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APPENDIX I

Estimation of Nutrients

1. Moisture content

Moisture content of the samples was determined as per the A.O.A.C. (2010) procedure.

Procedure

Two grams of sample in triplicates were placed in a pre-dried and weighed aluminium dish spreading as thinly as possible over the base of the dish and oven dried at 105°C for 1 hour and transferred to dessicator to cool and weighed. Continued drying until a constant weight has been reached and the moisture content was calculated from the weight loss of the sample.

$$\text{Moisture(g/100g of sample)} = \frac{\text{Initial weight} - \text{final weight(g)}}{\text{Weight of the sample (g)}} \times 100$$

2. Crude protein

Crude protein content of the samples was determined with modification of the A.O.A.C. (2010) method.

Reagents

1. Digestion mixture – Sodium sulphate (96 parts) and Copper sulphate (4 parts) were mixed thoroughly.
2. Concentrated sulphuric acid
3. 40% sodium hydroxide.
4. 0.1N sulphuric acid
5. 4 % boric acid
6. Mixed indicator: A mixture of bromocresol green and methyl red indicator (4:1).

Procedure

One gram of sample in triplicates was digested using one gram of digestion mixture and 10ml concentrated sulphuric acid. Digestion was done by using an automatic digestion unit (KEL PLUS, model KES O6L), till all the particles got digested and a clear bluish green solution was obtained. The digested samples was distilled using an automatic distillation unit (KEL PLUS, model DISTYLEM). The distillate was collected in 4% boric acid solution containing mixed indicator and titrated against 0.1N sulphuric acid. Crude protein content was calculated using a conversion factor of 6.25 and expressed in percentage.

Calculation

$$\text{Protein in g/100g} = \frac{\text{Titrate value} \times 0.014 \times 6.25}{\text{Weight of the sample (g)}} \times 100$$

3. Crude Fat

Crude fat was determined with modification of the A.O.A.C. (2010) procedure.

Chemicals

Petroleum ether (B.P. 40-60°C)

Procedure

Two grams of samples in triplicates were taken into thimble and plugged with cotton wool. The thimble was placed in Soxhlet apparatus and fat was extracted for 16 hours using petroleum ether (B.P. 40-60°C). The ether extract collected in pre-weighed flasks of the extraction apparatus, was distilled and condensed ether was collected separately. The residue in the flasks were dried in an oven at 80-100°C, cooled and weighed. From the difference between initial and the final weight of the flasks, the fat contents were calculated and expressed in percentage.

Calculation

$$\text{Fat in g/100 g of sample} = \frac{\text{Weight of the oil (g)}}{\text{Weight of the sample (g)}} \times 100$$

4. Crude Fibre

Crude fibre content of the samples was determined with modification of the A.O.A.C. (2010) method.

Reagents

1. 0.255 N sulphuric acid
2. 0.313 N sodium hydroxide
3. Alcohol and ether

Procedure

Four grams of the samples in triplicates were weighed and put in a 500ml beaker and 200ml of boiling 0.255 N sulphuric acid was added to it. The mixture was boiled for 30 minutes by keeping the volume constant. Evaporation of the mixture was prevented by keeping round bottomed flask filled with cold water at the top of the beaker. The round bottomed flask acted as a condenser and it was changed with cold water

frequently once the water of the flask was heated. After 30 minutes the mixture was filtered through muslin cloth and the residue was washed with boiling water until washings are no longer acidic. The material was transferred to a 500ml beaker, 200ml of boiling 0.313N NaOH was added to it and boiled for 30 minutes. The residue was filtered through muslin cloth and washed with 25ml of boiling 1.25% H₂SO₄, three 50ml portions of water and 25 ml alcohol. The residue was removed and transferred to a pre weighed ashing dish (W1). The residue was dried at 130±2°C for 2 hrs, cooled in a dessicator and weighed with ashing dish (W2). Ignited for 30 min at 600±15°C, cooled in a desiccator and weighed (W3). The fibre content was calculated using the formula given below:

$$\text{Crude fibre (g/100g of sample)} = \frac{\text{Weight of fibre (W2 - W1) - (W3 - W1)}}{\text{Weight of the sample (g)}} \times 100$$

5. Available carbohydrate

The available carbohydrate content was estimated by the method of Hedge and Hofreiter, 1962.

Principle

Carbohydrate is first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with absorption maximum at 630 nm.

Reagents

1. Glucose stock standard:

100 mg of glucose was dissolved in 100 ml of water in a standard flask.

1. Working standard:

10 ml of the stock was diluted to 100 ml. 1.0 ml of this solution contains 100µg of glucose.

Anthrone reagent:

0.2% anthrone was dissolved in ice cold concentrated sulphuric acid.

Prepared fresh before use 4. 2.5 N HCl.

Procedure

100mg of sample were weighed into a boiling tube and hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl and cooled to room temperature. The solution was neutralized it with solid sodium carbonate until the

effervescence ceased and the volume was made upto 100 ml and centrifuged. The supernatant was collected and 0.2 to 1.0 ml were taken for analysis. The standards were prepared by taking 0.2-1.0 ml of the working standards. 1.0 ml of water serves as a blank. The volume is made upto 1.0 ml in all the tubes with distilled water, then 4.0 ml of anthrone reagent is added and heated for eight minutes in a boiling water bath and cooled rapidly. The green to dark green colour is read at 630 nm.

Calculation

A standard graph was drawn by taking the concentration of glucose on X axis and spectrophotometer reading on Y axis. From the graph the concentration of glucose in the samples were calculated.

6. Estimation of Calcium

Procedure:

- The sample was treated with concentrated hydrochloric acid, transferred to a volumetric flask and made up to 100 ml.
- Take above 100ml in conical flask.
- Add 2-3 drops of sodium hydroxide 1N solution and to raise the pH 12 -13.
- Add a pinch of Patton & Reeder indicator and stir well.
- Titrated against the solution with 0.01M EDTA
- The end point is appearance of blue colour.

Calculation:

$$\text{Calcium (Ca), mg/l} = \frac{A \times B \times 1000}{V}$$

Where

A = Volume in ml of EDTA solution used for titration,

B = Mass in mg of calcium equivalent to 1ml of EDTA solution, and

C = Volume in ml of the sample taken for the test.

Reference: AOAC/BIS/FSSAI

7. Determination of Zinc

This standard prescribes the Atomic absorption spectrophotometric method for the determination of zinc present in the sample.

Apparatus:

- Atomic absorption spectrophotometer with air acetylene flame.
- Hollow cathode lamp – 213.8 nm.

Reagent:

- Zn (*NIST traceable*).
- Nitric acid (1:499).
- Conc. HCL.

Procedure:

- Take 100 ml standard flask
- Prepare Zn standards (*Nist traceable*) to 0.05, 0.075, 0.1, 0.125, 0.15&0.2 mg/l in nitric acid (1:499).
- Prepare a blank solution in 100ml distilled water.
- Take 1-2gm of sample in a beaker and digest with 50 ml. of conc. HCL till the volume reduced to three fourth
- Cool and filter and make up to 100 ml. with distilled water.
- Process the blank also in the above manner.
- Set the AAS as per the specific work instruction.
- Aspirate the blank, standards and sample solutions.
- Measure the absorbance of the zinc at 213.8nm.

Calculation:

- Draw the standard calibration graph by plotting the absorbance Vs standard conc. for each Standard.
- Process a standard at detection level (0.01 ppm) as quality control check with every batch of samples and measure its conc. from the Calibration graph.

8. Iron

Preparation of mineral solution

The individual minerals were analyzed from the mineral solution prepared from ash. The ash was moistened with a small amount of glass distilled water (0.5-1ml) and 5ml of concentrated hydrochloric acid was added to it. The mixture was evaporated to dryness on a boiling water bath. Another 5ml of HCl was added again and the solution

was evaporated to dryness as before. Finally 4ml of HCl and 5ml of water were added and the solution was warmed over a boiling water bath and filtered into a 100ml volumetric flask using Whatman No. 40 filter paper. After cooling, the volume was made up to 100ml with distilled water. This solution was used for estimation of iron and calcium.

Iron content was determined according to the method described by Ranganna (1986) by using spectrophotometer (Model No. 2513).

Reagents

1. 30% sulphuric acid (H_2SO_4)
2. 7% potassium per sulphate solution ($K_2S_2O_8$)
3. 40% potassium thiocyanate solution (KCNS): Dissolved 40 g of KCNS in about 90ml distilled water, added 4 ml of acetone and made up to volume to 100 ml.
4. Standard iron solution: Dissolved 70.2 mg of ferrous ammonium sulphate in 100 ml of distilled water and after addition of 5 ml 1:1 HCl made up the volume to one litre. Prepared the standard solution into 10 folds.

Procedure

To an aliquot of the ash solution, water was added to make up the volume upto 6.5ml. Added 1 ml of 30% sulphuric acid, 1ml of 7% potassium per sulphate solution, 1ml of 40% potassium thiocyanate solution respectively. The absorbance of blood red colour was measured in spectrophotometer at 540 nm within 20min of colour development. A standard curve was prepared by taking different concentration of iron ranging from 10 μ g to 50 μ g. The concentration of iron present in the sample was calculated from the standard curve and expressed as mg Fe/100 g sample.

9. Phosphorus

Apparatus: UV-VIS spectrophotometer

Reagents

- Molybdovanadate reagent – Dissolve 40 g ammonium molybdate $4H_2O$ in 400 ml hot H_2O and cool. Dissolve 2 g ammonium metavanadate in 250 ml hot H_2O and cool; 2 2 add 250 ml 70% $HClO_4$. Gradually add molybdate solution to vanadate solution with stirring, and dilute to 2 litre.

- Phosphorus standard solutions – (i) Stock solution -2 mg/ml. Dissolve 8.788 g KH₂PO₄ in H₂O and dilute to 1 L (ii). Working solution – 0.1 mg/ml. Dilute 50 ml stock solution to 1 litre.
- Preparation of standard curve
- Transfer aliquots of working standard solution containing 0.5, 0.8, 1.0 and 1.5 mg P to 100 ml volumetric flasks. Treat as mentioned in determination.
 - Prepare standard curve by plotting mg P against per cent T on semi log paper.
 - Determination using UV-VIS spectrophotometer
 - Ash 2 g sample in 150 ml beaker about 4 h at 600°C. Cool, add 40 ml HCl (1+3) and several drops HNO₃ and bring to boiling point. Cool, transfer to 200 ml volumetric flask and dilute to volume with H₂O. Filter and place aliquot containing 0.5-1.5 mg P in 100 ml volumetric flask. Add 20 ml molybdovanadate reagent, dilute to volume with H₂O and mix well. Let stand 10 min; then read per cent T at 400 nm against 0.5 mg standard set at 100% T (Use 15 mm diameter cells.). Determine mg P from standard curve.

