3

METHODOLOGY

The methodology adopted for the study entitled **"Efficacy of Prebiotic food in the Management of Hyperlipidemia"** was conducted in four phases and is discussed under the following headings:

3.1. Phase I

Assessment of knowledge, awareness and practice of prebiotic food consumption pattern among selected population groups

3.2. Phase II

Formulation and standardization of a prebiotic food

3.3 Phase III

Identification of hyperlipidemic subjects

3.4. Phase IV

Supplementation of prebiotic food and study its efficacy on lipid profile

3.5. Statistical Analysis

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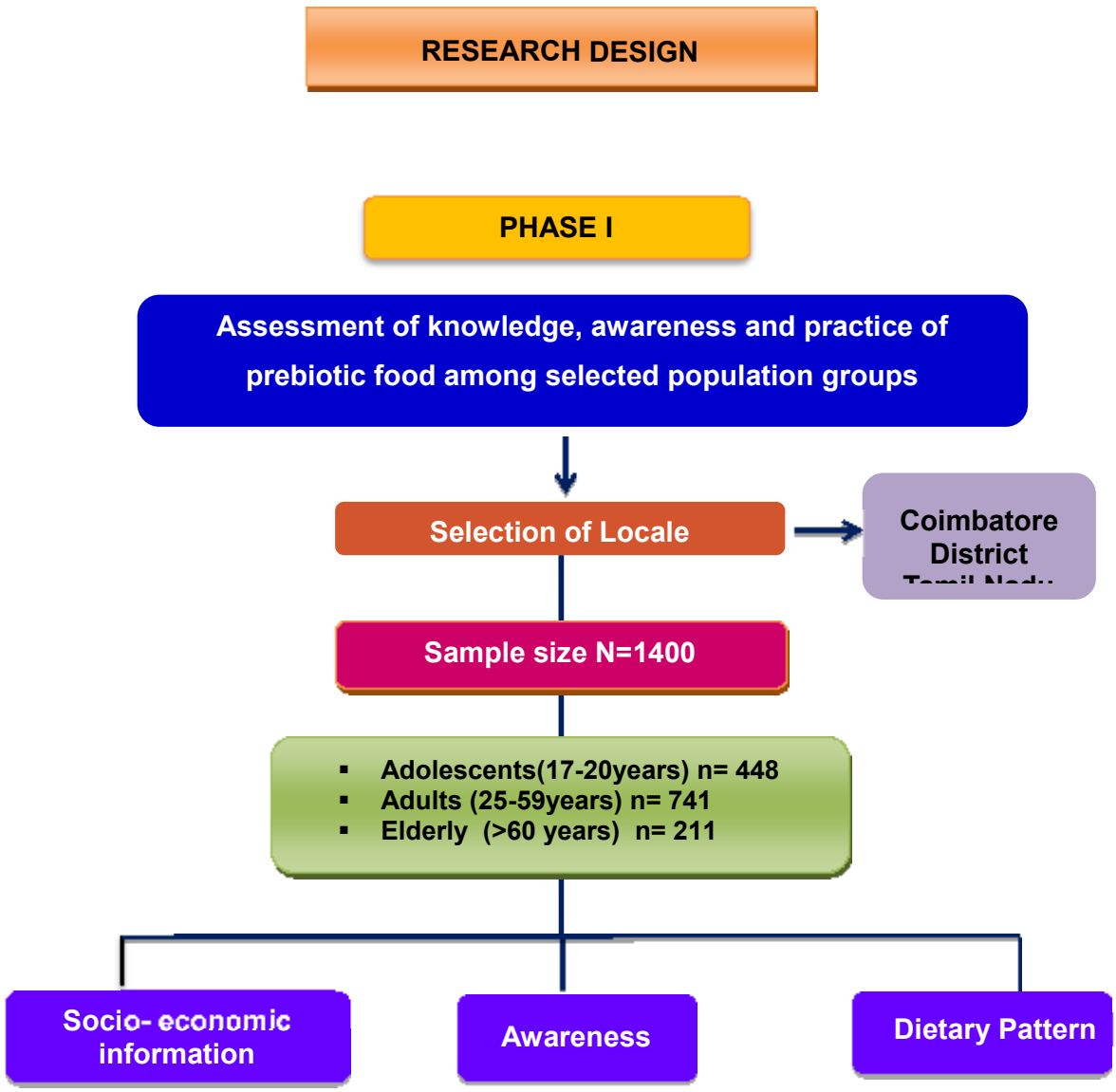
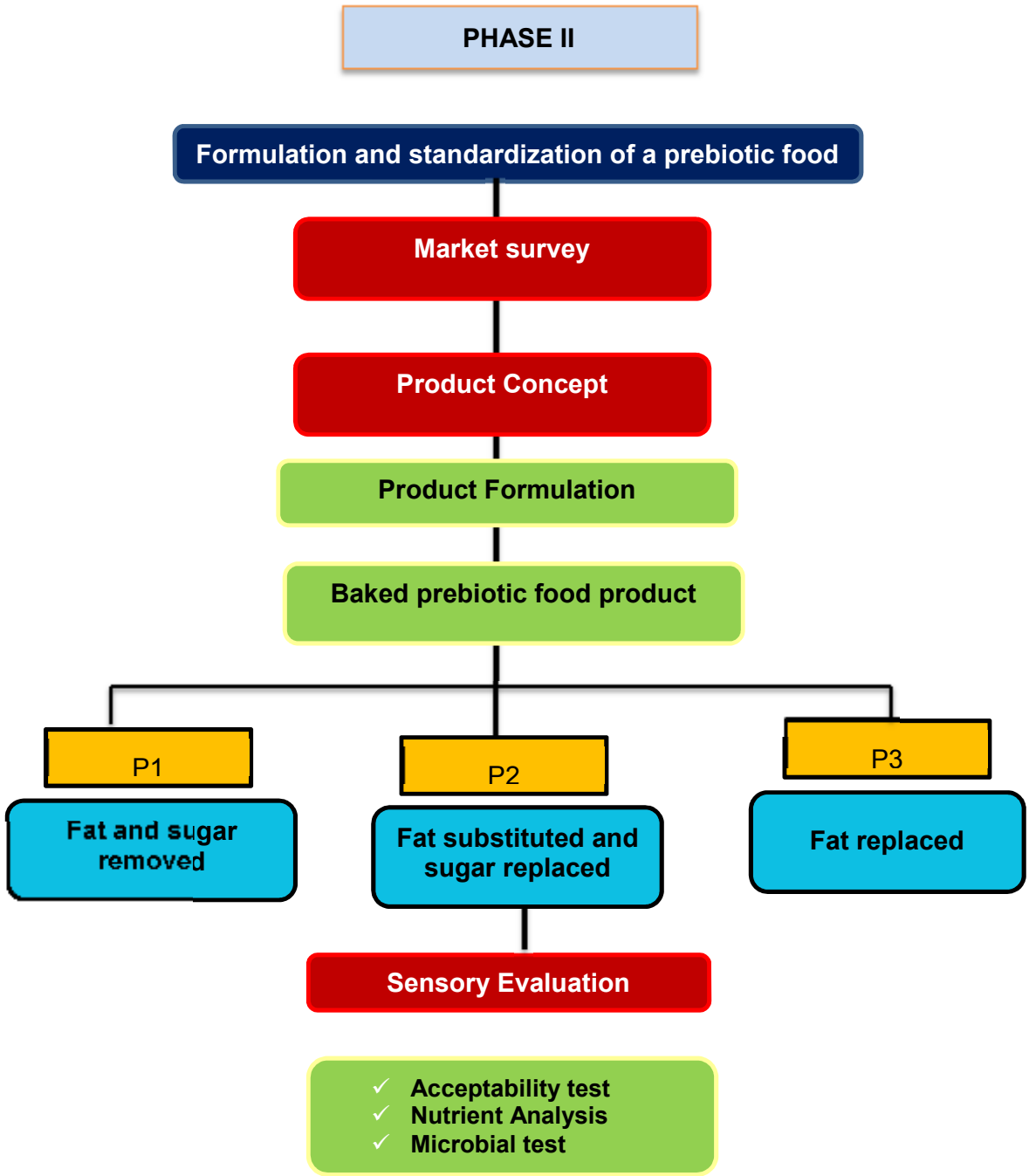
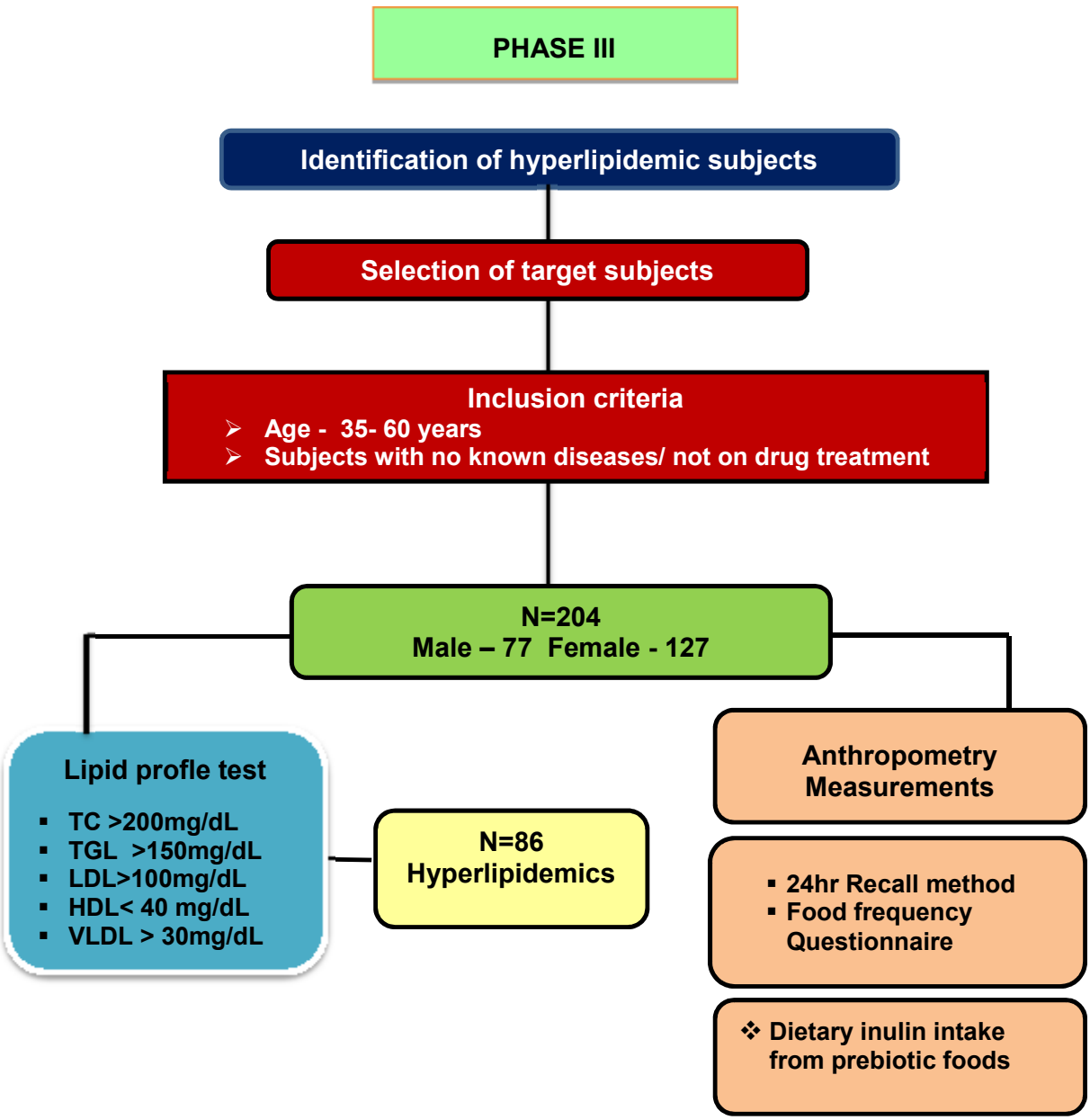
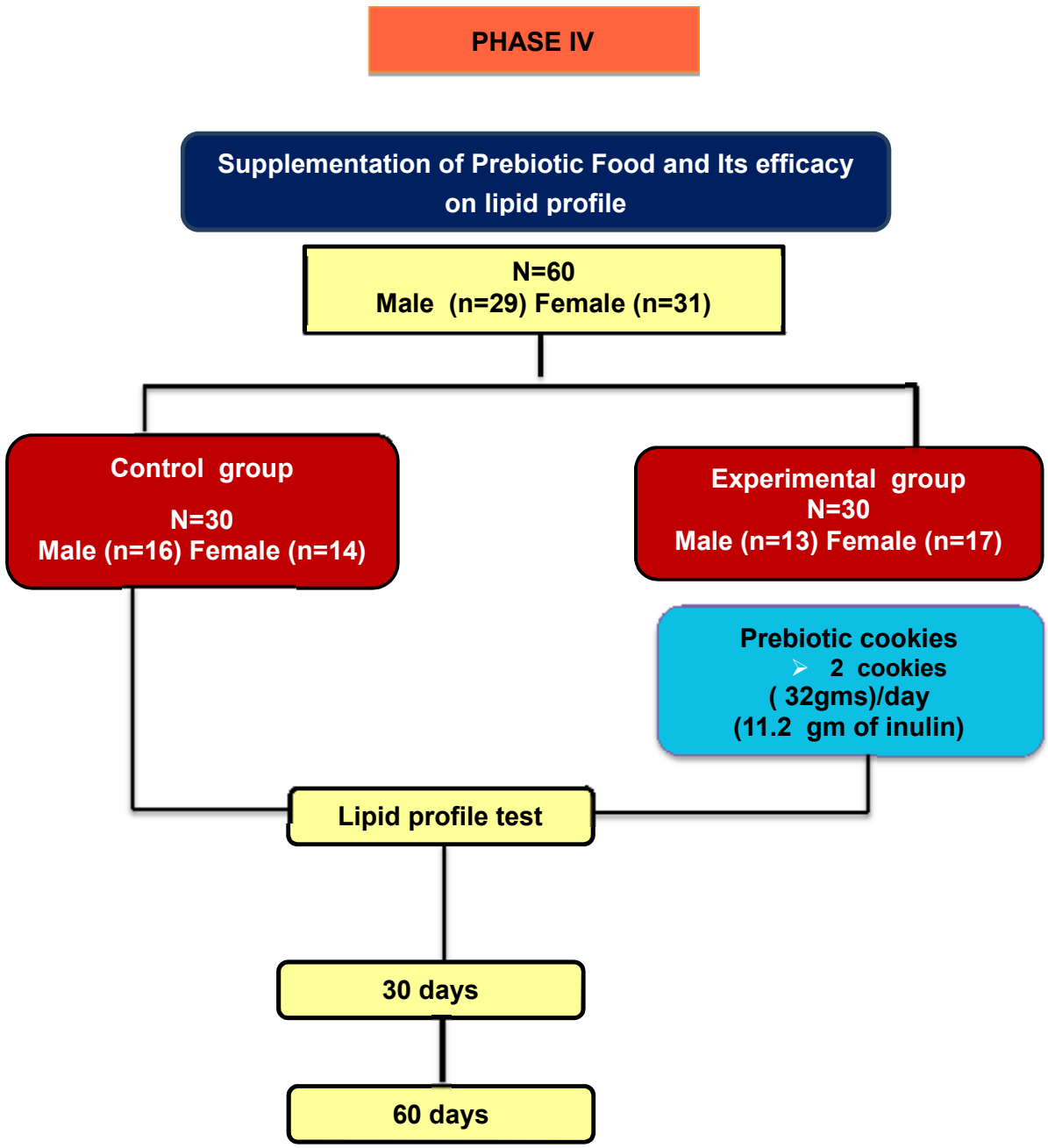
Ethical clearance for the study was obtained from Institutional Human Ethics Committee, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore with approval no. AUW/IHEC-13-14/FHP-22. An Informed consent was obtained for all the subjects who participated in this study (Appendix I). The research design followed for the study is given in Figure 4.

