



## **RESULTS AND DISCUSSION**

## **CHAPTER 5**

### **RESULTS AND DISCUSSION**

The present study was planned with a major aim to analyze selected Indian foods for inulin content using HPLC technique, acceptability trials of inulin incorporated recipes and the health effects of supplementing the diets of institutionalized elderlies with probiotic and synbiotic fermented milk. The results of the present study are described, discussed and interpreted in accordance with the objective of the study in the following three sections as follows:

- Section 5.1: Determination of Inulin content of selected raw and processed foods.
- Section 5.2: Development and acceptability trials of inulin incorporated products.
- Section 5.3: Effect of supplementation of probiotic and synbiotic fermented milk in diets of institutionalized elderlies.

#### **Section 5.1: Determination of Inulin Content of Selected Raw and Processed Foods.**

Inulins are polymers that are widely used in several industrial applications. In last few years they have received increasing interest because of its positive health benefits. So far, few studies are available reporting its presence in commonly consumed foods.

This section of the study focuses on the results obtained regarding the inulin content for 26 raw and cooked Indian food samples, using High Performance Liquid Chromatography, according to the methodology outlined in chapter 4. The major findings of this section of the study are presented under following subsections.

- Section 5.1.1. Standardization of HPLC technique for inulin analysis
- Section 5.1.2. Inulin content of cereals, pulses, vegetables, fruits and spices.
- Section 5.1.3 Determination of inulin content of the processed wheat based foods.

### **Section 5.1.1. Standardization of HPLC Technique for Inulin Analysis**

**Standardizing HPLC analysis:** The peak areas of repetitive injections of either samples or standard sugars differ to some extent due to concentration variation. The errors associated with repetitive injection are: (a) the precise injection of small quantities of samples is very difficult, and (b) detector responses fluctuate with time. In the following experiment an auto sampler was used to avoid the first error and an experiment with different levels of standards was carried out to check the second factor. Precisely water based standard solutions containing different concentrations 1000, 2000, 3000 and 4000 ppm of fructose was prepared and injected in replicates into HPLC with similar operating conditions. The chromatograms of various concentrations are depicted in appended in appendix I (i).

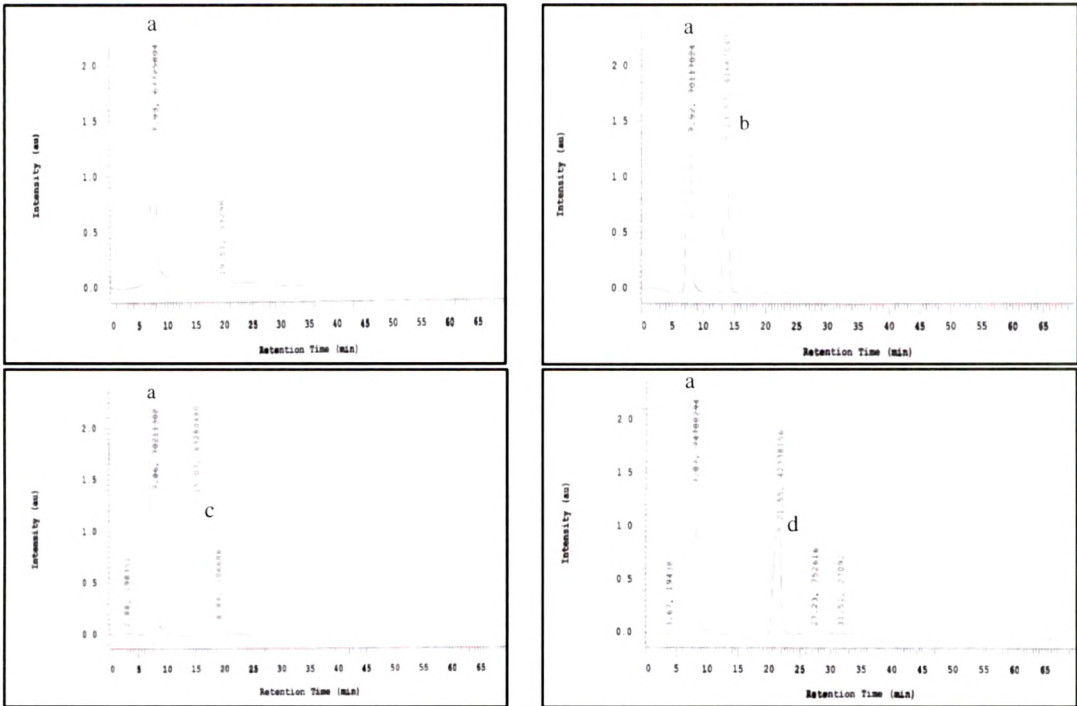
The standard curve plotted for various concentrations is depicted in appendix I (ii), which shows that there is no significant variation in the determined concentration, indicating that the detector response is valid at various concentrations of sugars. Such validation of detector response is essential to extrapolate the results in samples with different levels of sugars.

The standards of fructose (D-fructose extra pure Qualigens ltd.), glucose (D-glucose pure Qualigens ltd) and sucrose (Sucrose pure-merck chemicals ltd) were used as calibration samples (Fig 5.1.1 (i)-(iv)). Inulin ( $\beta$ -fructan) is the fructose remaining after hydrolysis as per the method mentioned in Methods and Materials, chapter four.

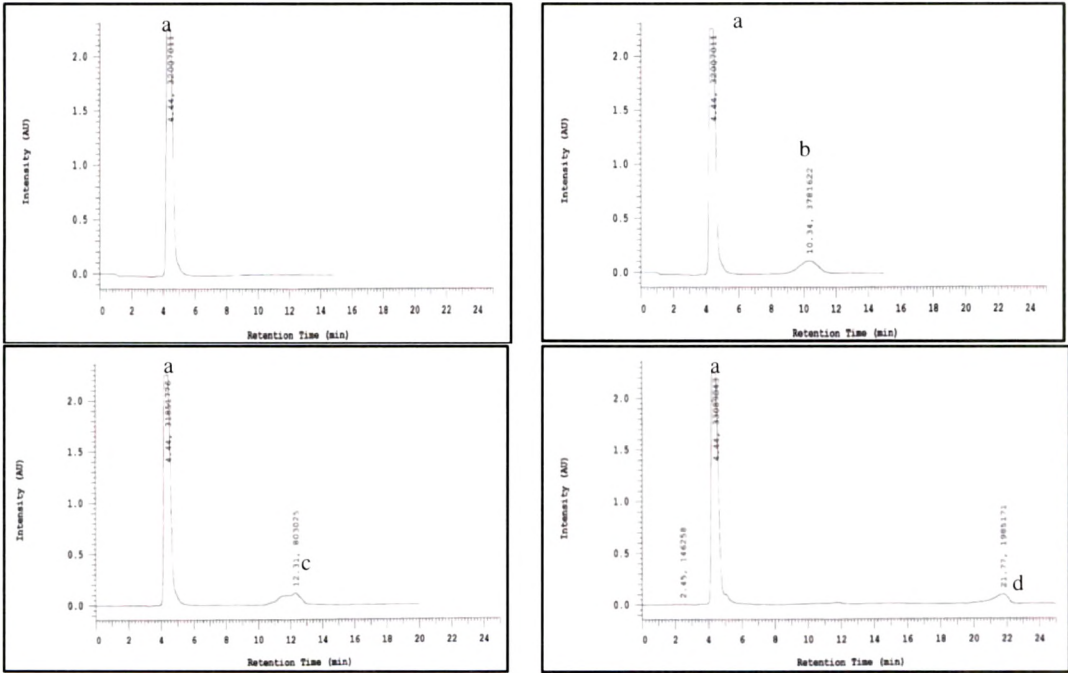
After standardizing and quantifying the standard sugars by HPLC, fructans (inulin) extracted from the five food groups namely cereal, pulses, fruits and vegetables and spices were determined. The concentration of inulin was calculated on dry matter basis taking into account the moisture content of foods estimated and the results of moisture content are presented in chapter 4.

### **Section 5.1.2. Inulin content of Cereals, Pulses, Vegetables, Fruits and Spices**

Samples of 6 variety of cereals, 5 variety of legumes, 8 samples of fruits, vegetables and spices and 7 samples of processed foods were analyzed using HPLC.

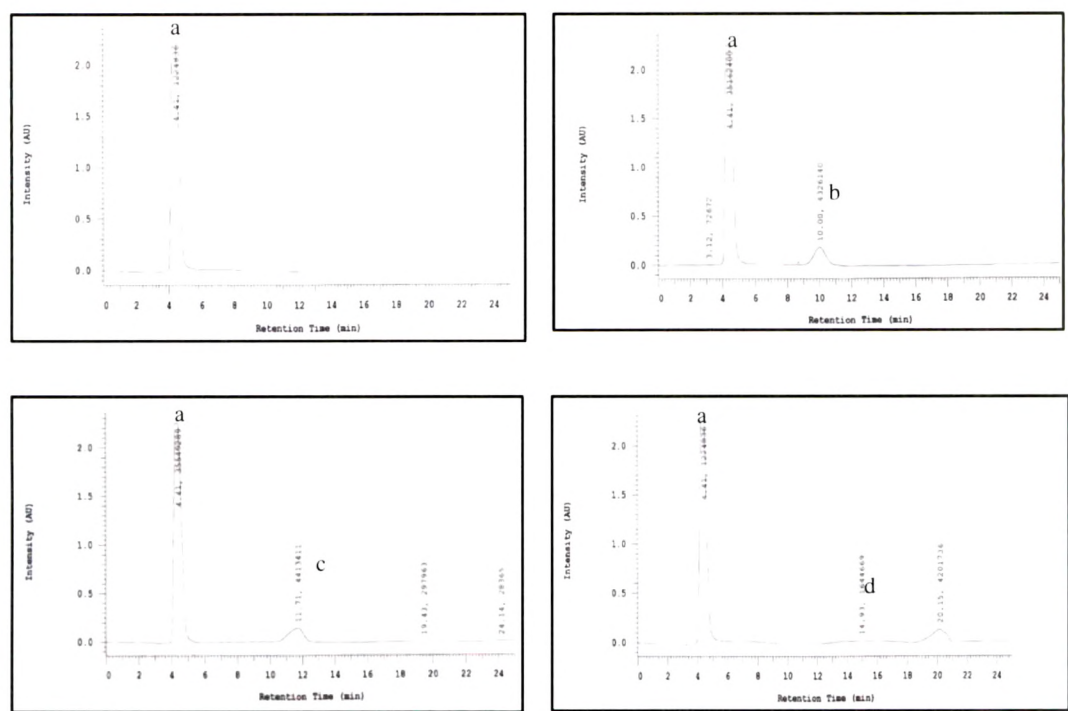


**Fig.5.1.1.(i) Chromatograms depicting standard for (a) blank: (b) Fructose (c) glucose; (d) Sucrose**

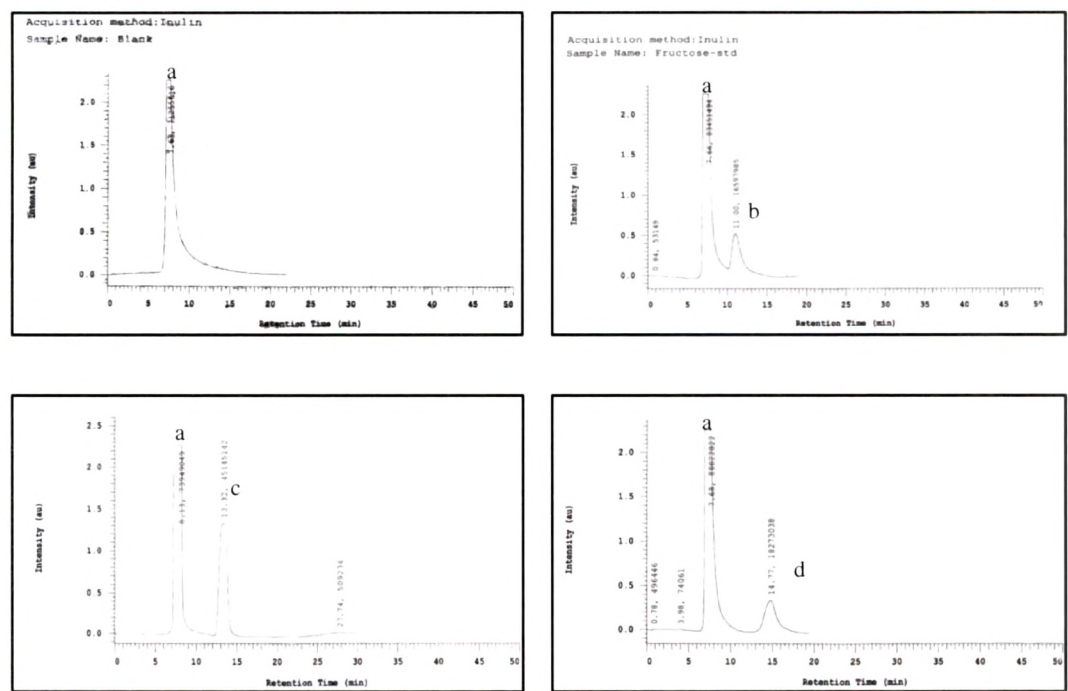


**Fig.5.1.1.(ii) Chromatograms depicting standard for (a) blank: (b) Fructose (c) glucose; (d) Sucrose**





**Fig.5.1.1.(iii) Chromatograms depicting standard for (a) blank: (b) Fructose (c) glucose; (d) Sucrose**



**Fig.5.1.1.(iv) Chromatograms depicting standard for (a) blank: (b) Fructose (c) glucose; (d) Sucrose**

**a) Inulin content of cereals**

As seen in Table 5.1.1.a and represented in chromatograms Fig.5.1.2, out of five cereals analyzed, wheat (*Triticum aestivum*) had highest content of inulin and it ranged from 0.01g% - 2.3g%. Out of three varieties of wheat, GW-496 had the highest content (2.3 g %). Only a trace amount of inulin was observed in rice sample whereas, the pearl millet and maize showed absence of inulin in the range of detection (0.001).

**Table 5.1.1 (a): Inulin Content of selected Cereals in Indian Diets**

Cereals	Inulin g%
Wheat-arnej8206	0.82
Wheat-GW496	2.30
Wheat -GW1	0.01
Pearlmillet	ND*
Rice	0.03
Maize	ND*

\*ND: Not Detected

**b) Inulin content of pulses and legumes**

Amongst the pulses viz; green gram, black gram, red gram, bengal gram and soyabean legumes analyzed, inulin was not detected in any of the five types of legumes [Table 5.1.1 (b) and Fig 5.1.3].

**Table 5.1.1 (b): Inulin content of selected raw pulses and legumes**

Pulses and Legumes	Inulin g%
Green gram	ND*
Bengal gram	ND*
Black gram	ND*
Red gram	ND*
Soyabean	ND*

\*ND: Not Detected

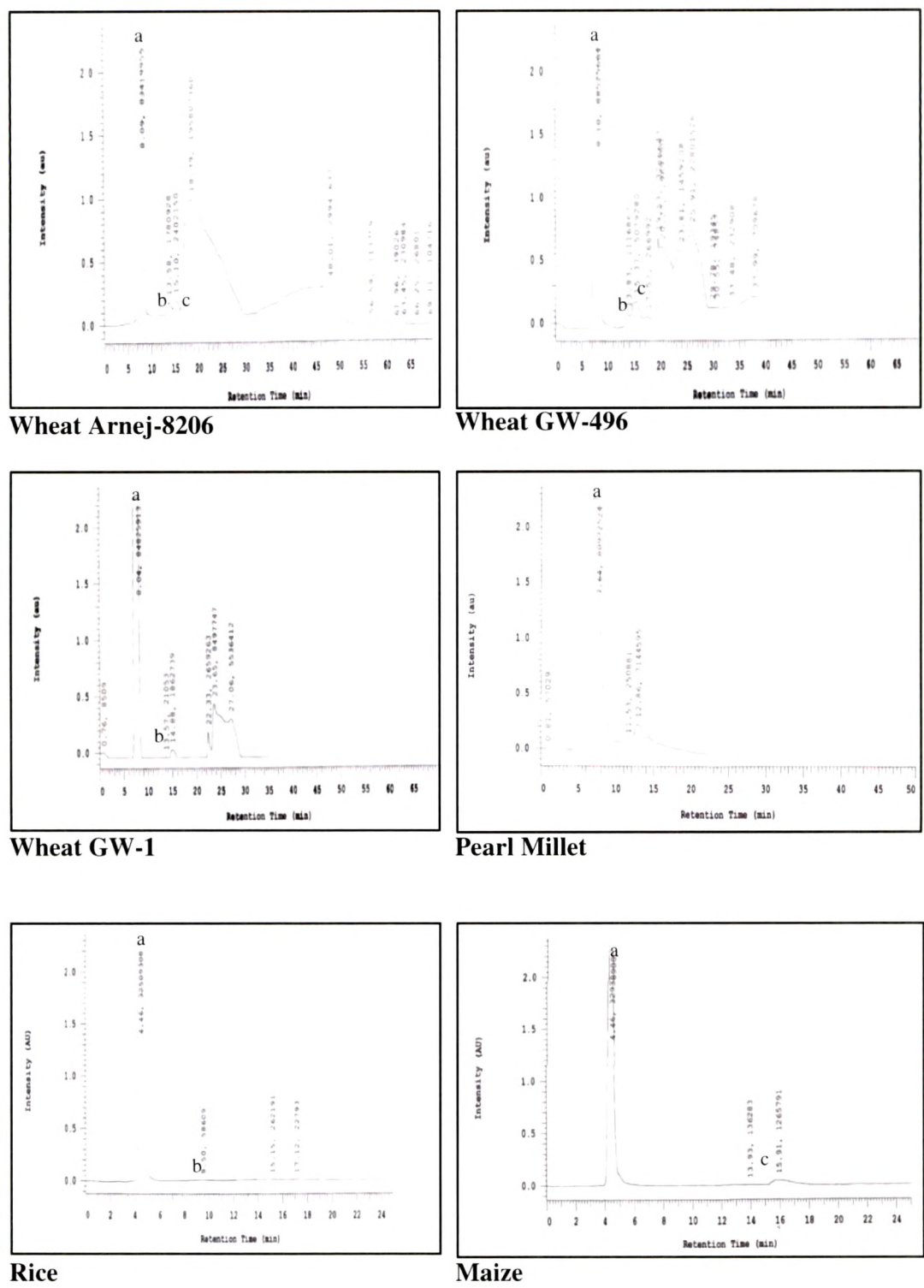
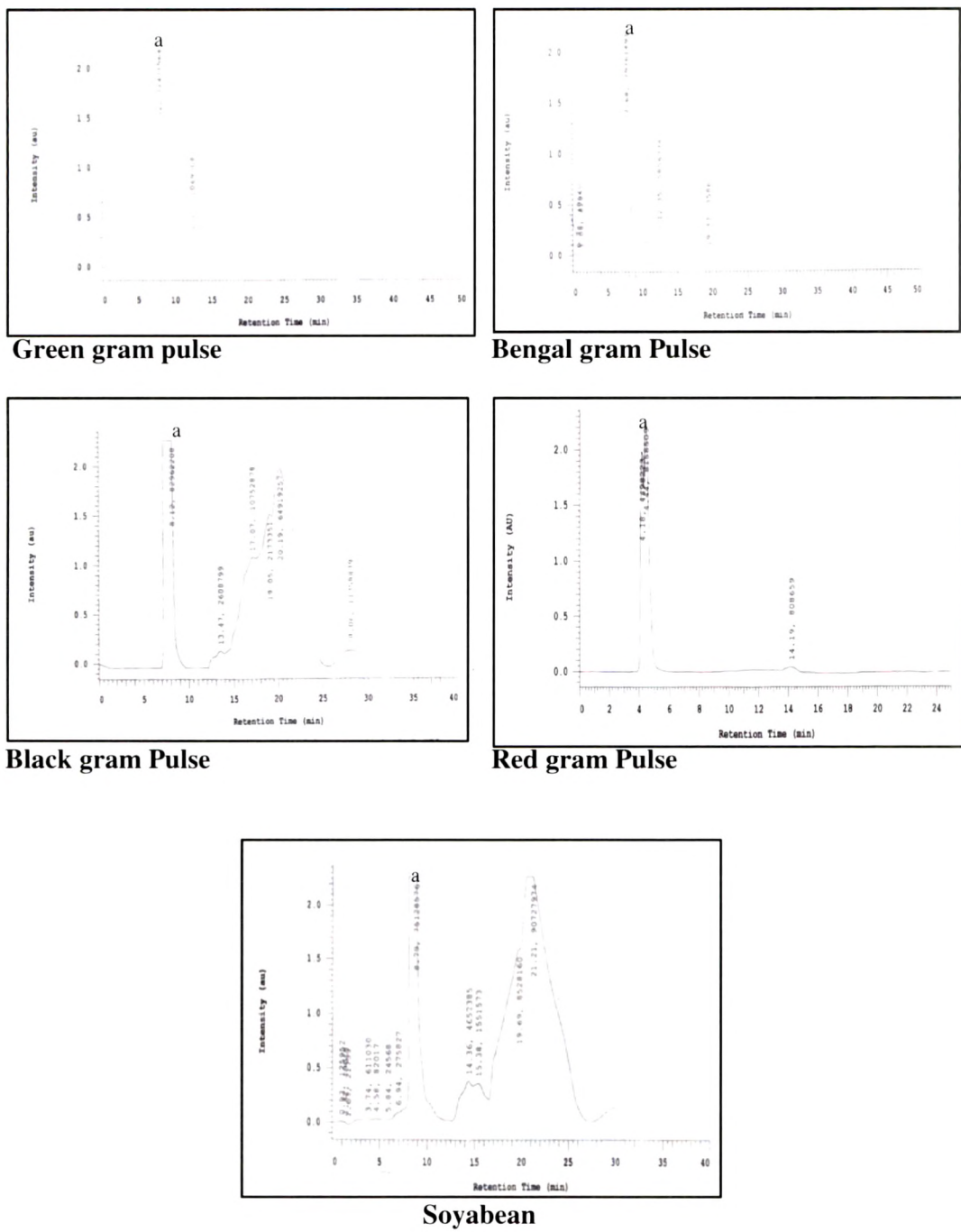


Fig.5.1.2 Chromatograms of the distribution of inulin chains in cereal grains (a) blank: (b) Fructose (c) glucose; (d) Sucrose



**c) Inulin content of vegetable, fruits and spices**

As depicted in Table 5.1.1.c and represented in Fig 5.1.4, vegetables like cabbage, spinach, brinjal and fenugreek leaves showed occurrence of inulin in the range of 0.02g% (Fenugreek) to 0.56g% (Spinach). Apple showed presence of 0.88g% inulin.

As seen in Table 5.1.1c and Fig.5.1.5, high levels of inulin were detected in garlic (12.71 g %) and onion (10.92g%) with negligible amount in fenugreek seeds.

**Table 5.1.1(c): Inulin content of selected raw fruits, vegetables and spices in Indian diets on wet weight basis**

Fruits vegetables and spices	Inulin g%
Apple	0.88
Fenugreek	0.021
Spinach	0.56
Brinjal	0.38
Cabbage	0.3
Garlic	12.71
Onion	10.92
Fenugreek seeds	0.002

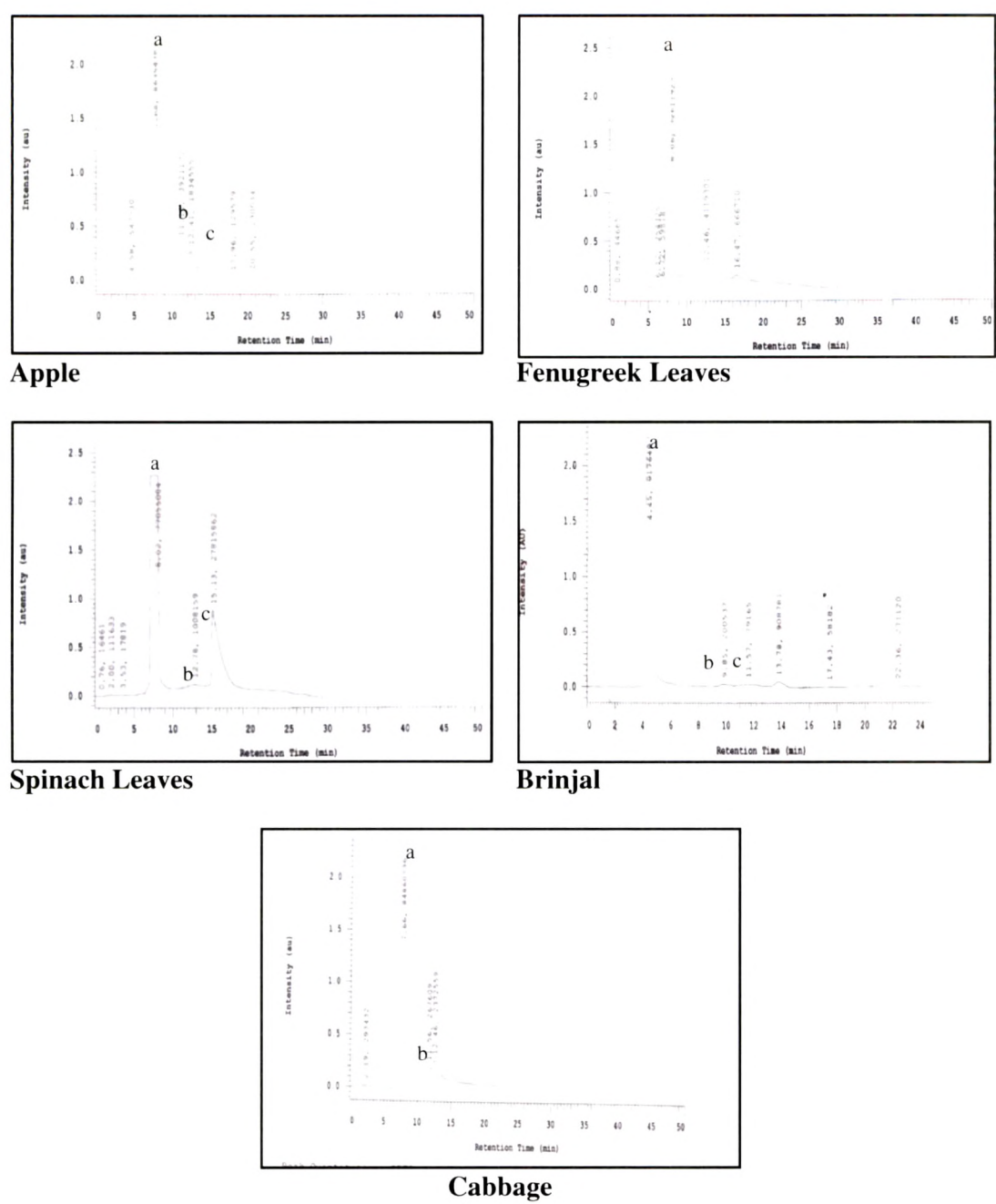
**d) Inulin content of selected processed foods**

Inulin content of selected processed foods such as oat bran, wheat bran and banana chips are represented in Table 5.1.1(d) and Fig. 5.1.6. Inulin was not detected in oat bran and banana chips however it was present in very small quantity in wheat bran (0.03g %).

