APPENDICES

APPENDIX – I

ETHICAL CLEARANCE CERTIFICATE

INSTITUTIONAL HUMAN ETHICS COMMITTEE

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Institute for Home Science and Higher Education for Women
University
(Estd. u/s 3 of UGC Act 1956)

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26th June 2015

To
Ms. Chingtiye Raiburg,
Department of Food Service Management and Dietetics
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear Madam,

Ref : Our letter dt. 21st February 2014 in response to your proposal No. AUW/IHEC/2013/22 entitled "Efficacy of selected prebiotic [illegible] foods in the management of hyperlipidemia"

With reference to the above letter, in continuation with the documents submitted by you in support of your proposal, as per the suggestions made by the IHEC, the Institutional Human Ethics Committee of our University hereby grants approval to your research proposal No.AUW/IHEC/2013/22 entitled "Efficacy of selected prebiotic [illegible] foods in the management of hyperlipidemia". The Approval number for the same is AUW/IHEC-13-14/TUD/22.

We wish you all the best in your research endeavours.

Regards,

Dr.P.R.Padma
Member Secretary

Institutional Human Ethics Committee

APPENDIX II

INTERVIEW SCHEDULE TO ASSESS KNOWLEDGE, AWARENESS AND PRACTICE OF PREBIOTIC FOODS

BACKGROUND DETAILS

1. Age _____(years)

❑<20 ❑ 20-29 ❑ 30-39 ❑ 40-49 ❑ 50-59 ❑>60

2. Gender

❑ Male ❑ Female

3. Occupation:

❑ Education ❑ Healthcare/medical ❑ Financial/banking ❑ IT/computer

❑Govt employee ❑ Housewife ❑ Student ❑ Self-employed ❑Others: _____

4. Highest educational level:

❑ Primary ❑ Secondary ❑ Diploma ❑ Degree ❑ Post-graduate

❑ Others: _____

5. Income level

❑>Rs. 5000 per month ❑Rs. 5000-10,000 per month ❑>Rs.10000 per month

SECTION I

1) Have you heard of the term prebiotic foods?

❑ Yes ❑ No

2) If Yes, from where did you know about prebiotic? *(You may select more than 1 response)*

❑ Internet ❑ Product brochure ❑ Magazines ❑ Newspapers ❑ TV/radio

❑ Doctors/Dietetian❑ Public talks ❑ Friends / colleagues

❑ Others: _____

3) According to you, Prebiotics are_____ *(Select the best possible answer only)*

❑Foods that promote health

❑Foods that stimulate the activity of the colon

❑Foods that are similar to probiotics

❑ Non-digestible food ingredients that stimulate the growth of bacteria in the colon and promote health

❑ Unsure / do not know

4) What is your idea about the usefulness of prebiotic foods? Prebiotics are used for conditions like_____: *(You may select more than 1 response)*

❑ Diabetes ❑Overweight/ Obesity ❑Heart health❑ to gain weight

❑those engaged in sports❑ for everyone❑ unsure / do not know

5) Have you consumed any prebiotic food product before?

❑ Yes ❑ No

SECTION II

1)Do you feel that prebiotics have a role in health

❑ Yes ❑ No

2) If you are aware about the health benefits, do you think you will purchase prebiotic foods

❑ Yes ❑ No

APPENDIX III

24 Hr. Recall Form

Meal timing	Menu	Ingredients	Quantity (ml/gm)
Early Morning			
Breakfast			
Mid-morning			
Lunch			
Evening tea			
Dinner			
Bed-time			

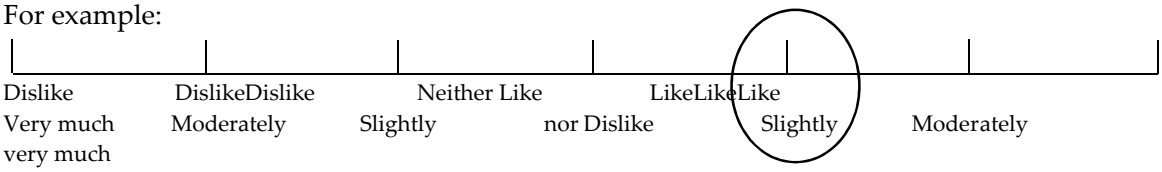
APPENDIX IV

Sensory Evaluation of _____(Name of the Product)