Figure 4


RESEARCH DESIGN
PHASE I
Assessment of knowledge, awareness and practice of prebiotic food among selected population groups
Selection of Locale
Coimbatore District
Sample size N=1400
Adolescents(17-20years) n= 448
Adults (25-59years) n= 741
Elderly (>60 years) n= 211
Socio- economic information
Awareness
Dietary Pattern


PHASE II
Formulation and standardization of a prebiotic food
Market survey
Product Concept
Product Formulation
Baked prebiotic food product
P1
Fat and sugar removed
P2
Fat substituted and sugar replaced
P3
Fat replaced
Sensory Evaluation
✓ Acceptability test
✓ Nutrient Analysis
✓ Microbial test


PHASE III
Identification of hyperlipidemic subjects
Selection of target subjects
Inclusion criteria
Age - 35- 60 years
Subjects with no known diseases/ not on drug treatment
N=204
Male – 77 Female - 127
Lipid profle test
TC >200mg/dL
TGL >150mg/dL
LDL>100mg/dL
HDL< 40 mg/dL
VLDL > 30mg/dL
N=86
Hyperlipidemics
Anthropometry Measurements
24hr Recall method
Food frequency Questionnaire
Dietary inulin intake from prebiotic foods


PHASE IV
Supplementation of Prebiotic Food and Its efficacy on lipid profile
N=60
Male (n=29) Female (n=31)
Control group
N=30
Male (n=16) Female (n=14)
Experimental group
N=30
Male (n=13) Female (n=17)
Prebiotic cookies
2 cookies
(32gms)/day
(11.2 gm of inulin)
Lipid profile test
30 days
60 days

3.1. PHASE I

ASSESSMENT OF KNOWLEDGE, AWARENESS AND PRACTICE OF PREBIOTIC FOOD AMONG SELECTED POPULATION GROUPS

3.1.1. Selection of area and subjects

3.1.1.1. Locale of study

The area selected for the study was urban areas of Coimbatore city. The subjects were selected from various near Saibaba colony and Ramalingam colony.

