EFFECT OF DIFFERENT TREATMENTS ON CHEMICAL COMPONENTSOF FERMENTED PROBIOTIC MILLET DRIEDMIX

ABSTRACT

Aims: The purpose of this study was to develop fermented probiotic millet dried mix by incorporating a probiotic *Lactobacillus acidophilus*and to ascertain how different processes used in the product's development affected its chemical components.

Study design: Fermented probioticmilletdriedmixwasdevelopedbyfermentationofgerminatedfingermilletflourandReconstituted Skim Milk (RSM) with *L. acidophilus.*The impact of various treatments on moisture, crude protein, crude fiber, total ash, alcoholic acidity and phytate content were investigated.

Place and Duration of Study: Department of Dairy Microbiology, Dairy Science College, Hebbal, Bengaluru, Karnataka, India between June 2023 and April 2024.

Methodology: Moisture, crude protein, crude fiber, total ash, alcoholic acidity and phytate content were examined after various treatments such as soaking, germination, autoclaving, fermentation and drying of millet dried mix.

Results: At 10% *L. acidophilus* inoculum level and a 1:1.5 millet to RSM ratio, a maximum viability of 9.52 $\log_{10}$cfu/g was reported after 30 h of fermentation.After freeze drying of fermented probiotic millet mix with 2%Skim Milk Powder (SMP) as a cryoprotective agent, viability was retained to 9.50 $\log_{10}$cfu/g.The optimized fermented probiotic millet dried mix had 4.76% moisture, 11.94% crude protein,6.50%crudefiber,4.74%totalash,1.90%alcoholicacidityand1.73mol/kgofphytate.

Conclusion: The resultant fermented probiotic millet dried mix was rich in nutrients and the probiotic *L.acidophilus* helps to improve gut health. Thus, the product had combined therapeutic and nutritional benefits of probiotics with millet.

Keywords:*Fingermillet; L.acidophilus;RSM;freezedrying*

1. INTRODUCTION

Globalproductionofmilletwas30,945thousandmetric tons (1 MT=1000 kg).With40%oftheworld'smilletproduction, Indiawasthe largestmilletproducer with12,500thousandMT[1].Milletsarerichsourceofcalcium(10-348mg/100g),iron(2.2-17.7mg/100g),zinc(32.7-60.6 mg/100 g) and phosphorus (200-339 mg/100 g), vitamins such as thiamine (0.15-0.60mg/100 g), niacin (0.09-1.11 mg/100 g) and riboflavin (0.28-1.65 mg/100 g) that makes them almost a perfect energy food[2]. In addition to their potential to decreasecholesterol,milletsalsohaveadditionalhealthbenefitsincludingantioxidative,anti-ulcerative,hypoglycemicand anti-inflammatoryqualities [3].

Thefingermilletgrainshavethehighestconcentrationsofphosphorus(130.0-283.0mg/100g) and calcium (162.0-358.0 mg/100 g). About 44.7% of essential amino acids such as lysine,isoleucine, leucine, phenylalanine, methionine, cysteine and tryptophan are found in fingermillet grains. Additionally, the important fatty acids such aspalmitic and linolenic acid that arenecessary for the growth of the brain and neural tissue are present in finger millet grains[4].Antinutrient levels can be decreased by using processing techniques such as decortication,heating, soaking, germination and fermentation as they restrict the bioavailability of minerals[5].

Functional foods are defined as "those foods and food components that offer a health benefitabove and beyond basic nutrition," and in particular, "a food that resembles, or may be aconventional food that is consumed as apart of a regular diet and is demonstrated to havephysiologicalbenefitsandreducetheriskofchronicdiseasesaboveandbeyondbasicnutritionalfunctions"[6].

Probiotics are the good example of functional food. Probiotics are "live microorganisms thatwhen administered in sufficient amounts confer a health benefit on the host''[7].Probioticfoodsmadefromcerealcanbeasignificantglobalsourceofcheapcaloriesandother

nutrients.Theyareusefulfortheproduction of functionalmealsastheycontainavariety of phytochemicals including phytoestrogens, phenolic compounds, antioxidants, phytic acidandsterols [8].Cereal grains offer typical benefits of whole cereal together with probiotic and prebioticbenefits. Additionally, fermentation has been shown to boost nutrients including methionine,lysine and tryptophan in a variety of cereal grains. A dietary mixture based on cereal that was fermented with a probiotic strain increased the amount of B complex vitamins specificallythiamineand niacin[9].