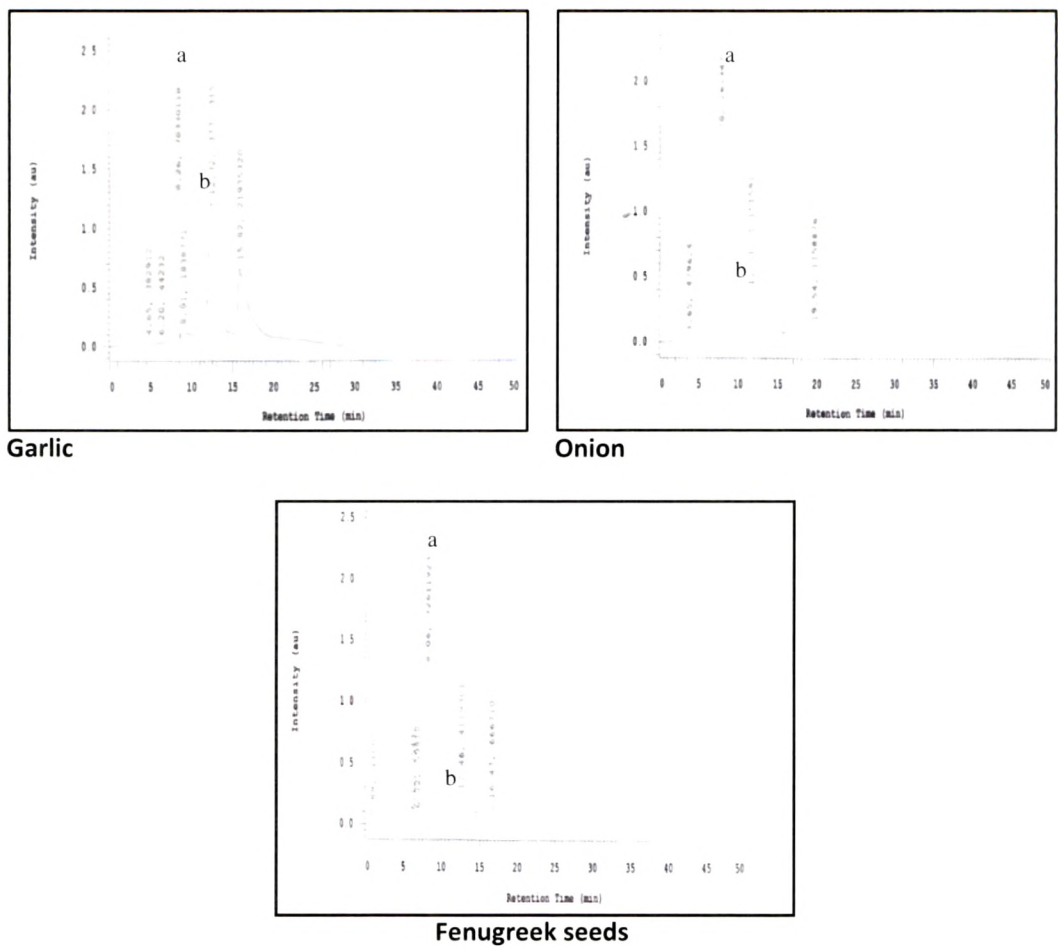
**Table 5.1.1(d): Inulin content of selected processed foods**

Processed Foods	Inulin g%
Oat bran	ND*
Wheat bran	0.03
Bread	2.2
Banana chips	ND*

\*ND: Not Detected



**Fig.5.1.4 Chromatograms of the distribution of inulin chains in fruits and vegetables (a) blank: (b) Fructose (c) glucose; (d) Sucrose**



**Fig. 5.1.5** Chromatograms of the distribution of inulin chains in roots and tubers, spices (a) blank: (b) Fructose (c) glucose; (d) Sucrose



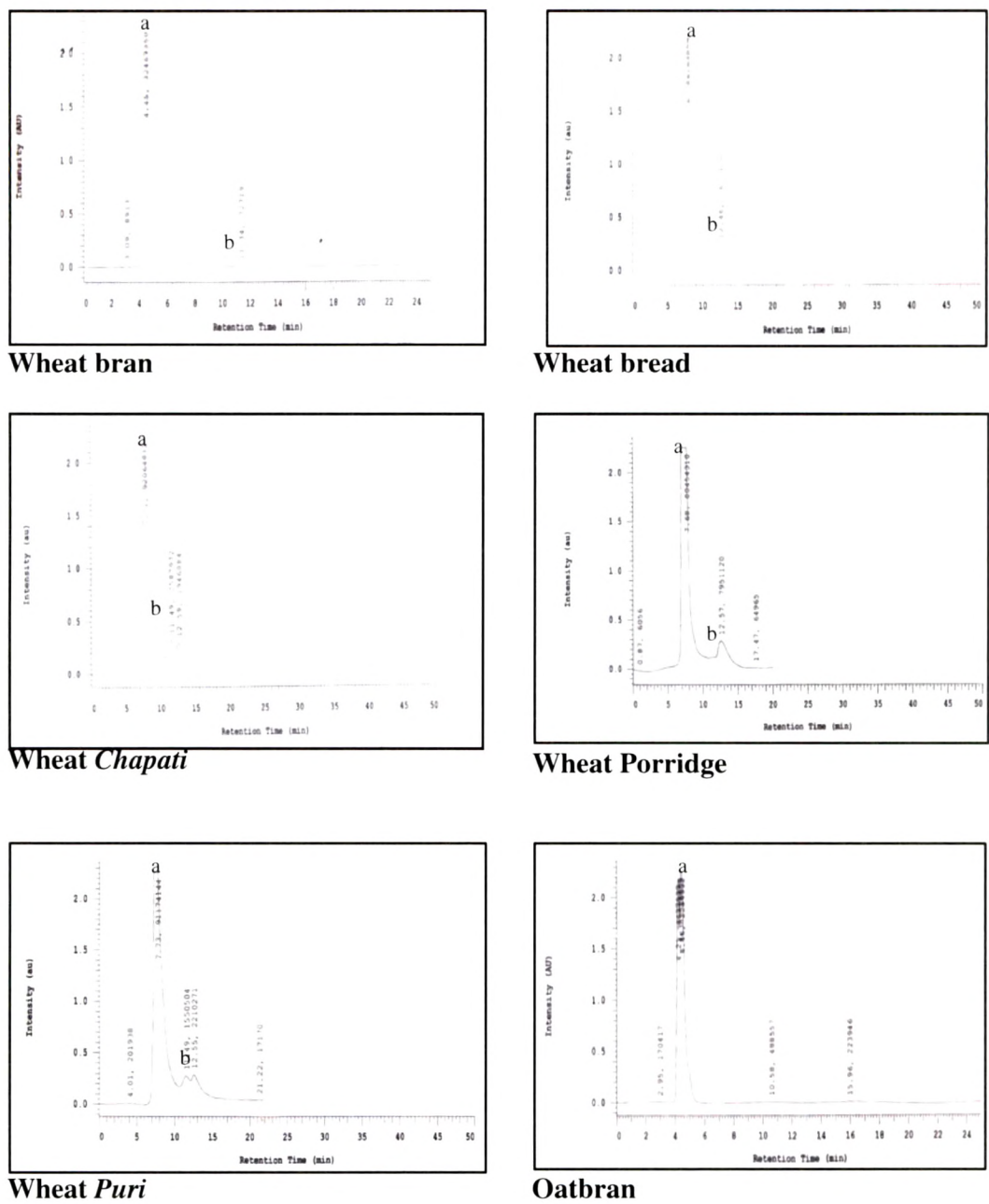


Fig. 5.1.6. Chromatograms of the distribution of inulin chains in processed wheat products (a) blank: (b) Fructose (c) glucose (d) Sucrose



Section 5.1.3 Processing Changes in Inulin Content of Wheat and Wheat Products

Processing of wheat is popular worldwide and the most popular processed wheat product in India is '*chapati*'. Mishra et al (1998) reported that 85 % of the wheat produced in India is consumed in the form of '*chapati*'. The inulin content of the processed wheat products prepared from GW-496 variety (Table 5.1.2) ranged from 1.9 g % for fried wheat product (puri) to 2.4 g% for roasted wheat product (*chapati*) Bread procured from the market prepared by unknown variety of wheat also showed high content of inulin (2.2 g%) (Fig.5.1.6).

Table 5.1.2: Processing changes in inulin content of wheat and wheat products

Brand	Process	Inulin content(g%)	Percent Change
Wheat –GW 496	unprocessed	2.3	-
Chapati(GW 496)	Roasting	2.4	4g%
Porridge (GW 496)	Steaming	1.2	47g%
Puri (GW 496)	Frying	1.9	17g%

\*ND: Not Detected

DISCUSSION

Whole grains provide a wide range of nutrients and phytochemicals that optimize health. Epidemiologic studies support the protectiveness of whole grain consumption for cardiovascular disease and cancer. Dietary guidance endorses increased whole grains in our diet. Among whole grains, wheat is an important food grain and a major source of calories, protein and minerals of the people of this region. Hill et al (1978) mentions wheat as a natural source of inulin. Nilsson and Dahlquist (1989) and Rumessen (1983) demonstrated the presence of 1 to 4 g% (on DM) fructans in wheat flour. According to Van Loo et al (1995) inulin content of wheat is 2.5 g %. Varietal differences were present in inulin content in wheat. The results of the present study are in line with the values reported in literature.

Rice is the most common cereal consumed all over the world. Low content of inulin was detected in rice in the present study. No published literature on inulin content of rice is available for comparison. However studies show that rice has soluble dietary

fibre content of 0.92 g % on dry matter basis (Ramulu et al 1997). Inulin although categorized as a soluble dietary fibre was absent in most of these cereals. Ramula et al 1997 reported very low content of 0.27 g %, 0.26 g % and 0.14 g % of soluble dietary fibre in maize, bajra and sorghum respectively.

Although food legumes contains oligosaccharides (31%) (Asami et al 1989). Inulin, a non digestible oligosaccharides was absent in the legumes under study. Ruparez Pilar (2004) also reported very small amounts of inulin in the processed and raw legumes when analyzed using HPLC method.

Fruits and vegetable contain constituents, notably vitamins, minerals and dietary fiber, essential to a healthy, well balanced diet. Furthermore it has been shown that some of the secondary metabolites of fruits and vegetables, such as flavonoids and carotenoids are beneficial to health in directly combating onset of various diet related NCDs. However when selected vegetables and fruit like fenugreek leaves, spinach, cabbage, brinjal and apple were analysed for inulin content (being classified as a soluble dietary fibre) using HPLC in the present study, the results showed occurrence of inulin in the range of 0.02 - 0.88 %. There is no published data reported for the inulin content of these vegetables and fruit. Few researches have analyzed soluble dietary fibre and prebiotic oligosaccharide content of few of these vegetables and fruits (Ramulu et al 2004, Siddeshwar et al 2008).

The inulin content is naturally high in onion and garlic (Van Loo et al 1995). Espinos and Rico (2006) showed inulin was high in garlic (13.5 g %), followed by onion (1.4 g %). Inulin is a precursor to produce a number of oligosaccharides by hydrolysis. Inulin content for onion in the present study is slightly higher to the results reported by the other researchers (Van loo et al 1995, Espino and Rico 2000, Homme et al 2003). The average inulin content of onion as reported by Van loo et al 1995, is 9.6 g% whereas in a study carried out by Rodriguez et al 2008, percent inulin content of onion ranged from 4.23 to 1.33 g% depending upon the source of cultivation.

Inulin content of wheat subjected to processing treatments like cooking by boiling, frying, steaming and roasting have been reported in the present study. Of the various processes adopted for the preparation of wheat product, steaming led to the maximum loss of inulin followed by frying and roasting. Processes involving heat-treatment may affect the dietary fibre in different ways. An increased temperature leads to a breakage

of weak bonds between polysaccharide chains. Also glycosidic linkages in the dietary fibre polysaccharides may be broken. During heat treatment there may be decreased association between fibre molecules and / or depolymerization of the fibre, resulting in solubilization. If the depolymerization is extensive, alcohol soluble fragments may be formed, resulting in a decreased content of dietary fibre. During wet heat treatment, as in blanching, boiling and canning of vegetables and fruits, there is a considerable loss of low molecular weight carbohydrates (i.e. mono- and disaccharides) as well as micronutrients, into the processing water for example, during blanching of carrots and swedes (rutabagas) a loss of 25 g% and 30 g% of these carbohydrates was observed respectively. With subsequent boiling another 20g% was lost (Nyman et al 1987). The loss of glucose and fructose at boiling was higher than that of sucrose (Svanberg et al 1997). Bekers et al 2007 evaluated the influence of temperature and action time on the extraction rate of carbohydrates of Jerusalem artichoke concentrate powder and inactivation of inulin during boiling and sterilization. Results showed that inulin was not lost during evaporation but as content of total carbohydrates it decreased during boiling. Ramulu et al 1997 reported no effect of processing wheat flour into *chapati* on the total dietary fibre content. Effect of food processing on fructooligosaccharides (FOS) in fruits such as stewed apple and banana was studied and the results showed the content of 1- ketose was much higher in raw material than the finished product indicating cooking is responsible for FOS loss (Homme et al 2003). A study carried out by Parchure et al 1997 showed decrease in RS content of starches of rice flour fried as *puris* attributing to the formation of more favored lipid amylase complex. Effect of temperature changes on sucrose, fructose and glucose contents in rice grain of two different varieties was studied by Cheng et al 2006 showed a significant increase in sucrose content occurred without any increase in fructose and glucose contents indicating no change in fructose content of rice due to processing at high temperatures. It must be noted that there are limitations to this study. There are many factors that will influence inulin content of fruits, vegetables and food products, including time of harvesting, ripening, and cooking temperature (Lessin et al 1997, Torregrosa et al 2005). Further, foods were purchased from local grocers and may differ in inulin content from one provider to another. Despite this limitation, for those foods common to this study and the study by Van Loo et al 1995 database, values for inulin are

comparable. Another limitation to present study is that the raw foods selected were from Indian subcontinent. These foods are likely to differ amongst the countries due to environmental and genetic factors.

## **Section 5.2: Development and Acceptability Trials of Inulin Incorporated Products**

Functional foods are considered to be those whole, fortified, enriched or enhanced foods that provide health benefits beyond the essential nutrients, when they are consumed at efficacious levels as a part of varied diet on a regular basis. Recently a lot of interest has been generated in prebiotics as functional food. Inulin is claimed to have prebiotic activity because of its ability to serve as a substrate for the gut microflora. This phase of the research work was carried out to study the acceptability trials of varying levels of inulin incorporation by substitution or addition in bread, cookies, *chapati*, *dhokla*, porridge, *potato bonda*, juice and fermented milk.

The results of this phase are presented and discussed under the following subsections:

- Section 5.2.1 Effect of substitution of refined wheat flour in bread with varying levels of inulin
- Section 5.2.2 Effect of substitution of fat in cookies with varying levels of inulin
- Section 5.2.3 Effect of substitution of whole wheat flour in *chapati* with varying levels of inulin
- Section 5.2.4 Effect of substitution of rice flour in *dhokla* with varying levels of inulin
- Section 5.2.5 Effect of varying levels of inulin addition to the *potato bonda*
- Section 5.2.6 Effect of varying levels of inulin addition to the porridge
- Section 5.2.7 Effect of varying levels of inulin addition to the sweet orange juice
- Section 5.2.8 Effect of varying levels of inulin addition to the fermented milk
- Section 5.2.9 Proximate composition of all the products.

### **Section 5.2.1 Effect of substitution of refined wheat flour in bread with varying levels of inulin**

Five types of experimental bread were prepared wherein refined wheat flour was substituted at 5%, 10%, 15%, 20%, 22% level of inulin and its effect was studied on physicochemical and organoleptic characteristics of bread.

### a) Assessment of Physico-chemical properties of the bread

The results of physical characteristics of the standard and experimental breads are depicted in Table 5.2.1.1

The loaf weight of the bread ranged from 228g (standard) to 205g (22% of inulin substituted bread). There was gradual decrease in the loaf weight of bread as the level of inulin substitution increased.

**Table 5.2.1.1: Mean values for physicochemical evaluation of standard and inulin incorporated breads**

Characteristics	Level of substitution					
	Standard Bread	5%	10%	15%	20%	22%
Loaf Volume(ml)	525±5.8	527±8.4	507±7.0	497±9.4	470±9.1	450±6.6
Loaf weight (g)	228±1.4	225±2.4	221±1.4	215±2.1	210±2.0	205±2.2
Water retention (ml)	30.7±0.05	30.5±0.32	28.7±0.21	27.2±0.44	26.1±0.65	25.9±0.58
Total baking loss (%)	8.8±0.55	10±0.49	11.6±0.35	14±0.47	16±0.27	18±0.23

Values are Mean± S.D. of three independent determinations

There was gradual decrease in the loaf volume from 525 (standard) to 450 (22 % inulin incorporated bread).

Water retention in inulin incorporated breads ranged from 30.5 ml (5% level) to 25.9 ml (22% level) against 30.7 ml in standard bread. The level of retention decreased with increasing level of inulin substitution. Baking loss increased as the level of inulin substitution increased. The baking loss was 8.8% in standard bread compared to 16% in 20% level of substitution whereas in 22% level of inulin substituted bread there was 18% decrease in baking loss percent (Table 5.2.1.1)

### b) Organoleptic evaluation of the bread

The results of all organoleptic attributes of breads are presented in Table 5.2.1.2 and Fig.5.2.1

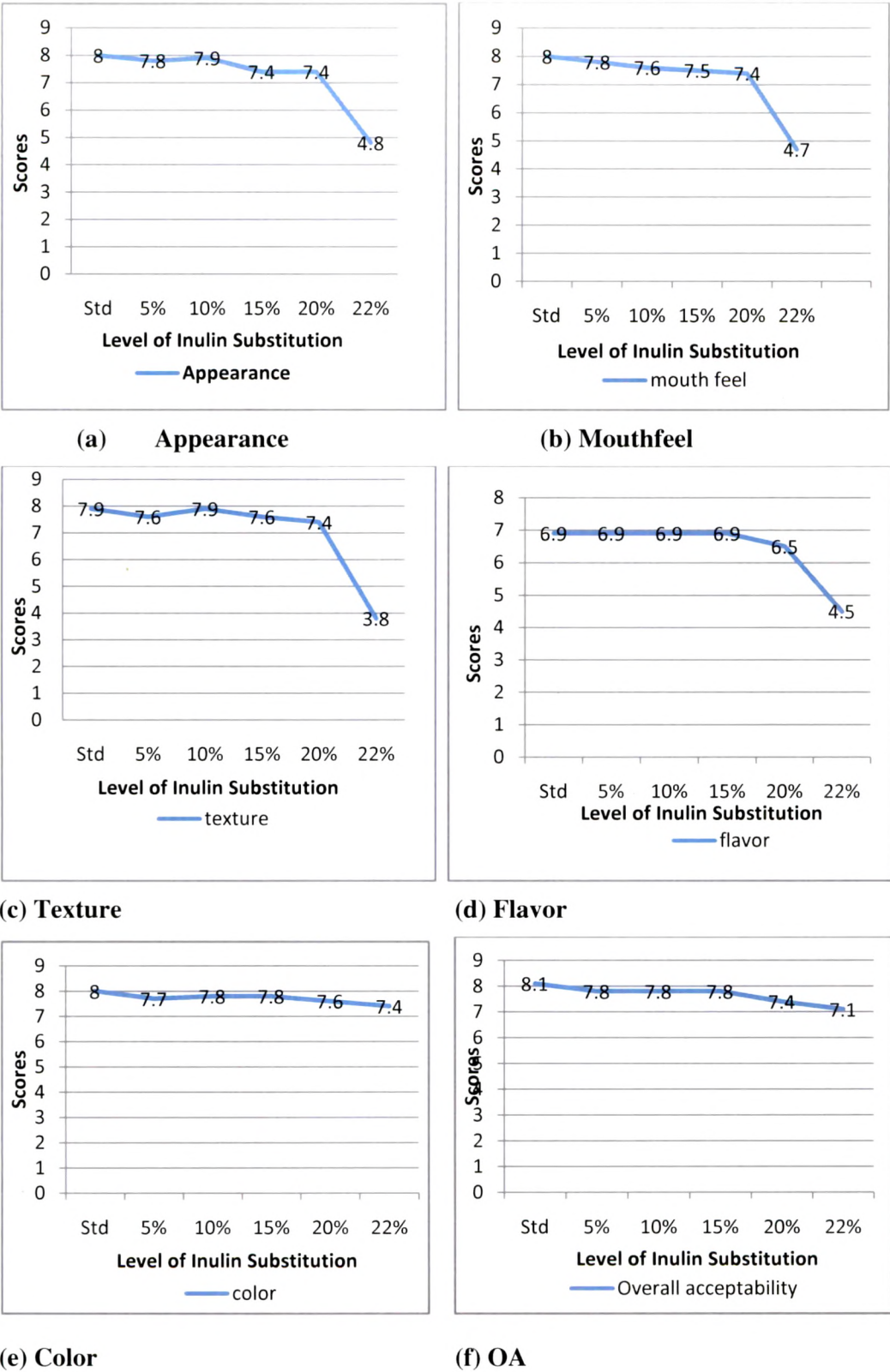
1. **Appearance:** As indicated in Table no 5.2.1.2 and Fig. 5.2.1 (a), for all the samples of bread mean scores ranged from 8.0 (Standard) to 4.8 (22% level of inulin substituted bread). Significant difference between the standard bread and bread with inulin substitution at 22% level was seen ( $p < 0.05$ ).

Table 5.2.1.2: Effect of Varying Levels of Inulin Substitution on the Organoleptic Quality of Bread

% Substitution		Organoleptic attributes											
Appearance		Color	Cell Size	Crust	Texture	Flavor	Mouth Feel	Aftertaste	Chew Ability	Ease Of Swallow	Absence Of Defects	Size &Shape	OA
Std	Mean	8.0 <sup>a</sup>	8.0	7.7	7.8	7.9 <sup>a</sup>	8.0 <sup>a</sup>	7.9 <sup>a</sup>	8.0	8.1	7.8	8.1	8.1
	SD±	±0.7	±0.8	±0.5	±1.0	±0.9	±0.6	±1.0	±0.9	±0.6	±1.1	±0.7	±0.7
	Range	5-10	5-10	5-10	5-10	5-10	4-10	5-10	6-10	6-10	5-10	5-10	6-10
5%	Mean	7.8 <sup>a</sup>	7.7	7.3	7.6	7.6 <sup>a</sup>	7.9 <sup>a</sup>	7.8 <sup>a</sup>	7.8	7.9	7.8	7.8	7.8
	SD±	±0.9	±0.7	±0.8	±0.9	±0.8	±0.7	±0.7	±0.9	±0.6	±1.1	±0.8	±0.7
	Range	4-10	5-10	5-10	5-10	5-10	5-10	5-10	5-10	6-10	5-10	4-10	6-10
10%	Mean	7.9 <sup>a</sup>	7.8	7.4	8.0	7.9 <sup>a</sup>	7.6 <sup>a</sup>	7.9 <sup>a</sup>	7.8	7.6	7.7	8.1	7.8
	SD±	±0.9	±0.7	±0.8	±0.9	±0.6	±1.2	±0.6	±0.7	±0.8	±0.9	±0.7	±0.8
	Range	5-10	6-10	4-10	6-10	7-10	3-10	6-10	5-10	5-10	5-10	5-10	5-10
15%	Mean	7.4 <sup>a</sup>	7.8	7.4	7.8	7.6 <sup>a</sup>	7.5 <sup>a</sup>	7.7 <sup>a</sup>	7.7	8.0	7.6	7.9	7.8
	SD±	±0.9	±0.9	±1.0	±0.8	±1.0	±0.9	±0.6	±0.8	±0.9	±0.9	±0.5	±0.8
	Range	4-10	6-10	4-10	6-10	5-10	5-10	6-10	6-10	5-10	5-10	5-10	5-10
20%	Mean	7.4 <sup>a</sup>	7.6	7.5	7.7	7.4 <sup>a</sup>	7.5 <sup>a</sup>	7.4 <sup>a</sup>	7.6	7.8	7.5	7.8	7.4
	SD±	±0.7	±0.8	±1.0	±0.6	±0.7	±1.1	±1.1	±0.7	±0.8	±0.8	±0.8	±0.9
	Range	5-10	6-10	4-10	6-10	5-10	5-10	6-10	5-10	5-10	5-10	5-10	5-10
22%	Mean	4.8 <sup>b</sup>	7.4	6.9	7.4	3.8 <sup>b</sup>	5.5 <sup>b</sup>	4.7 <sup>b</sup>	7.0	7.7	7.5	7.6	7.1
	SD±	±1.6	±1.1	±1.7	±1.5	±1.5	±1.8	±1.5	±2.1	±1.8	±1.4	±1.5	±1.4
	Range	4-9	6-9	4-9	6-10	4-9	5-10	5-9	5-10	5-10	6-10	5-10	5-9
ANOVA		*	NS	NS	*	*	*	*	NS	NS	NS	NS	NS

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b- The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.

2. **Color:** The crumb color score [Fig. 5.2.1(e)] decreased gradually from 8.0 (standard) to 7.4 (22% level of inulin substituted bread). No significant difference was noticed between the samples though a decreasing trend for color was noticeable as the level of inulin substitution increased.
3. **Cell size:** The mean scores for the cell size of the bread ranged from 7.7 (standard) to 6.9 (22% level of inulin substituted bread). No significant difference was observed in cell size scores of all the breads as the substitution of inulin increased from 0% to 22% level.
4. **Crust:** The crust scores of bread ranged from 7.8 (standard) to 7.4 (Inulin substituted bread at 22% level). No significant difference in the crust scores was observed as the level of substitution increased.
5. **Texture:** The mean scores for texture of bread ranged from 7.9 (standard) to 3.8 (Inulin substituted bread at 22% level). No significant differences were perceived between standard and modified bread except bread with 22% level of substitution [Fig.5.2.1 (c)]
6. **Flavor:** As seen in Table no 5.2.1.2 and Fig 5.2.1 (d) for all the samples of bread mean scores ranged from 7.9 (standard) to 5.5 (Inulin substituted bread at 22% level). The flavor of bread upto 20% level of inulin incorporation was well acceptable. The scores significantly decreased as the level of substitution increased (22 % level).
7. **Mouthfeel:** The mean scores for mouthfeel [Fig.5.2.1(b)] ranged from 8.0 (standard) to 4.7 (inulin substituted bread at 22% level) as the level of substitution increased. There was significant difference between 22% level of inulin substitution with all the other samples and standard bread.
8. **Aftertaste:** For 'after taste' of the breads mean scores ranged from 8.1 (standard) to 5.4 (inulin substituted bread at 22% level). The experimental sample with 5% level showed higher scores whereas 10% level of substitution scored similar to the standard. There was significant difference observed at 22% level of substitution when compared with all the other samples and standard.
9. **Chewability:** No significant difference was noticed between the breads substituted with varying levels of inulin and the standard for the chewability of breads. The scores for the standard bread were highest whereas overall decrease was noticed for this attribute for all the experimental samples.



**Fig.5.2.1(a)-(f) Organoleptic attributes of bread substituted with varying levels of inulin**



Table 5.2.1.3: Difference in Organoleptic Attributes of Individual Test Samples in Comparison with the Standard Bread

% Substitution		Organoleptic attributes											
Std test value=5		Appearance	Color	Softness	Size & shape	Crust	Texture	Absence of defects	Flavor	Mouth feel	Chew ability	Ease of swallow	Overall acceptability
5%	Mean	4.64 <sup>a</sup>	5.45	5.18 <sup>a</sup>	5.45 <sup>a</sup>	5.09 <sup>a</sup>	5.00 <sup>a</sup>	5.18 <sup>a</sup>	5.45	5.09 <sup>a</sup>	5.00 <sup>a</sup>	4.73 <sup>a</sup>	5.00 <sup>a</sup>
	SD±	±1.43	±1.70	±1.94	±1.51	±2.66	±2.15	±1.66	±1.86	±1.76	±1.73	±1.96	±1.67
	t value	0.84	0.89	0.31	1.00	0.11	0.00	0.36	0.81	0.17	0.00	0.46	0.00
10%	Mean	4.64 <sup>a</sup>	5.36	5.00 <sup>a</sup>	4.82 <sup>a</sup>	5.00 <sup>a</sup>	4.73 <sup>a</sup>	4.64 <sup>a</sup>	4.82	4.64 <sup>a</sup>	4.64 <sup>a</sup>	4.73 <sup>a</sup>	4.82 <sup>a</sup>
	SD±	±1.91	±1.29	±1.84	±1.17	±1.73	±1.27	±1.43	±1.60	±1.96	±1.69	±2.28	±1.60
	t value	0.63	0.94	0.00	0.52	0.00	0.71	0.84	0.38	0.61	0.71	0.40	0.38
15%	Mean	4.45 <sup>a</sup>	4.91	4.82 <sup>a</sup>	4.64 <sup>a</sup>	4.45 <sup>a</sup>	4.55 <sup>a</sup>	4.64 <sup>a</sup>	4.55	4.36 <sup>a</sup>	4.64 <sup>a</sup>	4.45 <sup>a</sup>	4.36 <sup>a</sup>
	SD±	±1.21	±1.70	±1.72	±1.75	±1.04	±1.04	±0.92	±1.37	±1.57	±1.50	±1.50	±2.01
	Range	1.50	0.18	0.35	0.69	1.75	1.46	1.31	1.10	1.35	0.80	1.14	1.05
20%	Mean	4.27 <sup>a</sup>	4.55	3.73 <sup>b</sup>	3.82 <sup>b</sup>	3.73 <sup>b</sup>	4.18 <sup>b</sup>	4.00 <sup>b</sup>	4.18	4.00 <sup>b</sup>	4.27 <sup>a</sup>	4.36 <sup>a</sup>	4.00 <sup>b</sup>
	SD±	±1.80	±1.57	±1.74	±1.40	±1.62	±1.17	±1.41	±1.66	±1.55	±2.05	±1.86	±1.48
	t value	1.35	0.96	2.43	2.78	2.61	2.32	2.35	1.63	2.41	1.17	1.20	2.24
22%	Mean	3.45 <sup>b</sup>	3.91	3.00 <sup>b</sup>	3.64 <sup>b</sup>	3.55 <sup>b</sup>	3.82 <sup>b</sup>	3.64 <sup>b</sup>	3.55	3.73 <sup>b</sup>	3.45 <sup>b</sup>	3.64 <sup>b</sup>	2.73 <sup>b</sup>
	SD±	±1.29	±1.87	±1.10	±1.12	±1.29	±1.17	±1.43	±1.21	±1.56	±1.75	±1.86	±0.47
	t value	3.97	1.94	6.06	4.04	3.73	3.36	3.16	3.98	2.71	2.92	2.43	6.14
ANOVA		*	NS	*	*	*	*	*	NS	*	*	*	*

Note:

- Mean values represent the average of 10 determinants in triplicates.
- a, b, c The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.