3.1.1.2 Selection of subjects

Sampling technique and sample size

The knowledge and awareness on prebiotic foods was studied among a total of 1400 subjects were selected from households by random sampling technique. Random sampling is defined as every unit of population which has the same probability of being selected into the sample (Kothari 2011).

Adolescents in the age group of 10-19 years, adults in the age group of 20-60 years and elderly in the age of 60-70 years (WHO) were included in the study. The inclusion criteria followed was that subjects should be literates and both the genders were selected for the study. Adolescents comprised of 173 males and 275 females. In the adult group 278 males and 463 female subjects participated in the study while the elderly group comprised of 98 males and 113 females.

3.1. 2. Validation of interview schedule

Validity is the extent to which a test measures what it is intended to measure.Interview schedule validation procedure allows accurate measurement of the aim of the study and helps to collect better quality data with high comparability which reduces the effort and increase the credibility of data. An interview schedule was used to assess the knowledge, awareness and practice of prebiotic food. It was validated through a pilot study among doctors, dietitians and nutritionists to check the content validity and reliability.

An interview schedule is a method of collecting data where the questions are asked and filled in by the interviewer with a face to face contact with the samples from whom the information are elicited (Gupta, 2004).

3.1.3. Assessment of knowledge, awareness and practice

The validated interview schedule (Appendix II) was administered to find out the knowledge, attitude and practice of prebiotic foods among the selected subjects. Background details such as education, occupation and income status was elicited from the subjects. Knowledge was tested through questions like prebiotic term and its concept . Awareness on prebiotics and sources of information was recorded and consumption of prebiotic foods was assessed through questions on intake prebiotic foods like wheat, barley onion, garlic, banana and leeks and consumption of coffee (Plate I). Inulin and oligofructose content of different natural food ingredients given by Van Loo *et al.,* 1995 was used to list out the commonly available prebiotic foods given in the interview schedule.

Plate I

Interviewing the subjects to elicit details on knowledge, awareness and practice of prebiotic foods

Based on the results that majority were unaware of prebiotic foods and an effort was taken to develop a product with prebiotic functional quality.

3.2. PHASE II

FORMULATION AND STANDARDISATION OF A PREBIOTIC FOOD

3.2.1 Market survey

Market survey on the availability of prebiotic food products was carried out in four well known department stores of Coimbatore to find out the availability of common food products incorporated with prebiotic ingredient. The labels were studied in about four convenience food products and mainly looked for inulin as it is a prebiotic ingredient (Slavin 2013).

3.2.2 Diet survey to assess the snacking pattern

A diet survey was carried out among 50 adults (33 females and 17 males) working in a government sector using purposive sampling technique to understand their diet behavior so that a suitable product can be decided.

A 24 hour recall survey (Appendix III) was administered and the consumption pattern was recorded for three consecutive days. As the subjects were working, the snacking behavior was studied and therefore information was collected on the menu offered in the canteen. The subjects visited the canteen twice a day during their mid-morning and tea time and the results were consolidated. The findings of the survey showed a snacking behavior of fried foods among the subjects and hence incorporating a prebiotic food in a snack was decided.

3.2.3. Product concept

The product concept was decided on the basis that the

i. Product that can be commonly consumed by the target groups i.e. the working adults.

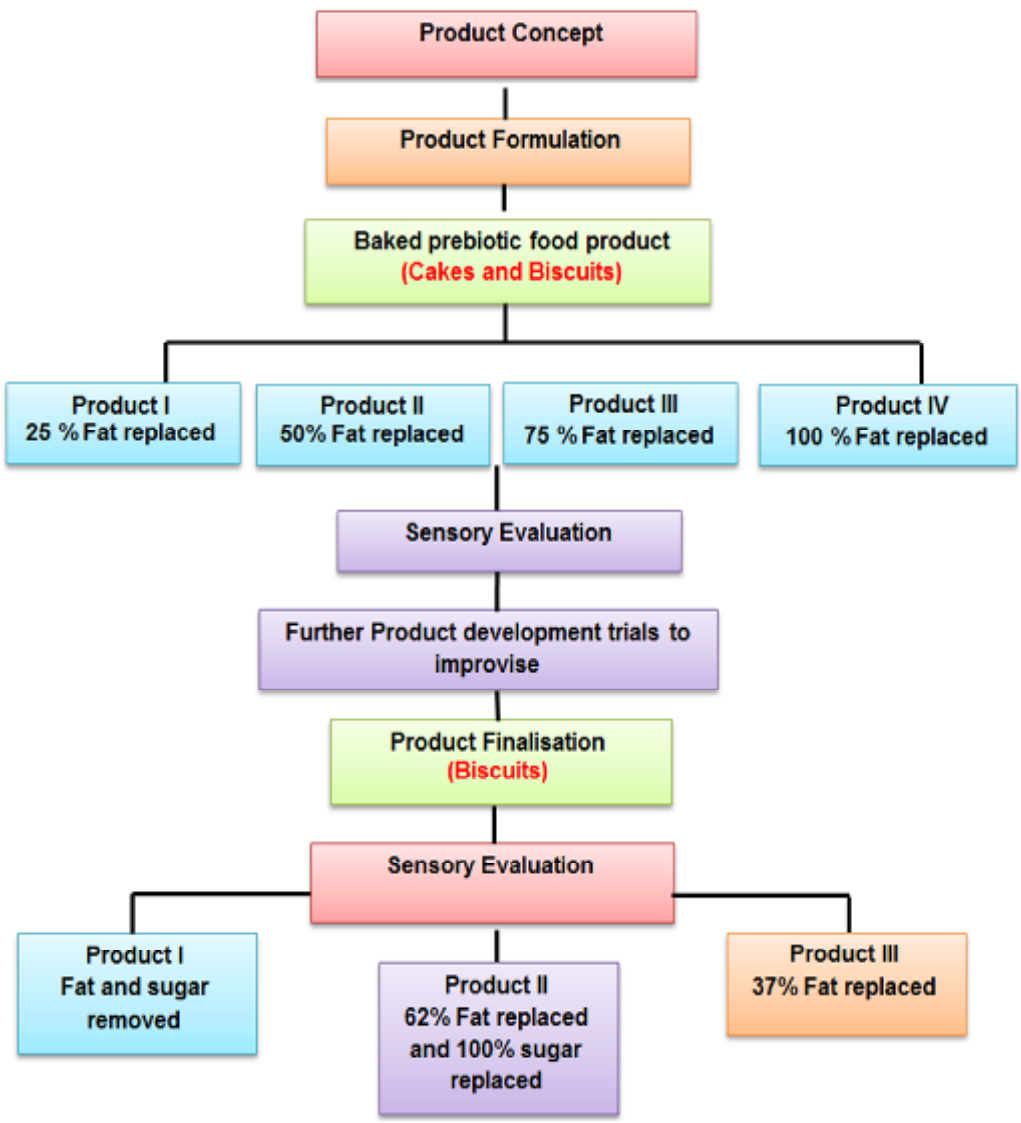
ii. Product which does not deviate much from the target group's diet.