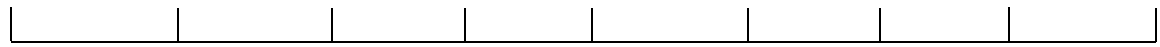
Hedonic Test

Panelist # :_____ Date: _____

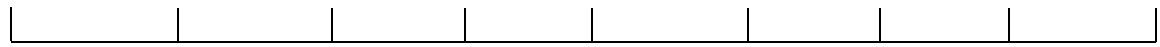
Instructions: Rinse your mouth and wait for 30 seconds before evaluating the sample.
Circle at the scale applicable to your liking.
For example:



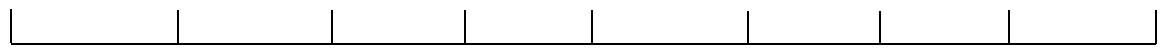
1. APPEARANCE –



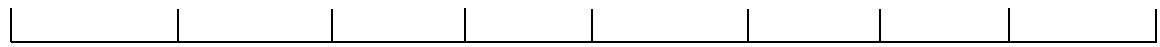
2. AROMA —



3. TASTE —



4. TEXTURE —



5. OVERALL ACCEPTABILITY —

APPENDIX V

PROCEDURE USED TO ANALYSE NUTRIENT CONTENT OF THE BISCUITS

ESTIMATION OF CARBOHYDRATE BY ANTHRONE METHOD

Aim

To estimate the carbohydrate content of the given food sample

Principle

Carbohydrate are first hydrolysed in to simple sugar using dilute HCl. In hot acidic medium, glucose is dehydrated for hydroxy methyl furfural. This compound forms with anthrone green coloured product with an absorption maximum at 630 nm.

Materials

- 2.5 N – Hydrochloric acid
- Anthrone reagent : Dissolve 200 mg anthrone in 100ml of ice cold 95% H_2SO_4 prepare fresh before use
- Standard glucose stock: Dissolve 100 mg of glucose in 100ml water
- Working standard: 10 ml of stock diluted to 100 ml with distilled water, store refrigerated after adding a few drops of toluene.

Procedure

- Weigh 100 mg of the sample into a boiling tube
- Hydrolyse by keeping it in a boiling water bath for 3 hours with 5 ml of 2.5N HCl and cool to room temperature
- Neutralise it with solid sodium carbonate until the effervescence ceases.
- Make the volume to 100 ml and centrifuge
- Collect the supernatant and take 0.5 and 1 ml aliquots for analysis

- Prepare the standards by taking 0.2, 0.4, 0.6, 0.8 and 1ml of working standards and '0' as blank.
- Make the volume of 1 ml in all the tubes including the sample tubes by adding distilled water.
- Then add 4 ml of anthrone reagent
- Heat for 8 minutes in a boiling water bath
- Cool rapidly and read the green to dark green colour at 630nm
- Draw a standard graph by plotting concentration of the standard on X-axis Vs absorption on Y – axis
- From the graph calculate the amount of carbohydrate present in the sample.

DETERMINATION OF PROTEIN BY KJELDHAL METHOD

Principle: Sample is digested with boiling sulphuric acid; the nitrogen of sample is transformed to ammonium sulphate. The acid digest is strongly alkaline using sodium hydroxide (NaOH). The ammonia released is distilled into boric acid solution; it is then titrated with standard sulphuric acid. The results are transformed by calculation into a percentage of protein in original sample.