10. **Ease of Swallow:** For 'ease of swallowing' of breads mean scores ranged from 8.1 (standard) to 7.7 (inulin substituted bread at 22% level). No significant difference was perceived between experimental samples and the standard bread. The highest scores were obtained by 15% level of inulin substituted bread compared to the standard
11. **Absence of Defects:** For absence of defects in breads, mean scores ranged from all levels of substitution (7.8) to (7.5). There was no statistically significant difference among the experimented samples.
12. **Size and shape:** In all cases, the size and shape scores indicated that the incorporation of inulin at varying levels brought about no significant difference in the size and shape of breads.
13. **Overall acceptability:** The overall acceptability scores of all the bread ranged from 8.1 (control) to 7.1 (22% level of inulin). The breads with inulin substitution up to 20% level were well within acceptable limits.

**(c) Difference in organoleptic attributes of individual test samples in comparison with the standard Bread**

As seen in Table 5.2.1.3, organoleptic attributes on difference test scale depicted that all the samples scored lower for all attributes compared to the standard bread. Bread substituted with 5 % level of inulin scored similar to the standard for attributes like texture, chewability and overall acceptability whereas 10% level of inulin incorporated bread scored similar to the standard bread for crust and softness attributes. Experimental bread with 20% level of inulin substitution scored significantly lower for softness, size and shape, crust, absence of defects, aroma and mouthfeel in comparison to standard bread ( $p < 0.05$ ). Significant difference ( $P < 0.05$ ) was perceived for appearance, softness, size and shape, crust, absence of defects, aroma, mouthfeel, ease of swallow, chewability and overall acceptability for 22% level of inulin substituted bread.

**Bread was well accepted up to 20% level of inulin substitution whereas as the level of inulin substitution was increased up to 22%, significant differences were perceived for appearance, flavor, mouthfeel, aftertaste.**

### Section 5.2.2 Effect of varying levels of fat substitution with inulin on physico - chemical and organoleptic qualities on cookies

Cookies are food products with high content of sugar and fat and generally low in moisture content. Fat in cookies was substituted by varying levels of inulin and the products have studied for physicochemical and organoleptic properties. The detailed recipe for preparing cookies is appended in appendix II (ii).

#### a) Assessment of Physico-chemical properties of the Cookies

The results of the all physicochemical properties of cookies are presented in Table 5.2.2.1.

**Table 5.2.2.1 Physical evaluation of standard and inulin incorporated cookies**

Characteristics	Level of substitution						
	Standard cookies	5%	10%	15%	20%	22%	25%
Diameter (cms)	4.50±0.02	4.51±0.01	4.62±0.02	4.75±0.02	4.61±0.03	4.50±0.01	4.50±0.06
Thickness (cms)	0.88±0.001	0.88±0.002	0.90±0.015	0.92±0.011	0.92±0.03	0.94±0.02	0.95±0.01
Spread ratio (SR)	5.11	5.12	5.13	5.16	5.01	4.78	4.73
Percent SR (%)	100	100.10	100.39	100.97	98.04	93.54	92.56

Values are Mean± S.D. of three independent determinations

The average diameter of the control (without inulin) was 4.50 cm whereas that of inulin substituted cookies was from 4.51 cm (5 % level of inulin substitution) to 4.75 cm (20 % level of inulin substitution) whereas for 22% and 25% level the diameter was same as control. The average thickness of standard cookie was 0.88 cm and for inulin incorporated cookies ranged from 0.88 cm (5 % level of inulin substitution) to 0.95 cm (25 % level of inulin substitution). The spread ratio for the standard cookie was 5.11. The spread factor of cookie containing up to 15% inulin as a substitute of fat was slightly higher than the control thereafter a decrease in the spread ratio was observed for 22 and 25% of inulin substitution.

### Organoleptic evaluation of the Cookies

The results of inulin incorporation on all the organoleptic attributes of cookies are presented in Table 5.2.2.2. and Figure 5.2.2 (a-f).

- 1) **Softness:** Softness scores for cookies ranged from 8.9 (standard) to 4.5 (Inulin substituted cookies at 25% level) which indicates that the cookies increased with increasing level of inulin substitution. Significant difference was perceived between the standard cookies and cookies with inulin substitution at 22% and 25% level ( $p < 0.05$ ) indicating increase in hardness in cookies.
- 2) **Color:** The crumb color score decreased gradually from 8.0 (standard) to 7.4 (Inulin substituted cookies at 25% level) with increasing level of inulin substitution. No significant difference ( $P < 0.05$ ) was noticed between the standard and experimental samples.
- 3) **Cell size:** No significant difference was observed within all the levels of substitution as the cell size scores ranged from 7.5 (5% level of inulin incorporated cookies) to 6.8 (25% level of inulin incorporated cookies) as against 7.8 scored by the standard sample.
- 4) **Crust:** Crust scores for all the cookies ranged from 8.2 (0% level) to 7.3 (25% level of inulin incorporated cookies). No significant difference was observed in crust scores of all the cookies as the substitution level increased from 0% to 25% level of inulin.
- 5) **Texture:** The mean scores for texture of cookies are presented in Fig 5.2.2(a), ranged from 8.3 (standard) to 4.6 (25% level of inulin incorporated cookies). A significant difference in the texture scores of inulin incorporated cookies at 22 % and 25% level and this was true particularly between standard and 22% and 25% level of substitution.
- 6) **Flavor:** The flavor scores of all the cookies [Fig. 5.2.2(b)] ranged from 4.3 (25% level of inulin incorporated cookies) to 8.1 (standard). In general flavor scores decreased as the level of substitution increased from 0% to 25% level.
- 7) **Mouthfeel:** The mean scores for mouthfeel ranged from 8.1 (0% level) to 6.6 (25% level of inulin incorporated cookies) as the level of substitution increased. There was no significant difference up to 25% level of inulin substitution with standard sample [Fig 5.2.2(c)].

**Table 5.2.2.2: Effect of Varying Levels of Inulin Substitution on the Organoleptic Quality of Cookies.**  
**% Substitution**

Organoleptic Attributes																		
	Softness	Color	Cell Size	Uniformity In Baking	Texture	Flavor	Mouth Feel	Aftertaste	Chew Ability	Crispness	Absence Of Defects	Size And Shape	Overall Acceptability					
Std	Mean	8.9 <sup>a</sup>	8.0	7.8	8.2	8.3 <sup>a</sup>	8.1 <sup>a</sup>	8.2 <sup>a</sup>	8.1	7.8	7.9	7.8	8.1					
	SD±	±0.9	±0.9	±1.0	±0.8	±0.6	±0.7	±0.7	±0.9	±0.8	±1.0	±0.8	±0.6					
	Range	6-10	6-10	5-10	5-10	6-10	6-10	6-10	6-10	5-10	5-10	5-10	6-10					
5%	Mean	7.7 <sup>a</sup>	7.8	7.5	7.4	7.4 <sup>a</sup>	7.4	7.4 <sup>a</sup>	7.7	7.5	7.5	7.4	7.7					
	SD±	±0.6	±1.1	±0.9	±1.2	±0.9	±1.0	±1.1	±1.1	±1.4	±0.6	±0.8	±1.1					
	Range	5-10	2-10	5-10	4-10	3-10	4-10	2-10	4-10	2-10	5-9	5-10	3-10					
10%	Mean	7.4 <sup>a</sup>	7.1	7.3	7.4	7.2 <sup>a</sup>	7.5	7.7 <sup>a</sup>	7.4	7.5	7.5	7.3	7.6					
	SD±	±0.9	±1.2	±1.3	±1.0	±0.8	±0.8	±0.8	±0.9	±1.1	±1.0	±0.8	±0.9					
	Range	6-9	2-10	5-10	4-10	3-10	5-10	5-10	4-9	3-10	5-9	5-9	3-10					
15%	Mean	7.6 <sup>a</sup>	8.0	7.9	7.8	7.8 <sup>a</sup>	7.5	7.6 <sup>a</sup>	7.7	7.6	8.1	7.6	7.5					
	SD±	±0.7	±1.0	±1.2	±1.1	±1.1	±1.0	±1.3	±1.1	±1.0	±1.0	±1.0	±1.2					
	Range	5-10	3-10	4-10	5-10	3-10	3-10	3-10	3-10	4-10	6-10	5-10	3-10					
20%	Mean	7.2 <sup>a</sup>	7.1	7.3	7.3	7.2 <sup>a</sup>	7.5	7.6 <sup>a</sup>	7.3	7.4	7.5	7.3	7.3					
	SD±	±0.8(a)	±1.2	±0.8	±1.2	±0.6	±0.7	±0.8	±0.9	±1.1	±1.0	±0.7	±1.2					
	Range	5-10	2-10	5-10	2-10	4-10	6-9	5-10	5-10	5-10	4-9	4-9	3-10					
22%	Mean	4.7 <sup>a</sup>	6.8	7.1	7.3	4.8 <sup>b</sup>	7.2	5.0 <sup>b</sup>	7.3	7.4	7.1	7.3	7.1					
	SD±	±1.0	±1.2	±0.8	±1.1	±0.8	±0.8	±1.0(b)	±0.8	±1.0	±1.2	±1.2	±0.9					
	Range	5-9	3-10	5-9	3-10	4-10	4-10	5-10	5-9	5-10	2-9	4-9	5-10					
25%	Mean	4.5 <sup>b</sup>	7.2	6.8	7.3	4.6 <sup>b</sup>	6.6	4.8 <sup>b</sup>	6.8	7.0	6.8	6.8	6.7					
	SD±	±0.9	±0.9	±1.1	±0.9	±1.0	±1.5	±1.3	±1.4	±1.5	±1.3	±1.0	±1.5					
	Range	5-9	3-10	4-10	4-10	3-10	2-9	5-10	2-9	4-10	4-9	4-9	3-10					
ANOVA												*	NS	NS	NS	NS	NS	NS

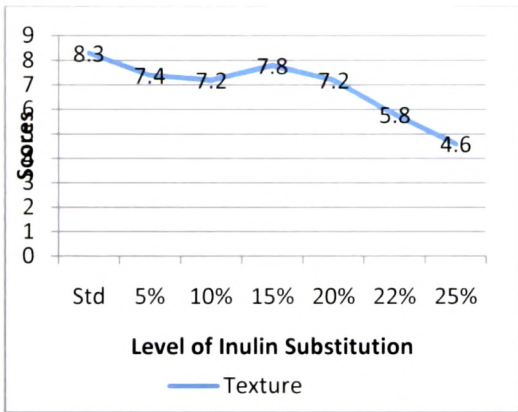
• Note: Mean values represent the average of 10 determinants in triplicates.

• a, b, c – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.

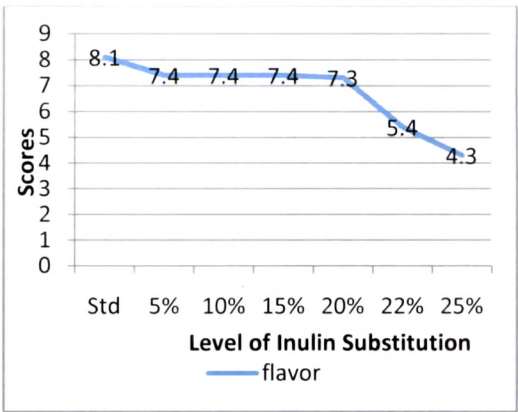
• NS – The difference between the mean values within the columns is not significant.

• Maximum score for all the organoleptic attributes was 10.

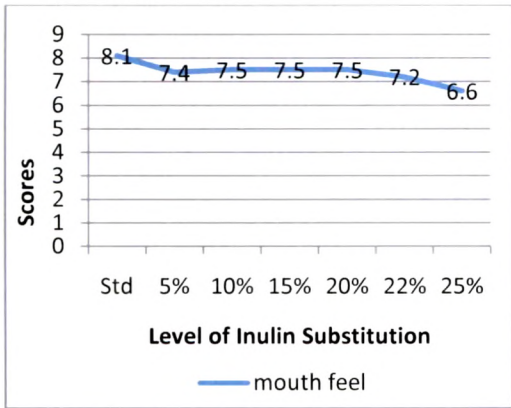
- 8) **Aftertaste:** Large differences were observed [Fig 5.2.2(d)] in aftertaste scores of cookies substituted with inulin, ranging from 8.2 (standard) to 4.8 (25% level of inulin incorporated cookies). Significant differences ( $P < 0.05$ ) in aftertaste scores were observed between cookies prepared from 22% and 25% level of inulin incorporated with all other samples (0%, 5%, 10 %,15% and 20% level) . However no significant difference was observed among the samples and with the standard up to 20% level of inulin incorporation.
- 9) **Chewability:** No significant difference was noticed between all the inulin substituted samples and standard sample for the chewability scores of cookies. The scores for the standard cookies were highest, ranging from 8.1 to 6.8 (25% level of inulin incorporated cookies). An overall decrease was noticed for this attribute for all the samples.
- 10) **Crispness:** For crispness of cookies the mean scores ranged from 7.5 (5% level of inulin incorporated cookies) to 7.0 (25% level of inulin incorporated cookies). As against 7.8 scored by the control sample. 15% level of inulin incorporated cookies scored highest among the samples, there was no significant between all the samples of cookies within the levels of substitution. 5% and 10% level of inulin substituted cookies scored similar whereas 20% and 22% level of inulin substituted cookies scored similar on organoleptic scale by the panel members for crispness [Fig 5.2.2(e)].
- 11) **Absence of Defects:** For absence of defects in cookies, no significant difference was observed among the samples and the mean scores ranged from 7.9 (standard) to 6.8 (25% level of inulin incorporated cookies). Mean score for 15% level of inulin incorporation scored higher for absence of defects than the standard sample. 5% and 10% level of inulin substituted cookies scored similar for this attribute however less than the standard cookies.
- 12) **Size and shape:** All the samples scored low compared to the standard sample however 15% level of inulin substituted cookies scored highest among the samples. No significant difference was seen for all the levels of inulin substitution among the samples of cookies.
- 13) **Overall acceptability:** As illustrated in Fig.5.2.2(f), the overall acceptability scores (OA scores) of all the cookies ranged from 8.1 (control) to 6.7 (25% level of inulin). The 25% and 22% level of inulin incorporated cookies rated low on score card and were considered unacceptable.



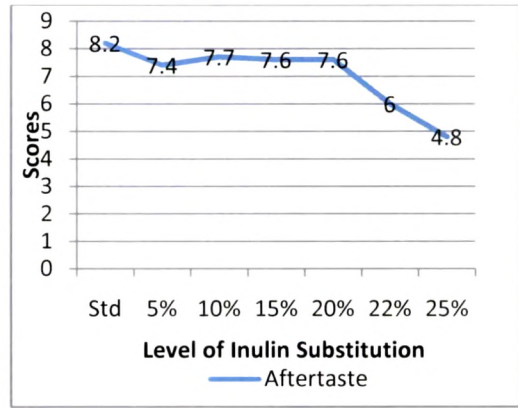
(a) Texture



(b) Flavor



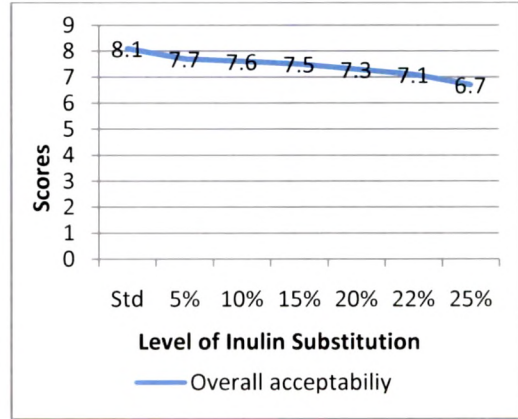
(c) Mouthfeel



(d) Aftertaste



(e) Crispness



(f) OA

Fig.5.2.2(a)-(f) Scores for organoleptic attributes of cookies substituted with varying levels of inulin

Table 5.2.2.3: Difference in Individual Test Samples in Comparison with Standard Cookies

% Substitution		Organoleptic Attributes											
	Std test value=5	Appearance	Color	Softness	Size & Shape	Burn Spots	Texture	Absence of Defects	Flavor/Aroma	Mouth Feel	Crispness	Ease of Swallow	Overall Acceptability
5%	Mean	3.40 <sup>b</sup>	4.70	3.70	4.50	3.90 <sup>a</sup>	4.30 <sup>a</sup>	4.40	4.40 <sup>a</sup>	4.80 <sup>a</sup>	4.60	4.40 <sup>a</sup>	4.40 <sup>a</sup>
	SD±	±1.58	±2.67	±1.70	±2.12	±2.38	±2.83	±2.07	±2.59	±1.93	±1.90	±1.85	1.96
	t value	3.21	0.36	2.41	0.75	1.46	0.78	0.92	0.73	0.33	0.67	1.03	0.97
10%	Mean	4.50 <sup>a</sup>	5.10	4.70	5.70	5.50 <sup>a</sup>	4.30 <sup>a</sup>	4.30	4.90 <sup>a</sup>	4.70 <sup>a</sup>	5.40	5.50 <sup>a</sup>	5.30 <sup>a</sup>
	SD±	±0.76	±2.23	±2.05	±2.06	±2.17	±2.05	±1.95	±2.28	±2.16	±2.01	2.01	2.16
	t value	0.66	0.14	0.46	1.08	0.72	1.08	1.14	0.14	0.44	0.63	0.79	0.44
15%	Mean	4.40 <sup>a</sup>	4.20	3.70	4.40	4.80 <sup>a</sup>	4.30 <sup>a</sup>	4.70	4.40 <sup>a</sup>	4.40 <sup>a</sup>	4.80	4.70 <sup>a</sup>	4.60 <sup>a</sup>
	SD±	±1.43	±2.20	±1.83	±2.17	±2.20	±2.35	±2.79	±2.01	±2.12	±1.81	±1.64	±2.12
	Range	1.33	1.15	2.25	0.87	0.29	0.94	0.34	0.94	0.90	0.35	0.58	0.60
20%	Mean	3.60 <sup>b</sup>	4.80	4.00	4.00	4.00 <sup>a</sup>	4.50 <sup>a</sup>	3.90	4.10 <sup>a</sup>	5.10 <sup>a</sup>	4.90	4.80 <sup>a</sup>	4.70 <sup>a</sup>
	SD±	±0.34	±2.04	±1.95	±1.94	±2.54	±2.84	±1.85	±1.91	±1.97	±1.85	±1.81	±2.06
	t value	4.12	0.31	1.63	1.63	1.25	0.56	1.88	1.49	0.16	0.17	0.35	0.46
22%	Mean	3.10 <sup>b</sup>	4.70	3.90	3.90	3.50 <sup>b</sup>	4.10 <sup>a</sup>	4.70	3.80 <sup>b</sup>	3.90 <sup>a</sup>	4.40	4.30 <sup>a</sup>	3.80 <sup>b</sup>
	SD±	±1.29	±2.11	±1.73	±1.91	±1.51	±1.73	±2.05	±1.62	±2.08	±1.65	±1.77	±1.93
	t value	4.67	0.45	2.01	1.82	3.14	1.65	0.46	2.34	1.67	1.15	1.25	1.96
25%	Mean	3.20 <sup>b</sup>	4.30	4.00	5.30	3.30 <sup>b</sup>	3.50 <sup>b</sup>	4.60	3.70 <sup>b</sup>	3.40 <sup>b</sup>	4.00	3.80 <sup>b</sup>	3.40 <sup>b</sup>
	SD±	±1.03	±1.95	±1.76	±1.89	±1.77	±1.65	±2.22	±1.57	±1.17	±1.41	±1.62	±2.07
	t value	5.51	1.14	1.80	0.50	3.04	2.88	0.57	2.62	4.31	2.24	2.34	2.45
ANOVA		*	NS	NS	NS	*	*	NS	*	*	NS	*	*

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.



**c) Difference in organoleptic attributes of individual test samples in comparison with the standard cookies**

As seen in Table 5.2.2.4, 10% inulin incorporated cookies scored higher for attributes like color, size and shape, softness, flavor, burnspots, ease of swallow and crispness against the standard cookies. Cookies substituted with 25 % level of inulin scored significantly lower compared to the standard sample for attributes like appearance, burn spots, texture, flavor, mouthfeel, ease of swallow and overall acceptability.

**Cookies were acceptable up to 20% level of inulin substitution. As the level of substitution increased, significant differences were perceived for softness, texture, flavor and after taste. Acceptability scores were higher for 15% level of inulin incorporated cookies based organoleptic scores whereas 10% level of inulin substituted cookies scored higher for most of the attributes.**

**Section 5.2.3 Effect of Varying Levels of Inulin Substitution to *Chapati***

The results of physicochemical properties of *chapati* is presented in Table 5.2.3.1

**a) Assessment of Physico-chemical properties of the *Chapati***

*Chapati* (Unleavened flat bread) is staple food of the Indian population. Almost 90 per cent of wheat produced in the country is used for *chapati* preparation. Such heavy dependence on a single food (*chapati*) has resulted in their use as a vehicle for fortification. Therefore *chapati* was substituted with inulin at varying levels and was studied for physicochemical and organoleptic properties.

Cooked weight of the *chapati* decreased 20 g (standard) to 19.39 g (20% level of inulin substitution) with increasing levels of inulin substitution. The ready dough weight decreased from 351 g (0% level) to 302 g (20% level of inulin substitution) as

the level of substitution of inulin increased. This may be due less absorption of water during preparation of dough making due to decreased solubility of inulin in water.

**Table 5.2.3.1 Physical Evaluation of Standard and Inulin Incorporated *Chapatis***

Characteristic	Standard <i>chapati</i>	5%	10%	15%	20%
Dough weight g	351±0.12	335±0.17	320±0.14	311±0.23	302±0.22
Cooked weight g	20 ±0.03	20±0.05	19.56±0.02	19.45±0.02	19.39±0.03
WAP %	75%	71%	69%	67%	65%
Puffing	Full	Full	Full	partial	Partial

Values are Mean± S.D. of three independent determinations

The percent water absorption showed significant differences with increasing level of inulin incorporation. The water absorption power (WAP) varied from 71% (5% level of inulin incorporation) to 65% (20% level of inulin incorporation). The WAP of more than 68% is generally considered suitable for *chapati* making. The *chapatis* prepared from flours with WAP less than 60% tend to be stiff and semi stiff with poor keeping quality. The difference in WAP may be attributed to addition of inulin because the inulin is moderately soluble in water and inulin used for the preparation was high performance inulin with 25 g/l solubility in water compared to standard inulin which solublizes in water at the rate of 120 g/l.

**b) Organoleptic evaluation of the *chapati*:**

The organoleptic scores of *chapati* prepared by substitution of flour with varying levels of inulin are presented graphically in Figure 5.2.3 (a-f) and the data is detailed in Table 5.2.3.2

- 1) **Softness:** The softness scores of *chapati* (Fig 5.2.3(a)) incorporated with inulin ranged from 8.0 (standard) to 6.1 (inulin substituted *chapati* at 25% level). These scores tended to decrease with an increase in level of substitution. Significant difference was perceived between the standard *chapati* and *chapati* with inulin substitution at 15% and 20% level ( $p < 0.05$ ).

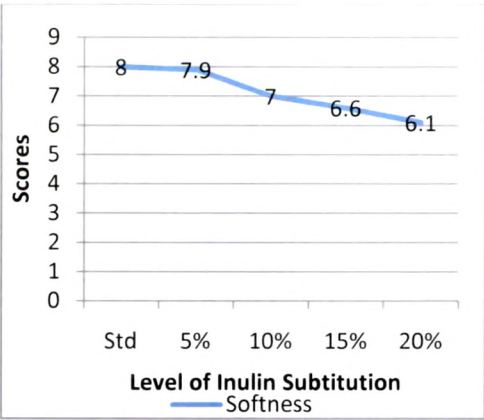
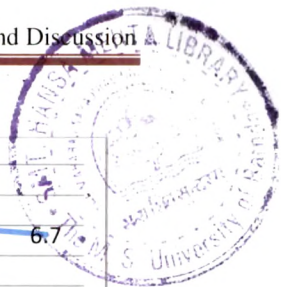
- 2) **Color:** In all cases, the color scores indicated that the incorporation of inulin at varying levels brought about no significant difference in the color of *chapatis*.
- 3) **Breakability:** Breakability scores ranged from 7.9 (5% level of inulin incorporated *chapati*) to 6.8 (20% level of inulin incorporated *chapati*) as against 7.9 scored by the standard sample. There was significant difference between standard and *chapati* with 20% level of inulin substitution also difference was observed between 5% level of inulin incorporated *chapati* and *chapati* with 20% level of inulin substitution.
- 4) **Size and Shape:** The size and shape scores for all the *chapati* samples ranged from 8.3 (0% level) to 7.5 (20% level of inulin incorporated *chapati*). No significant difference was observed in size and shape scores of all the *chapati* as the substitution level increased from 0% to 20%.
- 5) **Crust:** The mean scores for crust of *chapati* are presented in Fig 5.2.3(b), ranged from 8.0 (standard) to 6.7 (20% level of inulin incorporated *chapati*). A significant difference was noted in the crust scores of inulin incorporated *chapati* at 15 %and 20% level with all the levels of substitution.
- 6) **Appearance:** There was appearance of more number of brownish and black spots on the surface of *chapatis*. The appearance scores of all the *chapati* (Fig 5.2.3(c)) ranged from 6.8 (20% level of inulin incorporated *chapati*) to 8.3 (standard). In general appearance scores decreased as the level of substitution increased from 0% to 25% level of inulin. Appearance of the *chapatis* was slightly deteriorated with the incorporation of inulin. There was a significant difference in scores between standard and 20% level of inulin incorporated *chapati*.
- 7) **Absence of defects:** The mean scores for absence of defects ranged from 8.1 (0% level) to 6.2 (20% level of inulin incorporated *chapati*) as the level of substitution increased. There was no significant difference up to 15% level of inulin substitution with standard sample.

