



# Materials and Methods

## **CHAPTER 4**

### **MATERIALS AND METHOD**

Lactose intolerance is an ailment resulting in the occurrence of gastrointestinal symptoms such as abdominal pain, vomiting, nausea etc after milk consumption. The most commonly prescribed treatment for lactose intolerance is avoidance of milk and milk-based products. The present study was conducted to assess the presence of lactose intolerance among children, adults and elderly of urban Vadodara and evaluating the impact of supplementing lactose hydrolysed milk on their quality of life and nutritional Status. This chapter describes the experimental design of the study (Fig 4.1) and elaborates the materials and methods used to conduct the study.

#### **Phase I- Statutory clearance, study design and Screening and Identification of Lactose intolerant subjects**

Section 4.1.1 Statutory clearance of the study

Section 4.1.2 Type of study and determining sample size

Section 4.1.3 Screening on the basis of gastrointestinal symptoms

Section 4.1.4 Exclusion and Inclusion criteria to participate in Hydrogen Breath Analyser test

Section 4.1.5 Identification of lactose intolerant subjects by means of Hydrogen Breath Analyser test

Section 4.1.6 Statistical analysis

#### **Phase II- Impact of supplementing Lactose hydrolysed milk (LHM) to lactose intolerant subjects.**

Section 4.2.1- Baseline information and post supplementation data on BMI of the lactose intolerant subjects

Section 4.2.2- Baseline information and post supplementation data on dietary intake of the lactose intolerant subjects.

Section 4.2.3- Baseline information and post supplementation data on quality of life of the lactose intolerant subjects.

Section 4.2.4- Supplementation with Lactose Hydrolysed Milk (LHM)

Section 4.2.5- Statistical analysis

**Phase III- Organoleptic evaluation of food products developed from standard and lactose hydrolysed milk**

Section 4.3 Development of food products from standard and lactose milk

Section 4.3.1 Procurement of Lactose Hydrolysed Milk (LHM) and standard milk

Section 4.3.2 Selection of food products

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**Phase IV- Development of IEC material**