Apparatus: Micro kjeldahl digestion flask, Micro kjeldahl distillation apparatus, Erlenmeyer flask (250 ml), volumetric flask (250ml), Pipette, Burette (50ml)

Reagents: Standard sulphuric acid (0.1 N), Standard sodium hydroxide solution (0.1N), Mixed indicator – (a part of 0.2% methylred and 2 parts of 0.2% bromocresol green), Digestion mixture – (prepared by mixing 10 parts potassium sulphate, 0.3 parts copper sulphate and 0.02 parts selenium powder), 4% boric acid solution, 40% sodium hydroxide solution

Procedure:

1. Weigh accurately 0.5g of the sample on a butter paper and transfer in a clean and dry kjeldahl flask.
2. Add to the sample in the flask 2g of the digestion mixture and about 25ml of the concentrated sulphuric acid.
3. Shake the contents of the flask thoroughly.
4. Heat inside the hood on a low flame and then slowly raise the flame.
5. Continue to digest till the solution becomes clear and acquires a greyish blue or greenish color, and no carbon particles are seen adhering to the side of the flask (usually takes about 2 to 3 hours).
6. Cool and add slowly with intermittent shaking about 50 – 70ml of water. Cool again.
7. Transfer in 100ml volumetric flask and wash the kjeldahl 3-4 times with 1-2ml water and collect in volumetric flask, make volume with washings.

Distillation:

a) Set the ammonia distillation apparatus. Transfer 10ml digested solution in flask. Add 50ml of distilled water.

b) Carefully pour about 10ml of the concentrated sodium hydroxide by the side of the flask so that it does not mix at once with acid solution but forms a layer below.

c) Pipette 25ml of 4% boric acid solution in receiver flask; add two drops of mixed indicator.

d) Connect the flask to the bulb tube and see that the dip tube dips into the acid in the receiver flask.

e) Light the burner and regulate to a low flame. Increase the flame.

f) Continue distillation for about 45 minutes or at least 50ml distillate is collected in receiver flask. At the end, without putting out the burner, disconnect the dip

tube from the condenser. Rinse it with distilled water into the receiver flask and titrate the boric acid solution against standard acid.

Observation :

Volume of 0.01 NH_2SO_4 for the sample = A

Volume of 0.01 NH_2SO_4 for the blank = B

Weight of sample = W (g)

Volume of made (V) = 250 ml.

Aliquot distilled = 10 ml.

Calculation :

1ml of 0.01 NH_2SO_4 = 0.0014 (g) N

Titre value = A-B ml

$$\% \text{ Nitrogen (N)} = \frac{A-B \times 0.0014 \times V \times 100}{W \times V_1}$$

Percentage Protein = N x Conversion factor

Conversion factor = 6.2

ESTIMATION OF FAT BY SOXHLET METHOD

Aim

To determine the percentage of fat present in the developed breakfast bars

Principle

Ether extraction of crude fat in vegetable product is carried out in a continuous extractor. An apparatus in which ether after dissolving a portion of the fat in the food sample and discharging it into the extraction flask.Steps in the process being repeated automatically and continuously until the extraction is complete. The ether gradually condenses into extraction tube containing the food sample, until it rises to the top of siphon when it is discharged in the extraction flask.

Procedure

The soxhlet flask is weighted for consecutive concordant weight (0.005 difference was allowed).

The moisture free sample of 5g is transferred into the extraction thimble. The thimble is put into the extractor, which is fixed into the soxhlet flask. Pour ether into the extraction till it siphon, once into the flask. Pour ether again into the extractor till the thimble is soaked the ether. The entire setup is kept over the electric mantle and the extractor is connected to the condenses consisting of spiral coil around which water flows continuously. The thimble and contents are allowed to soak in ether for 24 hours. The nose of the condenser is always plugged by moistened cotton. The temperature is maintained at 60°c. The condenses is trued on so that the water flows continuously through the condenser. The evaporated ether would rise up but owing to condenser arrangement fall back into the extractor is free fro any yellow color indicating the presence of fat. The soxhlet flask is then disconnected and ether in flask is fully evaporated. The flask was weighted again for consecutive concordant weigh. By the difference in weight, the fat content may be calculated.

Calculation

$$\text{Crude fat or ether extract} = \frac{\text{Weight of fat} \times 100}{\text{Weight of sample}}$$

ESTIMATION OF INULIN

Inulin is a polymer made of fructose units with β-2-1 linkage. It is found in onion, garlic and in many other plant parts.

SAMPLE EXTRACTION

Grind the sample and extract in 80% ethanol for six hours to remove free sugars. Dry the sample and take 500 mg in a 100 ml conical flask. Add 20 ml of water and heat it in a water bath at $90^{O}C$ for 10 min. collect the extract and then add 70 ml of water. Replace the flask for another 30 min with occasional shaking to dissolve the fructosan, then remove and cool it at room temperature. Combine

the extracts and filter the solution if it is not clear and make up to 100 ml in a standard flask.