iii. Product which can be consumed daily during their work time and is cost effective.

iv. Product which can lend itself for cooking without fat.

v. Product which can be enriched with inulin.

Taking into consideration the product concept a baked prebiotic product was developed and is given in Figure 5.



Product formulation flow chart

Figure 5

3.2.4. Selection of prebiotic foods

A baked food product was formulated using inulin as an adjunct to incorporate the prebiotic functionality into the product. Inulin extracted from the chicory root (*Cichoriumintybus*) was used as it offers dual benefit of improving the organoleptic quality and offer functional benefit (Gupta, 2003).

A thorough search on the availability of inulin was sourced from ingredient suppliers with product specifications in India and Generally Recognized As Safe (GRAS) certified Fructafit inulin was purchased from DKSH India Private Limited Bengaluru, Karnataka. Fructafit® HD inulin is a natural powdered ingredient extracted from chicory roots. It is classified as a dietary fibre and used in food industry for its remarkable functional properties such as the ability to act as a fat or sugar replacer (Zahn, 2010).

3.2.5. Choice of ingredients used in the formulation

The choice of ingredients used in the formulation of cakes and biscuits is given below.

TABLE II

CHOICE OF INGREDIENTS

Ingredients	Reasons	References
Wheat flour	Whole-wheat flour contains bran and germ which makes it a healthier choice for baking than refined white flour.	(NIN, 2010)
Olive oil	Olive oil is often used to substitute cooking oils in most baked recipes. It has higher monounsaturated fatty acids (MUFA) and less prone to oxidation.	(USDA 2004)
Inulin	Fructafit® HD inulin is a natural powdered ingredient extracted from chicory roots. It is classified as a dietary fibre and used in food industry	(O'Brein, *et al*., 2003; Jardine, 2009)

Ingredients	Reasons	References
	for its) remarkable functional properties. Inulin is considered to be a fat and sugar substitute carbohydrate or a dietetic fiber	
Skimmed milk powder	The milk solids have a binding effect on the flour protein, creating a toughening effect and contain lactose which helps to regulate crust color. They improve the flavour and are important moisture retaining agents	Lauterbach and Albrech (2011)
Butter	Fat aids in giving volume and open texture or flakiness. Butter increases tenderness, which may also be attributed to lubrication in the mouth while eating biscuit	Rogers 2014.
Egg	They add flavor, colour and give structure to the product. It acts as an emulsifier. In cakes, it gives a uniform flavor and texture.	Lauterbach and Albrech (2011)
Sugar	Sugar tenderizes dough and batter products and may help the baked product to brown	Lauterbach and Albrech (2011)

3.2.6. Standardisation of prebiotic food

According to the U.S. Department of Agriculture, a standardized recipe is one that “has been tried, adapted and retried several times for use and has been found to produce the same good results and yield every time when the exact procedures are used with the same type of equipment and the same quantity and quality of ingredients.”

A baked prebiotic product namely cake was formulated. Four variations of cakes were made using inulin to replace baking fat on a weight basis at 0% (standard) 25% (T1), 30% (T2), 75% (T3) and 100%(T4) replacement levels.

Traditional creaming method of preparation was used for mixing of ingredients (Gisslen 2014) for the cakes and the method followed for the preparation is outlined and given in Table III.

TABLE III

FORMULATION OF CAKES

Ingredients	Standard	Replacement levels			
		T1 25%	T2 30%	T3 75%	T4 100%
Wheat flour	128	128	128	128	128
Egg	2	2	2	2	2
Inulin	nil	16	19.2	48	64
Butter	64	48	44.8	16	nil
Sugar	80	80	80	80	80
Salt	¼ tsp	¼ tsp	¼ tsp	¼ tsp	¼ tsp
Vanila essence	½ tsp	½ tsp	½ tsp	½ tsp	½ tsp
Baking powder	½ tsp	½ tsp	½ tsp	½ tsp	½ tsp

Cakes with four variations namely T1, T2, T3 and T4 were formulated. These variations were standardised by test and trial method three times to get consistent quality and yield.

Method of preparation:

1. All the dry ingredients such as wheat flour, salt and baking powder were sieved.
2. Butter was mixed slowly until the fat was smooth and creamy.
3. In fat replacement variation, inulin was added with butter before creaming
4. Sugar was added in this mixture and again mixed till a creamy mixture is obtained.
5. Eggs were added to the creamed mixture in several parts, beating well after each addition until fluffy.
6. The dry ingredients were added to the creamed mixture.
7. Vanilla essence was added for flavouring.
8. The cake was baked at 180^{o}C for 30 minutes, a yield of 200gm was achieved and 8 portions of cake was obtained.

