bifidobacterium lactis* and *Lactobacillus sp.* (*Lactobacillus acidophilus* and *Lactobacillus bulgaricus*) are the most frequently used probiotics (Bergogne, 2017). These were isolated from the meal samples; the isolation was done using Transgalactosylated oligosaccharides-mupirocin lithium salt (TOS-MUP) media for *Bifidobacterium lactis* and De Man Rogosa Sharpe (MRS) agar media (Oxoid, Italy) for selective isolation of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* species in accordance with the method described by Wang et al. (2019). An aliquot of the meal samples was serially diluted (10^{-3}) in saline water before it was pour plated and then, anaerobically incubated at 37 °C for 48 h.

Characterisation and identification of probiotics

Bacterial colonies isolated from the meal samples were characterised and identified according to the method described by Orban and Patterson (2000).

Enumeration of bacteria (probiotics)

The total bacterial load in the samples was determined by the serial dilution-pour plate method as described by Wang et al. (2019). Fresh meal samples were serially diluted (10^{-1}-10^{-3}) and

aliquots of the samples were placed on the fresh plate count agar media (TOS-MUP; MRS) and incubated at 37 °C for 24 hours. After the incubation period, the total bacterial load was counted using a digital colony counter (Gallenkamp, England) and expressed in colony-forming unit per gramme (CFU/g).

Determination of proximate composition and energy values

Proximate composition (%) of the processed and prepared meal samples was done according to standard procedures of AOAC (2005) for the determination of moisture, protein, fat, ash, and fibre contents (method numbers: 925.10, 960.52, 2003.05, 923.03, and 2009.01, respectively), while carbohydrates were calculated by the difference i.e. (100 – (%moisture + %protein + %ash + %crude fat + %crude fibre)). The total energy value (kcal/100

g) of the meal samples was calculated using Atwater factors.

Results

Probiotic potential of the prepared meals from the local fermented foods

The isolated probiotics from the selected local foods with their concentrations are depicted in Table 1. The generally regarded as safe (GRAS) LAB organisms as probiotics; *Bifidobacterium lactis, Lactobacillus acidophilus and Lactobacillus bulgaricus* were present in all meal samples, which is in accordance with the report of Liu et al. (2009). These LAB strains were the most frequently used probiotics that provide relief in diarrhoea and gastrointestinal afflictions (Borges et al., 2006; Bergogne, 2017).

Table 1. Identification and concentration (CFU/g) of the isolated probiotics from the prepared meals

Meal samples	*Bifidobacterium lactis* Identification	Concentration	*Lactobacillus acidophilus* Identification	Concentration	*Lactobacillus bulgaricus* Identification	Concentration
90% 'Ogi'+10% soya (TS)	+	5.28×10^{4e}	+	5.01×10^{4c}	+	3.49×10^{4d}
80% 'Ogi'+20% soya (PS)	+	7.77×10^{6a}	+	5.16×10^{4c}	+	6.57×10^{3e}
70% 'Ogi'+30% soya (ST)	+	5.79×10^{6b}	+	3.69×10^{6a}	+	7.39×10^{4a}
Sorghum 'Ogi' only (SP)	+	2.19×10^{6d}	+	6.17×10^{4c}	+	1.40×10^{4d}
'Ogi' liquor (E)	+	4.70×10^{6c}	+	2.48×10^{6b}	+	2.59×10^{4c}
'Garri' liquor (F)	+	3.41×10^{2ef}	+	1.48×10^{3d}	+	1.91×10^{2f}
Soaked 'garri' (G)	+	1.78×10^{3f}	+	4.19×10^{3d}	+	1.01×10^{2f}
RDA		10^6		$10^8 - 10^{10}$		10^3

Values represent the mean ± SD of three determinations. Values with different superscripts in the same column are significantly different (p≤0.05). Key: TS = Meal made from 90% sorghum 'ogi' flour+10%soya flour; PS = Meal made from 80% sorghum 'ogi' flour +20% soya flour; ST = Meal made from 70% sorghum 'ogi' flour+30% soya flour; SP = Meal made from sorghum 'ogi' flour only; E = 'Ogi' liquor, F = 'Garri' liquor, G = Soaked 'garri'; RDA = Recommended daily allowance (Andrade et al., 2009); + = Present

Table 2. Proximate composition of the processed fermented local foods

Sample	Moisture	Protein	Ash	Fat	Crude fibre	Carbohydrates
Sorghum-'ogi' flour	9.21^a	6.13^c	4.67^b	3.86^b	11.38^a	64.43^c
Soya-bean flour (soya)	8.07^c	37.79^a	4.29^c	28.23^a	5.44^c	16.3^d
80% 'ogi'+20% soya flours	8.69^b	6.44^b	5.81^a	4.02^b	10.29^b	65.59^b
'Garri'	7.30^d	2.58^b	1.98^d	0.00^c	1.95^d	86.28^a

Values represent the mean ± SD of three determinations. Values with different superscripts in the same column are significantly different (p≤0.05)