PRINCIPLE

The hydroxymethyl furfural formed from fructose in acid medium reacts with resorcinol to give a red colour product.

MATERIALS

- Resorcinol reagent: Dissolve 1 g resorcinol and 0.25 g thiourea in 100 ml of glacial acetic acid. This solution is indefinitely stable in the dark.
- Dilute HCl: Mix five parts of conc. HCl with one part of distilled water.
- Standard fructose solution: Dissolve 50 mg of fructose in 50 ml of water. Dilute 5 ml of this stock to 50 ml for a working standard.

PROCEDURE

1. To 2 ml of the solution containing 20-80 µg of fructose and add 1 ml of resorcinol reagent.
2. Then add 7 ml of dilute hydrochloric acid.
3. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and make up the volume to 2 ml with water. Add 1 ml of resorcinol reagent and 7 ml of dilute HCl as above.
4. Set a blank along with the working standard.
5. Heat all the tubes in a water-bath at $80^{O}C$ for exactly 10 min.
6. Remove and cool the tubes by immersing in tap water for 5 min.
7. Read the colour at 520 nm within 30 min.
8. Draw the standard graph and calculate the amount of fructose present in the sample using the standard graph.

DETERMINATION OF ENERGY

Energy value of food is often calculated from the analysis of foods for protein, fat and carbohydrate and multiplication of the content of these components with appropriate factors, one gram of carbohydrate and protein yield 4 kcal and one gram of fat yield 9 kcal of energy.

Calculation:

Physiological Energy value -
Kcal / 100g 4 × Protein + 9 × at + 4 × carbohydrate

ESTIMATION OF FREE FATTY ACID

A small quantity of free fatty acids is usually present in oils along with the triglycerides. The free fatty acid content is known as acid number/acid value. It increases during storage. The keeping quality of oil therefore relies upon the free fatty acid content.

PRINCIPLE

The free fatty acid in oil is estimated by titrating it against KOH in the presence of phenolphthalein indicator. The acid number is defined as the mg KOH required to neutralize the free fatty acids present in 1 g of sample. However, the free fatty acid content is expressed as oleic acid equivalents.

MATERIALS

- 1 % phenolphthalein in 95% ethanol.
- 0.1 N potassium hydroxide.
- Neutral solvent: mix 25 ml ether, 25 ml 95% alcohol and 1 ml of 1 % phenolphthalein solution and neutralize with N/10 alkali.

PROCEDURE

1. Dissolve 1-10 g of oil or melted fat in 50 ml of the neutral solvent in a 250 ml conical flask.
2. Add a few drops of phenolphthalein.
3. Titrate the contents against 0.1 N potassium hydroxide.
4. Shake constantly until a pink colour which persist for fifteen seconds is obtained.

CALCULATION

$$\text{Acid value } (\text{mg}\frac{\text{KOH}}{\text{g}}) = \frac{\text{Titrate x Normality of KOH x 56.1}}{\text{Weight of the sample (g)}}$$

The free fatty acid is calculated as oleic acid using the equation 1 ml N/10 KOH=0.028 g oleic acid.

DETERMINATION OF CRUDE FIBRE

Crude fibre content of dry sample was determined by using AOAC, 2005 method.

Principle

The sample is allowed to boil with 1.25% dilute H_2SO_4, washed with water, further boiled with 1.25% dilute sodium hydroxide and the remaining residue after digestion was taken as crude fibre.

Equipments

Fibretherm, muffle furnace and hot air oven.

Chemicals:

1. Sulphuric acid (1.25%): 6.7 ml in 1 litre distilled water.
2. NaOH(1.25%): 12.5 g in 1 litre distilled water.

Procedure

1. 1 g of moisture and fat free sample was weighed and kept in the fibre bags.
2. The glass spacer was put in to the bags.
3. The bag in the sample carousel was loaded at the previewed positions (positions 1-12).
4. The sample carousel was put into the glass container carefully.
5. The glass container was placed axial on the previewed position of the hot plate.
6. A method was created to estimate crude fibre.
7. The programme was started in the fibretherm.
8. After completion of the programme, the fibre bags were removed.
9. The residue was transferred to weighed crucible (W_1) and drier over night at 80^{o}C- 100^{o}C and weighed (W_2).
10. The crucible was heated in muffle furnace at 600^{o}C for 2-3 hours.