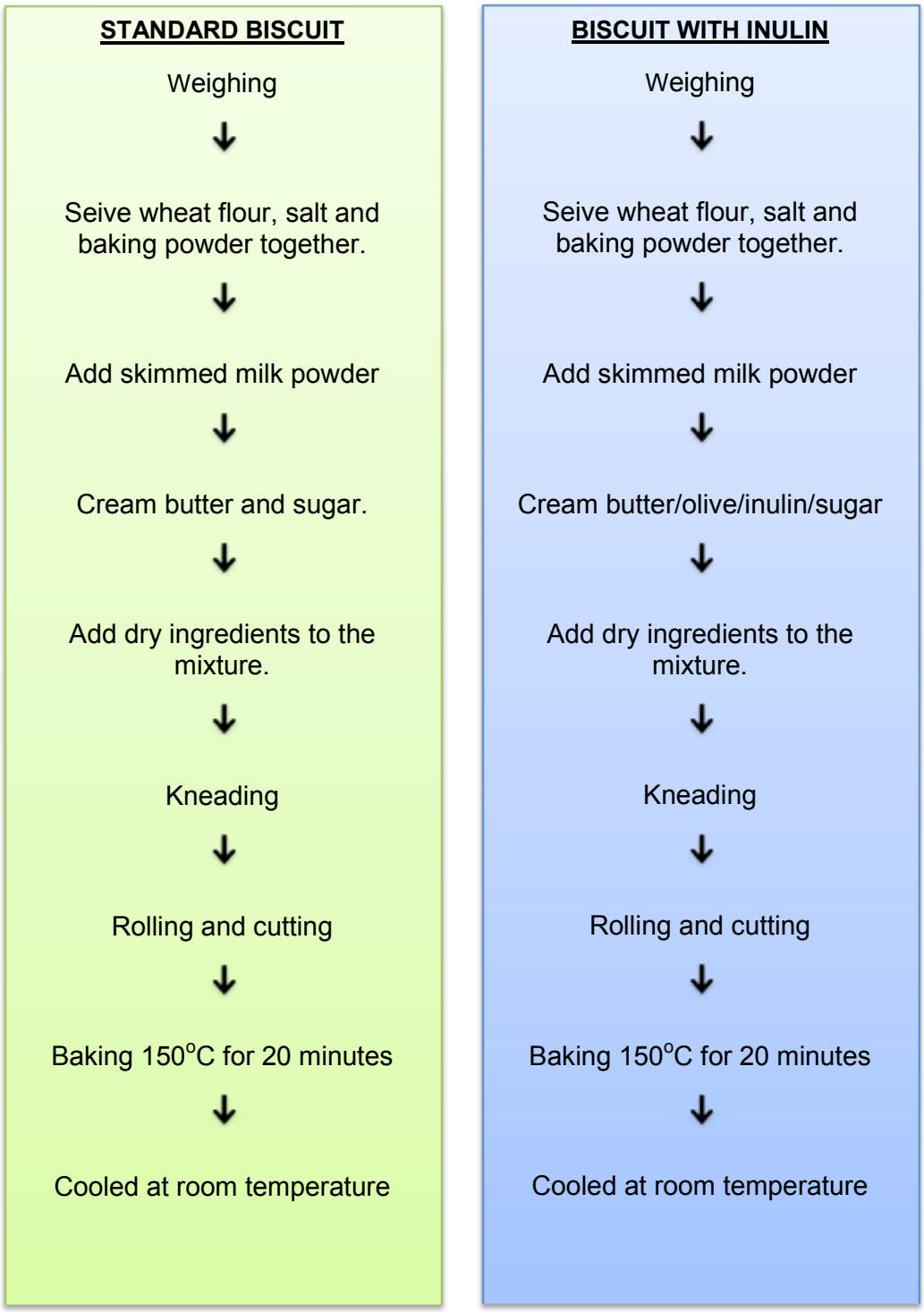
Sensory evaluations were carried out to measure overall acceptability, detect differences between formulations and measure intensity attributes.

Sensory evaluation is a scientific method to evoke, measure, analyse and interpret responses to products as perceived through the senses of sight, smell, touch, taste, and hearing'(Kemp and Hort 2015)

The sensory evaluation and the quality of the products was assessed by 15 panelists and based on the scores and overall acceptability the cake variation was not acceptable. Hence another baked product namely biscuits was developed.

Biscuits were formulated with three variations using inulin as fat and sugar replacer. A standard biscuit and three variations namely P1 (Fat and sugar removed), P2 (62% fat replaced and 100% sugar replaced) and P3 (37% fat replaced)

The formulations were prepared by standard creaming method and method of preparation is outlined below in Figure 6.



Flowchart for biscuit formulation

Figure 6

Method of preparation:

1. Wheat flour, salt and baking powder were sieved together. Add skimmed milk powder to the other dry ingredients.

2. Butter/ olive oil/sugar were creamed together separately

3. In case of biscuit variations, inulin was added with fat and creamed together.

4. The dry ingredients were then mixed with the creamed mixture to make a dough

5. The biscuit dough obtained was sheeted on a metal platform to a thickness of 3 mm using wooden rolling pin.

6. The dough was cut into circular shape using a metallic cutter and arranged on a baking sheet and baked in a pre-heated oven to 150°C until golden brown.

7. After baking biscuits were cooled to room temperature

The biscuits was baked at 150^{o}C for 20 minutes and yield of 200gm was achieved and 12 portions of biscuits were obtained. (Plate II)

The biscuits were evaluated for sensory attributes and was compared with standard. Fifteen judges evaluated the biscuits using a 9 point Hedonic Scale. Each attribute was assigned a numerical value for a subjective factor i.e. appearance, color, flavor, taste, texture, and overall acceptability. The biscuits were evaluated and the results were recorded. (Appendix IV)