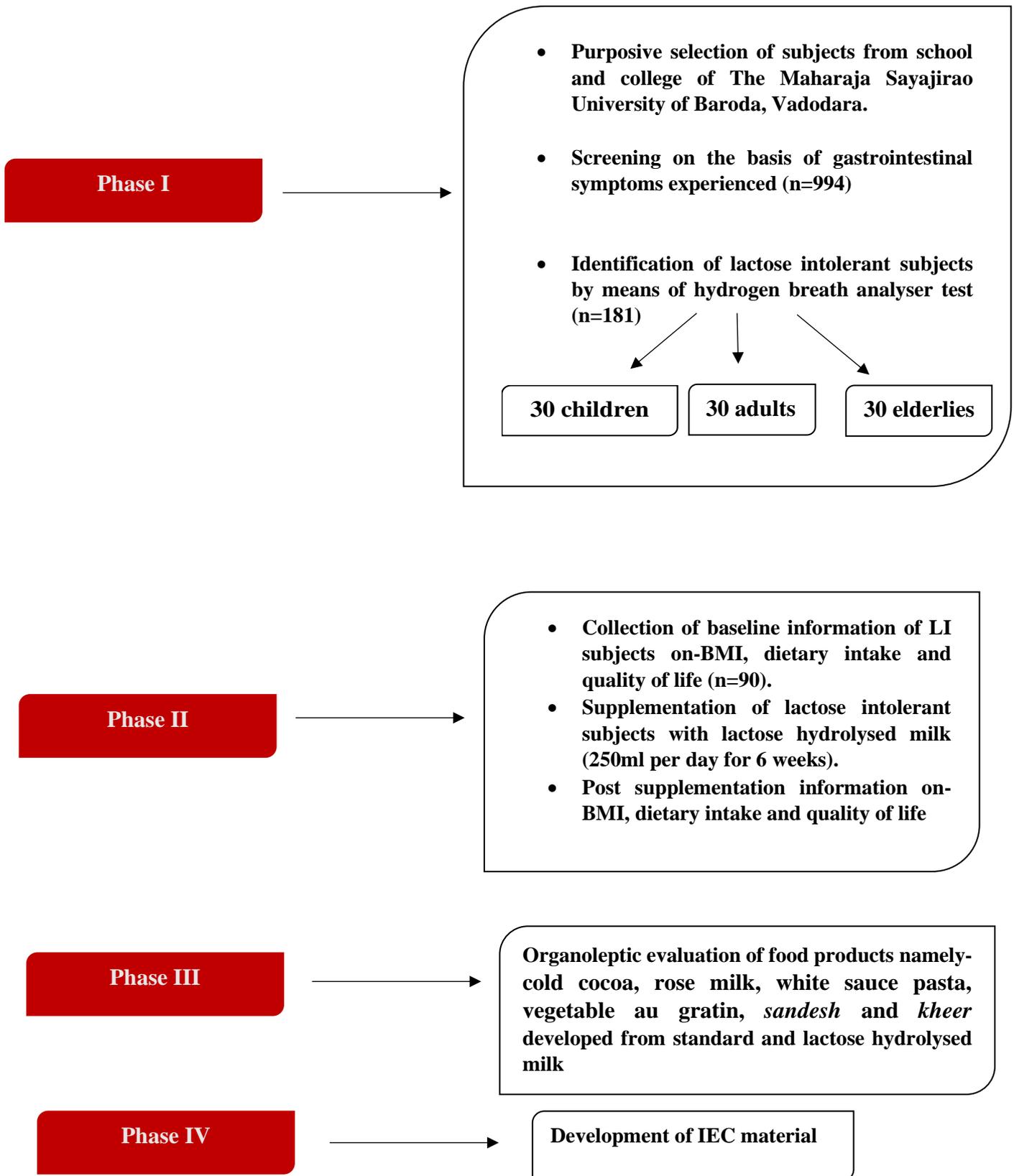


Figure 4.1 Experimental design of the study

## **Phase I- Screening and Identification of Lactose Intolerant subjects**

In this phase we determined the study design, sample size and identified lactose intolerant subjects using Hydrogen breath Analyser test. This phase is explained in the following sections.

4.1.1 Statutory clearance of the study

4.1.2 Type of study and determining sample size

4.1.3 Screening on the basis of gastrointestinal symptoms

4.1.4 Exclusion and Inclusion criteria to participate for Hydrogen Breath Analyser test

4.1.5 Identification of lactose intolerant subjects by means of Hydrogen Breath Analyser test

4.1.6 Statistical analysis

### **4.1.1 Statutory clearance of the study**

Ethical clearance was obtained from the ethical committee- Institutional Ethics Committee for Human Research (IECHR), Faculty of Family and Community sciences, The Maharaja Sayajirao University of Baroda, Vadodara. Ethical approval number IECHR/2018/18 was obtained for conducting this study.

### **4.1.2 Type of study and determining sample size**

Study design used in this study is cross-sectional in nature and sampling technique applied here is purposive sampling technique.

Sample size is calculated using the following formula

$$n = N \times \frac{Z^2 \cdot p \cdot (1-p)}{(N-1) E^2 + Z^2 \cdot p \cdot (1-p)}$$

p= Estimated proportion (from previous knowledge), E= % of error permitted

N= finite size of the population, (1- a) = confidence level (this formula is used when we have a finite population), Considering prevalence as 50% and error as 5%.

Total number of staff members in the university is 1742.

The sample size of adults, elderly and children are- Adults= 346 (N=3484),

Children= 346 (N=3484), Elderly= 183 (N=348)

### **4.1.3 Screening on the basis of gastrointestinal symptoms**

Total of 3000 adults comprising of staff members and their families working in a university of Vadodara, school going children (272) of the age group 10 to 17 years old and 950 people from elderly population were screened (Annexure 1).

#### **4.1.3.1 Administration of interviewer-based questionnaire**

Respondents were briefed about the objective of the study and were motivated to participate in the study. Questions were administered to the respondents and baseline information was obtained.

#### **4.1.3.2 Background information**

General information regarding age, gender, types of family, presence of disease, medication details was gathered.