11. Cooled in desiccator and weight of the crucible was taken after cooling (W_3).

Observations

Weight of the sample = W_1 g

Weight of the crucible + sample before heating at 600^oC = W_3 g

Weight of the crude fibre = ($W_2 - W_3$) g

Crude figre (g %) = 100-(moisture + fat) X weight of fibre / Weight of the sample taken (Moisture and fat free) (W1)

DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY

Principle

The total antioxidant activity was determined by phosphomolybdenum method, it is based on the reduction of MO (VI) to MO(V) by the sample and subsequence formation of a green Phosphate/ MO(V) complex at acidic pH. The absorbence is measured at 695nm using an UV/Vis spectrophotometrically. The antioxidant capacity was expressed as Ascorbic acid equivalent(AAE) by using the standard Ascorbic acid.

Reagents required

1.Standard solution:

50mg of Ascorbic acid is dissolved in 50ml standard flask using distilled water.(conc., 1mg/ml)

2. Extract solution:

50mg of methanolic dried extract is dissolved in 50ml standard flask using distilled water.(conc., 1mg/ml)

3. Phosphomolybdenum Reagent:

0.6M H2S04.

28mM sodium phosphate.

4mM ammonium molybdate.

Procedure

1. 1.Prepare (50-250µg) concentration of standard & extract solution, from that take 0.3ml of each sample respectively.
2. To all the tubes add 3.0ml of Phosphomolybdenum reagent.
3. 0.3ml of water and 3.0 ml of reagent alone serves as blank.
4. All the tubes incubate at 97 ^{0}C for 90minutes.
5. Cooled and the absorbance was measured at 695nm using an UV/V is spectrophotometrically against the blank . The antioxidant capacity was expressed as Ascorbic acid equivalent(AAE) by using the standard Ascorbic acid

APPENDIX VI

DETERMINATION OF THE AEROBIC COLONY COUNT IN FOODS BY HPB METHOD

Materials and Special Equipment

1. Plate count agar (PC)
2. Peptone water diluents (0.1%) (PW)
3. 2% sodium citrate (tempered to 45°C) (for cheese samples only)
4. Sodium 2, 3, 5 triphenyltetrazolium chloride (0.1%) (optional)
5. 1N HCl and 1N NaOH
6. pH meter or paper capable of distinguishing to 0.3 to 0.5 pH units within a range of 5.0 to 8.0
7. Stomacher, blender or equivalent.
8. Incubator capable of maintaining the growth temperature required for the specific type of aerobic bacterial being enumerated (i.e., for psychrophillicbacteria : 15 - 20°C, for mesophilic bacteria : 30 - 35°C and for thermophilic bacteria : 55°C) and 45° water bath.
9. Colony counting device (optional).

Procedure

Determine which type of aerobic bacteria are being enumerated. Analyze each sample unit individually. The test shall be carried out in accordance with the following instructions.

Handling of Sample Units

- During storage and transport, the following shall apply with the exception of shelf-stable products, keep the sample units refrigerated (0 - 5°C). Sample units of frozen products shall be kept frozen.

- Thaw frozen samples in a refrigerator or under time and temperature conditions which prevent microbial growth or death.

- Analyze sample units as soon as possible after receipt in the laboratory.

Preparation of Media

- Prepare plate count agar and dispense in appropriate quantities. Sterilize.
- Temper prepared melted agar in a waterbath to 45°C ensuring that the water level is 1 cm above the level of the medium in the bottles.
- Clean surface of working area with a suitable disinfectant.
- Clearly mark the duplicate Petri plates.