Calculation: $P (\%) = \frac{\text{mg P in aliquot}}{\text{g sample in aliquot} \times 10}$ Reference: AOAC Official Method 965.17

10. Determination of tannin Content

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 % Na₂CO₃ solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Tannic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of Tannic acid /g of extract. 10 ml of bromine water was added to the 0.5 g aqueous extract. Decoloration of bromine water showed the presence of tannins.

11. Test for Saponins

Saponins occur widely in plant species and exhibit a range of biological properties, both beneficial and deleterious. Saponins are group of natural products possessing the property of producing lather or foam when shaken with water. These are glycosides of high molecular weight. Saponins have been reported in soyabean, sword bean, jack bean

and ricebean. Toxic saponins cause nausea and vomiting. These toxins can be eliminated by soaking prior to cooking.

Method

Saponin content was determined by the modified method of Fenwick and Oakenfull (1981). Saponin was extracted for two hours in a reflux condenser containing pure acetone. Exhaustive re-extraction over heating mantle with methanol in the soxlet apparatus was done for two hours. The extract was weighed after allowing the methanol to evaporate. The saponin content was calculated as a percentage of the sample.

12. Test for Phytates

Phytate content was determined by the method of Wheeler and Ferrel (1971).

Reagents

1. 3% Trichloroacetic acid (TCA)
2. 3% sodium sulphate (Na_2SO_4) in 3% TCA
3. 1.5 N Sodium Hydroxide (NaOH)
4. 3.2 N Nitric acid (HNO_3)
5. FeCl_3 Solution(Dissolve 583mg FeCl_3 in 100 ml of 3% TCA)
6. 1.5 M Potassium thioicynate (KSCN)
7. Standard $\text{Fe}(\text{NO}_3)_3$ solution

Procedure

Samples were accurately weighed (5g) and transferred into 100 ml conical flasks. A total of 40-50 ml of 3% TCA was added and shaken vigorously for 45 minutes on a mechanical shaker. Centrifuged the suspension and 10 ml aliquot of the supernatant was transferred to a 40 ml conical centrifuge tube. 4 ml of FeCl_3 solution was added to the aliquot by blowing rapidly with the pipette. The content was heated in a boiling water bath for 45 minutes. If the supernatant was not clear after 30 minutes, 1-2 drops of 3% sodium sulphate (Na_2SO_4) in 3% TCA were added and then continued heating. Centrifuged (10-15 minutes) and the clear supernatant was decantant carefully. The precipitate was then washed twice by dispersing well in 20 to 25 ml 3% TCA heated in a boiling water bath for 5 to 10 minutes and centrifuged. Repeated washing with water. The precipitate was dispersed in a few ml of water and 3 ml of 1.5 N Sodium Hydroxide was added with mixing. Volume was brought to approximately 30 ml with water and heated in

boiling water for 30 minutes. Filtered hot(quantitatively) through a moderately retentive paper whatman 2. The precipitate was washed with 60-70 ml hot water and discarded the filtrate. Dissolved the precipitate from the paper with 40 ml hot 3.2 N HNO₃ into a 100 ml volumetric flasks. Paper was washed with several portions of water, collecting the washings in the same flasks. Cooled flasks and contents to room temperature and diluted to volume with water. A 5 ml of aliquot was transferred to another 100 ml volumetric flasks and diluted to approximately 70 ml. 20 ml of 1.5 M KCNS was added and volume was made up and colour was read immediately (within 1 minute) at 480 nm. A reagent black was run with each set of sample.

Standard

433mg Fe(NO₃)₃ was dissolved in 100ml distilled water in a volumetric flask. Diluted 2.5ml of this stock standard and volume was made up to 250 ml in a volumetric flask. Pipetted out 2.5, 5, 10, 15 and 20 ml of this working standard into a series of 100ml volumetric flasks and proceed from step 16.

Calculation

$$\text{Phytate (mg/100g sample)} = \frac{\text{ugFe} \times 15}{\text{Weight of sample (g)}} \times 100$$

13. Morphological and biochemical characterization of bacterial cultures

To perform a Gram stain, the technician applies bacteria to a slide then passes it over a flame to ensure the bacteria stay on the slide. Next, crystal violet dye is applied, which stains all of the bacteria purple. Iodine is then applied, which helps the dye bind to the peptidoglycan layer of the cell wall, and this is followed by acetone, which washes away the dye. The purple dye stays on gram-positive bacteria as a result of the strong bond between the bacteria and a thick peptidoglycan layer, but it washes away from gram-negative bacteria, which has a thin peptidoglycan layer. Lastly, a dye such as safranin is applied, which stains gram-negative bacteria pink. After this the propagated strains were further identified under optical microscope for morphological and biochemical characterization of bacterial cultures through gram staining reaction.

14. Tolerance to acidic pH values

Strains were grown in MRS broth at 37 °C overnight, 0.1 mL aliquots of each active cultures were adjusted to pH 3.0, 2.0 with 5 N Hcl and incubated at 37 °C for 3 hours. Samples were taken every hour for 3 hours and the viable number of bacteria was

enumerated by pour plate counts of all samples using 10-fold serial dilutions prepared in 0.1% peptone water. Simultaneously, bacterial growth was monitored by measuring absorbance with a spectrophotometer (Nova Spec II, Pharmacia) at 600 nm. All the experiments were replicated twice.

15. Bile Tolerance

Strains were grown in MRS broth at 37 °C overnight; saturated bile solution was prepared separately by dissolving powdered bile extract (Oxoid). Bile solution was then filter sterilized by 4 micron filter and was added to two of the cultures to achieve a final concentration of 0.3 % and the second culture with 0 % bile served as a control sample. The cultures were incubated at 37 °C for 3 hours and then every hour for 3 hour. Viable counts of *Lactobacillus* strains were determined by pour plate counts of all the samples using 10-fold serial dilutions prepared in 0.1% peptone water. Simultaneously bacterial growth was monitored by measuring absorbance with a spectrophotometer (Nova Spec II, Pharmacia) at 600 nm. All the experiments were replicated twice.

16. Antimicrobial assessment

The inhibitory effect of *Lactobacillus* strains on selected clinical reference strains was determined by the well-diffusion method. For the agar well diffusion assay, an overnight culture of the indicator strain (*Escherichia coli*, *Bacillus cereus*, *Salmonella enteritidis* and *Listeria monocytogenes*) was used to inoculate to (Brain Heart Infusion) BHI agar growth media at 37 °C (approximately 10^6 cells mL⁻¹ of indicator strains were overlaid onto BHI agar plates). Wells of 5mm diameter were cut into agar plates and 50 µL of *Lactobacillus* culture supernatant fluid that probably containing antibacterial activity was added to each well. Inhibitory zone of *Lactobacillus* were checked after 24 hour incubation at 37 °C.

17. Standard stock solution to estimate the accurate bacterial count

McFarland Equivalence Turbidity Standards have been used since the method was proposed by McFarland. Original McFarland Turbidity Standards were made of precipitates of barium sulphate (BaSO₄) prepared by adding barium chloride to sulphuric acid. By adjusting the volumes of these two reagents, standards of varying degrees of turbidity were prepared to represent different bacterial concentrations. For visual comparison, determination of bacterial density using McFarland Turbidity Standards uses a Whickerham Card which is a white card with contrasting black lines. When the distortion

of the black lines is equal in both, the Standard and the bacterial suspension, the turbidity matches and the approximate number of bacteria in the liquid suspension can be calculated.

18. Preparation of pure inoculum of probiotic strains prior to inoculation.

Overnight broth cultures of test isolates were centrifuged at 10,000 rpm for 15 min. The pellets were rinsed out thrice with 10 ml phosphate buffer saline (PBS) into sterilized universal bottles and kept as stock cultures in the refrigerator at $4 \pm 2^\circ\text{C}$. The total viable cells in the stock solution was then determined using serial dilution and pour plate methods.

19. Fermentation/Probiotification of Complementary food mixes by inoculation of bacterial cultures.

For fermentation, 100 g of the samples from each flour and mixture were introduced into 500 mL glass bottle and autoclaved at 121°C for 15 min. After cooling at room temperature in aseptic conditions, each flour was hydrated using sterile distilled water (1 : 3, w : v). The slurry obtained was inoculated with 3 mL of suspension of *Lb. plantarum* and *L. casei* to achieve a final concentration of 10^8 CFU/g, then homogenized aseptically with a sterile glass rod and incubated at 37°C for 72 h.

20. Bulk density

Bulk density was measured by the method suggested by Lewis, (1987)

A known weight of Food Multi Mix is placed into a measuring cylinder and tapping the cylinder for a fixed number of times. Resultant bulk volume was measured by using following formula. Bulk density (g/ml) = mass/bulk volume

21. Swelling capacity

Swelling capacity of FMM were also determined with the method suggested by Appiah *et al.* 2011. Approximately 1.0 g of sample was weighed and mixed with 10 ml distilled water in a centrifuged tube and heated in a hot water bath at $80 \pm \text{C}$ for 30 minutes while continuously shaking the tube. After heating, it was centrifuged at 1000 rpm for 15 minutes. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated using the formula

Swelling capacity (%) = Weight of paste (g) / Weight of dry flour (g).

22. Viscosity

Viscosity was measured by the modified method of Hallic and Kelly, (1959) by using Visco basic plus viscometer

Sample were reconstituted in water at different concentration (2, 4, 6 and 8 %), heated for 20 minutes on a boiling water bath. Cooled at ambient temperature and the viscosity was measured in Visco basic plus viscometer with 20, 30 and 60 varying appropriate spindles. Viscosity was measured in centipoises (cps).

23. Water holding capacity

Water absorption capacity of the flours was determined by the method of Sosulski *et al.* (1976).10ml of distilled water was added to 1.0 g of the sample in a tube and allowed to stand at ambient temperature ($30\pm 2^{\circ}\text{C}$) for 30 min, then centrifuged for 30 min at 3,000 rpm or $2000\times g$. Water absorption was examined as percent water bound per gram flour.

24. Fat holding capacity

The same procedure was repeated for oil absorption except that oil was used instead of water.

25. Dispersibility

Dispersibility was measured by placing 10 g of the sample in a 100-ml stoppered measuring cylinder, adding distilled water to reach a volume of 100 ml, stirring vigorously, and allowing it to settle for three hours. The volume of settled particles was subtracted from 100 and the difference reported as percentage dispersibility.