#### **4.1.3.3 Food groups specific questionnaire**

A detailed questionnaire was developed to gather information upon occurrence of gastrointestinal symptoms such as vomiting, diarrhoea, nausea etc post consumption of various food types like: milk and milk products, cereal products, pulses, fruits, vegetables and fermented foods (Annexure 1).

### **4.1.4 Exclusion and Inclusion criteria to participate for Hydrogen Breath Analyser test**

#### **4.1.4.1 Exclusion criteria**

- Ingestion of laxatives, or prebiotics 4 weeks before participation
- Taking supplementation of calcium or vitamin D
- Presence of any chronic disease
- Lactase supplementation
- SAM children

#### **4.1.4.2 Inclusion criteria**

- Children, adults and geriatric individuals suffering from lactose intolerance based on the HBA test
- Subjects giving their consent to participate in the study.

#### **4.1.5 Identification of lactose intolerant subjects by means of Hydrogen Breath Analyser test and their enrolment for the study.**

Subjects who reported to suffer from gastric discomfort after consumption of milk and milk products and who met the inclusion criteria were enrolled for conducting **Hydrogen breath analyser test** (HBT) post obtaining their consent (annexure 2).

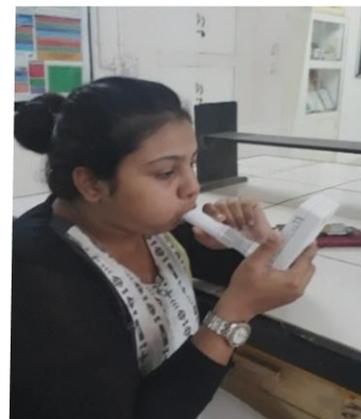
##### ***4.1.5.1 Hydrogen Breath Analyser Test Procedure***

Hydrogen breath analyser test is conducted using a Hydrogen breath analyser. In this the individual is required to exhale out into the mouth piece of the breath analyser post consumption of specific substrate. We administered orally 25gm of lactose powder dissolved in 250ml of water to subjects and they were asked to breath out six times into the device after a period of 30 minutes each (wbm health sciences). (Pate 1 and plate 2).



**Plate 1**

**Plate 1- HBT test being conducted on children**



**Plate 2**

**Plate 2- HBT test being conducted on adults**

**4.1.5.2 Subject preparation prior to conducting Hydrogen Breath Analyser test.**

Subject preparation is a vital aspect of conducting the test. Several factors such as medications, foods consumed before conducting the test might have detrimental effects on the results obtained. So, precautions need to be taken.

**4.1.5.2.1 Four weeks prior to test following measures were asked to be taken by the subjects.**

- i. Making sure that no antibiotics are taken four weeks prior conducting the test.
- ii. Having a colonoscopy within four weeks before taking the test can cause contraindications
- iii. Having fluoroscopy within four weeks before taking the test can cause contraindications

**4.1.5.2.2 One week prior to test following measures were asked to be taken by the subjects.**

- i. Non usage of stool hardeners or softeners
- ii. Avoidance of vitamins or supplements containing fructose flavouring or lactose fillers.
- iii. Avoidance of fibre supplements, Imodium or pepto bismol
- iv. Avoidance of pre and probiotics
- v. Avoid proton pump inhibitors and hydrogen blockers

**4.1.5.2.3 Three days prior the test following measures were asked to be taken by the subjects.**

Around three days prior to the test certain type of diet needs to be followed and foods need to avoided.

**i. Foods to be avoided**

Lactose intolerant subjects have been asked to avoid foods which are high in fructose, foods high in refined sugar, fats and oil and spices.

**ii. Foods to be consumed**

Lactose intolerant subjects have been asked to consume foods which are

grilled, low in spices and fats.

#### **4.1.5.2.4 Previous night before conducting test**

Lactose intolerant subjects were instructed to avoid high fats and spice rich food the previous night.

#### **4.1.5.2.7 On the day of test**

- i. Hydrogen breath analyser test was conducted on an empty stomach in the morning.
- ii. Subjects were allowed to drink water however no food was allowed to consume during the entire period of test.
- iii. Do not take any medication
- iv. No smoking
- v. No chewing gums
- vi. No sleeping during the procedure
- vii. If any discomfort was observed during the test, provision was made to attend to the patient.