Preparation of dilutions

- Prepare sterile 0.1% peptone water diluents.
- To ensure a truly representative analytical unit, agitate liquid or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion from several locations within the sample unit.
- Prepare a 1 : 10 dilution o the food by aseptically blending 25 g or ml (the analytical unit) into 225 ml of the required diluents, as indicated in Table 1. If a sample size other than 25 g or ml, is used, maintain the 1 : 10 sample to dilution ratio, such as 11 (10) g or ml into 99 (90) ml.
- If a homogeneous suspension is to be obtained by blending, the blending time should not exceed 2.5 min in order to prevent over-heating. With foods that tend to foam, use blender at low speed, and remove an aliquot from below the liquid / foam interface. If a homogeneous suspension is to be obtained by shaking, shake the dilution bottles 25 times through a 30 cm arc in approximately 7 sec.
- In some instances it may be advantageous to prepare the initial dilution on a percent basis to obtain a more accurate test material weight than is attained by the dilution ratio method. i.e., a 10 solution (suspension) is represented by 10 g (ml) per 100 g (ml) of solution (suspension), whereas a 1 : 10 dilution is based on 10 g (ml) of product (solute) plus 90 g (ml) of diluents (solvent).
- Check the pH of the food suspension. If the pH is outside the range of 5.5 – 7.6, adjust the pH to 7.0 with sterile NaOH or HCl.
- Prepare succeeding decimal dilutions as required, using a separate sterile pipette for making each transfer.
- Shake all dilutions immediately prior to making transfers to ensure uniform distribution of the microorganisms present.

Plating

- Agitate each dilution bottle to resuspend material that may have settled out during preparation.
- Pipette 1 ml or 0.1 ml of the required dilutions to appropriately marked duplicate petri plates.
- In the case of products that tend to adhere to the bottom of the plants, add the inoculums to 1.0 ml of sterile diluents previously placed in the Petri plate.
- Pour 12 – 15 ml of tempered agar into each plate and mix by rotating and tilting. Allow to solidify. Plates should be poured not more than 15 min after preparation of dilutions.

Incubation

Incubate plates in the inverted position for 48 h $\pm$ 4 h. Incubation temperature is dependent on the growth temperature requirements of the target organisms (for psychrophilic bacteria : 15 – 20°C, or mesophilic bacteria : 30 – 35°C, and for themophilic bacteria : 55°C). The plates used to enumerate psychrophilic and thermophilic bacteria may be incubated upto 5 days. Other combinations of time and temperature may be used, if the lab has verified their suitability. Avoid crowding or excessive stacking of plates to permit rapid equilibration of plates with incubator temperature.

Counting Colonies

- Count colonies promptly after the incubation period.
- If possible, select plates with 20 – 200 colonies (including pinpoint colonies). If counts do not fall within this range select plates that fall nearest to the 20 – 200 range.
- If plates contain colonies which spread, select a representative portion of the plates free from spreaders, if possible, and count the colonies in this area. The total count of the entire plate is estimated by multiplying the count for the representative area counted by the reciprocal of the fraction of the plate counted. E.g. 30 colonies counted on 1/ of area of the plate, count for the whole plate : 30 x 4 = 120 colonies.
-

Differentiation of Colonies from Interfering Particles

- Alternatively, after incubation flood plates with 2 ml of 0.1% 2, 3, 5, triphenyltetrazolium chloride. Gently rock plates from side to side to cover the entire area with solution. Pour off excessive solution and allow the plates to remain at room temperature for 3 hrs. in an inverted position. The bacteria reduce the indicator to a formazan which colours the colonies red and aids in distinguishing the food particles. Colonies cannot be picked for isolation after this method has been used.

Recording Results

- Calculate the average count (arithmetic mean) of the duplicate plates.
    - When reporting results (Table II) round-off the counts to two significant figures and record only the first two left hand digits : (e.g., record 2,850 as 2,900).