The main goal of the current study was to ferment finger millet with *L. acidophilus*probioticculture,enhancingthenutritionalandfunctionalbenefitsoftheproducttherebyresultingi nthedevelopmentof a functional product withmillets and probiotics benefits.

2. MATERIALSANDMETHODS

The finger millet was obtained from thereputed local market. Skim milk powder (Nandini brand) wassourced from localmarket, Bengaluru, Karnataka,India.

2.1 Cultures

Probiotic *L. acidophilus* culturemaintained in the department of Dairy Microbiology,DairyScienceCollege,Hebbal,Bengaluruwasusedfortheproduct.Theworkingcultureswere maintainedin sterilereconstituted skim milk and sub cultured atweeklyintervals.

2.2 Preparationofmillet

Finger millet was soaked for 24 h in sterile potable water at a ratio of 1:3 (millet: water) andthen germinated for an additional 48 h at 30℃. Following a 24 h drying in an open incubatorset at 45℃, the germinated millet was then ground and sieved in a sterile environment. Theresultinggerminated millet flour wasautoclavedfor15min at 121℃.

2.3 Growthstudyof*L.acidophilus* ingerminatedfinger milletflour

Autoclavedgerminatedfingermilletflourwasmixedwith9%sterileRSMatvariousratiosof 1:0.5, 1:1 and 1:1.5 to prepare germinated millet paste. Probiotic milk culture *L. acidophilus*was incorporated at inoculum levels of 5 and 10% into germinated millet paste and incubatedat 37℃ for 72 h under anaerobic condition. Throughout the 72h fermentation period, theviability of *L. acidophilus* was assessed for every 6 h. Viability was determined by usingstandard procedure given by Harrigan[10]. The period of fermentation at which maximumviability was seen with corresponding inoculum level and ratio of germinated millet flour and RSM wereoptimized.

2.4 Dryingofoptimizedfermentedprobioticfingermilletmix

The optimized fermented probiotic finger millet mix was dried using air drying and freezedrying techniques to lower the moisture content, extend the shelf life and to preserve themaximumviablecount.

2.4.1 Airdryingofoptimizedfermentedprobioticfingermilletmix

The fermented probiotic fingermillet mix was air dried for 24 h at 20, 25 and 30℃ in a sterile Biological Oxygen Demand (BOD) incubator. Moisture content (Food Safety and Standards Authority of India - FSSAI 03.005:2022), viable count, spores, coliforms, yeast andmoldcounts [10]wereassessed beforeand after airdrying.

2.4.2 Freezedryingofoptimizedfermentedprobioticfingermilletmix

Additionofskimmilkpowderat therateof 2%wasaddedtotheoptimizedfermentedprobiotic millet mix as a cryoprotective agent. It was then freeze dried at -40℃ for 8 h at 0.01 mm Hgvacuum.Moisturecontent(FSSAI03.005:2022)[11],viablecount,spores,coliformsandyeastand mold counts [10] were analysed before and after freeze drying with and withoutadditionofSkim Milk Powder (SMP). Thedryingconditionwithhighestprobioticviabilitywas optimized.

2.5 Effect of different treatments of fermented probiotic finger millet dried mix on

chemical components

Optimized samples were taken after soaking, germination, autoclaving, fermentation anddrying in order to ascertain the change inchemical constituent during the various stepsfollowed in the manufacturing of fermented probiotic millet dried mix and were analysed formoisture (FSSAI 03.005:2022),crude protein(FSSAI 03.016:2022),crude fiber (Association of Official Analytical Chemists - AOAC978.10)[12], total ash (FSSAI 03.010:2022), alcoholic acidity (FSSAI 03.014:2022) and phytate[13].