Table 5.2.3.2: Effect of Varying Levels of Inulin Addition on the Organoleptic Qualities of Chapati

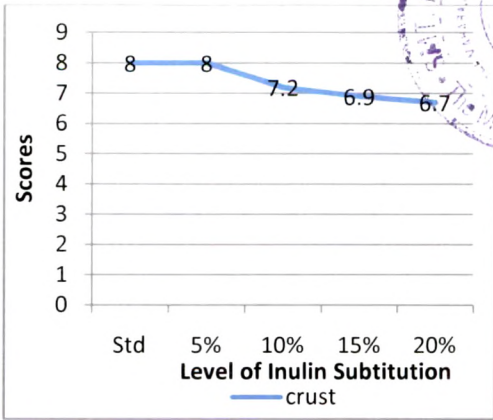
% Substitution	Organoleptic Attributes														
	Softness	Color	Breakability	Size n Shape	Crust	Appearance	Absence of Defects	Aroma	Taste	Texture	After taste	Chew Ability	Ease of Swallowing	Overall Acceptability	
Std	Mean	8.0 <sup>a</sup>	8.4	7.9 <sup>a</sup>	8.3	8.0 <sup>a</sup>	8.3 <sup>a</sup>	8.1 <sup>a</sup>	8.1	8.2 <sup>a</sup>	8.4 <sup>a</sup>	7.3 <sup>a</sup>	8.0 <sup>a</sup>	8.0	8.5 <sup>a</sup>
	SD±	±0.8	±0.9	±0.9	±0.6	±1.1	±0.8	±0.6	±0.7	±0.7	±0.9	±0.8	±0.7	±0.7	±0.7
	Range	5-10	6-10	6-10	6-10	6-10	6-10	6-10	7-10	7-10	6-10	6-10	6-10	6-10	7-10
5%	Mean	7.9 <sup>a</sup>	8.1	7.9 <sup>a</sup>	8.1	8.0 <sup>a</sup>	8.1	8.1 <sup>a</sup>	7.9	8.0 <sup>a</sup>	7.8 <sup>a</sup>	7.8	7.9 <sup>a</sup>	8.0	8.2 <sup>a</sup>
	SD±	±0.9	±0.8	±1.1	±0.7	±0.9	±0.8	±0.8	±0.9	±0.6	±0.7	±0.9	±0.7	±0.8	±0.5
	Range	6-10	7-10	5-10	7-10	6-10	6-9	7-10	4-10	7-10	7-10	6-10	6-10	7-10	7-10
10%	Mean	7.0	7.6	7.1	7.7	7.2 <sup>a</sup>	7.3	7.5	7.7	7.4	7.3	7.4	7.2	7.5	7.5
	SD±	±0.8	±0.9	±0.7	±1.0	±1.0	±1.0	±1.0	±0.6	±0.6	±0.8	±0.7	±0.7	±0.9	±0.8
	Range	5-9	5-10	6-10	5-10	6-10	6-10	6-10	6-9	6-9	6-9	5-9	6-10	6-9	6-9
15%	Mean	6.6 <sup>b</sup>	7.4	6.8	7.6	6.9 <sup>b</sup>	7.1	7.2	7.2	6.8 <sup>b</sup>	6.8 <sup>b</sup>	7.3	6.7 <sup>b</sup>	7.2	6.9 <sup>b</sup>
	SD±	±1.0	±1.0	±1.4	±1.2	±1.0	±1.2	±1.1	±1.3	±0.9	±1.2	±1.0	±0.9	±1.1	±1.1
	Range	4-9	5-10	4-10	4-10	5-10	6-10	5-10	4-9	4-9	4-10	5-10	4-10	4-10	4-10
20%	Mean	6.1 <sup>b</sup>	7.2	6.2 <sup>b</sup>	7.5	6.7 <sup>b</sup>	6.8 <sup>b</sup>	6.2 <sup>b</sup>	7.2	6.9 <sup>b</sup>	6.3 <sup>b</sup>	7.0 <sup>b</sup>	6.5 <sup>b</sup>	7.0	6.6 <sup>b</sup>
	SD±	±1.1	±1.2	±1.3	±1.1	±1.1	±1.3	±1.5	±0.8	±0.9	±1.1	±0.8	±0.8	±0.8	±0.8
	Range	4-9	4-10	4-10	5-10	4-10	4-10	4-10	4-9	4-9	3-9	5-9	5-9	5-9	5-9
ANOVA		*	NS	*	NS	*	*	*	NS	*	*	*	NS	*	*

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b, c – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.

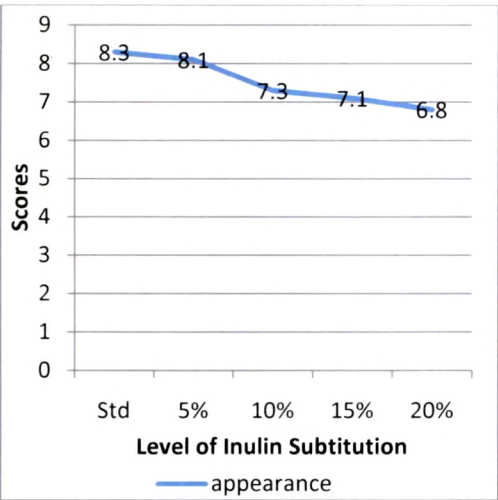
- 8) **Aroma:** No significant difference was noticed between all the inulin substituted samples and standard sample for the aroma scores of *chapati*. The scores for the standard *chapati* were highest, ranging from 8.1 to 7.2 (20% level of inulin incorporated *chapati*). An overall decrease was noticed for this attribute for all the *chapati* samples.
- 9) **Taste:** Taste scores of *chapati* substituted with inulin, ranged from 8.2 (standard) to 6.9 (20% level of inulin incorporated *chapati*). Significant differences ( $P < 0.05$ ) in taste scores were observed between *chapati* prepared from 15% and 20% level of inulin incorporated with all other samples (0%, 5%, and 10% level). However no significant difference was observed among the samples and with the standard up to 10% level of inulin incorporation (5.3.2(d)).
- 10) **Texture:** As indicated in Fig 5.2.3 (e), no significant difference was noticed between all the inulin substituted samples and standard sample for the texture scores of *chapati*. The scores for the standard *chapati* were highest, ranging from 8.1 to 6.8 (25% level of inulin incorporated *chapati*). An overall decrease was noticed for this attribute for all the samples.
- 11) **After taste:** Mean scores for after taste of *chapati* ranged from 7.8 (5% level of inulin incorporated *chapati*) to 7.0 (20% level of inulin incorporated *chapati*) as against 7.3 scored by the control sample. 5% and 10% level of inulin incorporated *chapati* scored higher than the standard, there was no significant between all the samples of *chapati* within the levels of substitution. 20% and 22% level of inulin substituted *chapati* scored significantly lower on organoleptic scale by the panel members for after taste.
- 12) **Chewability:** For chewability in *chapati*, mean scores ranged from 8.0 (standard) to 6.5 (20% level of inulin incorporated *chapati*). Mean score for 15% and 20% level of inulin incorporation scored significantly lower for chewability than the standard sample.
- 13) **Ease of swallow:** All the samples scored low compared to the standard sample for ease of swallow. However 5% level of inulin substituted *chapati* scored similar to the standard. No significant difference was seen for all the levels of inulin substitution among the samples of *chapati* for scores of ease of swallow.



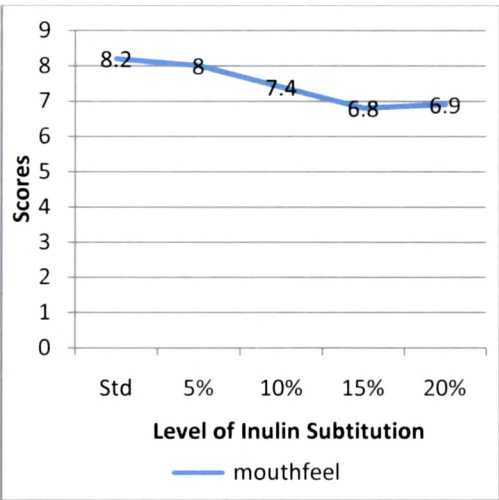
(a) Softness



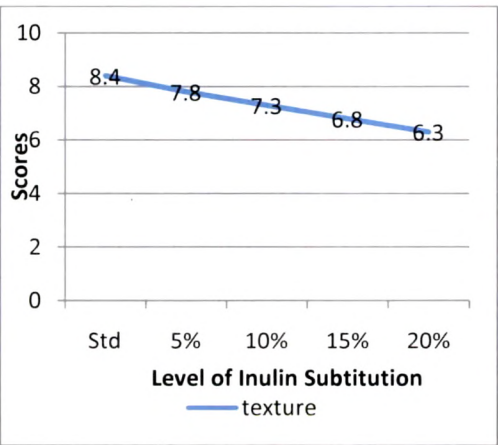
(b) Crust



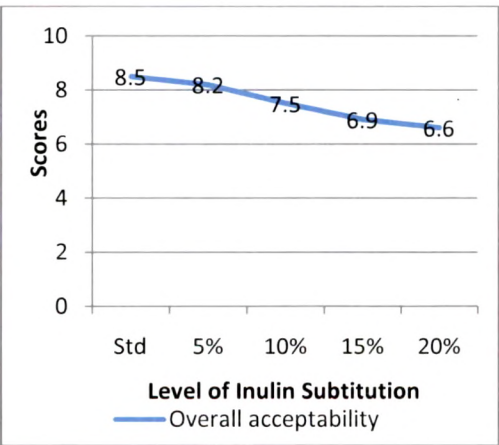
(c) Appearance



(d) Mouthfeel



(e) Texture



(f) OA

**Fig. 5.2.3(a)-(f) Scores for organoleptic attributes of *chapati* substituted with varying levels of inulin**

Table 5.2.3.3: Difference in Organoleptic Attributes of Individual test Samples in Comparison with the Standard Chapati

% Substitution		Organoleptic Attributes											
	Std test value=5	Appearance	Color	Softness	Size n Shape	Burn Spots	Texture	Absence of Defects	Aroma	Taste & Mouthfeel	Chewability	Ease of Swallowing	Overall Acceptability
5%	Mean	4.91	4.18	4.73	4.18	4.18	4.18	4.18	4.45	4.82	4.18	4.18	4.91
	SD±	±1.81	±2.09	±2.20	±2.09	±2.09	±2.18	±2.09	±2.25	±2.27	±2.09	±2.09	±2.43
	t value	0.17	1.30	0.41	1.30	1.30	1.24	1.30	0.80	0.27	1.30	1.30	0.12
10%	Mean	4.64	4.09	4.27	4.09	4.09	4.18	4.36	4.0	4.36	4.09	4.09	4.73
	SD±	±2.73	±2.02	±1.62	±2.02	±1.81	±2.29	±1.96	±1.79	±2.0	±2.02	±2.02	±1.74
	t value	0.44	1.49	1.49	1.49	1.66	1.22	1.08	1.85	1.05	1.49	1.49	0.52
15%	Mean	4.55	4.09	4.45	3.64	4.45	3.91	4.09	4.27	4.27	4.27	4.09	4.45
	SD±	±2.38	±2.02	±2.30	±2.34	±2.30	±1.97	±2.02	±2.24	±2.45	±2.01	±2.02	±2.58
	t value	0.63	1.49	0.79	1.94	0.79	1.83	1.49	1.08	0.98	1.20	1.49	0.70
20%	Mean	4.27	4.09	4.18	4.09	4.18	4.64	4.18	4.09	3.64	4.18	4.09	4.00
	SD±	±2.24	±2.02	±2.09	±2.02	±2.09	±2.16	±1.83	±1.64	±1.43	±1.83	±2.02	±1.84
	t value	1.08	1.49	1.30	1.49	1.30	0.56	1.48	1.84	3.16	1.48	1.49	1.80
ANOVA		NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b, c – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.

14) **Overall acceptability:** As illustrated in Fig.5.2.3(f), the overall acceptability scores (OA scores) of all the *chapati* ranged from 8.5 (control) to 6.6 (20% level of inulin). The 15% and 20% level of inulin incorporated *chapati* rated below fair and were considered unacceptable whereas the rest of the *chapati* (at all levels of substitution) were acceptable.

**c) Difference in organoleptic attributes of individual test samples in comparison with the standard *chapati***

As seen in Table 5.2.3.3, for all the attributes when individual samples of inulin incorporated *chapatis* at varying levels were compared with the standard sample, no significant difference was observed with the standard sample except for taste and mouthfeel for 20% level of inulin substitution.

*Chapatis* were well accepted up to 10% level of inulin substitution. As the level of inulin substitution increased to 15 and 20 % there was a significant decrease in scores for most of the attributes. No significant difference was observed in experimental *chapatis* for overall acceptability when compared to the standard.

**Section 5.2.4 Effect of Varying Levels of Inulin substitution to the *Dhokla***

The results for this sub-section are presented in Table 5.2.4.1 to 5.2.4.3 of inulin addition at varying levels on the physicochemical and organoleptic properties of *Dhokla*.

**d) Assessment of Physico-chemical properties of the *Dhokla***

The *dhoklas* were assessed for bulk density and time of steaming. The bulk density of the prepared *dhokla* varied from 1.85 g/ccs (0% level of inulin substitution) to 1.73 g/cc (20% level of inulin substitution). The bulk density of the steamed *dhokla* decreased with increasing levels of inulin substitution. It was found that the steaming time of about 20 minutes was sufficient to eliminate uncooked odour. There was no difference in time recorded for steaming of standard *dhokla* and *dhoklas* at all levels of inulin addition.



**Table 5.2.4.1 Physical Evaluation of Standard and Inulin Incorporated *Dhokla***

Characteristics	Standard <i>Dhokla</i>	5%	10%	15%	20%
Time of steaming	20 mins	20 mins	20 mins	20 mins	20 mins
Bulk density g/cc	1.85	1.83	1.81	1.81	1.73
Weight g	15±0.2	15.11±0.37	15.15±0.26	15.18±0.14	15.16±0.11
Volume cc	8.07	8.23	8.36	8.54	8.73

Values are Mean± S.D. of three independent determinations

a) **Organoleptic evaluation of the *Dhokla* :**

*Dhoklas* prepared with addition of inulin at varying levels were subjected to organoleptic evaluation. The results on organoleptic quality are recorded in Table no. 5.2.4.2 and are presented in Fig. 5.2.4(a-d)

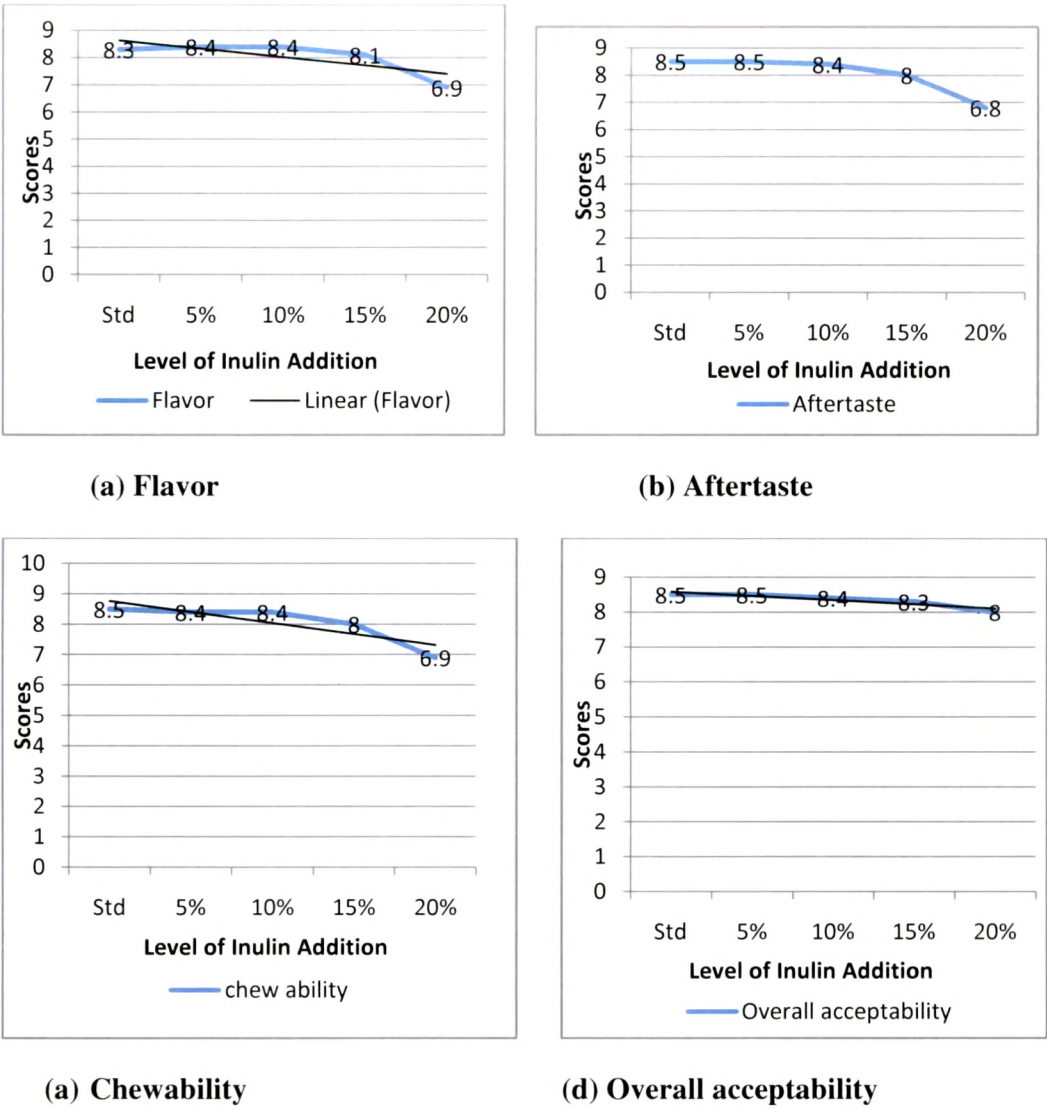
1. **Softness:** The softness scores of *dhokla* incorporated with inulin ranged from 8.3 (standard) to 8.3 (Inulin substituted *dhokla* at 20% level). The scores were high and constant with an increase in level of inulin addition up to 15%. No significant difference was perceived between the standard *dhokla* and *dhokla* with inulin substitution up to 20% level ( $p < 0.05$ ).
2. **Color:** In all cases, the color scores indicated that the incorporation of inulin at varying levels brought about no significant difference in the color of *dhoklas*. The mean scores for color were 8.3 that remained same with varying levels of inulin incorporation.
3. **Appearance:** The appearance scores of all the *dhokla* ranged from 8.4 (20% level of inulin incorporated *dhokla*) to 8.6 (standard). In general appearance scores decreased as the level of substitution increased from 0% to 20% level. No significant difference was noted in scores between standard and varying levels of inulin incorporated *dhokla*.
4. **Absence of defects:** The mean scores for absence of defects ranged from 8.4 (0% level) to 8.4 (20% level of inulin incorporated *dhokla*) as the level of substitution increased. There was increase in scores up to 10% level of inulin substitution whereas 15% and 20% level of inulin incorporated *dhoklas* scored similar to the standard sample.

Table 5.2.4.2: Effect of Varying Levels of Inulin Addition on the Organoleptic Quality of *Dhokla*

% Substitution		Organoleptic Attributes										
		Softness	Color	Appearance	Texture	Flavor/ aroma	Mouth feel	Aftertaste	Chew ability	Ease of swallow	Absence of defects	OA
Std	Mean	8.3	8.5	8.6	8.4	8.3	8.4	8.5	8.5 <sup>a</sup>	8.4	8.4	8.5
	SD±	±0.8	±0.8	±0.8	±0.8	±0.8	±0.8	±0.7	±0.8	±0.8	±0.8	±0.8
	Range	7-10	7-10	7-10	7-10	7-10	6-10	6-10	7-10	7-10	6-10	7-10
5%	Mean	8.4	8.5	8.5	8.3	8.4	8.4	8.5	8.4 <sup>a</sup>	8.5	8.5	8.5
	SD±	±0.8	±0.8	±0.7	±0.9	±0.8	±0.8	±0.8	±0.7	±0.6	±0.8	±0.8
	Range	7-10	7-10	6-10	6-10	7-10	5-10	6-10	7-10	8-10	7-10	7-10
10%	Mean	8.4	8.5	8.4	8.3	8.4	8.4	8.4	8.4 <sup>a</sup>	8.5	8.5	8.4
	SD±	±0.8	±0.8	±0.8	±0.9	±0.9	±0.7	±0.8	±0.8	±0.7	±0.8	±0.8
	Range	7-10	7-10	7-10	7-10	7-10	7-10	6-10	7-10	7-10	6-10	7-10
15%	Mean	8.4	8.5	8.4	8.4	8.5	8.3	8.4	8.4 <sup>a</sup>	8.4	8.4	8.3
	SD±	±0.7	±0.8	±0.8	±0.8	±0.8	±0.9	±0.7	±0.7	±0.7	±0.9	±0.7
	Range	7-10	7-10	6-10	7-10	7-10	6-10	6-10	7-10	7-10	6-10	7-10
20%	Mean	8.3	8.5	8.4	8.4	8.6	8.3	8.6	6.9 <sup>b</sup>	8.4	8.4	8.3
	SD±	±0.7	±0.7	±0.8	±0.8	±0.7	±0.9	±0.9	±0.8	±0.8	±0.8	±0.9
	Range	6-10	7-10	7-10	7-10	7-10	4-10	6-10	6-10	6-10	6-10	5-10
ANOVA		NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b, c – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.

5. **Flavor:** As indicated in Fig 5.2.4 (a), no significant difference was noticed between all the inulin incorporated *dhoklas* samples and standard sample for the flavor scores. The scores for the standard *dhokla* were highest, ranging from 8.3 to 8.6 (20% level of inulin incorporated *dhokla*). An overall increase was noticed for this attribute for all the samples.
6. **Mouthfeel:** Mouthfeel scores of *dhokla* substituted with inulin, ranged from 8.4 (standard) to 8.3 (20% level of inulin incorporated *dhokla*). *Dhokla* prepared from 5% and 10% level of inulin incorporated scored similar to the standard *Dhokla*. No significant differences ( $P < 0.05$ ) in mouthfeel scores were observed between all levels of inulin addition and standard *dhokla*.
7. **Texture:** No significant difference was noticed between all the inulin incorporated samples and standard sample for the texture scores of *dhokla*. The scores for the 20% level of inulin added *dhokla* were similar to the standard. Varying the level of inulin addition did not significantly affect texture scores.
8. **After taste:** As indicated in Fig 5.2.4(b), mean scores for *dhokla* ranged from 8.5 (5% level of inulin incorporated *dhokla*) to 8.6 (20% level of inulin incorporated *dhokla*) as against 8.5 scored by the standard sample. 5% level of inulin incorporated *dhokla* scored similar to the standard, there was no significant between all the samples of *dhokla* within the levels of addition. 20% level of inulin substituted *dhokla* scored higher on organoleptic scale by the panel members for after taste.
9. **Chewability:** As illustrated in Fig 5.2.4(c), for chewability in *dhokla*, mean scores ranged from 8.5 (standard) to 6.9 (20% level of inulin incorporated *Dhokla*). Mean score for 20% level of inulin incorporation scored significantly lower for chewability than the standard sample.
10. **Ease of swallow:** The mean scores for ease of swallow ranged from 8.4 (control) to 8.4 (20% level of inulin). 5% and 10% level of inulin added *dhokla* scored higher than the standard *dhokla* compared to 15% and 20% level of inulin added *dhokla* scored similar to the standard. No significant difference was seen for all the levels of inulin substitution among the samples of *dhokla*.
11. **Overall acceptability:** As illustrated in Fig 5.2.4(d), the overall acceptability scores (OA scores) of all the *dhokla* ranged from 8.5 (control) to 8.3 (20% level of inulin). All the *dhoklas* at all levels of substitution were acceptable.



**Fig.5.2.4(a)-(d) Scores for Organoleptic Attributes of *Dhokla* Substituted with Varying Levels of Inulin**

Table 5.2.4.3: Difference in Organoleptic Attributes of Individual Test Samples in Comparison with the Standard *Dhokla*

Organoleptic attributes													
% Substitution	Std test value=5	Appearance	Color	Softness	Size & shape	Burn spots	Texture	Absence of defects	Flavor/ aroma	Mouth feel	Chew ability	Ease of swallow	Overall acceptability
5%	Mean	5.64	5.0	5.0	5.09	5.0	4.82	5.0	5.27	5.09 <sup>a</sup>	5.55	5.45	5.73
	SD±	±1.12	±0.0e	±0.89	±1.76	±0.0e	±0.60	±0.0e	±0.65	±1.30	±1.21	±1.04	±1.27
	t value	1.88	00	00	0.17	00	1.0	00	1.40	0.23	1.49	1.46	1.90
10%	Mean	5.64	5.0	5.0	5.09	5.0	4.82	5.0	5.18	5.73 <sup>b</sup>	5.55	5.45	5.73
	SD±	±1.12	±0.0e	±0.89	±1.14	±0.0e	±0.60	±0.0a	±0.60	±1.10	±1.21	±1.04	±1.27
	t value	1.88	00	00	0.27	00	1.0	00	1.0	2.19*	1.49	1.46	1.90
15%	Mean	5.64	5.0	5.0	5.09	5.0	4.82	5.0	5.18	5.45 <sup>a</sup>	5.55	5.45	5.73
	SD±	±1.12	±0.0e	±0.89	±1.76	±0.0e	±0.60	±0.0e	±0.60	±1.04	±1.21	±1.04	±1.27
	t value	1.88	00	0.27	0.17	00	1.0	00	1.0	1.46	1.49	1.46	1.90
20%	Mean	5.36	5.0	5.09	5.36	5.0	5.18	5.0	5.0	5.45 <sup>b</sup>	5.55	5.0	5.45
	SD±	±0.8	±0.0e	±1.14	±1.43	±0.0e	±0.60	±0.0e	±0.0e	±1.04	±1.21	±1.95	±1.04
	t value	1.49	00	00	0.84	00	1.0	00	00	2.19*	1.49	0.0	1.46
ANOVA		NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b, c – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.
- e-t value cannot be computed.

**b) Difference in organoleptic attributes of individual test samples in comparison with the standard *Dhokla***

As illustrated in Table 5.2.4.3, for all the attributes individual samples of inulin incorporated *dhoklas* at varying levels were compared with the standard sample, no significant difference was observed with the standard sample except for mouthfeel wherein 10% and 20% level of inulin added *dhokla* scored significantly higher than the standard sample. All the *dhokla*'s were rated well within the acceptable limits. The product incorporated with inulin, obtained similar scores for all the other sensory attributes. The overall acceptability scores of the inulin substituted *dhokla* reduced from 5.45 (20% prebiotic incorporated *dhokla*) to 5.73 (control), however this was statistically insignificant.

**For all the organoleptic attributes, inulin substituted *dhoklas* were acceptable up to 20% level of substitution. Organoleptic attributes like softness, color, absence of defects, texture and mouthfeel scored similar to the standard *dhoklas* whereas scores of after taste increased with increasing level of inulin substitution.**

**Section 5.2.5 Effect of Varying Levels of Inulin Addition to the *Potato Bonda***

The results of this subsection of the study illustrated in Table 5.2.5.1 to 5.2.5.3, determined the effect of varying levels of inulin addition on the physicochemical as well as sensory characteristics of *potato bonda*

**a) Assessment of Physico-chemical properties of the *Potato Bonda***

Weight of the cooked preparation was 500 g whereas weight of one serving (2 in no) was 84 g for the standard *bonda*. Adding inulin at varying levels did gradually increase the cooked weight of the *bondas* from 500 g (standard) to 508(20 % level). The oil absorbed was 62 g for standard *bondas* where there was decrease in absorption of oil as the level of inulin addition increased.

**Table 5.2.5.1: Physical Evaluation of Standard and Inulin Incorporated *Potato Bonda***

Characteristics	Standard Bonda	5%	10%	15%	20%
Cooked weight	500±0.1	500.2±0.2	504.3±0.2	506.35±0.2	508.4±0.2
Oil Absorption g/cc	62±0.01	62±0.03	60.5±0.02	60±0.02	59±0.03
Frying Time g	5 mins	4.5 mins	4 mins	3.5 mins	3 mins
Frying temperature cc	180	180	180	180	180

Values are Mean± S.D. of three independent determinations

Time for frying the *potato bondas* varied from 3 mins (20% level of inulin added bondas) to 5 mins (standard bondas).

The temperature of oil was 180°C for the cotton seed oil used for frying monitored with a digital thermometer

**b) Organoleptic evaluation of the *Potato bonda* :**

The results of the all organoleptic attributes of *potato bondas* are presented in Table 5.2.5.2

- 1) **Softness:** As indicated in Table no 5.2.5.2 for all the samples of *potato bonda* mean scores for softness ranged from 8.7 (standard) to 8.8 (20% level). No significant difference between the standard sample and sample with inulin addition up to 20% level was observed.
- 2) **Color:** All the samples of *potato bonda* had golden brown crumb color. The crumb color score ranged from 8.4 (standard) to 8.5 (20% level of inulin added *potato bonda*). The crumb color score remained constant with increasing level of inulin addition. No significant difference was noticed between the samples as the level of inulin addition increased.
- 3) **Crispness:** As illustrated in Fig 5.2.5(a), the mean scores for the crispness of the *potato bonda* ranged from 8.4 (standard) to 8.6 (20% level of inulin added *potato bonda*). According to the scores obtained, sample with 20% level of inulin addition had highest score followed by experimental sample with 15% level of inulin addition.