#### **4.1.6 Statistical Analysis**

Collected data were entered into a Microsoft excel sheet. Data analysis was performed using Statistical Package of Social Sciences (IBM SPSS Statistics 25). Results were expressed in terms of Mean  $\pm$  Standard deviation of lactose intolerant and non-lactose intolerant subjects. Pearson's correlation and chi-square test was used to analyse the data.

## **Phase II- Impact of supplementing Lactose hydrolysed milk (LHM) to lactose intolerant subjects.**

In this phase, baseline information is taken upon BMI, dietary intake and Quality of life and then lactose hydrolysed milk was supplemented to the lactose intolerant subjects post obtaining their consent. The methods used to collect above parameters are explained under the following sections:

Section 4.2.1- BMI of the lactose intolerant subjects.

Section 4.2.2- Dietary intake of the lactose intolerant subjects.

Section 4.2.3- Quality of life of the lactose intolerant subjects.

Section 4.2.4- Supplementation with Lactose Hydrolysed Milk (LHM)

Section 4.2.5- Statistical analysis

### **4.2.1- BMI of the lactose intolerant subjects**

Anthropometry is the science of obtaining systematic measurements of the human body. It is portable, accessible, non-invasive technique to measure human body.

**Height** – Body height is the head-to-toe measurement of a human body, from the feet to the head of the individual.

**Procedure**- A spring- loaded non-stretchable tape was used to measure the height of the subjects. we identified one convenient flat wall for the measurement of height. The subject was made to stand barefoot with the arms hanging freely by the side. Heels of the feet were placed together with the medial (inner) border of the feet at an angle of 60 degrees. The scapula and the buttock were ensured to be in contact with the measuring wall. The head was held in the Frankfort plane (with the tragus of the ear and the lateral angle of the eye in a horizontal line). Height was recorded to the nearest 0.1 cm after the subject inhaled fully and maintained the erect position without altering the load on the heels. In this position, a mark was made on the wall and height was recorded with a measuring tape. Two consecutive reading were taken.

School had their own stadiometer so children were instructed to stand straight upon it with their hands on both the side. The measuring bar was brought down upon the child's head and reading was taken. Two readings were taken to avoid error.

**Weight-** Body weight it is the most commonly used anthropometric measurement. Also, convenient to execute.

**Procedure-** Karada scale (Omiron®) was used to calculate the body weight of the individuals. Subjects were asked to stand straight upon the weighing scale. Children's weight was analysed using the software WHO Anthroplus™.

This procedure was performed both before and after supplementation.

#### **4.2.1.1 Computing Body Mass Index (BMI) (Nutrition science, Srilakshmi B 2006)**

**BMI was calculated using the formula:**

$$\text{BMI (kg/m}^2\text{)} = \frac{\text{Weight (kg)}}{\text{Height (m}^2\text{)}}$$

**Table 4.2.1.1- BMI range (Asia pacific) (Karmacharya P et al 2019):**

<b>Categories</b>	<b>BMI range</b>
<b>Underweight</b>	<b>&lt;18.5</b>
<b>Normal</b>	<b>18.5-22.9</b>
<b>Overweight</b>	<b>23-24.9</b>
<b>Obese</b>	<b>≥ 25</b>

#### **4.2.2- Dietary intake of the lactose intolerant subjects.**

Dietary intake of the subjects was performed using 24 hr dietary recall method. Individuals were asked to mention their daily food intakes continuously for a period of 3 days. Three days comprising of 2-week days and 1 weekend. So, the interviewer gets an idea of all the food consumed during weekday and weekend by the subjects (annexure 3). This information was taken to determine the amount of energy, carbohydrate, protein, fat, carotenoids, calcium etc. The subjects were asked to mention detailed information of all the major foods consumed along with their quantity. The subjects were asked to mention snacks, beverages etc consumed (Nutrition science, Srilakshmi B 2006).

Nutrient content of diet was calculated using DietCal® Software (Gurdeep Kaur, RD, AIIMS) containing the data from the book- Longvah T, Ananthan R, Bhaskaracharya K, Venkaiah k. Indian Food Composition Tables. National Institute of Nutrition, Indian Council of Medical Research, 2017 (Referred commonly as IFCT 2017). This procedure was performed both before and after supplementation.