26. Texture analysis

Fifteen milliliters of each EWP printing mixture system (40°C) was transferred into small plastic jars (2.0 cm height and 3.0 cm diameter, flat bottom) and stored at 4°C overnight before measurements. The textural properties of the printing mixture systems were measured using a cylinder-measuring probe (P/0.5R) with a flat base of 12.7 mm diameter attached to a TA.TX2 texture analyzer (TA-XT plus, Stable Micro Systems, Ltd., Surrey, UK) at room temperature ($25 \pm 1^{\circ}\text{C}$). For Texture Profile Analysis (TPA), the sample was subject to two-cycle compression. A time of 5 s was allowed to elapse between the two compression cycles. The test settings were pre-test speed, 1 mm/s; test speed, 2 mm/s; post-test speed, 2 mm/s; target mode, distance; distance, 5 mm; time,

5 s; and trigger force, 0.049 N. The following parameters were quantified hardness, cohesiveness, springiness, and chewiness.

26. Estimation of Zinc, copper, manganese, potassium, magnesium, sodium, calcium, phosphorus.

Principle Products are digested with HNO₃ and H₂O₂ under pressure in a closed vessel heated by microwaves. Solution is diluted with H₂O. Zn, Mn, K, Mg and Na are determined by FAAS.

Apparatus (a) Atomic absorption spectrophotometer.—With air–acetylene burner or nitrous oxide–acetylene burner for flame (FAAS; see Table 999.10B) and a graphite furnace for electrothermal (GFAAS; see Table 999.10C) determinations, with appropriate background (nonatomic) correction.

(b) Hollow cathode or electrodeless discharge lamps.—For Pb, Cd, Zn, Cu, and Fe.

(c) Microwave oven.—Designed for laboratory use, e.g., MDS-2000, CEM Corp., PO Box 200, Matthews, NC 28106-2000, USA.

Microwave oven should be regularly checked for delivered power. If the measured effect does not agree with the specification, adjust the program: Fill a plastic beaker (polypropylene or Teflon) with 1.000 kg water (room temperature) and measure temperature (T_b). Place beaker in microwave oven and heat water at full power for 2 min. Take beaker out of oven, stir water, and measure temperature (T_a). The delivered power in watts: $P = 35 \times (T_a - T_b)$ (d) Teflon digestion vessels.—100 mL, withstanding a pressure of at least 1.4 MPa (e) Volumetric flasks.—25 and 1000 mL. (f) Funnels.—Glass or plastic. (g) Plastic bottles.—e.g., Polystyrene bottles with tightly fitting lids, 50–100 mL. (h) Drying oven.—Or equipment for freeze-drying. All glassware and plasticware should be carefully cleaned and rinsed, e.g., with HNO₃ or HCl, in order to avoid metal contamination.

Reagents should be of at least analytical reagent grade (p.a.), preferably ultrapure (suprapur) or equivalent. (a) Water.—Redistilled or deionized, ≥ 18 M Ω ·cm. (b) Nitric acid.—65% (w/w). (c) Nitric acid.—0.1M. Dilute 7 mL concentrated HNO₃, (d), with water to 1 L. (e) Nitric acid.—3M. Dilute 200 mL concentrated HNO₃, (f), with water to 1 L. (g) Hydrogen peroxide.—30% (w/w) (h) Zinc standard solution.—1 mg/. Dissolve 1.000 g Zn in 14 mL water + 7 mL nitric acid, (b), in 1 L volumetric flask. Dilute to volume with water. [Note: Commercially available standard solutions for AAS (e.g., BDH Chemicals Ltd., Poole, UK) may be used for all metal standard solutions.] (g) Copper standard solution.—

1 mg/mL. Dissolve 1.000 g Cu in 7 mL nitric acid, (b), in 1 L volumetric flask. Dilute to volume with water.

Working standard solutions.—(1) For flame analysis.—Dilute standard, (f)–(j), with 0.1M HNO₃, (c), to a range of standards that covers the concentration of the element to be determined. (2) For graphite furnace analysis.—Dilute standard solutions, (f)–(j), with 0.1M HNO₃, (c), to a range of standards that covers the linear range of the element to be determined. D. Procedures (a) Cleaning procedure.—(1) For glass and plastic ware.—Acid solution: 500 mL concentrated HNO₃, C (b), + 4500 mL deionized water, C(a). Wash first with water and detergent. Rinse with tap water, followed by deionized water, then with acid solution. Finally rinse 4–5 times with deionized water. (2) For Teflon digestion vessels.—Rinse with acetone, wash with deionized water, keep vessels covered with 0.1M HNO₃, C(c), for at least 30 min, rinse with deionized water, and let vessels dry. Use separate vessels for different applications, depending on the concentration of metals. If, however, the same digestion vessels are used for heavily contaminated products, e.g., sludge, it may be necessary to use a more severe cleaning procedure, e.g., heating vessels together with concentrated HNO₃.

If final result is based on fresh weight, weigh test portion before and after drying to obtain water content: $H_2O = \frac{W_f - W_d}{W_f} \times 100$ where H₂O, % = water content of the test portion (%); W_f = weight of the test portion (g); W_d = weight after drying (g). (d) Homogenization.—Homogenize products using non contaminating equipment. Check for leached metals if the apparatus consists of metal parts. (e) Digestion.—Weigh 0.2–0.5 g dry material into digestion vessel. If water-containing materials are used, maximum weight is restricted to 2 g, but dry matter content must never exceed 0.5 g. For example, if product has a water content of 50%, take a maximum of 1 g (= 0.5 g dry matter). If a product has a water content of 95%, take 2 g Remove digestion vessels from microwave oven and let cool thoroughly before opening them. Open vessel and rinse down lid and walls into container. Transfer solution to 25 mL volumetric flask and dilute to mark with deionized water. Then, transfer solution to plastic container. Treat blanks in the same way as tests. One blank should be included in every set. (f) Dilution.—If test solution needs to be further diluted (due to high metal concentrations), dilute with 3M HNO₃, C (d), in order to maintain same acid concentration prior to metal determination, (g). High acid concentration is environmentally undesirable and may depress the analytical signal. Reduce acid strength by diluting the test solution 1:2 with 0.1M nitric acid and standard solutions 1:2 with 3M nitric acid. The tests and standards are thereby brought to the same

acid concentration. Matching of acid concentrations is important when a calibration curve is used.

Atomic absorption spectrophotometry —Use of flame or graphite furnace technique is determined by the concentration of the metal to be determined. Flame technique should be used as far as possible, since this technique is less sensitive to interference than the GFAAS. The most appropriate wavelength, gas mixture/temperature program, and other instrumental parameters for each metal are found in the manual provided with the instrument. Always use background correction. Measurements must be within the linear range when the method of standard addition is used. A standard addition curve consists of at least 3 points, of which at least 2 are standards. The concentration of the highest standard should be 3–5 times the concentration in the test solution. The lower standard should have a concentration approximately half of the highest standard. A simplified version of the method of standard addition is to use a matrix-matched standard curve, which is applicable to products with the same matrix: The test and standard solutions are mixed and used to make a standard addition curve. This curve is then parallel transferred to origin and is used as the standard curve for the tests that followed and that have been diluted in the same proportions. The matrix-matched standard curve and the test solutions will thus have the same matrix concentration. On most modern instruments, this function is included in the software.

27. Estimation of free radical scavenging activity (RSA)

Free radical scavenging activity was measured by using DPPH method according to Vani *et al* (1997).

Reagents:

1. Methanol
2. 2.9 ml of DPPH solution (0.005 mM solution of 2,2 diphenyl-1-picryl-hydrazyl prepared in 99.5 % methanol)

Preparation of sample extract

Two grams of fresh sample was extracted with 20 ml of methanol (99.5%). The supernatant was filtered using whatman no.1 filter paper after centrifuging the suspension at 10,000 rpm for 15 minutes. Till analysis filtrated was stored at -20 ° C.

Procedure

100 µl of aliquot of sample extract was taken in a test tube and added 2.9 ml of DPPH solution (0.005 mM solution of 2,2 diphenyl-1-picryl-hydrazyl prepared in 99.5%

methanol) after added this solution vortex vigorously The test tube was incubated in dark for half an hour. The discolouration of DPPH methanolic solution was used as blank.

Molecular weight of DPPH = 394.33

1 M solution = 394.33g in 1000ml

1 Mm solution = 0.039 g in 100 ml methanol (stock solution)

From stock solution, 5 ml is taken and makeup volume up to 100ml by adding methanol. The concentration will be 0.05 mM)

Calculation

% inhibition = $(A_b - A_a) / A_b \times 100$

Where, A_b is absorbance of blank

A_a is the absorbance of sample

28. Determination of Phytochemical (Quantitative Method)

Determination of Alkaloid

The sample was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract.

Determination of Total flavonoid content:

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 mg of sample and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in

the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

Estimation for Saponin:

Total saponin determination was done using anisaldehyde reagent. Sample solution was prepared in water. Standard saponin solution, Weigh 10 mg of diosgenin, dissolve in 16 mL of methanol, and add 4 mL of distilled water. Standard solutions of diosgenin (20, 40, 60, 80 and 100 µg/ml) were prepared 80% aqueous methanol. Mix thoroughly and start pipetting immediately. For total saponins estimation 500 µg of sample, 500 µl of 0.5% anisealdehyde reagent, were mixed and kept aside for 10 min. Later, 2 ml of 50% sulphuric acid reagent was added and tubes were mixed. Tubes were then kept in water bath with constant temperature of 60°. After 10 min tubes were cooled and absorbance was taken at 435 nm. Same method for standard also. The amount of saponins was calculated as saponin equivalent from the calibration curve of standard.

Terpenoid determination:

Preparation of the reference solution: Linalool reference substance (10mg) was accurately weight, added in a 10ml volumetric flask, diluted with ethyl acetate to the marked line to afford a concentration of 1.0mg/ml standard solution.

Preparation of the test solution: The sample was precisely measured and placed in a 10ml volumetric flask, diluted with ethyl acetate to the marked line.

Chromogenic method: The color developing agent applied on this experiment was prepared by the procedure as follows, 5% vanillin-acetic acid solution plus 2mL of perchloric acid were heated at 65°C for 20min, then cooled in ice water and warmed up to room temperature after being shaken. Vanillin (500mg) was dissolved in acetic acid (10ml) to prepare the vanillin solution.

The standard curve 0.0,0.2,0.4,0.8,1.2,1.6,2.0 ml Linalool standard solution were precisely measured, placed in a 10 ml flask with ethyl acetate to volume marked line, The sample solution and standard mixture was then shaken, colored according to the chromogenic method. The absorbance (A) of each solution was measured at 210nm wavelength, a blank solution as the control reference.

Determination of Glycosides:

Glycosides of each generation of suspension culture were quantitatively determined according to Solich et al. by some modifications For determination of

glycosides, a 10% extract of seeds were mixed with 10 mL freshly prepared Baljet's reagent (95 mL of 1% picric acid + 5 mL of 10% NaOH). After an hour, the mixture was diluted with 20 mL distilled water and the absorbance was measured at 495 nm by UV/VIS spectrophotometer.

For preparation of the standard curve, 10 mL of different concentrations (12.5-100 mg/L) of securidaside were prepared. Total glycosides from were expressed as mg of securidaside per g of dried Sample.