Table 3. Proximate composition (%) and energy value of meals prepared from the formulated fermented foods

Meal samples	Moisture	Protein	Ash	Fat	Crude fibre	Carbohydrates	Energy value (kcal/100 g)
Sorghum 'ogi' flour (SP)	72.09^b	1.96^c	1.80^c	2.29^b	1.11^c	20.76^a	111.49
80% 'ogi'+20% soya (PS)	69.48^d	7.10^b	3.34^a	6.70^a	2.76^b	10.60^c	131.1
70% 'ogi'+30 % soya (ST)	71.10^c	8.26^a	2.50^b	6.79^a	3.05^a	8.30^d	127.35
Soaked 'Garri' (G)	80.65^a	0.88^d	2.24^b	1.68^c	1.09^c	13.46^b	72.48

Values represent the mean ± SD of three determinations. Values with different superscripts in same column are significantly different (p ≤ 0.05).SP = Meal prepared from sorghum 'ogi' flour only; PS = Meal prepared from 80% sorghum 'ogi' flour+20% soya flour; ST = Meal prepared from 70% sorghum 'ogi' flour+30% soya flour; G = Soaked 'garri'

The results of probiotic enumeration (Table 1) showed that the highest concentration of *Bifidobacterium lactis* (7.77×10⁶ CFU/g) in PS (80% 'ogi'+20% soya) was within the recommended dietary allowance (RDA) range (10⁶ CFU/g), which could confer health benefits on the host, especially as anti-diarrhea (Andrade et al., 2009). Also, meals from ST (70% 'ogi'+30% soya), SP (Sorghum 'ogi' only) and E ('ogi' liquor), had 5.79×10⁶, 2.19×10⁶, and 4.70×10⁶ CFU/g *Bifidobacterium lactis*, respectively, that falls within the standard range. On the other hand, TS (90% 'ogi'+10% soya), F ('garri' liquor), and G (Soaked 'garri') had 5.28×10⁴, 3.41×10², and 1.7×10³ CFU/g, respectively of *Bifidobacterium lactis* that were negatively below the acceptable RDA claims. None of the samples produced enough concentration of *Lactobacillus acidophilus* that matched the standard RDA (10⁸-10¹⁰ CFU/g) which could benefit health, as stated by Andrade et al. (2009). Only the formulated PS meal (80% 'ogi'+20% soya) also had *Lactobacillus bulgaricus* concentration (6.57×10³ CFU/g) close to the standard range, while all other samples were found to be negative either below or above the recommended values that provide health benefits. The excessive higher values above the RDA (10³ CFU/g) of *Lactobacillus bulgaricus* are indicative of a highly acidic meal (Andrade et al., 2009).

The PS meal (80% 'ogi'+20% soya) met the RDA standard of *Bifidobactarium lactis* and *Lactobacillus bulgaricus*, which is in agreement with the reports of Hong et al. (2005) and Andrade et al. (2009), and was also the most preferred among the samples from the conducted mini-preliminary sensory evaluation (results not presented). Thus, the formulated PS (80% 'ogi'+ 20% soya) is suggested as the best and most potent probiotic meal source that could be employed in the dietary treatment of diarrhoea among the local fermented foods studied. This corroborates the report of Akatsu et al. (2013). Among other factors used for evaluating health effects of probiotics, concentration remains the basic and preliminary prerequisite.

Nutritional composition of the processed foods and meal samples

In order to assess the nutritive quality of the food samples, the proximate composition of the processed and the prepared meal samples was determined. The 90% 'ogi'+10% soya (TS), based on the probiotic evaluation results was excluded in the nutritional evaluation of the processed foods, while 'Ogi' liquor (E) and 'Garri' liquor (F) were excluded among the meal samples based on the fact that they were considered majorly as water except for their probiotic contents, which is in consonance with the study of Okowa et al. (2016) on the quality of ogi water from maize fermentation.

Proximate composition of the processed fermented local foods

The proximate composition (moisture, protein, ash, fat, crude fibre, and carbohydrates) of the processed fermented local foods is presented in Table 2. The moisture content of all the samples ranges from 7.30-9.21% and is generally below 10%, which is an indication of good keep-ability of the products for ease of handling and a prolonged shelf-life, which concurred with the report of Awolu and Olabiran (2019). The lowest moisture value (7.30%) was found in 'Garri', which could be the result of roasting. The protein content was highest (37.79%) in 'soya flour only, followed by formulated meal of 80% 'ogi' flour+20% soya (6.44%), then, sorghum "ogi" flour, only 6.13%, while "garri" had the lowest protein content (2.58%). Soya flour is legume-based with high protein content (Adesokan et al., 2011), while 'garri' is starchy root-based. The ash content was highest (5.81%) in 80% sorghum 'ogi' flour+20% soya, followed by sorghum 'ogi' flour, only (4.67%) and lowest (1.98%) in "garri". The ash content observed in the soya bean and sorghum 'ogi' flours of the present study was higher than those reported by Igbua et al. (2019) and Awolu and Olabiran (2019). Ash content is an indicator of mineral composition in food material (Igbua et al., 2019) implying that the samples in the present study will be good sources of minerals.