#### **4.2.3- Quality of life of the lactose intolerant subjects.**

WHO defines Quality of Life (QOL) as an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standard and concerns. A semi-structured questionnaire (annexure 4) was developed in relation to lactose intolerance. This questionnaire was administered to our subjects. score obtained determined the subject's QOL. Higher score indicated poor QOL whereas less score referred to good QOL. This procedure was performed both before and after supplementation.

#### **4.2.4- Supplementation with Lactose Hydrolysed Milk (LHM)**

##### ***4.2.4.1 Procurement of Lactose Hydrolysed Milk (LHM)***

LHM was procured from GCMMF, Anand, Gujarat, India; commercially available in the name of Lactose free milk.

##### ***4.2.4.2 Dosage and duration of supplementation***

250ml of LHM was supplemented to lactose intolerant subjects daily for a period 6 weeks.

##### ***4.2.4.3 Compliance sheet***

Lactose intolerant subjects undergoing lactose hydrolysed milk supplementation were provided with a compliance sheet for a period of 6 weeks and were checked upon every 3<sup>rd</sup> day. They were instructed to put a tick mark to each day they consume the milk and not denote the day with cross, the day they haven't consumed it (Annexure 4).

#### ***4.2.4.4 Feedback from lactose intolerant subjects***

A feedback form was developed to assess lactose intolerant subject's response post supplementation (Annexure 6).

#### **4.2.5 Statistical Analysis**

Collected data was entered into a Microsoft excel sheet and data was analysed using Statistical Package of Social Sciences (IBM SPSS Statistics 25). Results were expressed in terms of Mean  $\pm$  Standard deviation for nutrient consumed. T-test was applied to analyse it.

### **Phase III- Organoleptic evaluation of food products developed from standard and lactose hydrolysed milk**

In this phase food products were developed from both lactose hydrolysed milk and standard milk and organoleptic evaluation of all the various food products was conducted. This phase is explained in the following sections.

#### 4.3.1 Development of food products from standard dairy and lactose hydrolysed milk

##### 4.3.1.1 Procurement of Lactose Hydrolysed Milk (LHM) and standard milk

##### 4.3.1.2 Selection of food products

##### 4.3.1.3 Procurement of raw ingredients

##### 4.3.1.4 Standardising recipe

##### 4.3.1.5 Selection and training of panellist for organoleptic evaluation

#### 4.3.2 Statistical Analysis

### **4.3.1 Development of food products from standard and lactose milk**

Preparation of two food products from each category viz beverage, main course and dessert was done. These included cold cocoa, rose milk, white sauce pasta, vegetable au gratin, *sandesh* and *kheer* respectively.

#### ***4.3.1.1 Procurement of Lactose Hydrolysed Milk (LHM) and standard milk***

Lactose hydrolysed Milk was procured from GCMMF (write full form), Anand, Gujarat, India in quantity of 250ml per tetra pack. It had beta galactosidase rendering less than 0.01g of lactose in it. However, all the other nutritive features were same. Standard milk from the same company were procured commercially.

#### ***4.3.1.2 Selection of food products***

Food products which are completely milk based were selected to understand the replacement of standard milk with lactose free milk. Care was taken to select food products from each category: beverage, main course and dessert to make sure lactose intolerance should not be a reason to

prohibit individuals from such food consumption. All the food products prepared from standard milk and lactose hydrolysed milk were studied for their organoleptic qualities (plate 3).