Determination of total phenolic content

The concentration of phenolics in plant extracts was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). One millilitre of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na₂CO₃) solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) /Visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract.

Determination of tannin Content

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 % Na₂CO₃ solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Tannic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of Tannic acid /g of extract.

28. Phytochemicals screening test: (Qualitative)

Test for Tannins

10 ml of bromine water was added to the 0.5 g aqueous extract. Decoloration of bromine water showed the presence of tannins.

Test for Saponins

5.0 ml of distilled water was mixed with aqueous extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

Tests for Flavonoids

Alkaline Reagent Test. 2 ml of 2.0% NaOH mixture was mixed with aqueous extract; concentrated yellow color was produced, which became colorless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

Tests for Glycosides

Liebermann's Test. We added 2.0 ml of acetic acid and 2 ml of chloroform with whole aqueous extract. The mixture was then cooled and we added H₂SO₄ concentrated. Green color showed the entity of aglycone, steroidal part of glycosides.

Test for Terpenoids

2.0 ml of chloroform was added with the 5 ml aqueous extract and evaporated on the water bath and then boiled with 3 ml of H₂SO₄ concentrated. A grey color formed which showed the entity of terpenoids.

Test for Alkaloids:

Sample is dissolved in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Test for Phenols: 2 ml of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the sample extract. Formation of blue or green color indicates presence of phenols.

29. Antidiarrheal Efficacy of Probiotic Complementary food mixes in Castor Oil Induced Diarrheal experimental rats

Time taken before the first defecation was considered as the 'latent period'. Total numbers of fecal output as well as the diarrheic feces (muddy or watery feces) excreted by the experimental animal for a period of 4 h after the latent period was determined. During the observation period of 4 h, latent period (time interval between the administration of castor oil and the first defecation in a minute), total fecal output and fecal water content were recorded for an individual rats. Because only after castor oil induction the feces of rats were converted to muddy or watery than the normal state in

castor oil induced rats. Percentages of diarrheal inhibition, as well as the weight of total and wet fecal output was determined according to the formula follows:

$$\text{Inhibition \%} = \frac{\text{ATFPC} - \text{ATFT}}{\text{ATFNC}} \times 100$$

where ATFPC is average number of wet feces in the positive control group, ATFT is average number of total feces in the test group, and ATFNC is average number of wet feces in the positive negative group.

$$\text{Total fecal output \%} = \frac{\text{Mean fecal weight of each group}}{\text{Mean fecal weight of positive control}} \times 100$$

Fecal output and fecal water content in mice: After administration of castor oil, when the faces became unformed, muddy, or watery was considered to as diarrhea. All of the faces were collected after each defecation and put into a covered vessel for each animal to prevent the faces from drying. All the faces collected over a 4 h period were dried for about 1 h at 100°C in a ventilated oven. Fecal water content was determined according to the following formula: Fecal water content = fecal wet weight - fecal dry weight

30. Peroxide value of developed probiotic complementary food mixes

Peroxide value of the samples were determined by following AOAC (1975) method.

Chemicals: All the chemicals were of analytical grade and purchased from MERCK Ltd., Mumbai.

Reagents

1. Solvent mixer: Two volume of glacial acetic acid were mixed with one volume of chloroform
2. 5% potassium iodide solution
3. 1% starch solution
4. N/500 sodium thiosulphate solution: N/10 solution were prepared and diluted to N/500 on the day of use.

Procedure

One gram of sample was weighed in a clean dry boiling tube. One gram of powdered KI was added. 20 ml of solvent mixture was added. The tube was placed in

boiling water so that the liquid boiled within 30 seconds. The contents were transferred to a 20 ml conical flask and titrated against N/500 sodium thiosulphate until yellow colour almost disappeared. 0.5 ml starch solution was added and shaken vigorously and titrated till blue colour just disappeared. A blank should be set at the same time.

31. Free fatty acid of developed Food Multi Mixes.

Free fatty acid content of the samples were determined by following AOAC (1970).

Chemicals: All the chemicals were purchased from MERCK Ltd., Mumbai.

Reagents:

1. 1% phenolphthalein in 95% ethanol
2. 0.1 N KOH
3. Neutral solvent- Mixed 25ml ether in 25ml 95% alcohol and 1ml at 1% phenolphthalein solution and neutralized with N/100 alkali (0.1 N KOH)

Procedure:

Two grams of sample were dissolved in 50 ml of neutral solvent in a 250 ml conical flask. A few drops of Phenolphthalein were added and the contents were titrated against 0.1 N KOH solution. It is shaken constantly until a pink colour which persists for 15 seconds was obtained.

Calculation

$$\text{Free fatty acid (mg KOH/g)} = \frac{\text{Titre value} \times \text{Normality of KOH} \times 56.1}{\text{Weight of sample (g)}}$$

The free fatty acid is calculated as oleic acid using the equation.

$$1 \text{ ml N/10 KOH} = 0.0028 \text{ g oleic acid}$$

32. Microbial viability and determination of pH across storage.

Total Plate Count (TPC) was enumerated using the conventional method. One gramme of sample was dissolved in 10 mL of distilled water. A serial dilution of the solution was prepared by pipetting 1 mL of solution in 9 mL of distilled water in a test tube. One mililitre of each dilution prepared was transferred into their corresponding sterilized Petri dishes. Plate Count Agar was melted in a water bath and 15 mL poured into the Petri dishes. It was swirled to disperse medium and sample solution evenly. It

was then allowed to solidify and then incubated at 37°C for 24 h. Colonies formed were then counted using a colony counter (LunaGuzman & Barrett, 2000).

To verify the viability of microbial food cultures in CFM I and CFM II, cfu count through serial dilution experimentations using Miles and Misra (1938) method were done. The objective of the serial dilution method is to estimate the concentration (number of colonies, organisms, bacteria, or viruses) of an unknown sample by counting the number of colonies cultured from serial dilutions of the sample.

Colony forming unit (CFU or cfu) is a measure of viable bacterial or fungal cells. In direct microscopic counts (cell counting using haemocytometer) all cells, dead and living are counted, but CFU measures only viable cells. For convenience the results are given as CFU/ml (colony forming units per milliliter) for liquids, and CFU/g (colony-forming units per gram) for solids. The CFU/ml can be calculated using the formula:

$$\text{cfu/ml} = (\text{no. of colonies} \times \text{dilution factor}) / \text{volume of culture plate}$$

Determination of pH

pH is the measurement of the acidity or alkalinity of a product commonly measured in the scale of 0 to 14. pH 7 is considered neutral, with lower pH values being acidic and higher values being alkaline or caustic.

The pH of probioticated FMM II and IV was measured with digital pH meter (Eutech Instruments, Germany).

33. Standard operating procedure for total fungal count (yeast and Mould count) by Colony Count Technique at 25°C

Scope

This standard specifies the method for viable fungal count in products intended for human consumption or feeding of animals by means of the colony count technique at 25°C.

References: 5403:1999 reaffirmed 2005

Principle: Two poured Plates are prepared using a specified culture medium with specified quantity of sample (if liquid) or Initial suspension (If solid) & other pair of Plates prepared under same condition with decimal dilution of test sample incubated aerobically at 25°C for 3, 4 or 5 days.

Culture media and dilution fluid

0.1% Peptone salt solution – Himedia M1748 Sterilized by autoclaving at 121° C/15 lbs pressure

Initial Suspension: *Food Homogenate*

1gm of the test sample (Grind if required) added to 9ml of diluents – 10^{-1} (or)

25gm of the test sample (Grind if required) added to 225 ml of diluents – 10^{-1}

Non viscous liquid measure volumetrically 10 ml sample added to 90ml of diluents- 10^{-1}

Viscous liquid weigh the sample 10 ± 1 g sample in 90ml of diluents- 10^{-1}

Decimal Dilution: 1ml of initial dilution to the 9 ml diluents 10^{-2} , 1ml from 10^{-2} to 9ml diluents 10^{-3} , 10^{-4} , 10^{-5} accordingly

Culture Media [Chloramphenicol Yeast Glucose Agar]

Media Preparation: Himedia M1008 - pH 6.6 \pm 0.2

Suspend 4.0 grams in 100 ml distilled water. Heat to boiling to dissolve the medium completely

Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Cool in water bath at 44°C to 47°C before use, Mix well before pour into sterile Petri Plates.

Preparation of test sample

Food Homogenate prepared as per procedure 4.1.1. Mix using vortex mixture (5 to 10 sec) allow the particles to settle and then transfer

Procedure.

Inoculation

- Take two sterile Petri dishes transfer to each dish, by means of a sterile pipette 1 ml of the test sample from 10^{-2} dilution or desired
- Take two other sterile Petri dishes transfer to each dish, by changing the tip 1 ml of the test sample from the next dilution .
- Follow similar procedure until the required dilution to be Plated
- Pour about 15 ml of the Chloramphenicol Yeast Glucose Agar (4.2) at $45^{\circ} \pm 1^{\circ}\text{C}$ into each Petri dish; carefully mix the inoculum with the medium by rotating the Petri dishes.
- Allow the mixture to solidify by leaving the Petri dishes standing on a cool horizontal surface

(Note: The time elapsing between the preparation of the initial suspension / dilution and the product is moment the medium poured into the dishes shall not exceed 15 min.)

Incubation: Invert the prepared dishes and incubate at $25^{\circ}\text{C} + 0.01^{\circ}\text{C}$ for 3,4,5 Days Do not stack the dishes more than six high. Stacks of dishes should be separated from one another and from the walls and top of the incubator.

Interpretation: Count the colonies on each Plate after 3, 4 and 5 days of incubation. After 5 days, retain those Plates containing fewer than 150 colonies If over growth

observed count the number of colonies at 3rd & 4th day and mention the incubation time in report. It is advisable to examine the Plates at the end of three days for yeast colonies as they are likely to be overgrown by mould growth. If only yeast counts are required, add 0.25 percent of sterile sodium propionate solution to the Plate at the time of pouring to inhibit the growth of moulds.

Count the colonies using colony counter after required incubation: Use counts from Plates containing fewer than, 150 colonies.

Method of calculation

The number *N* of microorganisms present in the test sample per ml (liquid products) or per gram (other Products).

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2) \times (d)]}$$

Where

. $\sum C$ = the sum of the colonies counted on all the Plates; n_1 = the number of Plates counted in the first dilution; n_2 = the number of Plates counted in the second dilution d = the dilution from which the first counts were obtained (for example, 10⁻¹). Round the result obtained to two significant figures. The result shall be expressed as a number between 1.0 and 9.9 multiplied by 10^x, where x is the appropriate power of 10.