2.5 Statistical analysis

R software (version 4.1.2) was used to analysethedataandperformstatisticalcomputations.For every treatment, three replications (n=3) ofthe corresponding variable data were gathered.The data were analysed using one way ANOVAand in cases where the F value is significant, thecritical differencewascalculatedwith95%confidencelevel(*P*=.05)todeterminethepresence of significant differences, which werethendisplayedinthetableswithsuperscripts.Theformulaforcriticaldifference(CD)

$$CD = \frac{\sqrt{2 \times MSS\ (E)}}{R}\ t\alpha \quad @0.05$$

Where, MSS (E) = Mean Sum of Squares of the error
R = number of replications
tα = table t value of the α level of significance

3. RESULTSANDDISCUSSION

3.1 Growthstudyof*L.acidophilus* ingerminatedfinger milletflour

After 30 h of fermentation, the finger millet paste that was prepared using autoclaved fingermillet flour and Reconstituted Skim Milk (RSM)of 1:0.5, 1:1 and 1:1.5 ratio with 5% *L. acidophilus* inoculum exhibited maximum viability of 7.78, 9.25 and 9.30 $\log_{10}$cfu/g. Similarly, addition of 10% *L. acidophilus*inoculum to finger millet paste exhibited maximum viability of 7.93, 9.28 and 9.52 $\log_{10}$ cfu/gafter30hoffermentation(Table1).Inordertoacclimatizetheculturestothemilkenvironment andtostimulatetheirgrowth,skimmilkwasadded.Thegrowthoftheculture increasedduringthefirst30hofferm entationafterwhichitsteadilydecreasedwithincreasedfermentationtime.This might be due to the inhibitory effect of excess lactic acid produced during fermentationon the growth of the culture. There were both significant and non-significant differences between the various hours of fermentation. Maximum viability of 9.52 $\log_{10}$cfu/g was observed after 30 h of fermentation period at 10% inoculum levelof*L.acidophilus*and1:1.5(millet: RSM)ratio.Henceitwas optimized.

On par with the above study, *L. acidophilus* growth during fermentation of ragikoozh for a period of 24 h was investigated. The viability had increased from 2 to 8 $\log_{10}$cfu/ml and further declined to 6.5 $\log_{10}$cfu/ml after 15 and 24 h of fermentation. Increased acidity and decreased nutrition availability might be the cause for the drop in viability [14].Similarly,growthstudyof*L.acidophilus*onsupplemented black gram dhal [*Vignamungo* (L.) Hepper] was conducted. Viable count increased from 7.80 to 9.57 $\log_{10}$cfu/g and further decreased to 6.50 $\log_{10}$cfu/g after 24 and 48 h of fermentation [15].

Table1:Growthstudyof*L.acidophilus*in germinatedfinger milletflour

Fermentationperiod (h)	Ratioof autoclavedgerminatedflourandRSM					
	1:0.5		1:1		1:1.5	
	L.acidophilus (%)					
	5	10	5	10	5	10
	$\log_{10}$cfu/g					
0	6.03^{e}	6.27^{fg}	6.03^{f}	6.27^{i}	6.03^{h}	6.27^{f}
6	6.48^{de}	6.62^{def}	6.72^{e}	6.75^{h}	6.78^{g}	7.28^{e}

12	7.06^{bc}	7.11^{bcd}	7.44^{d}	7.64^{f}	7.66^{ef}	8.00^{d}
18	7.36^{ab}	7.44^{abc}	8.23^{c}	8.24^{cd}	8.29^{c}	8.68^{bc}
24	7.47^{ab}	7.64^{ab}	8.70^{b}	8.76^{b}	8.79^{b}	9.02^{b}
30	7.78^{a}	7.93^{a}	9.25^{a}	9.28^{a}	9.30^{a}	9.52^{a}
36	7.46^{ab}	7.57^{abc}	8.42^{bc}	8.67^{bc}	8.84^{b}	9.01^{b}
42	7.30^{ab}	7.40^{abc}	8.37^{bc}	8.40^{bcd}	8.48^{bc}	8.75^{bc}
48	7.05^{bc}	7.18^{bcd}	8.16^{c}	8.18^{de}	8.22^{cd}	8.42^{cd}
54	6.73^{cd}	6.96^{cde}	7.69^{d}	7.78^{ef}	7.90^{de}	8.12^{d}
60	6.15^{e}	6.38^{efg}	7.40^{d}	7.43^{fg}	7.49^{f}	7.94^{d}
66	5.48^{f}	5.82^{gh}	6.99^{e}	7.05^{gh}	7.05^{g}	7.30^{e}
72	5.08^{f}	5.25^{h}	5.82^{f}	5.86^{i}	5.89^{h}	6.43^{f}
CD(*P*=.05)	**0.30**	**0.35**	**0.07**	**0.25**	**0.22**	**0.29**