**Plate 3- Preparation of Sandesh from standard dairy milk**

#### ***4.3.1.3 Procurement of raw ingredients***

All the ingredients for the preparation of various food products were procured from local market and online market. Detailed ingredients of each recipe and their commercial brand are mentioned in the table 4.3.3

**Table 4.3.3- Ingredients used along with their brand in each food preparation**

<b>Name</b>	<b>Ingredients</b>	<b>Source/Brand name</b>	
<b>Cold cocoa</b>	Cocoa powder	Hershey's	
	Sugar	Local market, Vadodara	
	Corn flour	Weikfield brand, Vadodara	
<b>Rose milk</b>	Rose syrup	Mapro brand, Vadodara	
	Sugar	Local market, Vadodara	
<b>White sauce pasta</b>	Butter	Amul brand, Vadodara	
	Refined oil	Fortune brand, Vadodara	
	Pasta	Bambino brand, Vadodara	
	Salt	Local market, Vadodara	
	Oregano	Keya brand, Vadodara	
	Chilli flakes	Keya brand, Vadodara	
	Black pepper	Catch brand, Vadodara	
	<b>Veg au gratin</b>	Butter	Amul brand, Vadodara
		Refined oil	Fortune brand, Vadodara
Salt		Local market, Vadodara	
Oregano		Keya brand, Vadodara	
Chilli flakes		Keya brand, Vadodara	
Vegetables- carrots, capsicum, onions, tomatoes.		Local market, Vadodara	
<b>Sandesh</b>	Clove	Local market, Vadodara	
	Sugar	Local market, Vadodara	
	Raisins	Local market, Vadodara	
<b>Kheer</b>	Vermicelli	MTR brand, Vadodara	
	Cashew	Local market, Vadodara	
	Raisins	Local market, Vadodara	

**4.3.1.4 Standardising recipe of all the food products:**

- a. **Cold cocoa-** It's a beverage made from milk of 500 ml and cocoa powder. In this milk is boiled with sugar and cocoa powder, and thickened with a mixture of cornflour dissolved in water.
- b. **Rose milk-** It's a beverage made from milk of 500 ml and rose syrup. Sugar is added to adjust in sweetness.
- c. **White sauce pasta-** It is an Italian main course delicacy in which 250 gm pasta is cooked in white sauce. The white sauce is prepared using 500ml of milk.
- d. **Vegetable au gratin-** It is an Italian main course in which vegetables are cooked in white sauce. The white sauce is prepared using 500ml of milk.
- e. **Sandesh-** It is a dessert prepared from 500ml of milk and sugar is added to sweetened it along with clove is added into it.
- f. **Kheer-** It is a dessert which has porridge like consistency. Vermicelli is cooked in 500 ml of milk and sugar is added to sweetened it.

**4.3.1.5 Selection and training of panellist for organoleptic evaluation**

- a. **Screening of semi-trained panellist:** In this section panel members were selected and trained further. Threshold test was conducted to select the panel members. Threshold test was conducted among students and staff members from the department of foods and nutrition to determine the panellists. Threshold is defined as a point on the stimulus scale at which a transition in a series of sensations or judgments occur. It measures an individual's ability to smell, taste or feel specific characteristics which is required for evaluating food products during research and development. In this study, while conducting threshold test, panel members were given different degree of four types of solutions namely – sweet, sour, salty and bitter. Individuals were asked to taste it and determine its strength in varying degree (mild, strong, very strong etc) and mention in the form (Annexure 7i, annexure 7ii) given to them. Subjects who pass the threshold test were included to conduct the organoleptic evaluations of the food products developed from standard milk and lactose hydrolysed milk (annexure 7) (Plate 4 and plate 5).



**Plate 4- Threshold test set up**



**Plate 5- Threshold test being conducted by the panellist**

- b. Development of score card for organoleptic evaluation:** Hedonic scale was used to score the food products developed from both standard and lactose hydrolysed milk on criteria such as: texture, aroma, odour, after taste etc (annexure 8).

#### **4.3.2 Statistical Analysis**

Collected data was entered into a Microsoft excel sheet and data was analysed using Statistical Package of Social Sciences (IBM SPSS Statistics 25). Results were expressed in terms of Mean  $\pm$  Standard deviation for nutrient consumed. Student t-test was applied to analyse the organoleptic evaluation of food products developed from standard and Lactose Hydrolysed Milk (LHM).

## **Phase IV- Development of IEC material**

It is being created to spread awareness and educate common people about lactose intolerance.

The IEC material pictorially depicts the following components:

- I. What is lactose intolerance
- II. Causes and types of lactose intolerance
- III. How to identify lactose intolerance
- IV. Recipes developed from lactose hydrolysed milk