34. Mean, standard deviation, paired T-test and ANOVA followed in the study are mentioned as under:

Mean: It is the arithmetic average and was used to measure the type of the observation as a whole. Formula used for calculating Mean:

$$\text{Mean } (\bar{X}) = \frac{\sum X_i}{n}$$

Where, X_i = total score obtained by the trainee

n = frequency of variable

Standard deviation: To find out the extent of variability shown by the variables i.e., the dispersion of the variables around the mean, standard deviation was used. Formula used for calculating Standard Deviation is mentioned below.

$$\text{Standard deviation} = \sqrt{\frac{\sum_{i=1}^n (X_i - \bar{X})^2}{n}}$$

Where, X_i = Total scores obtained by respondent

\bar{X} = Mean of variable

n = Frequency of variable

Paired T-test: A paired T-test is a statistical test that is used to compare the means of two groups. It is often used in hypothesis testing to determine whether a process or treatment actually has an effect on the population of interest, or whether two groups are different from one another. Formula for calculating paired T-test is mentioned below:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$
$$DF = n_1 + n_2 - 2$$

ANOVA: The Analysis of Variance (ANOVA) test allows a comparison of more than two groups at the same time to determine whether a relationship exists between them.

APPENDIX – II

**DEPARTMENT OF FOOD SCIENCE AND NUTRITION
SCHOOL OF HOME SCIENCE
AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION
FOR WOMEN, COIMBATORE
SENSORY EVALUATION SHEET**

Name :

Date :

Product :

Time :

You are provided with number of sample/samples. Please evaluate these samples for acceptability and allot a score from the hedonic scale as below :

- Liked extremely : 9
- Liked very much : 8
- Liked moderately : 7
- Liked slightly : 6
- Neither like nor dislike : 5
- Dislike slightly : 4
- Dislike moderately : 3
- Dislike very much : 2
- Dislike extremely : 1

Product Code	Colour	Flavour	Consistency	Appearance	Taste	Overall acceptability

Remark :

Signature

STANDARD 9-POINT HEDONIC SCALE

Liked extremely	: 9
Liked very much	: 8
Liked moderately	: 7
Liked slightly	: 6
Neither like nor dislike	: 5
Dislike slightly	: 4
Dislike moderately	: 3
Dislike very much	: 2
Dislike extremely	: 1

(Peryam and Pilgrim, 1957)

INSTITUTIONAL HUMAN ETHICS COMMITTEE



Avinashilingam

Institute for Home Science and Higher Education for Women
(Deemed to be University under Category 'A' by MHRD, Estd. u/s 3
of UGC Act 1956) Re-accredited with 'A+' Grade by NAAC.
Recognised by UGC Under Section 12 B
Coimbatore-641 043, Tamil Nadu, India

Chairman

Dr. Sudha Ramalingam
Director-Research & Innovation,
Professor-Community Medicine,
PSG Institute of Medical Sciences
& Research, Coimbatore

Member Secretary

Dr.S.Uma Mageshwari
Professor and Head,
Department of Food Service
Management & Dietetics

Members

Mr. K.Arunmoli (Legal Expert)
Dr.Subhashini K. Sripathi
Dr.A.Saraswathy (Medical Officer)
Ms.D.Kavitha
Dr.A.R.Sudamani Ramasamy
Dr.G.Victoria Naomi
Dr. Judith Justin
Dr.Anitha Subash

3rd January, 2022

To
Ms. Manisha Sharma
Department of Food Science and Nutrition
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear Manisha Sharma

Ref: Your proposal No. IHEC/21-22/FSN/33 entitled
“Development and Sensory Evaluation of Probiotic
Complementary Food Mixed Based on Cereals and Legumes”
submitted for approval of IHEC.

The Institutional Human Ethics Committee of our University
hereby grants approval to your research proposal
No. IHEC/21-22/FSN/33 entitled “Development and Sensory
Evaluation of Probiotic Complementary Food Mixed Based on
Cereals and Legumes” submitted by you. The Approval number for
the same is AUW/IHEC/FSN-21-22/XPD-33.

We wish you all the best in your research endeavours.

Regards,

Dr. Uma Mageshwari
Dr.S.Uma Mageshwari
Member Secretary



APPENDIX III

IAEC



Avinashilingam Institute for Home Science and Higher Education for Women
 (Deemed to be University under Category 'A' by MHRD, Estd. u/s 3 of UGC Act, 1956)
 Re-accredited with 'A+' grade by NAAC, Recognised by UGC under Section 12 B
 Coimbatore – 641 043, Tamil Nadu, India

Certificate

This is to certify that the project entitled "Assessment of Nutritional and Functional Properties of probiotic complementary food mixes from locally available traditional foods" has been approved by the IAEC having IAEC approval No AIW:IAEC.2020:FSN:01

Authorized by	Name	Signature	Date
Chairman:	Dr. S. KOWSALYA		19/02/2020
Member Secretary:	Dr. R. NIRMALADEVI		19/02/2020
Main Nominee of CPCSEA:	Dr. C. GUNASEKARAN		19.02.2020



APPENDIX IV

Application Details	
APPLICATION NUMBER	202241007163
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	10/02/2022
APPLICANT NAME	AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN
TITLE OF INVENTION	A PROCESS OF PREPARATION OF PROBIOTIC COMPLEMENTARY FOOD MIX AND PRODUCT THEREOF
FIELD OF INVENTION	FOOD
E-MAIL (As Per Record)	intellpat@gmail.com
ADDITIONAL-EMAIL (As Per Record)	
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	10/02/2022
PUBLICATION DATE (U/S 11A)	04/03/2022



Office of the Controller General of Patents, Designs & Trade Marks
Department of Industrial Policy & Promotion,
Ministry of Commerce & Industry,
Government of India

सत्यमेव जयते



APPENDIX- V

OPERATIONAL DEFINATIONS

- **Complementary food mixes-** Complementary feeding is the process by which infant progresses from a diet composed of only breast milk or infant formula milk to a family diet consisting of wide varieties of food which is necessary to ensure that nutrient intakes continue to be adequate for healthy growth and development throughout childhood.
- **Crude protein-**A measure of the amount of protein in a feed determined as the amount of nitrogen multiplied by 6.25. The factor 6.25 is the average grams of protein that contains 1 gram of nitrogen. The word "crude" refers to the fact that not all nitrogen in most feed is exclusively in the form of protein.
- **Crude fibre-** Crude fiber is a measure of the quantity of indigestible cellulose, pentosans, lignin, and other components of this type in present foods.
- **Available carbohydrate-** Available carbohydrate has been defined as the sum of free sugars (glucose, fructose, galactose, sucrose, maltose, lactose, and oligosaccharides) and complex carbohydrates (dextrins, starch, and glycogen).
- **Wistar strain albino rats-** The Wistar rat is an outbred albino rat. This breed was developed at the Wistar Institute in 1906 for use in biological and medical research, and is notably the first rat developed to serve as a model organism at a time when laboratories primarily used the house mouse.
- **Food conversion efficiency-** Food conversion efficiency (FCE) is a ratio measuring the efficiency with which food given to the animal is converted into mass gained by the animal body. It is a ratio of inputs to outputs.
- **Probiotics:** Probiotics are 'live microorganisms which when administered in adequate amounts confer a health benefit on the host.
- **ICMR:** Indian Council of Medical Research (ICMR) along with the Department of Biotechnology (DBT) to formulate guidelines for regulation of probiotic products in the country. These guidelines define a set of parameters required for a product/strain to be termed as 'probiotic'. These include identification of the strain, *in vitro* screening for probiotic characteristics, animal studies to establish safety and *in vivo* animal and human studies to establish efficacy.



Avinashilingam Institute for Home Science and Higher Education for Women

(Deemed to be University under Category A by MHRD, Estd. u/s 3 of UGC Act 1956)

Re-accredited with A+ Grade by NAAC. Recognised by UGC Under Section 12 B

Coimbatore - 641 043, Tamil Nadu, India

Appendix L2

(Item No 5 of Check List) Details of Research Publications

S.No	Article	Journal	Other Vol/No/Page Year	Details No/	Published in UGC-CARE / Scopus Indexed/ Web of Science (*List of Journals in that category including the particular Journal to be attached)
1	Comparative Study on Different Drying Techniques for determining the Moisture Content of Cereals /Millets and Pulses	<i>The Indian Journal of Nutrition and Dietetics,</i>	Supplement - 2, January - March 2021. UGC CARE list-Sciences S. No. 392.		UGC CARE list-Sciences S. No. 392.
2	A review on: functional foods and its growing trend in India	<i>Journal of Xi'an University of Architecture & Technology</i>	Volume XIII, Issue 8, 574-584. Scopus Indexed. Issn No : 1006-7930		Scopus Indexed. Issn No : 1006-7930

*Proof of list of Journals from Internet to be attached along with copies of reprints.

Scholar : Manisha Sharma

Supervisor : S. Kambhampati
28/2/22

M. S. Jais
28/2/22
Checked By :
HoD/Dean

Comparative Study on Different Drying Techniques for Determining the Moisture Content of Cereals / Millets and Pulses

Manisha Sharma and Kowsalya, S.

(Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore-641 043, Tamil Nadu, India)

e-mail: 2509manisha@gmail.com

Abstract

Cereals/millets and pulses are an important parts of healthy and balanced diet and hence helps in bridging the gap of protein hunger malnutrition and micronutrient deficiencies. To increase the shelf life of perishable and non-perishable food items, drying is one of the primary and oldest processing techniques that the food grains are subjected to. The convection oven drying method is one of the commonly used methods for the estimation of moisture in marketing enterprises and industries but as the time consumption is quite higher in this process a replacement but quick and dependable technique is essential. One variety rice (Luit variety), one variety of millets and one variety of pulses (samples) were used for the study. Experiments with temperature varying from 30-60°C and varied time intervals (15 minutes) were conducted in a microwave oven and convection oven using the samples. The main parameters used for the study were temperature and time which was recorded after a time interval of 15 minutes¹. The results stated that the process of drying takes place at a falling rate period i.e. with the rise in temperature the moisture content decreases. At a very long process the drying rate, the curve slop becomes less sharp and slowly tends to be horizontal at longer period of time. The values are calculated when the moisture content of the samples becomes constant. Drying food grains for a longer time period have adverse effect in the nature of the samples along with it can result in income loss of marketing industries. Salient findings from the study can be that the alternative means for the convection oven drying can be microwave oven drying as it can be a favorable method for the estimation of moisture content in food grains.

Keywords: Drying, moisture content, convection oven drying, microwave oven drying

Introduction

The oldest and simplest process of preserving food is through drying until there is not enough moisture to support microbial activity. Drying is a process of removing water by flowing hot air to prohibit the growth of molds, bacteria and yeasts to grow from food. The processing, marketing and storage of cereals and legumes is affected by the moisture content. The energy input in drying is comparatively less than that of freezing or canning and also the space for storage is minimized compared with other techniques such as freezing or canning. Drying affects the nutrient content of the food in a negligible amount². The primary objectives of drying are as follows: ease of storage and transport, protection from contamination, increasing the shelf-life and making it attractive for the consumers.