- 4) **Size and shape:** The size and shape scores of *potato bonda* ranged from 8.6 (standard) to 8.6 (inulin added *potato bonda* at 20% level). No significant difference in the size and shape scores was observed as the level of inulin addition increased.
- 5) **Crust:** The mean scores for crust of *potato bonda* ranged from 8.7 (standard) to 8.0 (20% level of inulin added *potato bonda*). No significant differences were perceived between standard and modified *potato bondas* up to 20% level of inulin addition.
- 6) **Appearance:** Small non significant differences were observed in the appearance scores of *potato bondas* prepared by addition of varying levels of inulin and it ranged from 8.7 (standard) to 8.3 (20% level of inulin added *potato bonda*).
- 7) **Absence of defects:** The mean scores for absence of defects ranged from 8.7 to 8.5 as the level of inulin addition increased. No significant difference was found among the inulin added at varying levels to *potato bonda* and the standard sample.
- 8) **Flavor:** For 'Flavor' of the *potato bondas* mean scores ranged from 8.0 (standard) to 8.5 (20% level of inulin added *potato bonda*). The experimental sample with 15% and 20% level showed higher scores whereas standard scored lowest. There was no significant difference among the samples and the standard *potato bonda* up to 20% level of inulin addition.
- 9) **Mouthfeel:** The mean mouthfeel scores ranged from 8.4 (standard) to 8.3 (20% level of inulin added *potato bonda*). No significant difference was noticed between the samples and standard for the mouthfeel scores of *potato bondas*.
- 10) **Texture:** As indicated in Table no 5.2.5.2, for all the samples of *potato bonda* mean scores for texture ranged from 8.7 (standard) to 8.7 (20% level of inulin added *potato bonda*). At all levels of inulin addition *potato bonda* scored similar to the standard. No significant differences were perceived between standard and modified *potato bondas* up to 20% level of inulin addition.
- 11) **Chewability:** For chewability in *potato bondas*, mean scores ranged from 8.3 (standard) to 8.2 (20% level of inulin added *potato bonda*). There was statistically no significant difference among the samples.

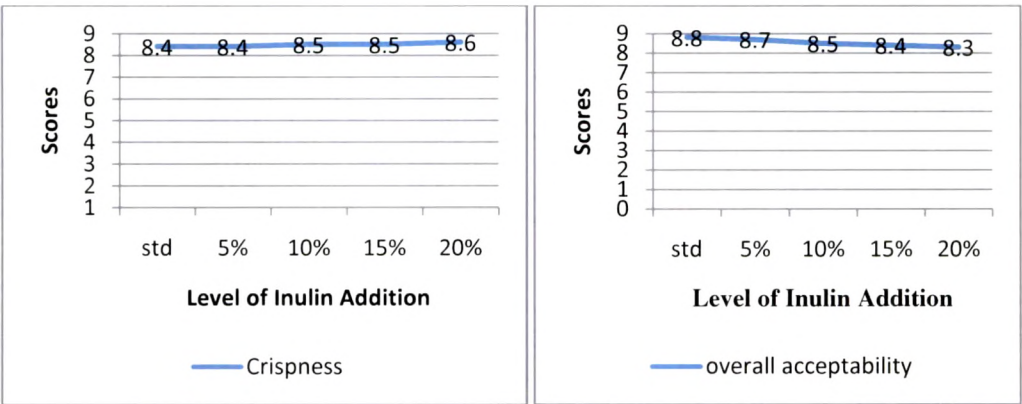


Table 5.2.5.2: Effect of Varying Levels of Inulin Addition on the Organoleptic Quality of *Potato Bonda*

% Addition		Organoleptic Attributes													
		Softness	Color	Crispness	Size & Shape	Crust	Appearance	Absence of Defects	Flavor	Taste	Texture	Chew Ability	After taste	Ease of Swallowing	Overall Acceptability
Std	Mean	8.7	8.4	8.4	8.6	8.7	8.7	8.7	8.0	8.4	8.7	8.3	8.6	8.8	8.8
	SD±	±0.4	±0.6	±0.4	±0.4	±0.4	±0.5	±0.5	±0.8	±0.7	±0.4	±0.6	±0.5	±0.5	±0.6
	Range	7-10	8-10	7-10	8-10	8-10	8-10	7-10	6-10	7-10	7-10	6-10	7-10	8-10	7-10
5%	Mean	8.7	8.5	8.4	8.5	8.3	8.6	8.7	8.2	8.5	8.6	8.5	8.6	8.8	8.7
	SD±	±0.4	±0.6	±0.4	±0.6	±1.3	±0.4	±0.6	±0.5	±0.6	±0.5	±0.5	±0.7	±0.5	±0.4
	Range	7-10	8-10	7-10	5-10	7-10	7-10	7-10	7-10	6-10	7-10	7-10	8-10	8-10	7-10
10%	Mean	8.7	8.5	8.5	8.5	8.2	8.5	8.6	8.4	8.4	8.7	8.5	8.6	8.8	8.5
	SD±	±0.3	±0.6	±0.4	±0.4	±1.3	±0.4	±0.7	±0.6	±0.6	±0.6	±0.5	±0.5	±0.5	±0.6
	Range	8-10	5-10	7-10	8-10	7-10	8-10	7-10	6-10	5-10	8-10	7-10	7-10	7-10	6-10
15%	Mean	8.8	8.5	8.5	8.6	8.1	8.4	8.5	8.5	8.3	8.7	8.3	8.7	8.7	8.4
	SD±	±0.5	±0.7	±0.4	±0.4	±1.4	±0.4	±0.6	±0.6	±0.7	±0.6	±0.7	±0.5	±0.5	±0.7
	Range	7-10	7-10	7-10	8-10	7-10	7-10	7-10	6-10	6-10	8-10	7-10	8-10	8-10	7-10
20%	Mean	8.8	8.5	8.6	8.6	8.0	8.3	8.5	8.5	8.3	8.7	8.2	8.8	8.7	8.3
	SD±	±0.6	±0.6	±0.6	±0.6	±1.3	±0.6	±0.6	±0.6	±0.7	±0.7	±0.9	±0.5	±0.6	±1.0
	Range	7-10	6-10	7-10	8-10	7-10	6-10	7-10	6-10	6-10	7-10	7-10	8-10	8-10	6-10
ANOVA		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b, c – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.
- e-t value cannot be computed

- 12) **After taste:** The aftertaste scores ranged from 8.6 (standard) to 8.8 (20% level of inulin added *potato bonda*). No significant difference was seen for all the levels of inulin addition among the samples.
- 13) **Ease of swallow:** The mean scores of ease of swallow ranged from 8.8 (standard) to 8.7 (20% level of inulin added *potato bonda*). At all levels of inulin addition *potato bonda* scored similar or higher to the standard. No significant difference was seen for all the levels of inulin added among the samples of *potato bonda*.
- 14) **Overall acceptability:** As illustrated in Fig 5.2.5 (b) the overall acceptability scores (OA scores) of all the *potato bonda* ranged from 8.8 (control) to 8.3 (20% level of inulin). All the samples scored low compared to the standard sample however no significant difference was seen for all the levels of inulin addition among the samples of *potato bonda*.



(a) Crispness

(b) OA Scores

**Fig.5.2.5(a)-(b) Scores for Organoleptic Attributes of *Potato Bonda* Substituted with Varying Levels of Inulin**

For quality characteristics like for texture, crispness, color and flavor inulin incorporated *bondas* assumed high overall acceptability compared to the standard. Analysis of variance revealed that the difference among the various characteristics such as crust, appearance, absence of defects, taste and mouthfeel and aftertaste had lower scores than the standard sample.

Table 5.2.5.3: Difference in Organoleptic Attributes of Individual Test Samples in Comparison with the Standard *Potato Bonda*

Organoleptic Attributes													
% Addition		Appearance	Color	Softness	Size n	Burn	Texture	Absence of Defects	Aroma	Mouth	Chew	Ease of Swallowing	Overall Acceptability
Std test value=5					Shape	Spots				Feel	Ability		
5%	Mean	4.09	4.18	4.09	4.0	3.82	4.73	4.0	4.73	5.0	4.18	4.09	4.45
	SD±	±1.64	±1.83	±2.02	±2.0	±1.94	±2.45	±1.84	±2.41	±2.90	±1.40	±2.02	±2.50
	t value	1.84	1.48	1.49	1.66	2.02	0.37	1.80	0.38	00	1.94	1.49	0.72
10%	Mean	3.91	4.09	3.82	4.09	3.82	3.82	3.73	4.27	4.73	4.0	3.91	4.09
	SD±	±1.97	±1.81	±1.89	±1.81	±2.23	±2.32	±1.62	±2.24	±2.01	±2.0	±2.02	±1.87
	t value	1.83	1.66	2.08	1.66	1.76	1.69	2.61	1.08	0.45	1.66	1.79	1.61
15%	Mean	3.82	3.73	3.55	3.73	3.55	3.64	3.82	4.09	4.09	3.82	3.82	3.91
	SD±	±1.99	±1.95	±1.44	±1.95	±2.02	±1.96	±2.09	±2.02	±1.87	±1.94	±1.60	±1.81
	t value	1.97	2.16	3.35	2.16	2.39	2.30	1.88	1.49	1.61	2.02	2.45	1.99
20%	Mean	3.45	3.64	2.82	3.73	3.45	3.45	3.27	3.82	3.82	3.55	3.73	3.82
	SD±	±1.92	±1.96	±1.33	±1.95	±1.86	±2.12	±1.79	±2.09	±1.25	±2.12	±1.95	±1.66
	t value	2.68	2.30	5.45	2.16	2.75	2.42	3.19	1.88	3.14	2.28	2.16	2.36
ANOVA		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b,c – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.

**c) Difference in organoleptic attributes of individual test samples in comparison with the standard *Potato bonda***

As seen in Table 5.2.5.3, No significant difference was perceived for all the organoleptic attributes on the difference test scale, compared to the standard *bonda*.

**For organoleptic attributes like crispness, flavor and after taste, 15% and 20% level of inulin added *potato bonda* scored higher compared to the standard. Inulin enriched *potato bonda* was accepted quite well among panel members up to 20% level of incorporation**

**Section 5.2.6 Effect of Varying Levels of Inulin Addition to the Porridge**

The results of acceptability trials of inulin incorporation in porridge is presented in Table 5.2.6.1 to 5.2.6.3

**a) Assessment of Physico-chemical properties of the porridge**

As can be seen in table 5.2.6.1, the water retention of the cereal pulse porridge is 70.04% for the standard porridge. As the level of inulin addition increased from 0% level of inulin addition to 20% there was no change in water absorption power. Cooked weight of the standard porridge was 806 g. Addition of inulin showed a gradual increase in the weight of porridge from 809 (5% level) to 819 (20% level). Time of cooking was 10 mins pressure cooked that was kept constant with regards to varying levels of inulin addition.

**Table 5.2.6.1: Physical Evaluation of Standard and Inulin Incorporated Porridge**

Characteristics	Standard Porridge	5%	10%	15%	20%
Water absorption power	70.04%	70%	70%	69.9%	69.85%
Cooked Weight g	806±0.2	809±0.37	811±0.26	815±0.14	819±0.11
Time of cooking	10 mins	10 mins	10 mins	10 mins	10 mins

**b) Organoleptic evaluation of the Porridge**

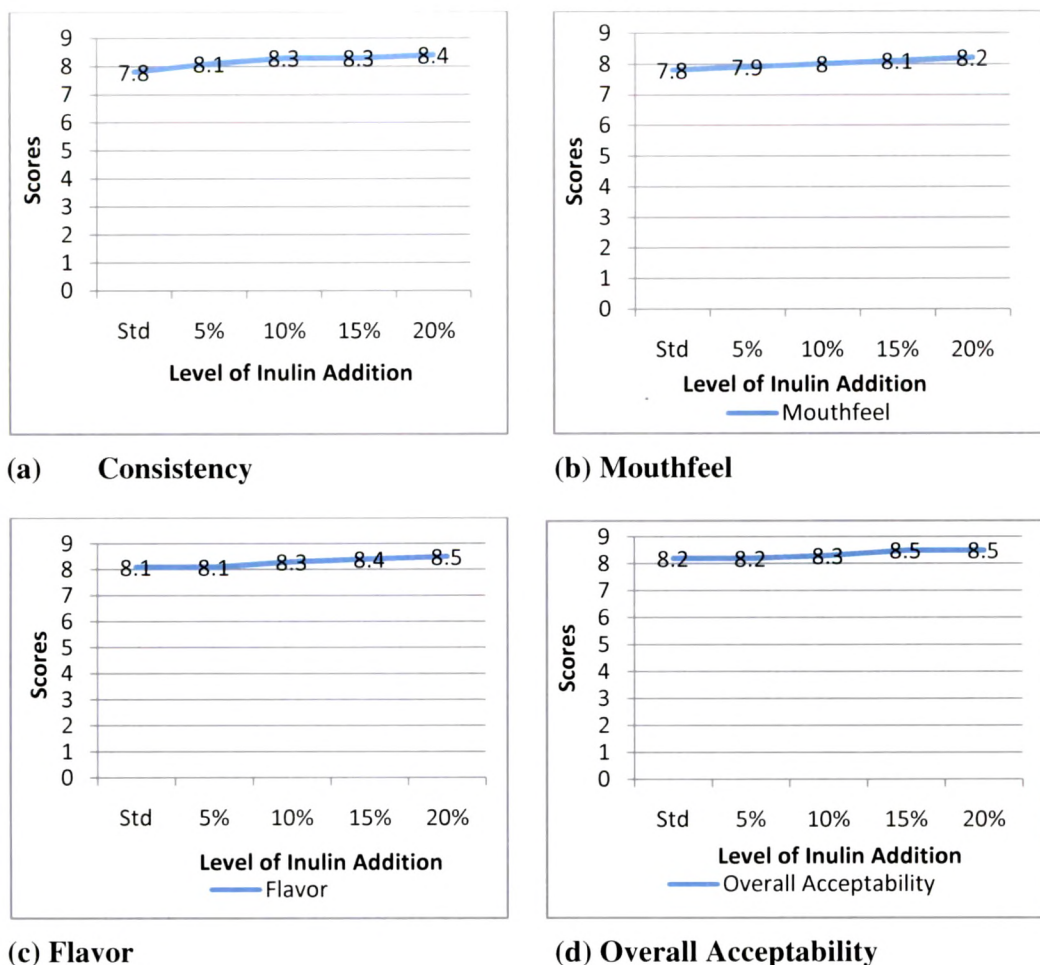
The effects of inulin incorporation on the organoleptic characteristics are presented in Table. 5.2.6.2

- 1) **Softness:** As indicated in Table no 5.2.6.2 for all the samples of porridge mean scores ranged from 8.0 (standard) to 8.5 (Inulin substituted porridge at 20% level). No significant difference was perceived between the standard porridge and porridge with inulin addition at 20% level. Therefore, as the level of inulin addition increased there was increase in softness scores.
- 2) **Color:** The color score increased from 7.9 (standard) to 8.5 (Inulin substituted porridge at 20% level) with increasing level of inulin addition. No significant difference was noticed between the samples though a increasing trend for color scores was noticeable as the level of inulin addition increased
- 3) **Consistency:** As indicated in Fig. 5.2.6, consistency scores ranged from 8.1 (5% level of inulin incorporated porridge) to 8.4 (20% level of inulin incorporated porridge) as against 7.8 scored by the standard sample. There was no significant difference in this quality characteristic of porridge within the levels of addition.
- 4) **Size and shape:** The size and shape scores for all the porridge ranged from 8.2 (0% level) to 8.5 (20% level of inulin incorporated porridge). No significant difference was observed in size and shape scores of all the porridge as the addition level increased from 0% to 20%.
- 5) **Burn spots:** The mean scores for burn spots of porridge ranged from 8.3 (standard) to 8.5 (20% level of inulin incorporated porridge). A non significant difference in the burn spots scores of inulin incorporated porridge upto 20% level was observed. There was increase in burn spot scores as the level of inulin addition increased.
- 6) **Appearance:** As can be seen from the Table 2.2.6.2, the appearance scores of all the porridge ranged from 8.4 (20% level of inulin incorporated porridge) to 8.2 (standard). In general appearance scores increased as the level of addition increased from 0% to 20% level.

Table 5.2.6.2: Effect of Varying Levels of Inulin Addition on the Organoleptic Quality of Porridge

% Addition		Organoleptic Qualities													
		Softness	Color	Consistency	Size and Shape	Burn Spots	Appearance	Absence of Defects	Flavor	Mouthfeel	Texture	After Taste	Chew Ability	Ease of Swallowing	Overall Acceptability
Std	Mean	8.0	7.9	7.8	8.2	8.3	8.2	8.2	8.1	7.8	8.0	8.0	8.2	8.3	8.2
	SD±	±0.9	±1.0	±0.9	±0.9	±0.8	±0.9	±0.9	±0.9	±0.9	±0.9	±0.8	±0.7	±0.8	±0.8
	Range	7-10	6-10	7-10	7-10	7-10	6-10	6-10	7-10	5-10	5-10	5-10	6-10	6-10	6-10
5%	Mean	8.0	8.1	8.1	8.4	8.3	8.2	8.3	8.1	7.9	8.1	8.0	8.1	8.3	8.2
	SD±	±0.9	±0.9	±0.9	±0.8	±0.9	±0.9	±0.8	±0.9	±0.9	±0.8	±0.9	±0.8	±0.8	±0.8
	Range	6-10	6-10	5-10	6-10	6-10	5-10	6-10	7-10	6-10	6-10	6-10	6-10	6-10	6-10
10%	Mean	8.3	8.4	8.3	8.4	8.4	8.3	8.4	8.3	8.0	8.2	8.0	8.2	8.3	8.3
	SD±	±0.7	±0.8	±0.8	±0.7	±0.7	0.8	±0.9	±0.8	±0.8	±0.8	±0.8	±0.8	±0.8	±0.8
	Range	7-10	7-10	7-10	7-10	7-10	7-10	6-10	7-10	8-10	7-10	7-10	7-10	7-10	7-10
15%	Mean	8.3	8.4	8.3	8.4	8.4	8.4	8.5	8.4	8.1	8.2	8.1	8.3	8.4	8.5
	SD±	±0.7	±0.8	±0.8	±0.7	±0.7	±0.8	±0.7	±0.7	0.8	±0.8	±0.9	±0.8	±0.8	±0.7
	Range	7-10	7-10	7-10	6-10	6-10	6-10	6-10	6-10	7-10	7-10	6-10	6-10	6-10	7-10
20%	Mean	8.5	8.5	8.4	8.5	8.5	8.4	8.5	8.5	8.2	8.2	8.2	8.4	8.4	8.5
	SD±	±0.7	±0.9	±0.8	±0.8	±0.8	±0.8	±0.7	±0.7	±0.8	±0.8	±0.8	±0.8	±0.8	±0.7
	Range	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	6-10	7-10	5-10	6-10	6-10	7-10
ANOVA		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b, c – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.



**Fig. 5.2.6.1(a)-(d) Scores for Organoleptic Attributes of Porridge Substituted with Varying Levels of Inulin**

- 7) **Absence of defects:** The mean scores for absence of defects ranged from 8.2 (0% level) to 8.5 (20% level of inulin incorporated porridge) as the level of addition increased. There was no significant difference up to 20% level of inulin addition with standard sample.
- 8) **Flavor:** As illustrated in Fig 5.2.6(c), inulin added at varying levels in porridge, resulted in porridge that were comparable to the standard for flavor scores. No significant differences were observed in flavor scores of porridge added with inulin, ranging from 8.1 (standard) to 8.5 (20% level of inulin incorporated porridge).

Table 5.2.6.3: Difference in Organoleptic Attributes of Individual Test Samples in Comparison with the Standard Porridge

Std test value=5		Appearance	Color	Softness	Size & shape	Texture	Absence of Defects	Flavor/ aroma	Mouth feel	After Taste	Ease of Swallow	Overall Acceptability
5%	Mean	5.27	5.36	5.27	5.45	5.09	4.82	5.00	6.00	5.64	5.27	5.55
	SD±	±1.74	±0.92	±1.68	±1.92	±1.58	±1.40	±1.48	±1.10	±1.12	±0.91	±1.21
	t value	0.521	1.305	0.539	0.787	0.191	0.430	0.00	3.028*	1.884	1.000	1.491
10%	Mean	5.36	5.64	5.27	5.09	5.27	4.55	5.00	5.55	5.64	5.27	5.82
	SD±	±2.06	±1.21	±2.05	±1.97	±2.24	±1.51	±1.48	±1.57	±1.12	±0.91	±1.40
	t value	0.585	1.750	0.440	0.153	0.404	1.00	0.00	1.150	1.884	1.000	1.936
15%	Mean	5.18	5.55	5.18	5.55	5.36	4.73	5.09	5.91	5.73	5.36	5.64
	SD±	±1.99	±1.04	±1.94	±2.21	±2.20	±1.68	±1.87	±1.14	±1.10	±0.92	±1.20
	Range	0.303	1.747	0.311	0.820	0.547	0.539	0.161	2.654*	2.185*	1.305	1.750
20%	Mean	5.55	5.64	5.45	5.73	5.55	4.64	5.64	6.27	6.00	5.73	5.45
	SD±	±1.92	±1.21	±1.81	±2.05	±2.07	±1.50	±1.12	±1.19	±1.41	±1.27	±2.02
	t value	0.944	1.750	0.833	1.174	0.875	0.803	1.884	3.545**	2.345*	1.896	0.747
ANOVA		NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b,c – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.



- 9) **Mouthfeel:** As indicated in Fig 5.2.6(b) mean scores for porridge ranged from 8.5 (5% level of inulin incorporated porridge) to 8.6 (20% level of inulin incorporated porridge) as against 78.5 scored by the control sample. No significant difference was noticed between all the inulin added samples and standard sample for the mouthfeel scores of porridge. The scores for the standard porridge were lowest, ranging from 7.8 to 8.2 (20% level of inulin incorporated porridge). An overall was noticed for this attribute for all the samples.
- 10) **Texture:** As can be seen from the Table 5.2.6.2, texture scores ranged from 8.0 (0% level of inulin incorporated porridge) to 8.2 (20% level of inulin incorporated porridge). 20% level of inulin incorporated porridge scored highest among the samples, there was no significant between all the samples of porridge within the levels of addition.
- 11) **Aftertaste:** For aftertaste in porridge, mean scores ranged from 8.0 (standard) to 8.2 (20% level of inulin incorporated porridge). Mean score for 20% level of inulin incorporation scored higher for aftertaste than the standard sample. 5% and 10% level of inulin incorporation scored similar to the standard for this attribute. There was statistically no significant difference among all the samples of porridge for aftertaste.
- 12) **Chewability:** The mean scores for chewability ranged from 8.2 (standard) to 8.4 (20% level of inulin incorporated porridge). Although small differences can be seen in the mean scores of chewability for varying levels of inulin addition, this difference was found to be statistically significant.
- 13) **Ease of swallow:** For 'ease of swallow' all the samples scored almost similar to the standard sample however 15% and 20% level of inulin added porridge scored highest among the samples. No significant difference was seen for all the levels of inulin addition among the samples of porridge.
- 14) **Overall acceptability:** As illustrated in Fig 5.2.6(d) the overall acceptability scores (OA scores) of all the porridge ranged from 8.2 (control) to 8.5 (20% level of inulin). The 15% and 20% level of inulin incorporated porridge rated above the standard though porridge at all levels of addition were quite acceptable.

c) **Difference in organoleptic attributes of individual test samples in comparison with the standard porridge**

As seen in Table 5.2.6.3, inulin incorporated at varying levels to porridge scored higher for attributes like appearance, color, softness, size and shape, texture, flavor, ease of swallow and overall acceptability against the standard sample with test score of 5. Scores for mouthfeel and aftertaste were significantly higher for 15% and 20% level of inulin incorporated porridge against the standard sample.

**Inulin added cereal pulse porridge was acceptable up to 20% level. Compared to the standard porridge a non significant increase for all the organoleptic attributes was observed in 15% and 20% level of inulin addition**

**Section 5.2.7 Effect of Varying Levels of Inulin Addition to the Sweet Orange Juice**

In the present section of the study sweet orange juice was selected as one of the source material to be developed as an innovative, nutritious drink and to examine the effects of level of inulin incorporation on the physical and sensory of juice to facilitate maximize inulin inclusion for nutritional enhancement.

a) **Assessment of Physico-chemical properties of the Sweet orange juice**

The data revealed that the juice recovery for the standard juice prepared by peeling, crushing and pressing the whole sweet oranges was 72%. The density was calculated as ratio of mass by volume of the juice and density of the standard sweet orange juice was 1.1. The pH of the sweet orange juice was 3.36.

**Table 5.2.7.1 Physical Evaluation of Standard orange Juice**

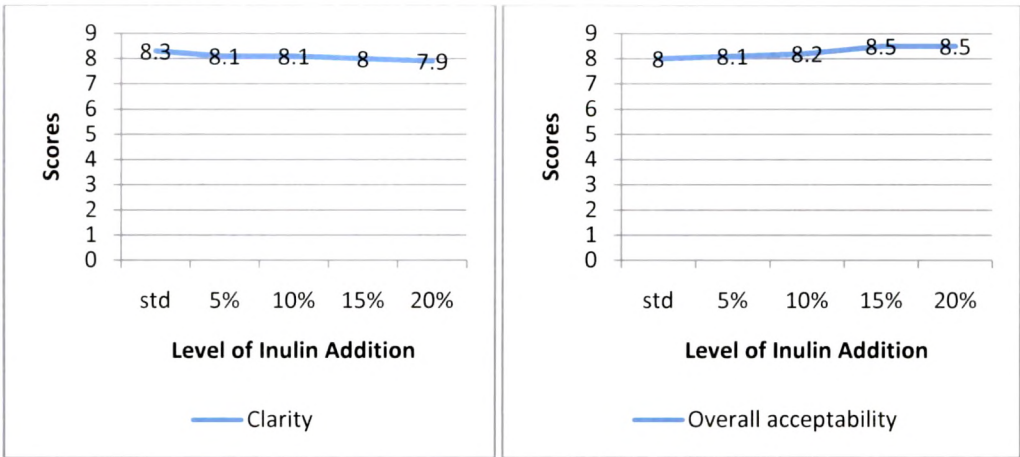
Characteristics	Standard juice
Juice Recovery	72%
Juice Density	1.1
pH of the juice	3.36

**b) Organoleptic evaluation of the Sweet orange juice:**

The organoleptic scores of juice prepared with addition of varying levels of inulin are presented graphically in Fig. 5.2.7 and the data is detailed in Table 5.2.7.1 to 5.2.7.3

- 1) **Color:** In all cases, the color scores indicated that the incorporation of inulin at varying levels brought about no significant difference in the color of sweet orange juice.
- 2) **Consistency:** Consistency scores ranged from 8.3 (5% level of inulin incorporated sweet orange juice) to 8.5 (20% level of inulin incorporated sweet orange juice) as against 8.4 scored by the standard sample. There was no significant difference between standard and sweet orange juice upto 20% level of inulin addition though there was increase in the consistency scores of the juice which may be due to increase in thickness because of addition of inulin.
- 3) **Clarity:** As illustrated in Fig 5.2.7(a), the clarity scores for all the sweet orange juice ranged from 8.3 (0% level) to 7.9 (20% level of inulin incorporated sweet orange juice). No significant difference was observed in clarity scores of all the sweet orange juice as the addition level increased from 0% to 20% level.
- 4) **Odour:** The mean scores for odour of sweet orange juice ranged from 7.8 (standard) to 8.4 (20% level of inulin incorporated sweet orange juice). No significant difference was noted in the odour scores of inulin incorporated sweet orange juice upto 20% level within all the levels of addition.
- 5) **Appearance:** The appearance scores of all the sweet orange juice ranged from 8.4 (20% level of inulin incorporated sweet orange juice) to 8.7 (standard). In general appearance scores decreased as the level of addition increased from 0% to 20% level of inulin incorporation. There was no significant difference in scores between standard and 20% level of inulin incorporated sweet orange juice.
- 6) **Absence of defects:** The mean scores for absence of defects were similar to the standard (8.4) and constant with varying levels of inulin addition.
- 7) **Flavor:** No significant difference was noticed between all the inulin added samples and standard sample for the flavor scores of sweet orange juice. The

scores for the standard sweet orange juice, ranged from 8.2 to 8.3 (20% level of inulin incorporated sweet orange juice).



(a) Clarity (b) Overall acceptability

Fig. 5.2.7(a)-(b) Scores for Organoleptic Attributes of Juice Substituted with Varying Levels of Inulin

- 8) **Mouthfeel:** Mouthfeel scores of sweet orange juice added with inulin, ranged from 8.1 (standard) to 8.4 (20% level of inulin incorporated sweet orange juice). No significant differences in mouthfeel scores were observed between standard sweet orange juice and juice prepared from addition of inulin up to 20% level of incorporation.
- 9) **Sweetness:** The sweetness scores of sweet orange juice incorporated with inulin ranged from 8.2 (standard) to 8.5 (Inulin added sweet orange juice at 20% level). No significant differences were perceived between the standard sweet orange juice and sweet orange juice with inulin addition up to 20% level.
- 10) **Sourness:** No significant difference was noticed between all the inulin added samples and standard sample for the sourness scores of sweet orange juice. The scores for the standard sweet orange juice were lowest, ranging from 7.7 to 8.0 (20% level of inulin incorporated sweet orange juice). An overall increase was noticed for this attribute for all the samples depicting that sourness decreased as the scores increased.