If the lowest dilution plated shows no colonies, the recorded value will be the lowest average obtainable with given volume plated onto a given set of replicate plates preceeded by a “less than” (<) sign, e.g., for one milliliter and a set of duplicate plates (1 ml/plate) the value is < 0.5. The lowest possible average with one colony on one of the two duplicate plates is : (1 + 0) / 2 = 0.5. This value is for a 10° dilution (Dilution Factor = 1). For other dilutions, the numerical value of 0.5 must be multiplied by the reciprocal of the dilution : i.e., the Dilution Factor, e.g. $1 / 10^{-1} = 10$. To compute the Aerobic Colony Count (ACC), use the formula : N = A x D, where N is the number of colonies per g (ml) of product, A is the average count per plate, and D is the respective dilution factor

APPENDIX – VII

INTERVIEW SCHEDULE TO ELICIT DETAILS FOR IDENTIFICATION OF SUBJECTS WITH HYPERLIPIDEMIA

I. SOCIO- ECONOMIC AND BACKGROUND INFORMATION

1. **Education qualification :**
2. **Occupation :**
3. **Income per month :**

4. COMPOSITION OF THE FAMILY:

Typeof the family: Nuclear ☐

Joint ☐

5. DETAILS ON FAMILY BACKGROUND

Sl. no.	Name of the family member	Age	Sex	Relationship to the head of the family	Education status						Occupation	Income per month
					1	2	3	4	5	6		

1.Illiterate 2.Primary 3.High school 4.Higher secondary 5.Graduate 6.Any other.

II. DIETARYPATTERN

Diet pattern

a) Vegetarian _____
Ova- vegetarian _____
Non-Vegetarian _____

b) Total amount of fat used for cooking/day.

<3tsp _____ 3-5 tsp _____ > 5tsp_____

c) Consumption of fats/oil

Type of fat	Frequency				Amount used (l/ml)
	Daily	Weekly	Monthly	Occasionally	
Butter					
Ghee					
Gingelly oil					
Rice bran oil					
Coconut oil					
Canola oil					
Soyabean oil					
Refinoil					

d) Consumption of junk foods/deepfriedfoods.

Food items	Daily				Weekly				Monthly			
	1	2	3	>3	1	2	3	>3	1	2	3	>3
Pastries												
Puffs												
Chatitems												
Chips												
Pizza												
Burger												
Manchurianitems												
ChillyGobi												
Carbonateddrinks												
Others												

f)Do you consume prebiotic foods?

☐ Yes ☐ No

Indicate whether you consume the following foods:

Prebiotic foods	Local name	Frequency of usage								Quantity (gm)
		Daily (gm)	Weekly (gm)			Monthly (gm)			Occasionally (gm)	
			1	2	3	1	2	3		
Wheat	Godumai									
Barley	Barley									
Onion	Thakkali									
Artichoke	Vengayam									
Garlic	Koonai Poo									
Leeks	Ullipoondu									
Banana	Vazhapazham									

g) Do you consume coffee?

☐ Yes ☐ No

If yes how many cups a day

☐ 2-3 cups ☐ 3-5 cups ☐ >5 cups

APPENDIX VIII

INFORMED CONSENT FORM

AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN, COIMBATORE – 641043, India

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings – including adverse events, if any, - whether directly or indirectly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements)

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date Witness:

APPENDIX IX
ESTIMATION OF LIPID PROFILE
ESTIMATION OF CHOLESTEROL

The serum cholesterol levels were determined using Zak's method (Zak, 1977)

Principle

Cholesterol reacts with ferric chloride in the presence of concentrated sulphuric acid to give a pink color. The intensity of color developed is directly proportional to the amount of cholesterol present and was read at 540 nm in a colorimeter.

Reagents

1. Stock ferric chloride: 840 mg of pure dry ferric chloride was weighed and dissolved in 100 ml of glacial acetic acid.
2. Ferric chloride precipitating reagent: 10 ml of stock ferric chloride reagent was taken in 100 ml of standard flask and made upto the mark with pure glacial acetic acid.
3. Ferric chloride diluting reagent: 8.5 ml of stock ferric chloride was diluted to 100 ml with pure glacial acetic acid.
4. Standard cholesterol solution: 100 mg of cholesterol was dissolved in 100 ml of glacial acetic acid.
5. Working standard: 10 ml of stock was dissolved in 0.85 ml of stock ferric chloride reagent and made up to 100 ml with glacial acetic cid. The concentration of working standard is 100 µg/ml.