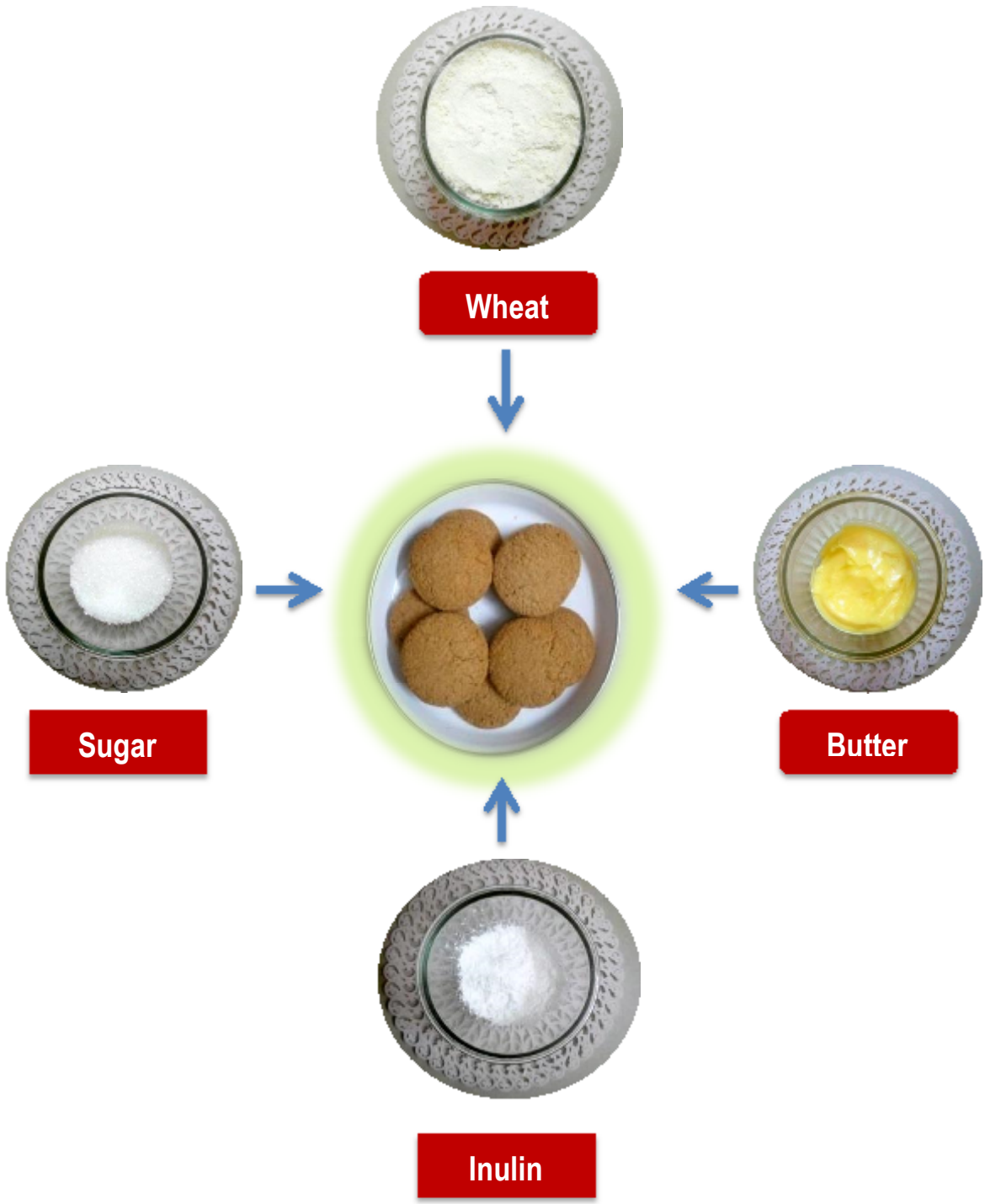


Plate II

Formulation of biscuits

(P2 –Fat replaced and sugar removed)

3.2.7. Nutrient Analysis

Analysis for P1, P2 and P3 was carried for nutrients such as energy, protein, carbohydrate, fat, fibre, free fatty acid and total antioxidant activity using standard procedures. Nutrient content in inulin powder was analysed for carbohydrate, fat, fibre and total antioxidant activity. (Appendix V)

METHODS OF ANALYSIS FOR NUTRIENTS

NUTRIENT	Method of analysis
Energy	Bomb calorimetry
Carbohydrates (g)	DGHS Method
Fat (g)	Soxhlet method
Protein (g)	AOAC 19th Edition 2012
Inulin (g)	DGHS Method
Free fatty acid(mg)	AOAC 19th Edition 2012
Crude Fibre (g)	DGHS Method
Total antioxidant activity (µg)	FRAP Method

3.2.8. Microbial Analysis

In order to find out the microbial quality of the developed products total plate count and mold count was carried out using standard procedures for the standard product and the variation which had a maximum overall acceptability. Tests for antimicrobial activity of the developed food was done for 0 and 7thday using standard plate count method (Appendix VI)

3.2.9. Cost calculation

The total cost incurred in the preparation of the biscuits based on the market prices prevalent during the specific time, was computed. The cost of unit weight of food ingredients was calculated and the economic feasibility was assessed.

3.3 PHASE III

IDENTIFICATION OF SUBJECTS WITH HYPERLIPIDEMIA

A government sector namely Bharat Sanchar Nigam Limited in Saibaba colony, Coimbatore was selected to identify the target groups i.e. hyperlipidemics through purposive sampling technique. Two hundred and four subjects were screened for their lipid profile. Inclusion criteria was that they should be in the age group of 30-60 years with no known disease. Those who are willing to take part in the study and were willing to take the prebiotic supplement were included in the study. Subjects with chronic diseases and those having lipid lowering drugs were excluded (Plate III).

Plate III

Eliciting details from screened subjects

Purposive sampling method is the deliberate selection of sample units that conform to some predetermined criteria(Krishnaswami and Ranganathan, 2005). The details of the subjects was elicited using an (Appendix VII) and is as in the following

3.3.1. Demographic profile

3.3.2 Anthropometry

3.3.3 Assessment of biochemical parameters

3.3.1 DEMOGRAPHIC PROFILE

The socio economic and background details of the selected subjects such as the education status, occupation and income status, type of family was elicited using an interview schedule.