Table 5.2.7.2: Effect of Varying Levels of Inulin Addition on the Organoleptic Quality of Juice

% Addition		Organoleptic attributes												
		Color	Consistency	Clarity	Odour	Appearance	Absence of defects	Aroma	Taste and mouthfeel	Sweetness	Aftertaste	Sourness	Bitterness	Overall acceptability
Std	Mean	8.4	8.4	8.3	7.8	8.7	8.4	8.2	8.2	8.2	8.2	7.7	8.0	8.0
	SD±	±0.9	±0.9	±0.8	±1.0	±1.0	±0.7	±0.9	±0.9	±1.0	±0.9	±1.1	±1.0	±0.7
	Range	6-10	7-10	7-10	5-10	5-10	7-10	6-10	6-10	5-10	7-10	7-10	7-10	6-10
5%	Mean	8.4	8.3	8.1	8.0	8.6	8.4	8.2	8.1	8.2	8.2	7.7	8.0	8.1
	SD±	±0.9	±0.8	±0.8	±0.9	±1.0	±0.7	±0.8	±0.9	±1.1	±1.0	±1.4	±0.9	±0.7
	Range	6-10	7-10	7-10	6-10	7-10	7-10	7-10	6-10	7-10	6-10	5-10	7-10	7-10
10%	Mean	8.4	8.4	8.1	8.1	8.5	8.4	8.2	8.2	8.3	8.3	7.8	8.1	8.2
	SD±	±0.8	±0.8	±0.8	±0.8	±0.7	±0.8	±0.8	±0.9	±0.8	±0.9	±1.4	±0.8	±0.5
	Range	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	5-10	6-10	7-10	5-10	5-10
15%	Mean	8.3	8.5	8.0	8.1	8.4	8.4	8.2	8.3	8.5	8.4	7.9	8.2	8.5
	SD±	±1.0	±0.8	±0.7	±1.0	±0.8	±0.7	±0.7	±0.8	±0.9	±0.9	±1.5	±0.8	±0.6
	Range	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10
20%	Mean	8.2	8.5	7.9	8.4	8.4	8.4	8.3	8.4	8.5	8.5	8.0	8.3	8.5
	SD±	±1.1	±0.8	±0.9	±0.9	±0.7	±0.7	±0.8	±0.7	±0.9	±0.8	±1.3	±0.8	±0.6
	Range	7-10	7-10	5-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	5-10	5-10	7-10
ANOVA		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.
- For consistency, clarity, sourness and bitterness absence is reported. Increase in scores depict there was decrease in these attributes.

- 11) **After taste:** Mean scores for after taste ranged from 8.2 (5% level of inulin incorporated sweet orange juice) to 8.5 (20% level of inulin incorporated sweet orange juice) as against 8.2 scored by the standard sample, except 5% level of inulin incorporated sweet orange juice all the samples scored higher than the standard, there was no significant between all the samples of sweet orange juice within the levels of addition.
- 12) **Bitterness:** For bitterness in sweet orange juice, mean scores ranged from 8.0 (standard) to 8.3 (20% level of inulin incorporated sweet orange juice). Bitterness scores depicted as the level of inulin incorporation increased in the sweet orange juice there was decrease in the bitterness of the juice.
- 13) **Overall acceptability:** As illustrated in Fig 5.2.7(b) the overall acceptability scores (OA scores) of all the sweet orange juice ranged from 8.0 (control) to 8.5 (20% level of inulin). The OA scores were highest for 15% and 20% level of inulin incorporation compared to the standard within all the levels of inulin addition

**c) Difference in organoleptic attributes of individual test samples in comparison with the standard sweet orange juice**

For all the attributes, individual samples of inulin incorporated sweet orange juices at varying levels were compared with the standard sample, no significant difference was observed with the standard sample. Sweet orange juice added with 5% and 10% level of inulin scored higher than the standard for attributes like color, sourness and overall acceptability whereas 5 % level of inulin incorporation scored similar to the standard for sweetness and flavor. 15% level of inulin incorporated sweet orange juice scored similar to the standard juice for mouthfeel and bitterness whereas scores were high for color, sourness and flavor attributes. Experimental juice sample with 20% level of inulin addition scored higher than the standard juice sample for color, sweetness, sourness, bitterness and overall acceptability compared to similar scores for sweetness. Significant increase in after taste scores for 5% and 10% level of inulin added juice. Significant lower scores were observed for clarity at 15% and 20% level of inulin added sweet orange juice.

Table 5.2.7.3: Difference in Organoleptic attributes of Individual Test Samples in Comparison with the Standard Juice

% Addition		Organoleptic Attributes										
Std test value=5	Appearance	Color	aftertaste	Sourness	Sweetness	clarity	consistency	aroma	Mouth feel	Absence of defects	bitterness	Overall acceptability
5%	Mean	4.91	5.36	6.64	5.64	5.00	4.55	4.91	4.82	4.73	4.55	5.27
	SD±	±0.70	±0.92	±1.36	±1.21	±0.89	±0.82	±1.14	±0.60	±0.65	±1.51	±0.65
	t value	0.43	1.31	3.99	1.75	0.00	1.84	0.27	1.00	1.40	1.00	1.40
10%	Mean	4.91	5.27	6.45	5.27	4.64	4.55	5.09	4.82	4.55	4.55	5.27
	SD±	±0.70	±0.65	±1.29	±1.27	±0.81	±0.82	±0.30	±0.60	±0.82	±1.04	±0.91
	t value	0.43	1.40	3.73	0.71	1.49	1.84	1.00	1.00	1.84	1.45	1.00
15%	Mean	4.91	5.27	6.18	5.45	4.82	.00	5.09	5.00	4.73	5.00	4.91
	SD±	±0.70	±0.65	±2.09	±1.04	±0.60	±1.00	±0.30	±0.89	±0.65	±0.89	±0.94
	t value	0.43	1.40	1.88	1.46	1.00	3.32	1.00	0.00	1.40	0.00	0.32
20%	Mean	4.82	5.09	4.55	5.36	5.00	4.27	4.73	5.00	4.55	5.27	5.27
	SD±	±0.75	±0.30	±1.64	±0.92	±0.89	±1.01	±0.65	±0.89	±0.82	±0.65	±0.91
	t value	0.80	1.00	0.92	1.31	0.00	2.39	1.40	0.00	1.84	1.00	1.00
ANOVA		NS	NS	*	NS	NS	*	NS	NS	NS	NS	NS

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b,c – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.

**Inulin addition at varying levels in sweet orange juice was accepted quite well among panel members up to 20% level of incorporation. A non significant decrease was observed for scores of color, clarity and appearance. Scores for Organoleptic attributes such as consistency, odor, sourness, bitterness and after taste, mouthfeel and overall acceptability were high for 15% and 20% added sweet orange juice when compared to the standard**

### **Section 5.2.8 Effect of Varying Levels of Inulin Addition to the Curd**

The results of acceptability trials of inulin incorporation in curd is presented in Table 5.2.8.1 and 5.2.8.2

#### **a) Assessment of Physico-chemical properties of the Curd**

**pH of the curd:** The curd sample procured had a pH of 5.5

#### **b) Organoleptic evaluation of the Curd**

The results of the all organoleptic attributes of curds are presented in Table 5.2.8.1

- 1) **Appearance:** As indicated in Table no 5.2.8.1, for all the samples of curd mean scores ranged from 8.5 to 8.4. No significant difference between the standard sample and sample with inulin addition upto 20% level was observed.
- 2) **Color:** The color score ranged from 8.5 (standard) to 8.6 (20% level of inulin added curd). No significant difference was noticed between the samples as the level of inulin addition increased.
- 3) **Consistency:** The mean scores for the consistency of the curd ranged from 7.8 (standard) to 8.4 (20% level of inulin added curd). According to the scores obtained, sample with 20% level of inulin addition had significant highest score for consistency, compared to the standard scores.
- 4) **Sweetness:** The sweetness scores of curd ranged from 8.0 (standard) to 8.2 (inulin added curd at 20% level). No significant difference in the sweetness scores was observed as the level of inulin addition increased.
- 5) **Absence of defects:** The mean scores for absence of defects ranged from 8.2 to 8.4 as the level of addition increased. No significant difference was found among the inulin added at varying levels to curd and the standard sample.



- 6) **Flavor:** For 'Flavor' of the curds mean scores ranged from 8.0 (standard) to 8.3 (20% level of inulin added curd). The experimental sample with 15% and 20% level showed higher scores whereas standard scored lowest. There was no significant difference among the samples and the standard curd upto 20% level of inulin addition.
- 7) **Mouthfeel:** The mean mouthfeel scores ranged from 8.3 (standard) to 8.5 (20% level of inulin added curd). No significant difference was noticed between the samples and standard for the mouthfeel scores of curds.
- 8) **Texture:** As indicated in Table no 5.2.8.1, for all the samples of curd mean scores ranged from 8.1 (standard) to 8.8 (20% level of inulin added curd). At all levels of inulin addition curd scored higher than the standard curd. Significant differences were perceived between standard and curd with 15% & 20% level of inulin addition.
- 9) **After taste:** The aftertaste scores ranged from 7.4 (standard) to 7.6 (20% level of inulin added curd). Mean score for 20% level of inulin incorporation scored higher for aftertaste than the standard sample. 5% and 10% level of inulin incorporation scored similar to the standard for this attribute. No significant difference was seen for all the levels of inulin addition among the samples.
- 10) **Ease of swallow:** The mean scores of ease of swallow ranged from 7.5 (standard) to 7.9 (20% level of inulin added curd). At all levels of inulin addition curd scored similar or higher to the standard. No significant difference was seen for all the levels of inulin added among the samples of curd.
- 11) **Overall acceptability:** The overall acceptability scores (OA scores) of all the curd ranged from 7.0 (control) to 8.5 (20% level of inulin). All the samples scored higher compared to the standard sample however, no significant difference was observed between the standard and inulin added curd at 15-20% level.

Table 5.2.8.1: Effect of Varying Levels of Inulin Supplementation on the Organoleptic Quality of Curd

% Addition		Organoleptic qualities									
Appearance		Color	Consistency	Sweetness	Texture	Flavor	Mouth feel	Aftertaste	Ease of swallow	Absence of defects	Overall acceptability
Std	Mean	8.5	7.8 <sup>a</sup>	8.0	8.1 <sup>a</sup>	8.0	8.3	7.4	7.5	8.2	7.0 <sup>a</sup>
	SD±	±0.8	±1.0	±0.8	±0.6	±0.9	±0.8	±0.7	±1.1	±0.9	±0.8
	Range	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10
5%	Mean	8.5	8.0 <sup>a</sup>	8.0	8.1 <sup>a</sup>	8.1	8.3	7.4	7.5	8.2	7.2 <sup>a</sup>
	SD±	±0.8	±0.9	±0.8	±0.7	±0.9	±0.9	±0.6	±1.0	±0.9	±1.1
	Range	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10
10%	Mean	8.4	8.1 <sup>a</sup>	8.2	8.5 <sup>ab</sup>	8.2	8.4	7.4	7.9	8.3	7.5 <sup>a</sup>
	SD±	±0.9 <sup>a</sup>	±0.9	±0.5	±0.6	±0.9	±0.7	±0.8	±0.7	±0.8	±1.0
	Range	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10
15%	Mean	8.4	8.1 <sup>a</sup>	8.2	8.7 <sup>b</sup>	8.3	8.4	7.6	7.9	8.4	8.2 <sup>b</sup>
	SD±	±0.9	±1.0	±0.6	±0.4	±0.8	±0.7	±1.3	±0.7	±0.8	±0.5
	Range	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10
20%	Mean	8.4	8.4 <sup>b</sup>	8.2	8.8 <sup>b</sup>	8.3	8.5	7.8	7.9	8.4	8.5 <sup>b</sup>
	SD±	±0.9	±0.9	±0.6	±0.6	±0.8	±0.8	±0.8	±0.7	±0.8	±0.7
	Range	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10	7-10
ANOVA		NS	*	NS	*	NS	NS	NS	NS	NS	*

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b,c – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.

Table 5.2.8.2: Difference in Organoleptic attributes of Individual Test Samples in Comparison with the Standard Curd

% Addition		Organoleptic qualities								
test value=5	Appearance	Color	Sweetness	Texture	Flavor	Mouth feel	Aftertaste	Ease of swallow	Absence of defects	Overall acceptability
5%	Mean	5.55	4.82	4.55	4.55	5.55	4.18	5.27	5.45	5.2
	SD±	±1.21	±0.60	±0.82	±1.04	±1.57	±2.09	±0.65	±1.04	±1.1
	t value	1.49	1.00	1.84	1.45	1.150	1.30	1.40	1.46	1.44
10%	Mean	5.55	4.82	4.73	4.55	5.91	4.55	5.27	5.45	5.5
	SD±	±1.21	±0.60	±0.65	±1.51	±1.14	±0.82	±0.65	±1.04	±1.0
	t value	1.49	1.00	1.40	1.00	2.654*	1.84	1.40	1.46	1.19
15%	Mean	5.55	5.00	4.73	5.00	6.00	4.82	5.09	5.0	6.2
	SD±	±1.21	±0.89	±0.65	±0.89	±1.10	±0.60	±0.30	±1.95	±0.5
	t value	1.49	0.00	1.40	0.00	3.028*	1.00	1.00	0.0	3.224*
20%	Mean	5.55	5.00	4.73	5.27	6.27	5.55	5.36	5.45	6.5
	SD±	±1.21	±0.89	±0.65	±0.65	±1.19	±1.21	±0.92	±1.04	±0.7
	t value	1.49	0.00	1.40	1.00	3.545**	1.49	1.31	1.46	3.010*
ANOVA	*	NS	NS	NS	NS	NS	*	NS	NS	NS

- Note: Mean values represent the average of 10 determinants in triplicates.
- a, b, c – The non identical letters in any two rows within the column denote a significant difference at a minimum of 5% level.
- NS – The difference between the mean values within the columns is not significant.
- Maximum score for all the organoleptic attributes was 10.

**c) Difference in organoleptic attributes of individual test samples in comparison with the standard Curd**

As seen in Table 5.2.8.2, when assessed for organoleptic attributes on difference test scale, all the samples scored lower for all attributes compared to the standard curd. Inulin enriched curd was accepted quite well among panel members upto 20% level of incorporation.

**The curd enriched with inulin up to the level of 20% was well within the acceptable limits. The addition of inulin at all levels had a remarkable improvement in scores of experimental curds for texture and overall acceptability.**

**Section 5.2.9 Proximate Composition of all the Food Products**

The proximate composition of the standard and inulin incorporated recipes are presented in table 5.2.9(a) and (b).

In the products wherein inulin was substituted for the base material i.e bread, *chapati* and *dhokla*, the total moisture, total ash, fat, protein content was low compared standard recipes. Whereas carbohydrate content and energy value was high in bread *chapati*, *dhokla* substituted with 20% inulin. Pasrachie and Rebello, 1998 reported similar nutrient content for standard recipes for bread, *chapati* and *dhoklas*. Fat replaced by inulin in cookies showed lower fat content and energy value than the standard cookies whereas addition of inulin increased the energy value of porridge, potato bonda, juice and curd without any significant changes in the other nutrients.

Table 5.2.9: (a) Percent Proximate Composition of Standard Recipes

Recipes	Ash (%)	Moisture(%)	Fat (g)	Protein (g)	CHO (g)	Energy(Kcal)
Bread	1.32±0.05	39.67±0.83	5.87±0.56	7.05±0.034	46.08±0.45	265.35
Cookies	0.87±0.34	3.64±1.91	19.57±0.20	6.66±0.04	69.26±0.26	479.81
Chappati	1.23±0.12	34.25±0.91	4.73±0.15	9.21±0.021	50.59±0.53	281.71
Dhokla	1.92±0.13	71.25±1.33	1.90±0.02	6.53±0.046	18.39±0.2	116.78
Daliya	1.96±0.23	62.92±1.65	4.04±0.05	5.29±0.01	25.79±0.25	160.68
Wada	1.87±0.08	63.62±1.55	8.38±0.32	5.16±0.01	20.97±0.05	179.94
Juice	0.29±0.05	83.5±1.42	0	0.50±0.01	15.7±0.05	64.81
Curd	0.80±0.02	88.36±3.12	3.375±0.02	3.9±0.26	4.96±0.02	65.55

\*Mean of three trials, Average± S.D.

Table 5.2.9: (b) Percent Proximate Composition of 20% Inulin Incorporated Recipes

Recipes	Ash (%)	Moisture(%)	Fat (g)	Protein (g)	CHO (g)	Energy(Kcal)
Bread	1.06±0.67	31.90±2.45	6.1±0.60	6.56±0.045	54.38±0.55	298.65
Cookies	0.40±0.04	3.28±1.55	13.37±0.10	5.77±0.05	77.18±0.35	452.13
Chappati	1.01±0.90	27.02±1.65	2.35±0.22	7.92±0.03	62.42±0.67	299.64
Dhokla	1.88±0.11	56.39±1.23	2.14±0.01	5.88±0.01	33.71±0.52	177.63
Daliya	1.78±0.05	62.46±1.08	4.45±0.55	4.35±0.66	26.96±0.45	165.29
Wada	2.42±0.09	69.49±3.12	6.18±0.04	3.9±0.071	18.09±0.36	143.59
Juice	0.23±0.01	78.7±2.78	0	0.43±0.01	20.63±0.11	84.26
Curd	0.57±0.05	57.7±3.33	4.07±0.03	3.35±0.24	16.89±0.02	117.64

\*Mean of three trials, Average± S.D.

**DISCUSSION:**

The effect of varying levels of inulin incorporation on the physicochemical and organoleptic properties of bread, cookies, chapatti, *Dhokla*, porridge, potato bonda, juice and fermented milk was studied.

Inulin incorporated bread showed decrease in water absorption, loaf weight and loaf volume with increasing level of inulin substitution. Water Absorption of dough decreased on adding inulin when compared to the standard. Chen et al (1998) suggested that the incomplete hydration of gluten would have deleterious effect on the dough development. Regarding the dough weight decrease, inulin enriched dough weight have the lower values compared to the standard sample because of lower water absorption in the dough. Nevertheless Jangchaimonta (2006) found that the application of wheat flour mix with 20% resistant starch in bread produced no significant differences ( $p < 0.05$ ) on the dough weight and loaf weight.

The loaf volume of the inulin substituted bread significantly reduced at 22% level of incorporation compared to the standard bread. The result is in agreement with previous findings that revealed a decrease in the loaf volume with increased inclusion of fibre in wheat flour. Brennan (2004) reported the reduction in volume increased with higher addition of  $\beta$ -d-glucans rich fraction from barley. An increase in elasticity and extendable nature of dough is related to increase in overall loaf volume. Addition of inulin might promote a physical disruption of gluten protein matrix which leads to a lower ability of the dough to enclose air during proofing. The result shows an expected decline in loaf weight associated with reduced water absorption and reduction of sugar content of bread formula. The higher inulin levels added gave a significant difference in loaf weight compared to the standard sample.

Regarding organoleptic characteristics, no significant difference was perceived for color, cell size, texture, flavor, mouthfeel, aftertaste, ease of swallow, chewability and overall acceptability up to 20% level of inulin substitution. Adding inulin to bread presents innumerable technological advantages such as stability of mass, modulation of water absorption, neutral flavor, uniformity and slicing improvement (Soate 2002).

Significant decrease in scores for appearance, texture, flavor, mouthfeel and after taste was perceived for bread with 22% level of inulin substitution.

Results of physico chemical properties of cookies showed that there was increase in diameter of the cookies up to 15% of inulin incorporation thereafter a decrease in diameter was seen. The thickness of inulin substituted cookies increased gradually with increasing level of inulin incorporation. As a result, the spread ratio of inulin substituted cookies increased up to 15% level of inulin incorporated cookies thereafter a decreasing trend was noticed. Cookies having higher spread ratio are considered most desirable (Finney et al 1950; Kissel and Prentice 1979). Vratannia and Zaibik reported reduction in spread ratio when wheat bran was substituted for wheat flour from 10 to 30% cookies. Contrary to this, Leelavathi and Rao 1993 reported an increase in spread ratio with increased level of wheat bran in cookies whereas as Chen et al 1988 did not find a significant difference between the control and cookies containing oat bran or wheat bran at levels ranging from 4 to 12%. Overall acceptability scores revealed that cookies upto 20% level were acceptable. Significant differences were perceived for texture, flavor, aftertaste at 22% and 25% level of inulin incorporation.

Low flavor scores are often associated with low fat content which are known as flavor enhancers in the cookies. In a study carried out by Devereux et al 2003, showed that when inulin was used as fat replacer in anzac cookies at a level ranging from 4 to 13% achieving a significant reduction of 20% fat, were rated as acceptable by a untrained taste panel though scored consistently lower than the control.

Results of the physicochemical properties of *chapati* revealed decrease in water absorption power and cooked weight of inulin substituted *chapatis*. The water absorption of the whole wheat flour decreased with increase in the level of inulin substitution. As the level of inulin substitution increased, the dough became sticky and was difficult to sheet. Moreover, it resulted in partial puffing of *chapatis* and presumably inulin weight might interfere and reduce the dough expansion. Poor dough development might consequently result in denser texture. There is gradual decrease in dough weight of the *chapatis*. This may be due less absorption of water during preparation of dough making due to decreased solubility of inulin in water.

Organoleptic evaluation of *chapati* showed that appearance of the *chapati* was slightly deteriorated with incorporation of inulin. It caused more brownish and blackish spots on the surface of the chapattis during baking on the tawa. The appearance of number

brownish and blackish spots on the surface of the chapattis could be because of enhanced maillard reaction. The overall acceptability scores were significantly lower for 15 and 20 % the level of inulin incorporation compared to the standard. Therefore inulin can be incorporated upto 10% for preparation of *chapattis* without significantly affecting organoleptic characteristics.

Addition of inulin to *dhokla* showed increase in weight and volume as the level of inulin addition increased. Therefore the results revealed a decrease in bulk density as level of inulin incorporation increased in *dhokla*. Organoleptic evaluation revealed that all the *dhoklas* were quite acceptable at 20% level of inulin incorporation. Adding inulin in the coating of *potato bonda* reduced oil absorption and frying temperature of the *potato bondas* with increasing level (0 to 20%). Inulin has a remarkable capacity to replace fat. When thoroughly mixed with aqueous liquid, it forms a particle gel network such a gel is composed of tri-dimable network of insoluble sub micron crystalline inulin in water. Large amount of water is immobilized in this network, which assures its physical stability. The cereal pulse porridge showed no change in the water absorption properties as the level of inulin addition increased. The extent of this effect dependent on the amount of inulin addition. Inulin that was used in this study had the high solubility and did not absorb much water as the required for the consistency of porridge resulting in no change in water absorption of the porridge. The overall acceptability scores of porridge increased with increasing level of inulin addition. No significant differences were perceived for organoleptic properties of cereal pulse porridge under study.

Addition of inulin to orange juice showed no significant difference in its overall organoleptic acceptability. However there was increase in the mean scores for aftertaste, mouthfeel, odour and acceptability with increase levels of inulin addition. Inulin has bland neutral taste, without any off flavor and it combines easily with other ingredients without modifying its delicate flavor and is moderately soluble in water. All these characteristics of inulin might have contributed to its acceptability in juice up to 20% inclusion. The present study showed that the mean pH of the curd ranged from 5.0 to 5.1. Rao and Dastur (1955) reported that a good quality curd has pH in the range of 4.6-5.0



The organoleptic attributes of inulin added curds (5% to 20%) showed no significant difference with the standard curd. However addition of Inulin had improvement in scores of texture and mouthfeel. In dairy products the incorporation of inulin improves the processability and upgrades the quality, imparts a better round flavor and a creamier mouthfeel. Therefore it can be concluded that inulin can be substituted ( bread, chapatti, *Dhokla*) and added as dietary fibre (cereal pulse porridge, juice, Potato bonda) in many products and as fat replacer in baked goods eg cookies.

### **Section 5.3: Effect of supplementation of probiotic and synbiotic fermented milk in diets of institutionalized older adults**

Worldwide, the population is aging, with estimates of 1 billion people age 60 years or over within the next 20 years. With aging comes a reduction in overall health and increased morbidity and mortality due to infectious disease. Mortality due to gastrointestinal infections is up to 400 times higher in the older adults compared with younger adults. Recent studies have shown that the gut microbiota changes in old age, with an increased number of bacterial groups represented in the predominant older adults gut microbiota. This change in species "evenness" coincides with parallel changes in immune function, diet, and lifestyle and may contribute to disease susceptibility and its severity in old age. The intestinal microbiota may thus be identified as an important target for improving health through reduced disease risk. Recent human intervention studies have confirmed the microbiota modulatory capability of the inulin-type fructans in the older adults and there is some evidence for reduced risk of disease. Therefore, a need was felt for human intervention study to determine the efficacy of prebiotics in the older adults, particularly the application of probiotic and prebiotics, especially the inulin-type fructans, and synbiotics (prebiotics combined with efficacious probiotic strains) in terms of microbiota modulation and impact on disease risk in the aged population. In view of this section of this phase of this present study was planned to see the effect of probiotic and synbiotic fermented milk supplementation on institutionalized older adult participants. The major findings of this section of the present study are presented under following subsections.

Section 5.3.1: Background information, activity pattern, anthropometry and disease profile of Institutionalized Older Adults.

Section 5.3.2: Effect of probiotic and synbiotic fermented milk supplementation in the diets of older adults

**Section 5.3.1: Background information, activity pattern, anthropometry and disease profile of Institutionalized Older Adults.**

A total of 100 participants were enrolled from old age institutions of Baroda. The detailed methodology of selection of participants is given in methods and material chapter. Data on background information, activity pattern, anthropometric measurements, biochemical parameters, disease burden, psychological background and nutrient intake was collected for all the participants and the results are presented in sections 5.3.1.1 to 5.3.1.8.

**Section 5.3.1.1: Background information of the participants**

Socio demographic data was collected using a pretested questionnaire; the assessment included present information on age, education, marital status and per capita of income. A comprehensive picture of the socio-demographic data is given in Table 5.3.1.1. Out of hundred institutionalized older adult participants enrolled 48% of the participants aged between 60 -70 years and 56% of them were females. Majority of participants (71%) were married and living with their spouses. Almost all the participants were Hindus. The economic data showed that per capita income of the majority of the participants ranged between 3000-7000 per month. Sixty one percent of the institutionalized older adults were literate up to secondary and higher secondary levels.

**Section 5.3.1.2: Activity pattern of the older adult participants**

Activity pattern of older adult participant is depicted in Table 5.3.1.2. Apart from sleep the institutionalized older adults remained idle for most of their time (7.88 hours), followed by social and religious activities, which included chatting with friends and neighbors, visiting relatives, doing prayers and attending functions.

**Table 5.3.1.1: Background Information of the Participants**

<b>Category</b>	<b>Percent Subjects (N=100)</b>
<b>Age</b>	
60-70 yrs	48(48)
71-80 yrs	43(43)
81-90yrs	8(8)
≥ 90yrs	1(1)
<b>Sex</b>	
Males	44(44)
Females	56(56)
<b>Marital status</b>	
Unmarried	4(4)
Married	71(71)
Divorced	3(3)
Widow/widower	22(22)
<b>Religion</b>	
Hindu	98(98)
Muslim	0(0)
Christian	1(1)
Jain	1(1)
Others	0(0)
<b>Education level</b>	
Illiterate	10(10)
Primary	17(17)
Secondary	34(34)
Secondary high	29(29)
Graduate/post graduate	10(10)
Others	0(0)
<b>Per capita income</b>	
a) Rs 1000-3000	18(18)
b) Rs 3000-7000	58(58)
Rs 7000-10,000	24(24)
Rs1,000and above	

(Figures in parenthesis denote the no. of subjects)

Table 5.3.1.2: Activity Pattern of the older Adult Participants

Activities	Mean hours spent (n=100)
Activities of daily living	1.64±0.69
Leisure activities	2.24±1.43
Exercise	0.32±0.30
Yoga	0.36±0.48
Social/religious activities	3.09±1.43
Idle/time	7.88±1.33
Sleep	8.26±2.06

Participants spent their leisure time by watching television, listening music, reading and writing. Of all the older adult participants studied, only (66%) had sedentary lifestyle as the mean hours spent in exercise is 0.32 hrs (Figure 5.3.1.1).Subjects spending half hour for exercise for 5 days in a week were termed to lead a active lifestyle.