Note:
RSM:ReconstitutedSkimMilk
CD= Critical Difference, all the values are average of three trials; same superscripts indicate non-significance while different superscript indicates significant difference at P=.05

3.2 Airdryingofoptimizedfermentedprobioticfingermilletmix

Viability of *L. acidophilus* had decreased from 9.52 to 8.50, 7.90 and 7.50 log_{10}cfu/g after air drying at 20, 25 and 30℃ (Table 2). A statistically significant (*P*=.05)differences in viability were noted throughout drying regimes with the exception of dryingbetween25and 30℃. The presence of spores, yeast and mold may be due to exposure of product to open air. Duetothedecreasedviabilityandpresenceofsporesandyeastand mold, this method was not opted for further studies.

On par with the above study, air drying of *L. acidophilus* incorporated black gram dhal resulted in decreased viability from 9.45 to 8.94, 8.71 and 8.04 log_{10}cfu/g after air drying at 20, 25 and 30℃respectively [16].Similarly, decline in viability from 10.80 to 8.92, 9.00, 8.87 and 8.40 log_{10}cfu/g was observed after air drying of *L. acidophilus* 111 incorporated rice husk at 15, 20, 25 and 30℃ respectively [17].

Table2:Airdryingofoptimizedfermentedprobioticfingermilletmix

Treatments	**Moisture (%)**	**Viability**	**Spores**	**Coliforms**	**Yeastand Mold**
		Log_{10}cfu/g			
Beforedrying	61.90^{a}	9.52^{a}	0.00^{c}	0.00	0.00^{b}
Afterairdrying(℃)					
20	7.00^{b}	8.50^{b}	2.70^{b}	0.00	1.30^{a}
25	6.80^{b}	7.90^{c}	3.07^{a}		1.48^{a}
30	5.74^{c}	7.50^{c}	3.30^{a}		1.60^{a}
CD(*P*=.05)	**0.00**	**0.39**	**0.33**		**0.33**

CD= Critical Difference, all the values are average of three trials; same superscripts indicate non-significance while different superscript indicates significant difference at P=.05

3.3 Freezedryingofoptimizedfermentedprobioticfingermilletmix

The viability of *L. acidophilus* in fermented probiotic finger millet mix had declined from 9.52 to 9.02 log_{10} cfu/g after freeze drying. Before freeze drying, 2% skim milk powder was added as a cryoprotective agent, which assisted to retain viable cells of 9.50 log_{10}cfu/g even after freeze drying (Table 3). There was no statistically significant difference noted between freeze drying with addition ofSMP and before drying, althoughthere wasa statistically significant(*P*=.05) differenceidentifiedbetween sample dried without SMP andsample beforedrying.As a cryoprotective

agent, skim milk powder might have created a viscous coating on the cell surface and thereby preventing the air crystals formation. Freeze drying proved ideal for drying probiotic mix since it produced higher viable count and hence it was optimized.
On par with the above study, survival rate of freeze dried *Lactobacillus* sp. using 5% skim milk powder as a cryoprotective agent was found to be 93.98% [18]. On contrary, viability of *L. acidophilus* incorporated black gram dhal declined from 9.50 to 7.47 log_{10}cfu/g after freeze drying[16].

Table3:Freezedryingofoptimizedfermentedprobioticfingermilletmix

Treatments	Moisture (%)	Viability	Spores	Coliforms	Yeastand Mold
		Log_{10}cfu/g			
Beforedrying	61.90[a]	9.52[a]	0.00		
Afterfreezedrying(8h)					
WithoutSMP	4.76[b]	9.02[b]	0.00		
With2%SMP	4.76[b]	9.50[a]			
CD(*P*=.05)	**0**	**0.41**			

CD= Critical Difference, all the values are average of three trials; same superscripts indicate non-significance while different superscript indicates significant difference at P=.05

3.4 Effect of different treatments of fermented probiotic finger millet dried mix on chemicalcomponents

In order to ascertain how the chemical components changed over the course of the probiotic millet dried mix manufacturing process, samples were taken at different points and chemically analysed for factors such as moisture, crude protein, crude fiber, total ash, alcoholic acidity and phytate (Table 4).