3.3.2. ANTHROPOMETRY

Using standard procedures, anthropometric measurements such as height, weight, waist and hip circumference were recorded and the corresponding Body Mass Index and Waist to Hip Ratio was calculated for all the subjects.

i. Measurement of Height

Height was measured to the nearest 0.5cm with the subject standing in an erect position with a fibre tape with the head position so that the top of the external auditory meatus is in level with the inferior margin of the body orbit (Plate IV a)

ii. Measurement of Body Weight

Body weight was measured to the nearest 0.5 kg with the subject standing motionless on the weighing scale. Body weight is the most widely used and the sensitive and simplest reproducible anthropometric measurement. Weight will be taken with the individual under basal conditions with minimum clothing and without shoes. (Plate IV b)

iii. Body Mass Index

Body mass index provides a quick and relatively easy indication of obesity or underweight. It is a simple index of weight for height that is commonly used to determine obesity in adults. Body mass index was calculated using the formula

BMI = Weight (kg)/Height (m^2) and was classified as per WHO (2004) classification for obesity and underweight.

CLASSIFICATION	BMI(kg/m^2)
Normal	18.50 - 24.99
Overweight	25.00-29.99
Obese	≥30.00
Obese class I	30.00 - 34.99
Obese class II	35.00 - 39.99
Obese class III	≥40.00

Source: WHO (2004)

Plate IV a
Measurement of height

Plate IV b
Mesurement of weight

iv. Measurement of Waist circumference

Waist circumference was measured at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest, using a stretch-resistant tape that provides a constant 100 g tension (Plate IVc).

According to WHO (2008), the normal waist circumference is 80 cm for women and 90 cm for men. Waist circumference greater than >80 cm in women and > 90 cm in men poses increased risk for metabolic complications such as obesity and cardiovascular diseases.

v. Measurement of Hip circumference

Hip circumference was measured in the horizontal plane at the level of maximal circumference including the maximum extension of the buttocks posterior (Bray, 2004). (Plate IV d).

vi. Waist to Hip Ratio

Waist hip circumference ratio is commonly used to predict the risk of obesity related morbidity and mortality as they account for regional abdominal adiposity (Welborn, 2003).

Waist to hip ratio (WHR) was calculated using the formula

$$\text{Waist to Hip Ratio} = \frac{\text{Waist (centimeters)}}{\text{Hip (centimeters)}}$$

Women with a ratio of 0.85 or less were considered safe and above 0.9 were classified with central obesity and men with waist hip ratio above 0.9 was included in at risk category.

Plate IV c

Measurement of waist circumference

Plate IV d

Mesurement of hip circumference

3.3.3 ASSESSMENT OF BIOCHEMICAL PARAMETERS

Biochemical assessment provides the most objective and quantitative data on the nutritional status of an individual. Due to objective accuracy that these biochemical tests provide, these can be used to verify the validity of the results obtained using dietary surveys.

i. Blood pressure

Blood pressure was measured for three readings using a sphygmomanometer and standard procedures of measurement were followed. Mean values of the reading was recorded.

ii. Lipid profile

An informed consent (Appendix VIII) was taken from all the subjects after full explanation of procedure and they were asked to keep 12-14 hours overnight fasting and the 5ml venous blood sample were collected next morning before breakfast for estimation of serum lipid profile. Lipid fractions like total cholesterol, triglycerides, HDL Cholesterol, LDL cholesterol and VLDL were estimated using standard procedure. Total cholesterol was estimated by Zak's method. Estimation of triglycerides was done using KIT method.. HDL cholesterol was measured by glycol method while LDL cholesterol and VLDL cholesterol was calculated using Freidewald's formula . (Appendix IX).

The obtained values were compared against reference values of National Cholesterol Education Programme and hyperlipidemic subjects were identified.(Plate VI a Plate VI b)

Hyperlipidemia is defined as elevation of cholesterol, lipoprotein fractions and triglycerides or both (Nelson, 2013)

LIPIDS	CLASSIFICATION
TOTAL CHOLESTEROL	
< 200	Desirable
200-239	Borderline
>239	High
TRIGLYCERIDES	
< 150	Normal
>150-199	Borderline
200-499	High
>500	Very High
HDL-C	
<40	Low
>60	High
LDL-C	
<100	Optimal
100-129	Near optimal
130-159	Borderline high
160-169	High
>190	Very High

Source: NCEP (2010)

Plate V

Assessment of biochemical parameters

On estimation of serum lipid among the subjects, 86 subjects were found to be hyperlipidemic. Their consent on participation in the intervention trials were obtained and 30 subjects formed the experiment group with 13 males and 17 females .and 30 were selected as the control group with 16 males and 14 females.

3.4. PHASE IV

SUPPLEMENTATION OF PREBIOTIC FOOD AND ITS EFFICACY ON LIPID PROFILE

Before the intervention, both the groups were counseled on diet and importance of prebiotic foods in promoting health. (Plate VII). The experimental group was advised to follow their regular diet pattern avoiding fried snacks and pastries during their midmorning and tea time regularly for 60 days. They were also asked to restrict high fat foods and deep fried foods on a regular basis.

Plate VI

Counselling of subjects before intervention

The diet pattern and consumption pattern of fats/oil by the selected hyperlipidemic subjects was recorded. The mean food intake of the subjects was recorded using a twenty four hour recall method for three consecutive days in order to find the dietary inulin intake of the subjects from prebiotic foods and coffee consumption.

Twenty-four hour diet recall interview is a quantitative research method used in nutritional assessment, and asks individuals to recall foods and beverage they consumed in the twenty-four hours prior to the interview (Ferrari, 2002).

The developed prebiotic biscuits was prepared fresh and packed for a week in air tight polyethylene packaging. Experimental group was supplemented two prebiotic biscuits of 16gm each for 60 daysas a snack to be taken as one during mid-morning and one at tea time instead of their usual snacks. From the percentage of chicory added to coffee powder the amount of chicory available in one cup of coffee was estimated and the corresponding amount of dietary inulin intake was calculated.

The subjects were advised to report of any digestive disturbance or allergy during the study period and no adverse effect was found among the subjects during the intervention period. Care was taken to ensure that the prebiotic biscuits were consumed on a regular basis throughout the study through phone call, personal visits and reminder through mails for pre intervention (0 day), mid intervention period (30^{th} day) and post intervention (60^{th} day) and the results were inferred and compared using by

3.5. Statistical analysis

The findings of the study was statistically analysed using chi-square test, Anova and Anacova test.