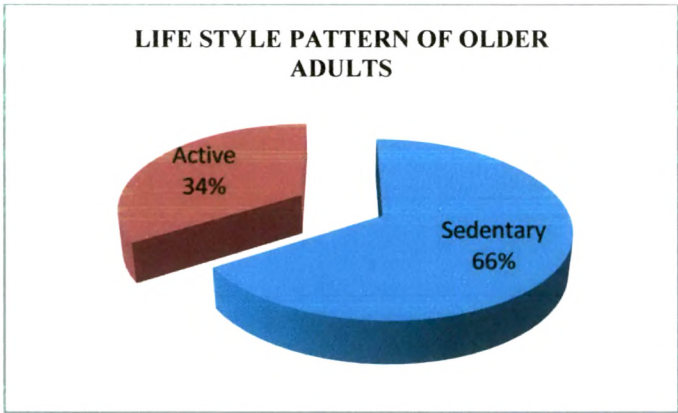


Fig. 5.3.1.1 Life style pattern of older adults

Section 5.3.1.3: Anthropometric profile and hypertension status of the older adult participants

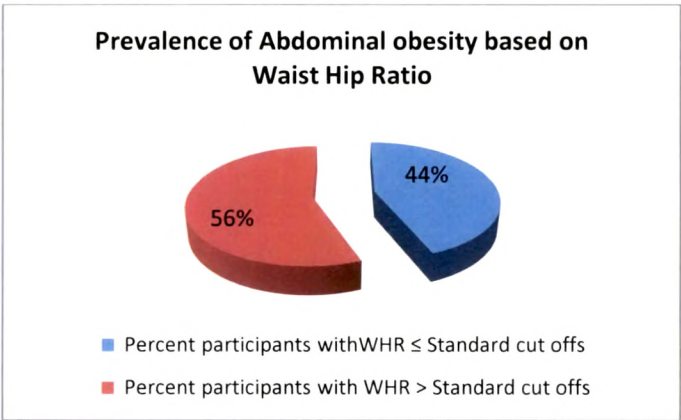
The anthropometric profile of the institutionalized older adults is illustrated in Table 5.3.1.3.

The mean age of the male participants were 70 yrs and female participants were 69 yrs. The waist hip ratio was slightly higher for males as compared to females in the older adult participants. About 56 % of the participants had WHR higher than the recommended standards by Asia Pacific for South Asians (fig 5.3.1.1). The frequency

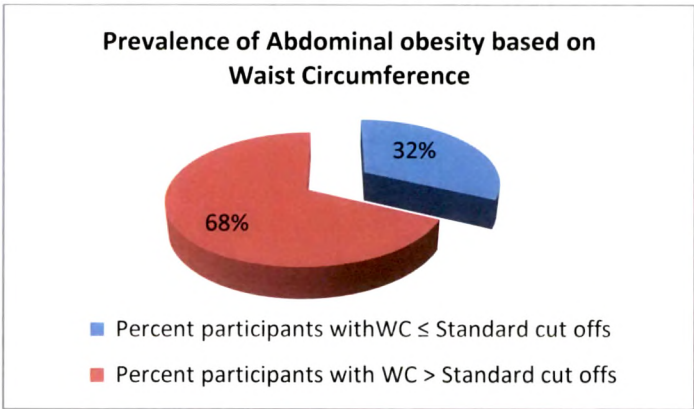
distribution of waist circumference values of the participants is given in fig 5.3.1.2. About 62 % of the participants had WC > cut offs for males and female

**Table 5.3.1.3: Anthropometric Profile of the older Adult Participants**

Variable	Subjects (N=100)		
	Male (n=44)	Female (n=56)	Total (n=100)
Age (Y)	70±7.08	69±8.30	70.38±7.01
Height (cm)	162.61±7.16	153.48±6.08	157.31±7.94
Weight (kg)	62.43±9.38	58.45±11.04	60.12±10.5
BMI	25.78±3.10	24.26±2.20	25.05±2.78
W.C (cm)	87.75±10.58	88.16±10.93	87.96±10.66
WHR	0.93±.060	0.92±.063	0.93±0.06
MUAC	23.45±3.00	22.82±2.16	23.12±2.60

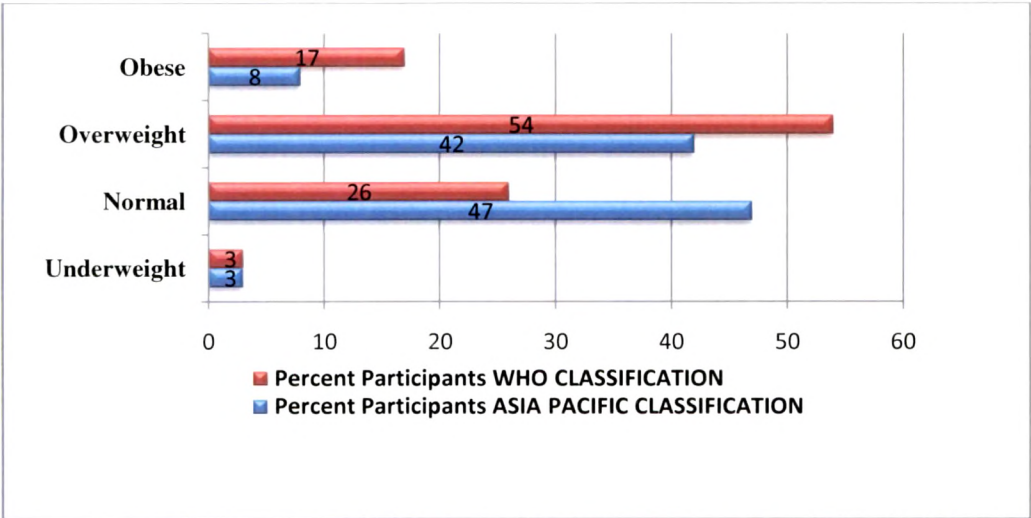


**Fig. 5.3.1.2 (a) prevalence of abdominal obesity based on waist-hip ratio**



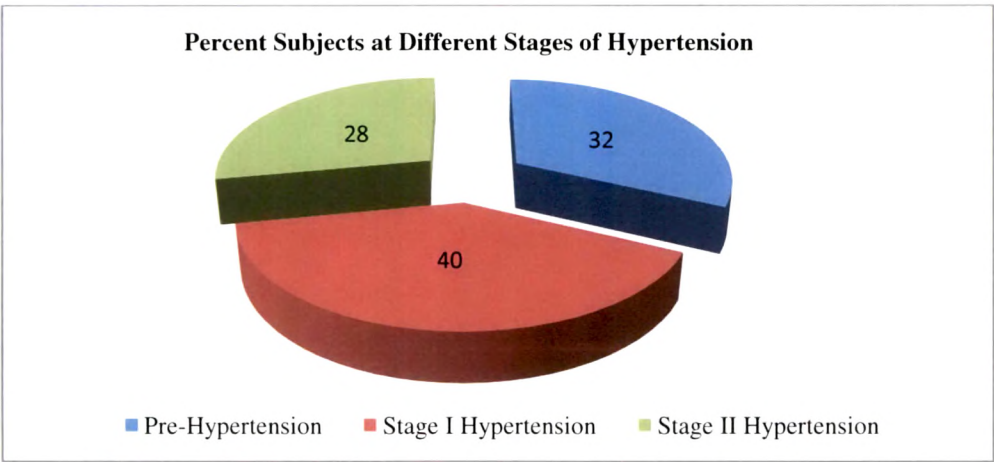
**Fig. 5.3.1.2 (b) Prevalence of abdominal obesity based on waist circumference**

As seen in Figure 5.3.1.3, prevalence of overweight was higher among institutionalized older adults using both, WHO and Asia Pacific (AP) classifications. Considering the overall picture, 54 % of the participants were overweight and 17 % were obese according to WHO cutoff. This trend was same when classified according to AP cutoffs where 42 % of the participants were overweight and 8 % obese. About 3% of the total population (n=100) was underweight with BMI <18.5 kg/m<sup>2</sup>. Only 47 % of the participant belonged to normal category with BMI ranging between 18.5 – 22.9 kg/m<sup>2</sup>.



**Fig. 5.3.1.3 Percent Participants under different categories of BMI Classification**

As seen in fig. 5.3.1.4, 32% subjects suffered from pre-hypertension where as 40% & 28% subjects fell in the category stage one and stage two hypertension respectively.



**Fig.5.3.1.4 Percent Participants at Different Stages of Hypertension**



**Section 5.3.1.4: Biochemical profile of the older adult participants**

Biochemical profile of the institutionalized older adults was studied by assessing various clinical parameters like hemoglobin values, blood glucose and lipid profile and the results are presented in table 5.3.1.4

**Table 5.3.1.4: Biochemical Profile of the Older Adult Participants**

Parameters	Normal values	Total subjects N=100
Hb (mg/dl)	12 mg/dl; 14 mg/dl	10.07±1.34
FBS (mg/dl)	80-110 mg/dl	117.35±29.6
TC (mg/dl)	160-200	207.17±35.25
HDL-C (mg/dl)	45-55	41.22 ±6.83
TG (mg/dl)	60-170	163.44 ±48.64
LDL-C(mg/dl)	<130	133.71± 32.39
VLDL(mg/dl)	<45	32.66±9.74
LDL/HDL	<2.5	3.32±0.88
TC/HDL	<3.5	5.13±1.00
BP Systolic ( mm Hg)	120 mmHg	135.55±14.2
BP Diastolic (mm Hg)	80 mm Hg	89.41±5.8

From the Table 5.3.1.4 and Fig.5.3.1.5, it can be observed that the mean hemoglobin values of both males and females were less compared to the normal range of standards. No cases of severe anemia were identified and majority of the participants were under normal and mild categories. Only 10 % of the participants were suffering from moderate anemia. The mean fasting blood glucose (117 mg/dl), total cholesterol (207mg/dl) and low density lipoproteins (133 mg/dl) were slightly above the normal range whereas triglycerides (163 mg/dl) and very low density lipoproteins (32 mg/dl) were under normal range. Atherogenic indices values (TC/HDL, LDL/HDL) reported by the older adults were higher above the normal range. Fig. 5.3.1.6(a) and Fig. 5.3.1.6(b) depicts percent participant under different categories for total cholesterol and triglycerides respectively.

The mean systolic and diastolic blood pressure values reported were 135 mm Hg and 89 mm Hg respectively (Table 5.3.1.4)

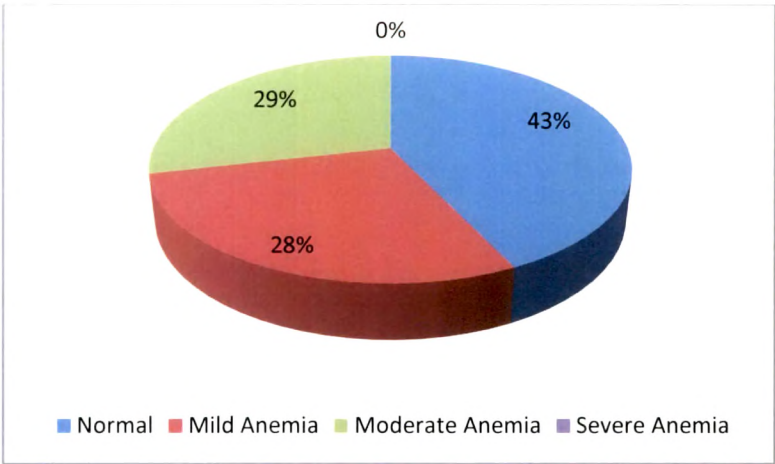


Fig.5.3.1.5 Percent Participants Suffering from Different Degrees of Anemia

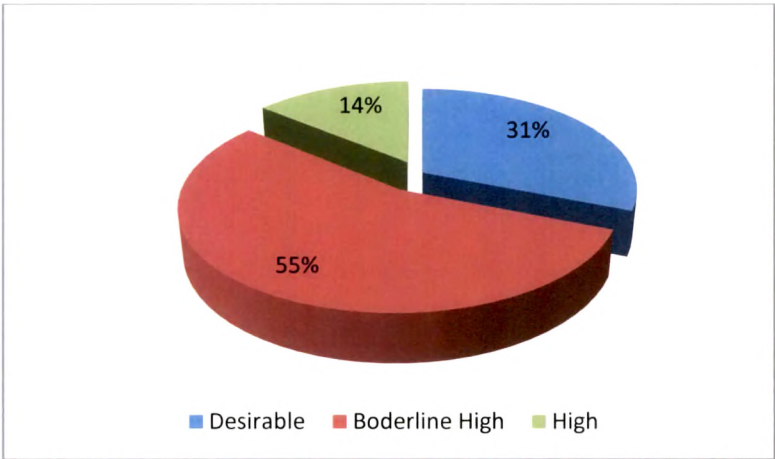


Fig.5.3.1.6(a)Percent Participants under Different Categories for Total Cholesterol

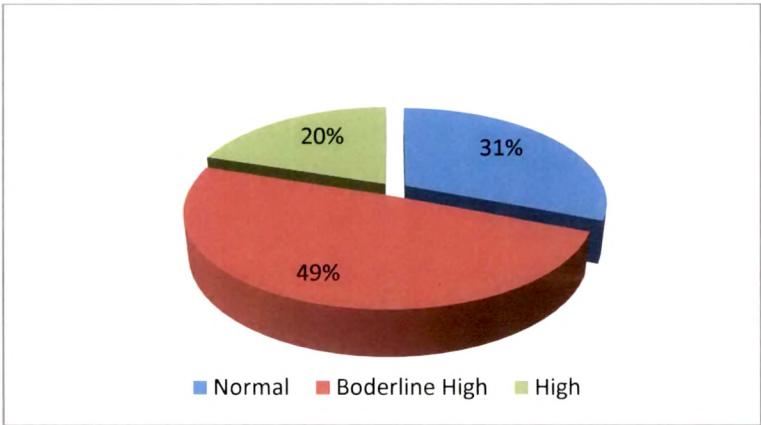


Fig. 5.3.1.6(b) Percent Participants under Different Categories for Triglycerides



Section 5.3.1.5: Dietary intakes of the older adult participants

The detailed nutrient analysis of diets of male and female institutionalized participants is shown in Table 5.3.1.5 and 5.3.1.6.

Table 5.3.1.5: Nutrient Intake of Male and Female Older Adults (Mean ± S.D)

Nutrient	RDA	Female (n =56)	RDA	Male (n =44)	Pooled (N=100)
Energy ( Kcal)	1350	1348.07±200.43	1750	1543.73±193.00	1434.17±219.15
Protein (gms)	50	40.41±14.77	60	42.26±7.43	41.23±12.08
Fat (gms)	30	48.35±11.82	30	45.53±8.75	47.11±10.63
Calcium	400	421.14±70.13	400	441.53±77.19	430.12±73.65
Fibre (mg)	20	6.18±1.80	20	6.21±1.44	6.20±1.64
Iron (g)	28	12.20±4.94	30	12.17±2.35	12.19±3.99
Vitamin C(mg)	40	37.44±28.24	40	33.63±13.34	42.08±70.30

Source: Natrajan 1991

Table 5.3.1.6: Frequent and Non Frequent Consumption of Different Food Groups By Percent Participants

Food group	Non frequent	Frequent
Cereals	0(0)	100(100)
Wheat Flour> Rice > Bajra > Puffed Rice > Rice Flakes		
Pulses And Legumes	0(0)	100(100)
Red Gram > Bengal Gram > Green Gram > Black Gram > Lentil		
Other Vegetables	44(44)	56(56)
Tomato > Cauliflower > Carrot > Pumpkin > Onion Stalks > Drumstick> Karamda		
Green Leafy Vegetable	44(54)	56(46)
Fenugreek Leaves > Spinach > Colocasia Leaves> Drumstick Leaves> Cauliflower Leaves		
Fruits	73(73)	27(27)
Banana > Guava > Grapes > Papaya		
Milk And Its Products	35(35)	65(65)
Milk > Butter Milk > Butter >Curd		
Nuts And Oilseeds	50(50)	50(50)
Snacks	58(58)	42(42)
Chevda, Samosa, Khari		
Sugar	40(40)	60(60)
Peda, Ladoo, Sukhadi		
Fats	30(30)	70(70)

The mean intake for energy matched the RDA of 1350 Kcal for females where as it was lower for male subjects. Lower intake for protein, fiber and iron were observed for both male and female subjects. Intake of fat was high whereas iron and fiber intake was low when compared to RDA in both male and female participants. 73% and 44% subjects consumed fruits and vegetable less frequently. Almost 60% of the participants had frequent consumption of fat and sugar rich foods (Table 5.3.1.6).

#### **Section 5.3.1.6: Disease burden of the older adult participants**

Under the disease burden, parameters like major health problems pertaining to various systems of the body, minor illnesses and factors related to mental health of the institutionalized participants were studied.

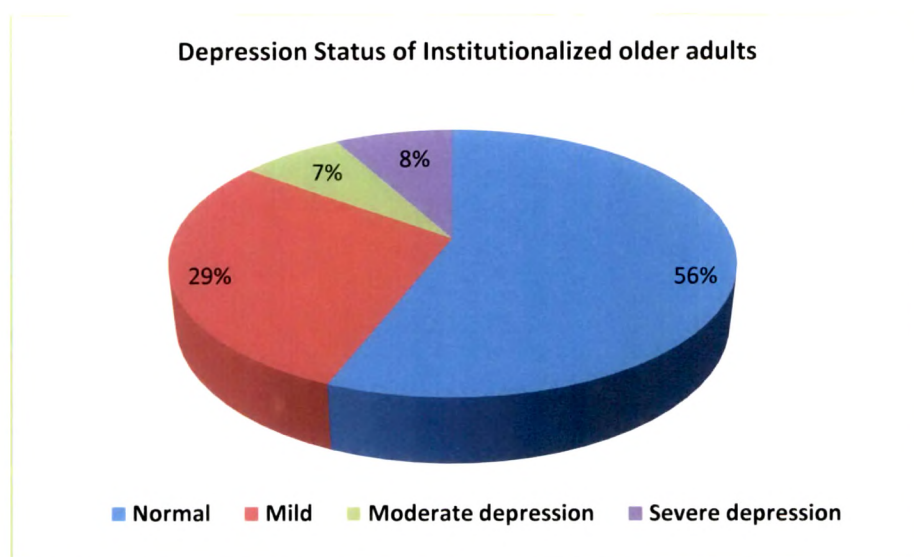
From the Table 5.3.1.7, it is clearly evident that 90% participants had oral cavity problems which included difficulty in chewing due to missing teeth or denture. The next rated was gastro intestinal problems in 86% of the total participant which included gas, acidity, indigestion and flatulence. 54% of the participants had respiratory problems like cold cough, running nose. Other problems like locomotor (67%), cardiovascular (15%) were also reported by the subjects.

**Table 5.3.1.7: Disease Burden of Older Adult Participants**

<b>Disease category</b>	<b>Percent subjects reporting the symptoms before supplementation (n=100)</b>
Problems of oral cavity	90(90)
Problems of gastrointestinal tract	86(86)
Problems of respiratory tract	54(54)
Problems of cardiovascular system	15(15)
Problems of locomotor system	67(67)
Problems of central nervous system	48(48)
Problems of endocrine system	13(13)
Miscellaneous problems	49(49)

(Figures in parenthesis denote the no. of subjects)

Geriatric depression scale revealed that 29% of the participants were suffering from mild depression whereas only 15% suffered from moderate and severe depression. (fig.



**Fig. 5.3.1.7 Depression Status of Institutionalized older adults**

#### **Section 5.3.1.7: Association of biochemical parameters and various risk factors in older adult participants**

Association of biochemical parameters and various lifestyle factors such as physical activity, BMI, WC, WHR, fruit consumption, green leafy vegetable consumption, fibre intake and fat intake are shown in Table 5.3.1.8 to Table 5.3.1.10. A significant positive correlation was observed for BMI with TG. Waist hip ratio and physical activity showed a positive correlation ( $p < 0.01$ ) with total cholesterol. Low intake of fruits and BMI was significantly correlated with HDL levels.

Fiber intake significantly co-related systolic and diastolic blood pressure also the GLV consumption co-related significantly with FBS.

WHR significantly co-related with atherogenic indices such as LDL/HDL, TG/HDL/, TC/LDL and TC/HDL. Also the GLV consumption significantly co-related with TG/HDL ratio.

**Table 5.3.1.8: Association between Lipid profile and life style factors of the institutionalized elderly subjects**

Life style variables	Lipid profile			
	TC	TG	HDL	LDL
Physical Activity	-.145	-.078	-.082	-.130
BMI	-.006	.181*	-.233*	-.111
WC	.077	.135	-.139	.024
WHR	.306**	.190*	-.119	.170
Fruit consumption	-.046	-.071	.177*	-.091
GLV consumption	-.003	-.110	.147	-.005
Fibre Intake	-.050	-.055	-.005	.003
Fat intake	.072	.042	-.145	-.120

\* Correlation values are significant

**Table 5.3.1.9: Association between Fasting blood glucose, blood pressure levels and life style factors of the institutionalized elderly subjects**

Life Style factors	FBS	BPD	BPS
Physical Activity	-.149	-.065	-.016
BMI	.108	.077	.137
WC	.072	-.040	.040
WHR	.029	.004	-.006
Fruit consumption	-.131	.001	.021
GLV consumption	.208*	-.047	-.079
Fibre Intake	-.017	.363***	.321***
Fat intake	.008	.122	-.126

\* Correlation values are significant

**Table 5.3.1.10: Association between Atherogenic indices and life style factors of the institutionalized elderly subjects**

Life style variables	Atherogenic indices			
	LDL/HDL	TG/HDL	TC/LDL	TC/HDL
Physical Activity	-.103	-.059	-.005	-.120
BMI	.046	.328***	.107	.149
WC	.077	.159	.159	.192*
WHR	.203*	.228*	.196*	.368***
fruit	-.201*	-.148	.055	-.124
GLV	-.113	-.189*	.031	-.105
Fibre Intake	.072	.082	.012	.058
Fat intake	-.060	.053	.083	.025

\* Correlation values are significant

### **Section 5.3.2: Effect of Probiotic and Synbiotic Fermented Milk Supplementation in the Diets of older Adults**

Out of 100 older adults enrolled, 66 participants were selected and assigned to each of the three groups as control group (n=20), Experimental group II (Probiotic fermented milk supplementation group) (n=20), Experimental group III (Synbiotic milk fermented supplementation group) (n=26). Pre and post intervention data of the selected older adults was collected.

#### **Effect of probiotic and synbiotic fermented milk supplementation on Lipid Profile of the participants:**

As seen Table 5.3.2.1 Fermented milk supplementation resulted in 3.57% reduction in probiotic supplemented group ( $p < 0.05$ ) and 6.9% reduction in synbiotic supplemented group ( $p < 0.001$ ) with respect to TC values, with non significant rise in the HDL levels. A significant reduction in TG (4.3%) and LDL levels (8.3%) in group III was observed. A non significant reduction was seen in serum triglyceride levels of group II participants (2.96%) In the control group the lipid profile remained unaltered.

Table 5.3.2.1: Lipid Profile of Older Adult Participants Before and After Probiotic and Synbiotic Fermented Milk Supplementation

Parameters	Group I (n=20)	Group II (n=20)	Group III (n=26)	Annova
TC				
Pre	222.49±21.3	224.11±20.44	226.68±23.94	0.211 <sup>NS</sup>
Post	223.37±20.92 <sup>a</sup>	216.16±24.16 <sup>b</sup>	209.30±25.18 <sup>b</sup>	2.008*
t' value	0.348 <sup>NS</sup>	2.906*	2.986***	
% change	0.39	3.57	6.93	
TG				
Pre	169.96±47.36	168.84±32.21	171.78±48.75	0.37 <sup>NS</sup>
Post	171.35±49.18	161.66±43.14	159.72±40.48	0.381 <sup>NS</sup>
t' value	0.158 <sup>NS</sup>	0.744 <sup>NS</sup>	2.573*	
% change	3.27	2.96	4.34	
HDL-C				
Pre	43.79±4.50	43.74±4.17	40.64±5.59	0.495 <sup>NS</sup>
Post	43.29±4.79	43.02±4.77	41.77±4.91	0.893 <sup>NS</sup>
t' value	0.921 <sup>NS</sup>	0.417 <sup>NS</sup>	0.933 <sup>NS</sup>	
% change	1.03	0.76	4.16	
LDL-C				
Pre	144.70±25.14 <sup>a</sup>	146.59±22.09 <sup>b</sup>	151.67±26.39 <sup>b</sup>	0.495 <sup>NS</sup>
Post	145.81±18.11	139.78±27.76	135.57±25.47	0.893 <sup>NS</sup>
t' value	0.404	1.651	2.248*	
% change	2.49	6.88	7.75	
VLDL-C				
Pre	33.99±9.47	34.76±7.35	34.35±9.75	7.054 <sup>NS</sup>
Post	34.27±9.83	33.33±9.58	31.94±8.09	7.505 <sup>NS</sup>
t' value	0.158 <sup>NS</sup>	0.744 <sup>NS</sup>	1.573 <sup>NS</sup>	
% change	3.27	2.06	4.0	

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- a,b,c- the non identical letters in any two coloums within a raw denote a significant difference at a minimum of 5% level.
- NS - Non Significant

Table 5.3.2.2 projects the lipid profile of the male and female older adult participants before and after the intervention of probiotic and synbiotic fermented milk. There was a significant reduction in TC, TG, LDL-C and VLDL levels in the male and female participants in synbiotic fermented milk group and a non significant increase in the HDL-C values in both male and the female participants in both the experimental groups (Fig. 5.3.2.1 and 5.3.2.2).

**Effect of probiotic and synbiotic fermented milk supplementation on atherogenic indices of the participants:**

Table 5.3.2.3 gives the atherogenic indices of the control and experimental groups. The favourable redistribution of lipoproteins with probiotic and synbiotic fermented milk supplementation had a significant positive impact on the atherogenic indices lowering the risk of CHD in the hypercholesterolemic older adult participants.

A significant reduction was seen in the atherogenic indices LDL: HDL, TC: HDL ratio in participants of group III whereas such reductions were not observed in group II participants. With regards to gender, reduction was observed for atherogenic indices in both male and female participants in group II and group III. Significant reduction was seen for TC: HDL ratio in male participants of group III (Table 5.3.2.4).

**Plasma glucose of the participants:**

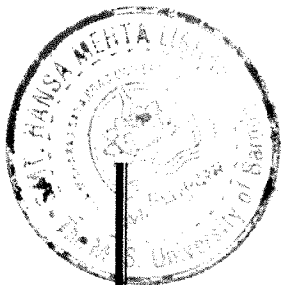
A significant reduction of 5.2 % and 7.8% was observed in fasting plasma glucose values in group II and group III respectively. No variation was seen in FBS levels with regard to gender, though the response was better in males of probiotic and synbiotic supplemented groups as compared to females (Table 5.3.2.5 and Fig. 5.3.2.3).