The moisture content of cereals/ millets and pulses are affected by the physical, chemical, thermal and mechanical properties. The moisture content of cereals and legumes in the tropics are affected by the fluctuations in the relative humidity rather than the varying temperature. The quantity and shape of the grains are not directly affected by the moisture content but is an important aspect in harvesting, storage, processing and transport in the market³. The moisture content during storage of grains should be lower than the storage environment to avoid growth of molds, bacteria and yeasts leading to the

spoilage of grains making it inappropriate for utilization. During storage of grains the un-equal distribution of moisture is generally found hence to reduce the loss of food grains after post harvest operations moisture estimation is an important step. One of the commonly used methods for drying is the convection air oven, but as the requirement of time period is quite higher to estimate the moisture content. Although the moisture estimation can be done through different scientific instruments such as moisture meters (electric) but it has to be adjusted constantly with the convection oven drying values. Hence for marketing enterprises and industries a quick and alternative technique is a need of the hour. For a long time the potential applications in relation to microwave heating (MW) includes estimation of moisture content, microbial safety, reduction non-uniform heating and cooking.

The time, space and energy used in microwave oven is considerably lesser than the convection air oven drying. The functioning cost of convection air oven is quite lower compared to that of microwave oven drying, but due to the extensive potential of microwave oven the moisture content of food grains can be estimated at a short span of time at a reduced amount. The present research was conducted to study the comparison of moisture content from two different drying techniques *i.e.* convection air oven method and microwave drying.

Comparative Study on Different Drying Techniques for Determining the Moisture Content of Cereals / Millets and Pulses

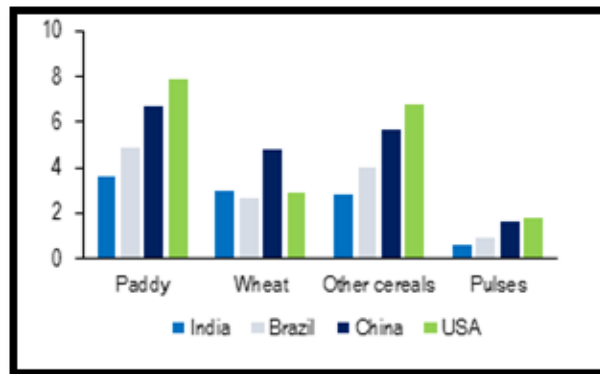


Figure 1
Grains production by different countries⁴

Materials and Methods

Procurement of raw materials

For carrying out the present study, fresh samples of rice (Luit variety), pearl millet and green gram were procured from local markets of Dibrugarh district of Assam and were selected for their

easy accessibility, availability and high therapeutic and nutraceutical properties.

Processing of raw materials

To conduct the experiment different levels of moisture (12, 14, 16, 18 and 20%) (wet basis) were taken according to previous studies done. To estimate

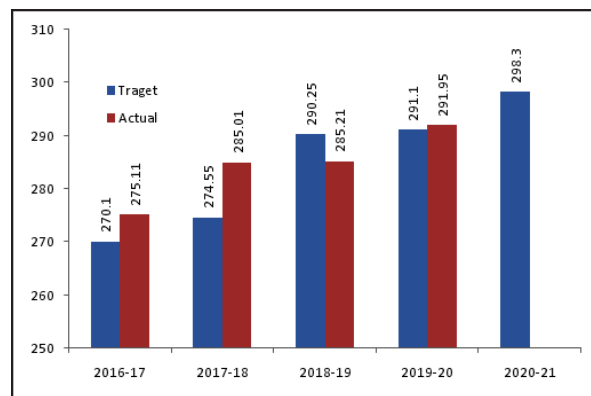


Figure 2
Food grains output⁵

the moisture content initially convection oven drying method was used. Based on the results, the samples were prepared by adding the amount of needed distilled water⁶. The samples were then stored in 4°C for a time span of 28 days into a glass container to achieve the equilibrium state. The glass containers were shaken for the uniform distribution of moisture all over the samples⁷.

Standard protocols of drying

Convection air oven drying

In convection air oven drying, the samples are kept in the oven and brought into touch with the hot air in the oven to ease the transfer of mass and heat. One of the chief feature of mass transfer are that the water get transferred to the bottom of the samples that are dried and transfer of water vapors from the surface of the samples. Electricity is the source of energy to warm the air in the convection air oven drying⁸.

Microwave Oven Drying

In microwave oven drying the samples are subjected to electromagnetic waves that are of very high frequency. The penetrating quality of microwave oven drying helps in the even drying of samples upon which the control on heating is easily done and can be made rapid if required along with it the radiation by liquid water is selectively absorbed⁹.

Packaging materials

High Density Polyethylene (HDPE) virgin containers were used for storing all the ingredients.

Determination of moisture content of cereals/ millets and pulses

The moisture content of cereals/ millets and pulses were estimated following A.O.A.C. (2000) method.

Procedure

Two grams of samples in triplicates were placed in a pre-dried and weighed aluminum dish spreading as thinly as possible over the base of the dish. In convection air oven and microwave oven, the samples were placed on the rotating shelf of glass, the samples were dried, cooled in a desiccator and weighed. The samples placed were then heated at 295 Watt, 552 Watt and 872 Watt for 4 min, 7 min and 22 min. Based on several studies conducted for the moisture determination of grains, the power of microwave along with the time intervals were selected^{2,6}. The moisture content of the samples was calculated by the weight loss after each microwave drying. Until a constant weight has not been achieved, the drying continues.

The moisture content of the samples was calculated by following method:

$$\text{Moisture (in grams) of 100 grams of samples} = \frac{\text{Difference in weight (in grams)}}{\text{weight of the samples (in grams)}} \times 100$$

Statistical analysis

The Statistical Package for Social Sciences (2006) was used to perform the Analysis of Variance (ANOVA) and at probability level of 5% the significance test was tested. The means were tested using Duncan's comparison tests.

Results and Discussion

Estimation of moisture

The moisture content of the rice sample initially was 3.23 ± 2.12 g/100 g, pearl millet was 9.5 ± 2.12 g/100 g and green gram was 8.25 ± 2.19 g/100 g. The estimated moisture content obtained after different temperature and time setup is shown in Table I. The convection air oven drying method was used as a standard protocol for determination of moisture content. The three temperatures and time setup in microwave oven drying was

not even. The results depicted at 550 W there was no statistical difference ($P \geq 0.05$) in the moisture content of convection air oven drying and microwave oven drying. At 870 W the estimated moisture content in microwave oven drying was significantly higher ($P \leq 0.05$) than the convection air oven drying. At 265 W the microwave (MW) drying showed the lowest moisture content in the samples compared to the other two MW drying temperature. Similar moisture values ($P \geq 0.05$) were shown for all the samples at 550 W by both microwave oven drying and convection air oven drying methods.

Moisture prediction

The rectilinear moisture content of convection air oven drying and microwave drying (w.b.%) of the samples were shown in Table II for the data obtained from

TABLE I
Mean Samples Values at Different Temperature and Time Obtained by Convection Air Oven Drying and Microwave Drying

Different moisture levels	Moisture content by convection air oven drying	Moisture content by microwave air oven drying		
		265 Watt	550 Watt	870 Watt
		20 Minutes	7 Minutes	4 Minutes
20%	19.96 ± 0.181	16.47 ± 0.202	21.97 ± 0.261	22.67 ± 0.603
18%	18.59 ± 0.661	15.66 ± 0.082	17.66 ± 0.111	20.60 ± 0.173
16%	17.27 ± 0.092	16.96 ± 0.202	15.87 ± 0.192	18.89 ± 0.221
14%	14.97 ± 0.261	12.87 ± 0.202	16.16 ± 0.201	15.97 ± 0.203
12%	$16.01 \pm 0.152^*$	$12.22 \pm 0.112^*$	$12.66 \pm 0.12a^*$	$16.82 \pm 0.101^*$

* Mean (\pm SD) different numbers within a row are significantly different ($P \geq 0.05$)

TABLE II

The Rectilinear Relationships of Microwave Oven Drying (X) and Convection Air Oven Drying (Y) of Moisture Content of Samples

MW oven power (in Watt)	265 W	550 W	870 W
MW oven time (in min)	20	7	4
Rectilinear equation	$Y = 1.12 + 1.01 X$	$Y = 0.25 + 1.00 X$	$Y = 3.25 + 0.98 X$
Adjusted value of r^2	0.40	0.95	0.36
F- value	19.19	250.62	10.10

* Y = Convection air oven drying moisture content (Standard)

X = MW oven drying moisture content (w.b.%)

** Probability level

apparent moisture content of microwave oven drying method and convection air oven drying method. As a result of heat generation a linear relationship was shown in all the three MW power of 265 W, 550 W and 870 W⁶. To avoid the uneven drying many numbers of trials and errors were done because of the non-uniform energy distribution during drying. As the samples have absorbed high energy leading to the rise in temperature the results were obtained in such manner. Several studies have revealed that as the temperature rises, moisture loss goes higher resulting in the overheating and roasting of samples. Hence significant number of researchers have been conducted for setting the desirable time and temperature to avoid the non-uniform distribution of heat⁹. Several studies have revealed that as conventional air oven drying is operated at a fixed temperature fluctuation in relative

humidity may have an important effect on the moisture determination of the grains, as drying through MW oven operates at a tolerable body temperature and it prevents the heat to distribute un-evenly to prevent roasting within the samples¹. A higher amount of energy was consumed by the samples because of the longer duration of drying time of samples along with low power of MW power¹⁰. The nature of the samples when heated for an ample amount of time period may change its characteristics because of the water being firmly enclosed in the seeds⁷. The physical and chemical nature of the grains with high moisture content changes when subjected to long period of MW drying at low MW power¹¹. From the physico-chemical parameters the moisture estimation is an important criteria required for the formulation and development of final product along with the shelf life.

Conclusion

To calculate the moisture content of varieties of grains MW oven can be used for reduction in time along with uniform heating. Temperature along with time combination in the MW drying can be used for the moisture estimation of the grains

rather than the time consuming convection air oven drying. From the study, it can be concluded that MW temperature and time at 7 minutes and 550 watt is highly significant ($P \leq 0.05$) than any other MW temperature and time combination to estimate the moisture content (12-20%).

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A REVIEW ON: FUNCTIONAL FOODS AND ITS GROWING TREND IN INDIA

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Abstract: In the 21st century, the new way of life adopted by coming generation had changed their basic food choices leading to expending more on packaged and processed food and also due to improper nutrition leading to number of metabolic diseases and lifestyle disorders such as diabetes, CVD, Cancer, atherosclerosis, High blood pressure etc. In the following review paper the terms that are discussed are “Traditional foods”, “Functional foods and nutraceuticals. Foods that have been eaten for many ages and are passed on through generations are termed as traditional foods. A food with specific benefitted affects on one or more functions of the body functions adding to the general nutritional properties resulting to a overall improvement in the health and reduction in the lifestyle disorders. In the following review article the concept, their use for promoting and improving public health of functional foods and nutraceuticals are discussed in a brief manner.