Procedure

To 0.1 ml of sample added 4.9 ml of ferric chloride precipitating reagent. Centrifuged and to 2.5 ml of supernatant added 2.5 ml of supernatant added 2.5 ml of ferric chloride diluting reagent. Added 4.0 ml of concentrated sulphuric acid. A blank was prepared simultaneously by taking 5.0 ml of diluting reagent and 4.0 ml of concentrated sulphuric acid. A set of standards (0.5 – 2.5 ml) were taken and made up to 5.0 ml with FeCl2 diluting reagent. Then added 4.0 ml of

con.H2SO4. After 30 mins, the intensity of colour developed was read at 540 nm against reagent blank.

The amount of cholesterol in the sample was expressed as mg/dl.

ESTIMATION OF TRIGLYCERIDES BY KIT METHOD

(GPO / PAP Method)

Summary

Triglycerides are a form of fatty acid esters. They are produced in the liver by binding glycerol and other fatty acids. They are transported by VLDL and LDL and act as a storage source for energy. Increased levels are found in hyperlipidemias, diabetes, nephrotic syndrome, hypothyroidism. Increased levels are risk factor for arteriosclerotic coronary disease and peripheral vascular disease. Decreased levels are found in malnutrition and hyperthyroidism.

Principle

Lipoprotein lipase hydrolases triglycerides to glycerol and free fatty acids. The glycerol formed with ATP in the presence of glycerol kinase forms glycerol 3 phosphate which is oxidized by the enzyme glycerol phosphate oxidase to form hydrogen peroxide. The hydrogen peroxide further reacts with phenolic compound and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colouredquinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of triglycerides present in the sample.

Triglycerides $\xrightarrow{\text{Lipoprotein Lipase}}$ Glycerol + Free fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol 3 Phosphate + ADP

Glycerol 3 Phosphate + O2 $\xrightarrow{\text{Glycerol 3 PO}}$ Dihydroxyacetonephos. + H_2O_2

H2O2 + 4 Aminoantipyrine + Phenol $\xrightarrow{\text{Peroxidase}}$ Red Quinoneimine dye + H_2O

Normal reference values

Serum / plasma : 150-200 mg/dl

Contents	**25 ml**	**2 X 75 ml**
L1 : enzyme Reageent 1	20 ml	2 X 60 ml
L2 : Enzyme Reagent 2	5 ml	2 X 15 ml
S : Triglycerides Standard (200 mg/dl)	5 ml	5 ml

Procedure

Wavelength / filter	:	505 nm / Green
Temperature	:	37°C / R.T.
Light path	:	1 cm

Pipette into clean dry test tubes labelled as Blank (B) & Test (T):

Addition sequence	B (ml)	S (ml)	T (ml)
Working reagent	1.0	1.0	1.0
Distilled water	0.01	-	-
Triglycerides Standard (S)	-	0.01	-
Sample	-	-	0.01

Mix well and incubate at 37°C for 5 min. measure the absorbance of the standard and test sample against the blank.

Calculations

Triglycerides in mg /dl = Abs.T /Abs. S x200

Cholesterol Assay:

Pipette into clean test tubes labeled as blank (B), Standard (S), and Test (T):

Addition sequence	B (ml)	S (ml)	T (ml)
Working reagent	1.0	1.0	1.0
Distilled water	0.05	-	-
HDL Standard (S)	-	0.05	-
Supernatant	-	-	0.05

Mix well and incubate at 37°C for 5 min. or at R.T. (25°C) for 15 min. measure the absorbance of the standard and test sample against the blank, with in 60 min.

Calculations

HDL Cholesterol in mg/dl = Abs.T / Abs.S X 25 X 2

(where 2 is the dilution factor due to the deproteinization step)

ESTIMATION OF LDL

Calculation of LDL Cholesterol (mg/dl) (Freidewald's formula):

=Total cholesterol – (triglycerides/5) + HDL cholesterol

Freidewald's formula is reliable provided that:

No chylomicrons are present i.e. it is a fasting sample. Triglyceride values are below 400 mg/dl

Type III hyperlipoproteinemia is absent

ESTIMATION OF VLDL

Calculation of VLDL Cholesterol (mg/dl) (Freidewald's formula):

VLDL cholesterol = triglycerides/5

Freidewald's formula is reliable provided that:

No chylomicrons are present i.e. it is a fasting sample. Triglyceride values are below 400 mg/dl

Type III hyperlipoproteinemia is absent