Finger millet flour had moisture content of 13.50%, 7.23% of crude protein, 4.00% crude fiber, 2.31% of total ash, 0.17% alcoholic acidity and 5.32 mol/kg of phytate.

3.4.1 Soakingoffingermillet

After soaking for 24 h, the moisture content of finger millet flour increased from 13.50 to34.47%andthiscouldbeduetotheabsorptionofmoisturefromthesoakingmedia.Thephytate level decreased from 5.32 to 4.06 mol/kg as a result of phytic acid breakdown by phytase andleachingintothesoakingmedium.Enzymaticactivitycausedthealcoholicacidity torisefrom0.17to0.19%. Becauseofthedropinphytatelevel,ashcontentincreasedfrom2.31to2.50%.Proteinincreased from 7.23 to 7.28% and crude fiber from 4.00 to 4.12% and this increase might bedueto thedeclinein phytate content.
On contrary, the influence of soaking circumstances on theproximate composition of finger millet flour was investigated. After 24 h of soaking at 30°C, the moisturecontentincreasedfrom7.08to8.89%,theproteincontentdroppedfrom9.47to5.78%,theash content increased from 1.10 to 1.94%, the crude fiber content reduced from 2.36 to 1.34% and the phytate content decreased from 0.75 to 0.44 mg/100g [19].

3.4.2 Germinationoffingermillet

The breakdown of complex organic molecules like lipids, phytin and protein into simplersubstancesmaybethecausefortheincreaseinalcoholicacidityfrom0.17to0.28%.Thetotal ash increased from 2.31 to 2.91% which could be attributed dueto the reduction of metal-binding water-solublesecondarymetabolitesortheconversionofphytatetoinositolbyphytase,whichwould have liberated the orthophosphate and released the minerals into the millet seeds thathadgerminated.Thesynthesisofproteins,thebreakdownofantinutritionalsubstancesandthesynthesis of enzymes by germinated grains may have contributed to the rise in crude proteinfrom 7.23 to 7.91%. Phytate dropped from 5.32 to 3.34 mol/kg and the phytase enzyme mayhave been activated to cause

this phytate to degrade and leak out. The amount of crude fiberincreased from 4.00 to 4.80% and the production of cellulose and hemicellulose may havecontributedto this rise.
Onparwiththeabovestudy,thephysicochemicalparametersof finger millet after soaking and germinating for 24 and 48 h was investigated. To create finger millet flour,germinated seeds were dried at 40°C for 24 h, then ground and sieved. The moisture contentincreased from 8.47 to 8.65% while the protein content increased from 7.36 to 8.51%, fiberincreased from 3.75 to 4.55%, ash content from 2.35 to 2.84%, drop in fat from 2.05 to 1.31%andthe amount of phytatedropped from 15.98 to9.77 mg/gwereobserved [20].

3.4.3 Autoclavingof germinatedfingermilletflour

Followinggermination,theseedswereground,sievedtocreategerminatedflourthenautoclavedfor15minat121℃.Moisture content increasedfrom8.00to8.33% and this increase might be due tomoistheatsterilization.Crudeproteindecreasedfrom7.91to7.54%,phytatefrom3.34to2.71mol/kg,totalashfrom2.91to2.70%,alcoholicacidityfrom0.28to0.20%andcrudefiber from 4.80 to 4.30%. The separation of the bran layer during milling could be the cause of thisdrop.

Theimpactofgerminationandfermentationonthenutritionalmakeupoffoodblendsincluding pearl millet was investigated. After autoclaving for 15 min at 121°C,the moisture content of the germinated pearl millet declined from 28.41 to 28.39%, the crudeprotein from 13.89 to 13.78, the ash from 2.93 to 2.90%, the titratable acidity increased from 2.15to2.22 glacticacid/100mlandthecrudefiber content declined from 0.79 to0.70% [21].

3.4.4 Fermentation ofgerminatedfingermilletflour

The addition of reconstituted skim milk caused the moisture content to increase from 8.33 to61.90% after fermentation. Alcoholic acidity increased from 0.28 to 1.62% as a result ofprobiotic fermentation, which might have convertedglucose to lactic acid. Totalashincreasedfrom2.70to3.06%duetothereductionofantinutrients.Thepossiblereasonforthe increasedcrude protein from 7.54 to 7.70% during fermentation might be due the solubilization of insolubleproteins. Phytate hydrolysis might have caused it to decline from 2.71 to 1.32 mol/kg. Crude fiber declined from4.30to4.21%,possiblyasaresultoflacticacidbacteriausingthefiberasacarbonsource duringthe fermentation processandenzymaticallybreakingdownthefiber'scomponents.