Key words: Traditional foods, nutraceuticals, functional foods.

Introduction

“To eat may be a necessity, but to eat intelligently is an art” these inspiring words of los angeles Rochefouald are a dictum today. Nutritionist, food technologists, scientists, manufacturers and most importantly customers have realized that the health benefits of the consumed foods is not onlyfor the specific functions of the body but also to improve the overall health and well being. Researchers have been done for understanding the health benefits of foods in the past few decades. Evidences have been found that foods and its ingredients can help heal, often unrecognized health benefits and can help reduce other non-communicable diseases such as cardio vascular disorders, obesity, diabetes, cancers etc. Efforts are being taken by researchers and scientist to understand the benefits of food and health (El Sohaimy, 2012). This efforts are driven by the escalated awareness of the relationship between food and optimal health among the consumers. These beliefs are well supported by scientific researches that increasingly shows that foods and their functional ingredients can help accord many overall and often unrecognized health benefits. In this context the terms that are usually discussed are “Functional foods” and “Nutraceuticals”. The article will provide brief insights of the definition, functional foods in India, components present in commonly consumed functional foods, latest findings and regulatory framework in Indian context.

Defining functional foods

Functional foods are broad term coined to describe substances which are derived from the food sources that provide extra health benefits. They don't fall into the legal category of food and drug and often inhabit a grey area between the two. Functional foods consist of both traditional and non-traditional foods. Traditional foods are simply whole foods with new information about their potential health quality. There has been no change to the actual foods, other than the way the consumer perceives them. Many of the cereals and legumes, vegetable and root tubers, fish, dairy and meat products contain several natural components that deliver benefit beyond the basic nutrition. Non- traditional functional foods resulted from agriculture breeding or added nutrients or ingredients. Agricultural scientists are able to boost the nutritional content of certain crop through the same breeding techniques that are used to bring out other beneficial traits in plants and animals. In recent years, the concept of foods specifically developed to scale back the danger of diseases was introduced, aiming at health promotion. Functional foods are considered those foods, fresh or processed, that are intended to be consumed as a part of the traditional diet and contain biologically active components which supply the potential of enhanced health or reduced risk of diseases. With the growing population, there is growing recognition of the potential role for functional foods in helping to reduce health risks and improve quality of health as well as life. Functional foods are food products that contains vital nutrients that transcend simply nurturing usual growth and development of a private . Fortified with nutritional and disease-preventing qualities, consumption of such food is with an intention towards improved wellbeing, prolonged existence and prevention of chronic diseases. In 2006, Food Safety and Standards Authority of India (FSSAI) defined functional foods, relevant in Indian context as a food which influences specific functions within the body which will provide added health benefits or remedy from some diseased condition following the addition/concentration of a beneficial ingredient, or removal/substitution of an ineffective or harmful ingredient. Functional foods are those food products which give essential nutrients needed permanently health and which potentially have a positive impact on human health besides providing the required nutritional requirements. Food is viewed as a product to reinforce health and wellbeing, and producers are responding proactively by supplying new goods that meet these needs (Shikha *et al.* 2014)

Functional foods in India

According to WHO, 2016 India's population is 132 crores and predominantly young; As per India's Census 2011, Youth (15-24 years) in India constitutes one-fifth (19.1%) of India's total population. India is expected to have 34.33% share of youth in total population by 2020. As younger generation moves toward middle age and income increases, the need to maintain and/or establish a healthy diet drives functional food consumption increasingly higher with its strong tradition of healthy eating, India ranks among the top ten nations in buying functional foods

(Watson, 2006). The functional food industry in India is strong and growing with aims of becoming a major force in the international health foods market (Japan Development Institute, 2006).

Nine out of ten urban Indian consumers have been reported to generally choose foods based on health and wellness benefits (Ciocca, 2003). The government is active in the development of the functional foods industry. Realization of functional properties of Indian traditional foods eventually lead to development of one of the world's oldest medicinal system, the Ayurveda (Sarkar *et al.*,2015).

Researchers have proved that consumer goods giants in India understand their consumer targets well and are successfully positioned in both mass-market and higher value-products (Japan Development Institute, 2006). In India, many suffer from deficiencies of iron, iodine and Vitamin A. To deal with these deficiency conditions, fortified foods viz. wheat flour, iodized salt, calcium, vitamin-enriched jams and soft drinks are included as the part of daily diet of the urban population. National Iodine Deficiency Disorders Control Programme (NIDDCP) formerly known as National Goitre Control Programme (NGCP) is being implemented from 1962 (NHRM, 2008). The Central Council of Health and Family Welfare in 1984 decided to implement compulsory iodisation of Salt for human consumption in the entire country. The Programme started in a phased manner with effect from 1st April, 1986. For the high value market, companies have launched products such as low-sodium salt, catering to blood pressure patients (Nutraingredients.com).

Cereals and millets

The most important of macronutrients and source of energy are cereals and millets. Cereals and millets are an important source of energy, carbohydrate, protein and fibre, as well as containing a range of micronutrients such as vitamin E, some of the B vitamins, magnesium and zinc. A range of bioactive substances are present in cereals and millets and there is growing interest in the potential health benefits these substances may provide. There is evidence to suggest that regular consumption of cereals and millets, specifically whole grains have a role in the prevention of chronic diseases such as coronary heart disease, diabetes and cancer. The exact mechanisms by which cereals and millets convey beneficial effects on health are not clear. Most likely a number of factors may be involved in conveying the beneficial effects of cereals and millets in Health, e.g. their micronutrient content, their fibre content and/or their glycaemic index. The two important cereals that are widely consumed in India are rice and wheat, although others like *jowar* (sorghum), *bajra* (pearl millet) and *ragi* (finger millets) and other minor millets are used as staples or substitutes in many regions.

Pulses and legumes

An important part of the traditional Indian food systems include pulses and legumes. Pulses provide energy and protein besides micronutrients, dietary fiber, and many vitamins and minerals. They also contain “phytochemicals” (plant chemicals), which may reduce the risk of certain types of cancer and other diseases. Pulses include chickpeas (also known as garbanzo

beans), lentils and dry peas, black gram, green gram, green peas and *rajmah* (Kidney bean), soy beans find important place in Indian food system alongside Bengal gram and red gram. The main mechanism by which Pulses and legumes moderate the glycemic response is due to the nature of the starch in legumes which is encapsulated and is higher in amylose than grains. This means it is less likely to be fully gelatinised during cooking which reduces the rate of starch digestion and therefore the glycemic response. It has also been proposed the protein in pulses and legumes stimulates insulin secretion, facilitating a more rapid extraction of glucose from the bloodstream into cells compared to other carbohydrate foods. Pulses and legumes particularly chickpea, grass pea, beans and peas also reduces the risk of diabetes through the second-meal effect. The second meal effect is the ability of legumes to lower both postprandial glycemia after the meal at which they are consumed and also at a subsequent meal later in the day or even on the following day.

Fruits and vegetables

Fruits and vegetables play an important role in human nutrition and health, particularly as sources of vitamin C, thiamine, niacin, pyridoxine, folic acid, minerals and dietary fibre (Wargovich, 2000). Consumption of low fruit and vegetable intake constitute a risk factor for chronic diseases such as cancer, coronary heart disease (CHD), stroke and cataract formation (Van Duyn & Pivonka, 2000). Scientific evidence indicates that frequent consumption of fruits and vegetables can prevent non communicable diseases such as diabetes, obesity, cardio vascular disorders and cancers such as oesophageal, stomach, pancreatic, bladder and cervical cancers. Some components of fruits and vegetables (phytochemicals) are strong antioxidants and modify the metabolic activation and detoxification/disposition of carcinogens and may even influence processes that may change the course of the tumor cell (Wargovich, 2000). Although antioxidant capacity varies greatly among fruits and vegetables (Kalt, 2002), it is better to consume a variety of them rather than limiting consumption to a few with the highest antioxidant capacity.

Spices

Spices and aromatic herbs are one of the major factors that set the Indian cuisine apart from rest of the World which not only enhances the taste but also the aroma of food. Spices and herbs have been used as medicines traditionally as well as flavor enhancers, preservatives, colorants. A number of bio-actives are being added to food through spices that render food functional. In a variety of experimental studies on both humans and animals, it was found that some of the commonly consumed spices and herbs have a hypocholesteromic action (Srinivasan et., 2004). Fenugreek, turmeric, or its active principle curcumin, onion or its active principle allyl propyl disulfide, garlic, and cumin were observed to improve glycemic status in diabetic animals noninsulin dependent diabetes mellitus (NIDDM) patients. Some of the commonly consumed spices were naturally evaluated for a possible hypocholesterolemic action in a variety of experimental situations in both animals and humans(Srinivasan et al., 2004). The spices fenugreek, red pepper, turmeric, garlic, onion and ginger were found to be effective as hypocholesterolemic agents under various conditions of experimentally induced hypercholesterolemia/hyperlipemia. Further, fenugreek, onion, and garlic are effective in

humans with hyperlipemic condition. Curcumin and capsaicin, the active principles of turmeric and red pepper, respectively, are also efficacious at doses comparable to calculated human daily intake.

Prebiotics and probiotics

The interaction between the host and the healthy bacteria have been indisputable established in the intestine. The healthy bacteria have been termed as probiotics that are live microbial food ingredients that provides benefits to the host. The microbes interact with the intestinal components of the host body to provide a wide range of beneficial biological effects (Roberfroid,2000). Probiotics include food such as yoghurts, curds and kefir. The importance of probiotics has been identified by the medical/ pharmaceutical industry and several strains of *lactobacilli* and *bifidobacteria* are currently available for treating gastrointestinal infections (Salminen *et al.*, 2005). Many strains of probiotics including *L. acidophilus LA-1*, *L. paracasei*, *B. lactis Bb-12*, and *L. Casei Shirota*, are currently available for commercial utility (Nagpal *et al.*, 2012).

Prebiotics are short-chain carbohydrates (SCCs) that are non-digestible by digestive enzymes in humans and that have been called resistant SCCs (Quigley, Hudson, & Englyst, 1999).

They are sometimes referred to as non-digestible oligosaccharides (NDOs) which are soluble in 80% ethanol. Prebiotic is a non-active food constituent that shifts to the colon and is then selectively fermented.