The fat content was highest (28.23%) only in 'soya flour, as shown in Table 2 but the formulation of 80% sorghum 'ogi'+20% soya flour reduced the fat content to 4.02%, although this reduced value was still higher than that observed in sorghum 'ogi', only 3.86%. Fat in 'garri' was below a significant detectable value relative to other samples. The fat content is an indicator of energy boosting and when combined with meals could add to weight so as to neutralise the weight lost during a case of diarrhoea (Ikya et al., 2013). Crude fibre is a measure of the quality of indigestible cellulose, pentose, lignin, and other components; it provides the bulk necessary for the proper peristaltic action in the intestinal tract (Johnson and Southgate, 2013). Processed sorghum 'ogi' flour had the highest crude fibre content of 11.38%, followed by (80% sorghum'ogi'+20% soya) with 10.29%, then, soya flour (5.44%); 'garri' had the lowest value of 1.95%. This indicated that sorghum contributed to the bulk of the fibre content of the formulated meals, which is at variance with the report of Oluwamukomi et al. (2005). However, carbohydrates were higher in 'garri' (86.28%)

followed by 80% sorghum 'ogi'+20% soya (65.59%), then sorghum 'ogi', only 64.43%, while soya flour had the lowest value (16.31%), which is expected for legumes and is in consonance with the previous literature (Ikya et al., 2013; Igbua et al., 2019).

Nutritional composition of the prepared meals

The proximate composition of the prepared meals from the processed fermented food samples is presented in Table 3. Generally, the results show a decrease in the nutritional characteristics of the meal samples with a significant decrease in the contents; protein (0.88-8.26%), ash (1.80-3.34%), fat (1.68-6.79%), crude fibre (1.09-3.05%), and carbohydrates (8.30-20.76%) in comparison with the processed fermented foods. The moisture content significantly increased with a range of 69.48% in 80% 'ogi'+ 20% soya (PS) to 80.656% in soaked 'garri' (G). However, there are significant differences among the proximate composition of the meal samples, which is in agreement with the report of Monti et al. (2008); except the fat contents of the ST (6.79%) and PS (6.70%) meals which were not significantly different from each other. This could be the result of the soya flour inclusion in the two samples.

The range of values for fat content was comparable to that of stiff porridge meals (4.16-5.82%) in the study of Igbua et al. (2019), while the obtained range for crude fibre contents was higher than those found in literature (Ikya et al., 2013; Igbua et al., 2019). Also, the obtained values for carbohydrates were considerably lower than the range (59.43-78.28%) reported by Igbua et al. (2019). This may be the result of the differences in the nature of the meals. Igbua et al. (2019) worked with stiff porridges, which involved less quantity of water in preparation of the meal than required in the preparation of thin-gelatinised gruels in the present work.

Consequently, the reduction in nutrient contents in the prepared meals (Table 3) with reference to the processed fermented foods (Table 2) was a result of the preparation process that has to do with a high dilution rate with water in the meals. This is advantageous for rehydration in cases of diarrhoea and emetics that involve the loss of body fluids, while the ash contents of the meals contributed to the total mass of the product to influence rehydration in such cases (Osungbaro, 2009). Notwithstanding, the result showed that meals of 70% 'ogi'+30%soya (ST) and 80% 'ogi'+20%soya (PS) were of better nutritional quality and comparable to that of Oluwamukomi et al. (2005). However, PS was the most acceptable from the preliminary mini sensory evaluation carried out, implying that soybean improved the nutritional quality

without adverse effects on the acceptability, which is in agreement with the report of Adesokan et al. (2011). Thus, the processing and preparation methods used in the present study did not impair the nutrient content of the meals. Moreover, good nutritional quality of PS (80% 'ogi' +20% soya) could enhance the fast recuperation of a convalescent of diarrhoea and gastroenteritis. Therefore, the meal prepared from 80% 'ogi'+20% soya in addition to its standard concentration of the determined probiotics, has a potential to support the treatment of diarrhoea.

Conclusions

Three species of LAB (*Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*) were isolated from the meals. The meal prepared from 80% sorghum 'ogi' flour+20% soya flour (PS) contains standard loads of *Bifidobacterium lactis* and *Lactobacillus bulgaricus*, which is in accordance with the RDA claims of health benefits and it is of good nutritional quality. Therefore, the consumption of PS meal in an appropriate proportion could serve the dual purposes of having the potential to treat and recover from diarrhoea. Consequently, regular intake of locally available fermented lacto-free foods could serve as a preventive measure for reducing the risk of diarrhoea and gastroenteritis. This, along with the literature records of probiotic potent in the stimulation of the immune system, will cumulate into building a strong natural body defence against infection and diseases, especially in the era of Covid-19. Hence, hygienically fermented, prepared, and served meals as PS should be advocated in the practical dietary therapy by dietitians in the management of diarrhoea or any gastroenteritis. Nevertheless, a clinical dietetic study on the suggested meal will be required to confirm their effectiveness.

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