On par with the above study, the impact of fermentation treatmentson the nutritional makeup of finger millet was explored. Fermentation was carried out with *L. brevis*for 12, 24and36h.After12,24and36h,themoisturecontentincreasedfrom9.7to10.97,11.40and11.85%.Fiberalso declinedfrom5.67to4.48,3.68and3.49%.Ashlevelsincreasedfrom3.10to 3.2, 3.53 and 3.63%. Protein content increased from 9.10 to 10.28, 10.44 and 13.13% from 9.10.Thephytic acid decreasedfrom 629to 250.49,230.88 and207.94 mg/100g [22].

3.4.5 Freezedryingoffermentedprobioticmilletmix

Due to the concentration of the mix caused by the elimination of moisture after freeze drying,themoisturecontentdecreasedfrom61.90to4.76%andthealcoholicacidityincreasedfrom1.62 to 1.90%. Drying caused increase in total ash of 3.06 to 4.74%, protein from 7.70 to11.94%, crude fiber from 4.21 to 6.50% and phytate from 1.32 to 1.73 mol/kg. This increaseinnutrients couldbedueto the result oftheconcentrationprocess duringfreezedrying.

On contrary to the above study, the impact of freeze drying onthenutritionalqualityofpowderedfingermilletporridge was assessed.Moisturelevelsdecreasedfrom15.63to9.5%,ashfrom4.53to4.486,fiberfrom3.61to3.59%,fatfrom 1.589to1.545%and protein content from 7.72 to 7.60%. Water loss from the interior surface to the surroundingcouldbe thecauseof thisloss [23].

Table 4: Effect of different treatments of fermented probiotic finger millet dried mix onchemicalcomponents

Treatments	Moisture	Crudeprotein	Crudefiber	Total ash	Alcoholic acidity	Phytate(mol/kg)
	(%)					
Fingermillet flour	13.50^{c}	7.23^{c}	4.00^{c}	2.31^{c}	0.17^{b}	5.32^{a}
Aftersoaking(24h)	34.47^{b}	7.28^{c}	4.12^{c}	2.50^{c}	0.19^{b}	4.06^{b}
48 h Germinatedfinger milletafter drying	8.00^{d}	7.91^{b}	4.80^{b}	2.91^{b}	0.28^{b}	3.34^{c}
Afterautoclaving (121^{0} C/15 mins)	8.33^{d}	7.54^{bc}	4.30^{bc}	2.70^{bc}	0.20^{b}	2.71^{d}
Afterfermentation (30h)	61.90^{a}	7.70^{b}	4.21^{c}	3.06^{b}	1.62^{a}	1.32^{e}
Afterfreezedrying (8 h)	4.76^{e}	11.94^{a}	6.50^{a}	4.74^{a}	1.90^{a}	1.73^{f}
CD(*P*=.05)	**0.00**	**0.36**	**0.36**	**0.36**	**0.29**	**0.36**

CD= Critical Difference, all the values are average of three trials; same superscripts indicate non-significance while different superscript indicates significant difference at P=.05

4. CONCLUSION

Fermented probiotic millet dried mix was developed by fermentation of germinated fingermilletflourandReconstitutedSkimMilkwith*L.acidophilus*.Maximumviabilityof9.52log$_{10}$cfu/g was seen after 30 h of fermentation period at 10% inoculum level of *L. acidophilus* and1:1.5millettoRSMratio.Airdryingresultedindecreasedviabilityandindicatedthepresence ofsporesandyeastandmoldintheproduct. Prior to drying, the viable count of *L. acidophilus* was 9.52 log$_{10}$cfu/g, however after freeze drying of fermented probiotic millet mix with 2% SMP as a cryoprotective agent, viability was retained to 9.50 log$_{10}$cfu/g. After freeze drying, the fermented probiotic finger millet dried mix had 4.76% moisture, 11.94% crude protein, 6.50% crude fiber, 4.74% total ash, 1.90% alcoholic acidity and 1.73 mol/kg phytate.Hence, the product serves as a functional food to the community.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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