Examples of some commonly consumed functional foods and its functional components (International food information council foundation, July 2011)

Sl. no.	Class/ Components	Source	Potential benefits
1	Beta- Carotene	Carrots, pumpkin, sweet potatoes, cantaloupe, spinach, tomatoes	It neutralizes free radicals which may damage cells; bolsters cellular antioxidant defenses; can be made into vitamin A in the body
2	Lutein	Kale, collards, spinach, corn, eggs, citrus fruits, asparagus, carrots, broccoli	Supports maintenance of eye health
3	Lycopene	Tomatoes and processed tomato products, watermelon, red/pink grapefruit	Supports maintenance of prostate health
4	Insoluble fiber	Wheat bran, corn bran, fruit skins	Supports maintenance of digestive health; may reduce the risk of some types of cancer
5	Beta glucan	Oat bran, oatmeal, oat	May reduce risk of

Sl. no.	Class/ Components	Source	Potential benefits
		flour, barley, rye	coronary heart disease (CHD)
6	Soluble fiber	Psyllium seed husk, peas, pulses, apples, citrusfruits	May reduce risk of CHD and some types of cancer
7	Whole grains	Cereal grains like sorghum, barley, wheat and rice, whole wheat bread, oatmeal, brown rice	May reduce risk of CHD and some types of cancers; supports maintenance of healthy blood glucose levels
8	Monounsaturated fatty acids(MUFAs)	Tree nuts, olive oil,canola oil	May reduce risk of CHD
9	Polyunsaturated fatty acids (PUFAs) Omega-3 fatty acids-ALA	Walnuts, flaxseeds, flaxseed oil	Supports maintenance of heart and eye health; supports maintenance of mental function
10	Anthocyanins	Berries, cherries, red grapes	Bolster cellular antioxidant defenses; supports maintenance of healthy brain function
11	Selenium	Fish, red meat, whole grains, garlic, liver, eggs	Neutralizes free radicals which may damage cells; supports maintenance of immune and prostate health
12	Magnesium	Spinach, pumpkin seeds, whole grain breads and cereals, halibut, almonds, brazil nuts, beans	Supports maintenance of normal muscle and nerve function, immune health and bone health
13	Potassium	Potatoes, low-fat dairy products, whole grain breads and cereals, citrus juices, beans, banana, leafy greens	May reduce the risk of high blood pressure and stroke, in combination with a low sodium diet
14	Caffeic acid	Apples, pears, citrus fruits, some vegetables, whole grains, coffee	Bolsters cellular antioxidant defenses; supports maintenance of eye and heart health
15	Lignans	Flax seeds, rye, some vegetables, seeds and nuts, lentils, triticale, broccoli, cauliflower, carrot	Support maintenance of heart and immune health
16	Vitamin A	Organ meats, milk, eggs,carrots, sweet potato,spinach	Supports maintenance of eye, immune and bone

Sl. no.	Class/ Components	Source	Potential benefits
			health; contributes to cell integrity
17	Pantothenic acid (Vitamin B5)	Sweet potato, organ meats, lobster, soybeans, lentils and certain fortified breakfast cereals	Helps regulate metabolism and hormone synthesis
18	Pyridoxine (Vitamin B6)	Beans, nuts, legumes, fish, meat, whole grains and certain fortified breakfast cereals	Supports maintenance of immune health; helps regulate metabolism
19	Folate or folic acid (Vitamin B9)	Beans, legumes, citrus fruits, green leafy vegetables and fortified breads, cereals, pasta, rice	May reduce a woman's risk of having a child with a brain or spinal cord defect; supports maintenance of immune health
20	B12 (Cobalamin)	Eggs, meat, poultry, milk and certain fortified breakfast cereals	Supports maintenance of mental function; helps regulate metabolism and supports blood cell formation

Some examples of components present in Functional foods

Turmeric

The active ingredient present in turmeric is curcumin. Curcumin exhibits anti-cancer, antioxidant, anti-diabetic, and anti-inflammatory activities and neuro-protective properties. Curcumin has been demonstrated to improve cardiovascular, reproductive and gastrointestinal health (Aggarwal and Harikumar, 2009; Noorafshan and Ashkani-Esfahani, 2013). Curcumin has been reported to delay the onset and progression of cataract in experimental studies (Suryanarayana *et al.*, 2003; 2005). It has been effectively used for stimulant or general tonic, food preservative, diuretic, blood purifier as well as for cold, pain, sinusitis, cough and liver and intestinal disorders in India (Krishnaswamy, 2009).

Ginger

Zingiber officinale, commonly known as ginger, is a spice consumed worldwide for culinary and medicinal purposes. Ginger is used for therapeutic purposes in many countries in Middle East, Asia and Europe and there are researches proving that ginger may exert advantageous effects against nauseating, discomforts, platelet aggregation & cardiovascular diseases, dyslipidemia, inflammation, oxidative stress and hypertension (Singletary, 2010). The plant has a number of chemicals responsible for its medicinal properties, such as antiarthritis, antiinflammatory, antidiabetic, antibacterial, antifungal, anticancer, etc. (Patrick J., 2018)

Fenugreek

In the traditional food system both the seeds as well as leaves of Fenugreek plant are used. Fenugreek seed which is a common spice in every household is known to exert large spectrum of therapeutic effects such as gastro-protectant, hepato-protectant, and antidiabetic effects in addition to exhibiting antioxidant activity and hypocholesterolemic activity (Srinivasan, 2006; Meghwal and Goswami, 2012). The antidiabetic effects of fenugreek are attributable to components such as 4-hydroxyleucine (potentiator of insulin secretion), disogenin (α-amylase and α-glucosidase inhibitor), and the fibre fraction comprising galactomannan (Smith 2003, Ghosh *et al.*, 2014; Fuller and Stephens, 2015). Most encouraging aspect of utility of fenugreek seeds as an anti-diabetic agent is its established efficacy in clinical studies (Sharma *et al.*, 1990; Sharma and Raghuram, 1990; Raghuram *et al.*, 1994).

Tomato

Tomato is the fourth most heavily consumed vegetable/fruit in the world. A healthy dose of carotenoids such as lycopene and pro-vitamin A and Vitamins E and C are present abundantly (Canene-Adams *et al.*, 2005). The functional effects of tomatoes are the carotenoids content. A case controlled study by Gann *et al.*, (1999) stated that consumption of tomato products and other lycopene containing foods might reduce the occurrence or progression of prostate cancer. High levels of lycopene has been linked to reduced risk of cardiovascular diseases (Canene-Adams *et al.*, 2005). Researches have proven since ages that tomatoes or its constituents may exert neuroprotective, antioxidant and anti-diabetic effects (Ali and Agha, 2009; di Matteo *et al.*, 2009; Gokul and Muralidhara, 2014; Hsiao *et al.* 2004; Lavelli *et al.*, 1999; Owwoeye and Onwuka, 2015).

Cinnamon

A colloquially term for the inner bark of trees *Cinnamomum zeylanicum* and *Cinnamomum cassia*, is a spice used world over. Evidences are present that states cinnamon is useful in control of hypertension along with glycaemia. It has also been reported to exert antioxidant, anti-microbial, anti-inflammatory, neuroprotective and hepato protective effects. Cinnamtannin B1 is believed to be a major molecule responsible for anti-diabetic effect of cinnamon (Ranasinghe and Galappaththy, 2016).

Garlic

Garlic is used as spice, herb and a vegetable whose biological properties are attributable to low molecular weight sulphur-compounds. Active principles of garlic are demonstrated to confer a wide array of therapeutic effects including anti-microbial, anti-cancer, anti-diabetic, anti-inflammatory, and antioxidant activities as well as ability to improve cardiovascular health (Rivlin, 2006; Milner, 2010; Bayan *et al.*, 2014).

Regulations Concerning Functional Food: Legislations on Functional Foods

In 1980, Japan's Ministry of Health and Welfare initiated a regulatory system, Foods for Specified Health Use (FOSHU) for development and commercialization of the concept of functional foods that could legitimately be labelled and categorised as possessing specific health promoting or disease preventing properties. In 1991, Functional food was given a formal legislative food category as "*Foods for Specified Health Uses (FOSHU)*". Safety in clinical and non-clinical studies and determination of active components in any food or ingredient are strict requirements as per law for food to be called Functional. To obtain a FOSHU label, an application containing scientific evidence supporting the proposed medical or nutritional link, the suggested dose of the functional food, safety and descriptions of the food's physical and chemical qualities, experimental methods, and composition must be filed by the producer. The process of taking approval typically requires a year and is subject to review by the Ministry of Health and Welfare (MHW) and local authorities. The completed FOSHU label contains: "the approved health claim; recommended daily intake of the food; nutrition information; guidance on healthy eating; a warning against excessive intake, if necessary; any other special precautions relating to intake, preparation or storage; and other information (Martirosyan and Singh, 2015).

Conclusion

In the 21st century the new lifestyle adopted by people has changed their basic food leading to consumption of more processed foods which leads to a number of lifestyle disorders and onset of metabolic diseases due to improper nutrition. In recent years, a new diet and health paradigm is evolving which places more emphasis on positive aspects of diet focusing more emphasis and demand for foods with additional health enhancing properties. In developing country like India with the increasing urbanization, technological, industrial and economic advances, the demand for functional foods are also increasing rapidly. It is not necessary that all traditional foods are functional foods. But it can be said that the common denominator for dietary supplements, nutraceuticals and functional foods is that they all are products designed to supplement the human diet by increasing the intake of bioactive agents that are thought to enhance health and well being. However, each term describes a different product category. Adequate scientific evidence and rational use of available scientific knowledge should form the basis for their choice, use and promotion for public health.

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Sharma, M., Das, M., & Alam, S. (2018). Development of functional flour using malted cereals and legumes.

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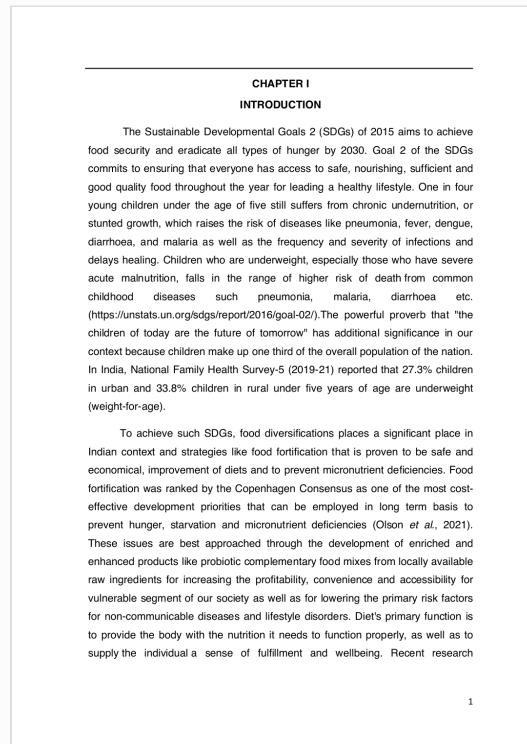


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