Table 5.3.2.2: Lipid Profile of the Male and Female Older Adult Participants Before and After Probiotic and Synbiotic Fermented Milk Supplementation

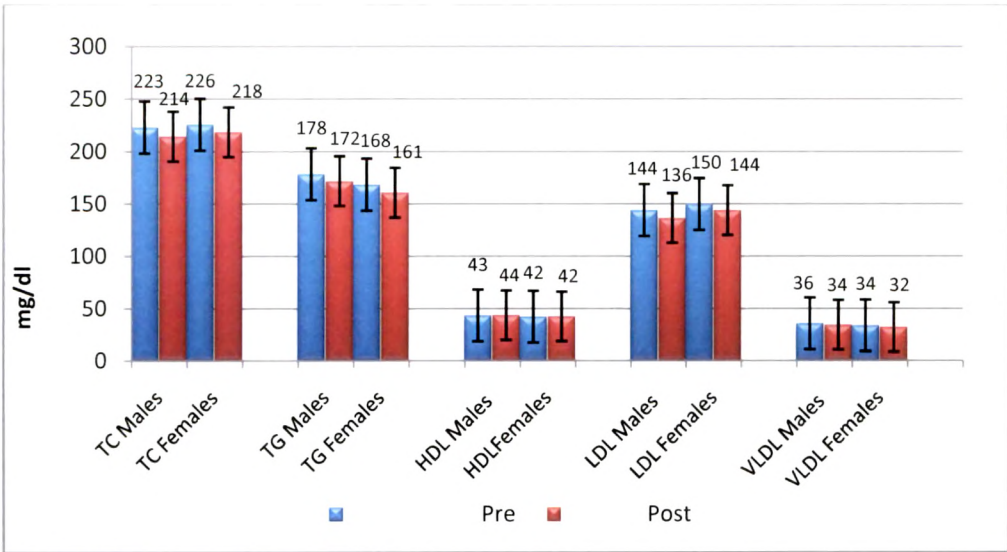
Parameters		Group I		Group II		Group III	
		Males (n=10)	Females (n=10)	Males (n=11)	Females (n=11)	Males (n=11)	Females (n=15)
TC	Pre	220.08 ± 11.98	224.89 ± 28.30	222.96 ± 8.67	225.51 ± 23.51	226.46 ± 28.25	226.87 ± 20.64
	Post t value	223.48 ± 15.99 1.089 NS	223.27 ± 25.85 0.400 NS	214.32 ± 9.38 0.982 NS	218.41 ± 17.23 2.191 NS	207.08 ± 26.70 2.499 *	211.19 ± 8.43 2.589 *
HDL-C	Pre	45.12 ± 2.34	42.46 ± 5.77	43.30 ± 4.85	42.06 ± 3.30	40.22 ± 6.50	41.01 ± 4.90
	Post t value	44.07 ± 3.87 1.507 NS	42.52 ± 5.68 0.086 NS	43.56 ± 5.49 0.551 NS	42.37 ± 3.93 0.275 NS	41.86 ± 3.74 0.753	41.7 ± 5.88 0.374 NS
TG	Pre	180.57 ± 50.84	155.35 ± 40.95	178.29 ± 5.20	168.40 ± 40.00	173.03 ± 53.89	170.02 ± 5.88
	Post t value	175.84 ± 53.48 0.618 NS	166.87 ± 46.91 1.104 NS	171.61 ± 7.89 0.564 NS	160.61 ± 34.50 0.448 NS	158.61 ± 45.47 2.802 *	160.66 ± 37.41 3.075 **
LDL-C	Pre	138.04 ± 17.69	151.36 ± 30.38	144.00 ± 17.63	149.76 ± 21.53	151.47 ± 34.95	151.85 ± 25.79
	Post t value	144.24 ± 18.02 1.981 NS	147.36 ± 29.58 1.003 NS	136.43 ± 34.51 0.710 NS	143.91 ± 20.77 1.792 NS	133.5 ± 32.69 2.248 *	137.35 ± 12.52 2.389 *
VLDL-C	Pre	35.91 ± 10.16	31.07 ± 8.91	35.65 ± 7.04	33.68 ± 8.00	34.76 ± 10.77	34 ± 9.17
	Post t value	35.16 ± 10.69 0.618 NS	33.37 ± 9.38 1.104 NS	34.32 ± 11.57 0.564 NS	32.12 ± 6.90 0.448 NS	31.72 ± 9.09 2.802 *	32.13 ± 7.48 3.075 **

Note

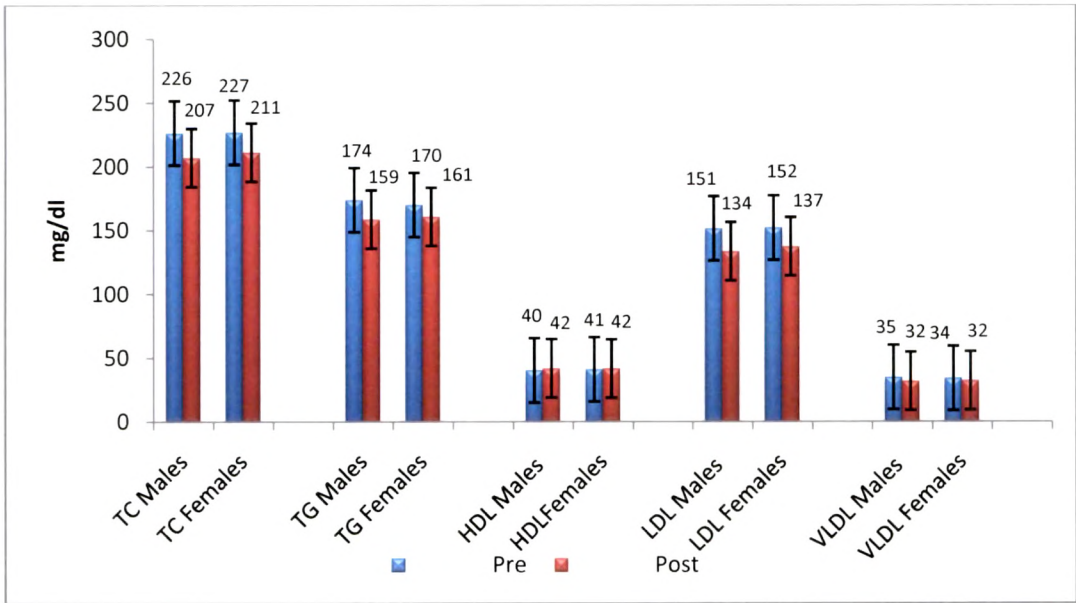
- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant







**Fig. 5.3.2.1 Lipid Profile of Male and Female Elderly Subjects Before and After Probiotic Fermented Milk Supplementation**



**Fig. 5.3.2.2 Lipid Profile of Male and Female Elderly Subjects Before and After Synbiotic Fermented Milk Supplementation**

Table 5.3.2.3: Atherogenic Indices of Older Adult Participants after Probiotic and Synbiotic Fermented Milk Supplementation

Parameters	Group I (n=20)	Group II (n=20)	Group III (n=26)	Annova
LDL/HDL				
Pre	3.35±0.76 <sup>a</sup>	3.42±1.16 <sup>a</sup>	3.87±0.98 <sup>b</sup>	9.227*
Post	3.36±0.66	3.23±1.07	3.30±0.81	0.207 <sup>NS</sup>
't' value	0.578 <sup>NS</sup>	0.227 <sup>NS</sup>	2.214*	
% change	0.007	3.32	9.5	
TG/HDL				
Pre	3.90±1.13	4.38±1.01	4.31±1.40	0.903 <sup>NS</sup>
Post	4.23±1.41	4.06±1.32	3.92±1.28	0.317 <sup>NS</sup>
't' value	1.648 <sup>NS</sup>	1.038 <sup>NS</sup>	1.913 <sup>NS</sup>	
% change	5.56	5.02	6.82	
TC/LDL				
Pre	1.55±0.15 <sup>a</sup>	1.95±0.59 <sup>b</sup>	1.51±0.15 <sup>b</sup>	10.420*
Post	1.55±0.14 <sup>a</sup>	2.00±0.67 <sup>b</sup>	1.57±0.18 <sup>b</sup>	8.840*
't' value	.380 <sup>NS</sup>	0.361 <sup>NS</sup>	1.269 <sup>NS</sup>	
% change	0	6.18	9.00	
TC/HDL				
Pre	5.13±0.75 <sup>a</sup>	6.49±1.29 <sup>b</sup>	5.75±1.09 <sup>a</sup>	7.927*
Post	5.21±0.73 <sup>b</sup>	6.08±1.47 <sup>a</sup>	5.08±0.93 <sup>b</sup>	5.663*
't' value	0.796	2.086	2.480*	
% change	1.55	10.40	10.95	

## Note

- \*Significant from the baseline value at  $p < 0.05$
- \*\* Significant from the baseline value at  $p < 0.01$
- a,b,c- the non identical letters in any two columns within a row denote a significant difference at a minimum of 5% level.
- NS - Non Significant

**Table 5.3.2.4: Atherogenic Indices of the Male and Female Older Adult Participants Before and After Probiotic and Synbiotic Fermented Milk Supplementation**

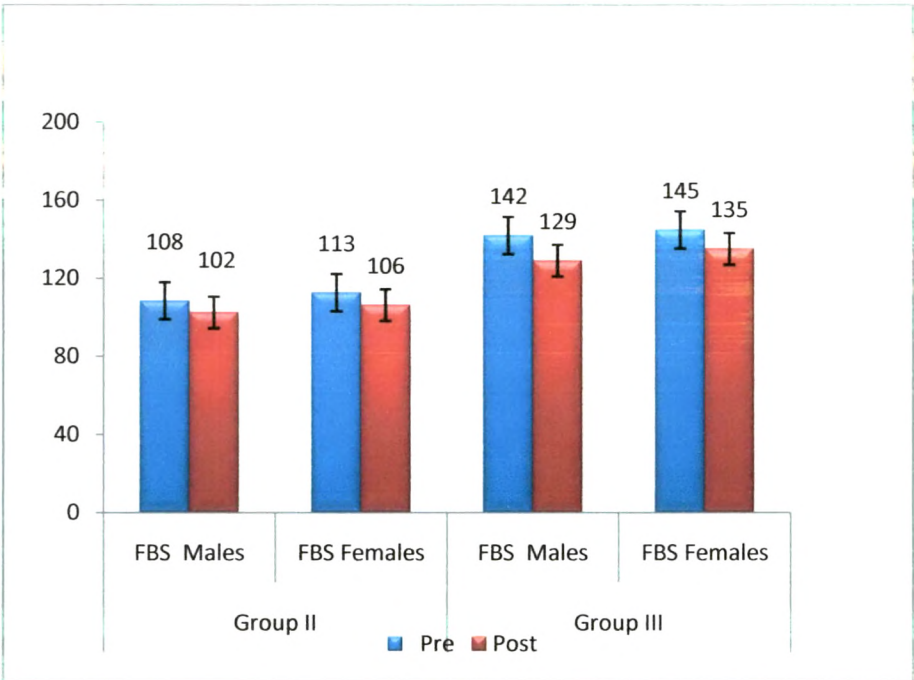
Parameters	Group I		Group II		Group III		
	Males (n=10)	Females (n=10)	Males (n=11)	Females (n=11)	Males (n=11)	Females (n=15)	
LDL:HDL	Pre	3.07 ± 0.45	3.64 ± 0.92	3.37 ± 0.61	3.57 ± 0.50	4.02 ± 1.18	3.76 ± 0.85
	Post	3.30 ± 0.54	3.51 ± 0.78	3.22 ± 1.81	3.42 ± 0.61	3.36 ± 1.03	3.36 ± 0.61
	t value	2.495 <sup>NS</sup>	0.923 <sup>NS</sup>	0.537 <sup>NS</sup>	1.303 <sup>NS</sup>	1.774 <sup>NS</sup>	1.684 <sup>NS</sup>
TG:HDL	Pre	4.09 ± 1.13	3.72 ± 1.15	4.13 ± 0.72	4.01 ± 0.97	4.46 ± 1.68	4.2 ± 1.33
	Post	4.78 ± 1.61	3.69 ± 1.13	3.94 ± 1.16	3.79 ± 0.78	3.89 ± 1.36	3.92 ± 1.19
	t value	1.869 <sup>NS</sup>	1.00 <sup>NS</sup>	0.668 <sup>NS</sup>	0.524 <sup>NS</sup>	1.885 <sup>NS</sup>	1.000 <sup>NS</sup>
TC:LDL	Pre	1.61 ± 0.14	1.50 ± 0.15	1.55 ± 0.12	1.51 ± 0.09	1.52 ± 0.18	1.51 ± 0.15
	Post	1.55 ± 0.11	1.54 ± 0.17	1.61 ± 0.22	1.53 ± 0.11	1.57 ± 0.12	1.54 ± 0.11
	t value	1.504 <sup>NS</sup>	1.433 <sup>NS</sup>	0.932 <sup>NS</sup>	0.515 <sup>NS</sup>	0.720 <sup>NS</sup>	1.085 <sup>NS</sup>
TC:HDL	Pre	4.88 ± 0.37	5.38 ± 0.96	5.20 ± 0.64	5.37 ± 0.53	5.96 ± 1.29	5.6 ± 0.84
	Post	5.11 ± 0.58	5.32 ± 0.80	5.01 ± 1.15	5.18 ± 0.54	5.04 ± 1.27	5.14 ± 0.68
	t value	1.864 <sup>NS</sup>	0.454 <sup>NS</sup>	0.735 <sup>NS</sup>	1.530 <sup>NS</sup>	2.094 <sup>*</sup>	1.635 <sup>NS</sup>

**Note**

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant.

**Table 5.3.2.5: Fasting Blood Glucose of Older Adult Participants after Probiotic and Synbiotic Fermented Milk Supplementation**

Parameters		Group I (N=20)	Group II (N=20)	Group III (N=26)	Annova
FBS	Pre	109.14±33.82 <sup>a</sup>	110.37±31.80 <sup>a</sup>	143.34±37.09 <sup>b</sup>	4.588*
	Post	109.09±34.35 <sup>a</sup>	104.55±29.31 <sup>a</sup>	132.18±50.52 <sup>b</sup>	2.969*
	t' value	0.347 <sup>ns</sup>	4.089*	2.445**	
	% change	0.24	5.2	7.78	



**Fig. 5.3.2.3 Fasting Blood Glucose of Male and Female Elderly Subjects Before and After Probiotic and Synbiotic Fermented Milk Supplementation**  
**Changes in hypertension status and body composition after supplementation of probiotic and synbiotic supplemented group:**

As seen in Table 5.3.2.6, there was a significant reduction in systolic and diastolic blood pressure from 142 to 137 and 92 to 89 mm Hg in the synbiotic supplemented group respectively whereas a non significant reduction was observed in the probiotic supplemented group. Significant variation was observed in blood pressure values with regards to gender, response was better in male participants of synbiotic fermented milk supplemented group as compared to females.

Table 5.3.2.6: Body Composition of Older Adult Participants After Probiotic and Synbiotic Fermented Milk Supplementation

Parameters	Group I (n=20)	Group II (n=20)	Group III (n=26)	Annova
<b>BMI</b>				
Pre	25.39 ± 2.29	24.63 ± 2.38	25.11 ± 3.40	3.77 <sup>NS</sup>
Post	25.40 ± 2.28	24.52 ± 2.32	24.92 ± 3.32	0.515 <sup>NS</sup>
't' value	0.748 <sup>NS</sup>	2.129 <sup>NS</sup>	5.114**	
% change	0.039	0.44	0.75	
<b>Systolic BP</b>				
Pre	135.00 ± 19.12	143.25 ± 15.91	142.12 ± 15.24	1.473 <sup>NS</sup>
Post	136.50 ± 12.25	142.25 ± 18.95	137.88 ± 13.35	0.815 <sup>NS</sup>
't' value	0.436 <sup>NS</sup>	0.183 <sup>NS</sup>	3.275**	
% change	1.1	0.69	2.98	
<b>Diastolic BP</b>				
Pre	90.0 ± 7.43	91.70 ± 7.46	92.46 ± 7.63	0.617 <sup>NS</sup>
Post	93.5 ± 8.75	90.50 ± 8.41	89.23 ± 7.16	1.626 <sup>NS</sup>
't' value	1.524	0.546	3.742**	
% change	1.66	1.3	3.49	

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- a,b,c- the non identical letters in any two coloums within a raw denote a significant difference at a minimum of 5% level.
- NS - Non Significant

There was 1.15% decrease in BMI values ( $p < 0.001$ ) in the synbiotic supplemented group compared to a non significant 0.44% decrease in BMI values in the probiotic group. Statically significant reduction was observed in male and female participants of group III.

**Effect of probiotic and synbiotic fermented milk supplementation on the lipid profile and atherogenic indices of older adult participants in relation to their initial TC values**

Impact studies have shown that initial values are an important determinant for the response in the supplementation studies. Therefore the lipid profile and atherogenic indices was seen in relation to the initial TC values of the older adult participants (Table 5.3.2.7 and Table 5.3.2.8).

As can be seen favorable response was seen in older adult participants who had TC > 210 mg/dl as compared to those having TC < 210 mg/dl in synbiotic group. There was a 10.73% fall in TC, 13.04% in LDL-C and 5.47 % in TG in synbiotic fermented milk supplemented group with initial level of TC > 210 mg/dl . The trend noticed in both the experimental groups was not observed in the control group. Since the initial value of TC had an influence an attempt was made to look at the response of probiotic and synbiotic fermented milk supplementation on the initial values of TG. In line with TC data a 7.29% reduction was observed in TG after 6 weeks of synbiotic fermented milk supplementation who had TG > 150 mg/dl (Table 5.3.2.9 and Table 5.3.2.10). Concomitantly in these participants a significant reduction in VLDL-C was observed (39.11mg/dl vs. 36 mg/dl). In group II and group III participants with normotriglyceridemia a decrease in TG and VLDL-C was also seen.

Table 5.3.2.7: Impact of Probiotic and Synbiotic Supplementation on Lipid Profile of Elderly Subjects Based on the Initial TC Values (Mean± SD, mg/dl)

Parameters		Group I			Group II			Group III		
		TC<210 N=11	TC≥ 210 N=9	TC<210 N=11	TC≥ 210 N=9	TC<210 N=14	TC≥ 210 N=12			
TC	Pre	209.45 ± 7.006	238.41 ± 22.30	211.19 ± 5.42	239.90 ± 21.11	205.78 ± 5.22	244.59 ± 18.17			
	Post	210.73 ± 6.79	238.83 ± 22.20	204.85 ± 21.40	229.98 ± 20.57	199.58 ± 31.98	223.34 ± 17.77			
	t value	1.371	0.073	1.039	1.174	1.134	2.790 **			
TG	Pre	169.09 ± 50.49	171.02 ± 46.25	178.53 ± 43.23	170.00 ± 32.20	182.26 ± 62.38	162.79 ± 32.99			
	Post	170.15 ± 51.53	172.83 ± 49.19	173.47 ± 50.12	161.06 ± 47.75	166.52 ± 52.90	153.88 ± 26.52			
	t value	0.067	0.295	0.275	0.831	2.792	3.325 **			
HDL-C	Pre	43.92 ± 4.78	43.62 ± 4.40	42.46 ± 4.37	42.93 ± 4.23	41.02 ± 6.05	40.32 ± 5.37			
	Post	43.69 ± 4.68	42.62 ± 5.17	42.59 ± 4.32	43.70 ± 5.40	42.39 ± 5.86	41.24 ± 4.09			
	t value	0.351	0.906	0.218	0.772	0.558	0.583			
VLDL-C	Pre	33.81 ± 10.09	34.20 ± 9.25	35.70 ± 8.64	34.00 ± 6.44	36.45 ± 12.47	32.55 ± 6.59			
	Post	34.02 ± 10.30	34.56 ± 9.83	34.69 ± 10.02	32.21 ± 9.54	33.30 ± 10.58	30.77 ± 5.30			
	t value	0.067	0.295	0.275	0.831	2.792	3.325 **			
LDL-C	Pre	131.70 ± 15.13	160.58 ± 26.43	134.60 ± 10.40	161.26 ± 17.26	128.30 ± 28.43	171.70 ± 24.07			
	Post	133.00 ± 12.63	161.44 ± 25.62	130.17 ± 22.95	151.57 ± 31.83	120.88 ± 12.47	149.31 ± 17.53			
	t value	0.397	0.180	0.644	0.922	1.060	2.300 **			

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant

Table 5.3.2.8: Impact of Probiotic and Synbiotic Supplementation on Atherogenic Indices of Elderly Subjects Based on the Initial TC Values (Mean± SD, mg/dl)

Parameters		Group I		Group II		Group III	
		TC<210 N=11	TC≥210 N=9	TC<210 N=11	TC≥210 N=9	TC<210 N=14	TC≥210 N=12
LDL/HDL	Pre	3.05 ± 0.65	3.72 ± 0.35	3.19 ± 0.47	3.78 ± 0.50	3.31 ± 0.61	4.36 ± 1.04
	Post	3.08 ± 0.50	3.80 ± 0.65	3.08 ± 0.54	3.59 ± 1.27	3.13 ± 0.96	3.47 ± 0.66
	t value	0.264	0.554	0.605	0.720	0.619	2.664
TG/HDL	Pre	3.88 ± 1.24	3.93 ± 1.05	4.00 ± 0.69	4.17 ± 1.00	4.58 ± 1.88	4.11 ± 1.04
	Post	3.93 ± 1.36	4.60 ± 1.47	3.81 ± 1.10	3.95 ± 0.88	4.07 ± 1.67	3.76 ± 0.77
	t value	0.242	1.961	0.689	0.514	1.244	1.957
TC/LDL	Pre	1.60 ± 0.15	1.50 ± 0.14	1.57 ± 0.12	1.49 ± 0.08	1.62 ± 0.17	1.43 ± 0.10
	Post	1.59 ± 0.14	1.49 ± 0.13	1.59 ± 0.16	1.55 ± 0.21	1.61 ± 0.13	1.51 ± 0.08
	t value	0.202	0.774	0.475	0.958	0.120	2.291
TC/HDL	Pre	4.83 ± 0.67	5.51 ± 0.71	5.00 ± 0.46	5.62 ± 0.56	5.27 ± 0.81	6.19 ± 1.10
	Post	4.83 ± 0.60	5.63 ± 0.68	4.84 ± 0.49	5.39 ± 1.22	4.95 ± 1.21	5.23 ± 0.75
	t value	0.452	0.631	1.005	0.820	0.970	2.694

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant



**Table 5.3.2.9: Impact of Probiotic and Synbiotic Supplementation the Lipid Profile of Elderly Subjects Based on the Initial TG Values (Mean  $\pm$  SD, mg/dl)**

Parameters	Group I			Group II			Group III		
	TG<150 N=12	TG $\geq$ 150 N=8		TG<150 N=12	TG $\geq$ 150 N=8		TG<150 N=9	TG $\geq$ 150 N=17	
TC	Pre	220.88 $\pm$ 16.08	224.90 $\pm$ 28.53	225.68 $\pm$ 22.4	217.82 $\pm$ 11.33		224.09 $\pm$ 20.61	231.58 $\pm$ 29.99	
	Post	222.04 $\pm$ 16.89	225.38 $\pm$ 27.99	220.48 $\pm$ 21.65	198.90 $\pm$ 29.26		218.51 $\pm$ 17.86	191.90 $\pm$ 28.67	
	t value	0.272	0.496	0.961	1.612		0.822	3.469	
TG	Pre	222.75 $\pm$ 25.13	134.77 $\pm$ 9.77	135.78 $\pm$ 7.51	183.36 $\pm$ 34.89		126.81 $\pm$ 22.37	195.58 $\pm$ 18.89	
	Post	220.25 $\pm$ 52.90	135.42 $\pm$ 25.73	144.95 $\pm$ 25.34	172.09 $\pm$ 51.22		118.93 $\pm$ 27.06	181.31 $\pm$ 27.63	
	t value	1.214	0.717	0.685	0.951		1.888	3.588	
HDL	Pre	42.98 $\pm$ 4.62	40.00 $\pm$ 4.30	39.90 $\pm$ 2.96	43.45 $\pm$ 4.19		40.32 $\pm$ 6.07	41.25 $\pm$ 4.82	
	Post	42.55 $\pm$ 4.38	44.42 $\pm$ 5.47	39.97 $\pm$ 3.97	43.78 $\pm$ 4.75		41.21 $\pm$ 4.74	42.83 $\pm$ 5.35	
	t value	0.713	0.562	0.110	0.497		0.436	1.217 *	
VLDL	Pre	26.95 $\pm$ 1.95	44.55 $\pm$ 5.02	27.15 $\pm$ 1.50	36.67 $\pm$ 6.97		25.36 $\pm$ 3.77	39.11 $\pm$ 3.47	
	Post	29.08 $\pm$ 4.74	42.05 $\pm$ 10.58	26.99 $\pm$ 5.06	34.14 $\pm$ 10.24		23.78 $\pm$ 5.41	36.26 $\pm$ 5.52	
	t value	1.214	0.717	1.661	0.951		1.888 *	3.588	
LDL	Pre	150.93 $\pm$ 17.73	135.35 $\pm$ 32.47	145.55 $\pm$ 21.14	150.77 $\pm$ 7.99		144.64 $\pm$ 27.00	164.96 $\pm$ 31.60	
	Post	150.40 $\pm$ 16.45	138.90 $\pm$ 32.15	142.27 $\pm$ 29.51	129.93 $\pm$ 26.39		141.03 $\pm$ 19.29	125.27 $\pm$ 28.4	
	t value	0.131	1.125	0.499	0.685		0.463	3.385 *	

**Note**

- \*Significant from the baseline value at  $p < 0.05$
- \*\* Significant from the baseline value at  $p < 0.01$
- NS - Non Significant

Table 5.3.2.10: Impact of Probiotic and Synbiotic Supplementation on the Atherogenic Indices of Elderly Subjects Based on the Initial TG Values (Mean± SD, mg/dl)

Parameters		Group I		Group II		Group III	
		TG<150 N=12	TG≥150 N=8	TG<150 N=12	TG≥150 N=8	TG<150 N=9	TG≥150 N=17
LDL/HDL	Pre	3.57 ± 0.43	3.02 ± 0.73	3.38 ± 0.60	3.78 ± 0.12	3.77 ± 0.98	4.08 ± 1.077
	Post t value	3.57 ± 0.58 0.007	3.16 ± 0.75 1.042	3.32 ± 1.02 0.322	3.26 ± 0.71 1.381	3.47 ± 0.68 1.055	3.02 ± 1.00 2.963 **
TG/HDL	Pre	3.17 ± 0.44	5.01 ± 0.91	3.41 ± 0.20	4.24 ± 0.83	4.87 ± 1.41	3.19± 0.54
	Post t value	3.46 ± 0.54 2.334	5.40 ± 1.55 0.799	3.61 ± 0.45 0.643	3.94 ± 1.08 1.066	4.38 ± 1.08 1.560	2.88 ± 0.68 2.651 **
TC/LDL	Pre	1.47 ± 0.07	1.69 ± 0.14	1.56 ± 0.11	1.44 ± 0.01	1.57 ± 0.17	1.41 ± 0.08
	Post t value	1.43 ± 0.06 0.665	1.65 ± 0.17 0.829	1.58 ± 0.19 0.523	1.54 ± 0.13 1.465	1.56 ± 0.10 0.338	1.56 ± 0.15 3.321 **
TC/HDL	Pre	5.20 ± 0.80	5.03 ± 0.72	5.23 ± 0.65	5.46 ± 0.13	5.80 ± 1.02	6.70 ± 1.12
	Post t value	5.27 ± 0.68 0.465	5.13 ± 0.84 0.657	5.11 ± 0.97 0.723	4.98 ± 0.71 1.302	5.37 ± 0.81 1.339	4.58 ± 1.10 3.026 **

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant

It is important to note TG/H which represents the small dense lipoprotein was significantly lowered in participants having TG  $\geq$  150 mg/dl (3.19 vs 2.88). A significant reduction was also observed in LDL/HDL and TC/HDL ratios.

**Effect of probiotic and synbiotic fermented milk supplementation on the lipid profile and atherogenic indices of older adult participants in relation to their initial BMI**

The effect of probiotic and synbiotic fermented milk supplementation was also studied in relation to the initial BMI of the individuals. Table 5.3.2.11 and Table 5.3.2.12 shows the effect of the probiotic and synbiotic fermented milk supplementation on lipid profile and atherogenic indices of the participants.

Favourable changes in the lipid profile and atherogenic indices of the experimental participants were seen in overweight and obese participants as compared to those who had BMI  $<23$  in group II and group III participants. There was a significant decrease in TG, VLDL, TG/HDL, LDL/HDL, TC/HDL synbiotic fermented milk supplemented group participants with initial BMI  $>23$ . However such a trend was not reflected in the control group, signifying the beneficial role of probiotic and synbiotic fermented milk supplementation in the lipid metabolism of overweight and obese older adult participants.

**Effect of probiotic and synbiotic fermented milk supplementation on lipid profile and atherogenic indices of older adults with hypertension as a complication.**

Table 5.3.2.13 depicts the supplementation effect on the lipid profile of the normotensive and hypertensive older adult participants. Higher reduction was observed for TC, TG, LDL-C, and VLDL-C in older adult participants who suffered from hypertension of group II and group III than normotensive subjects. There was a significant decrease in TC values with a corresponding increase in HDL values ( $P<0.05$ ) of hypertensive older adult participants in both the supplemented groups. Atherogenic indices were significantly lowered in hypertensive older adults after synbiotic fermented milk supplementation (Table 5.3.2.14).

**Table 5.3.2.11: Impact of Probiotic and Synbiotic Supplementation on the Lipid Profile of Elderly Subjects Based on the Initial BMI Values (Mean  $\pm$  SD, mg/dl)**

Parameters	Group I			Group II			Group III		
	BMI<23 N=7	BMI $\geq$ 23 N=13		BMI<23 N=12	BMI $\geq$ 23 N=8		BMI<23 N=14	BMI $\geq$ 23 N=12	
TC	Pre	222.97 $\pm$ 25.37	221.59 $\pm$ 12.14	215.60 $\pm$ 10.57	229.78 $\pm$ 23.72		224.96 $\pm$ 19.42	228.69 $\pm$ 29.12	
	Post	221.96 $\pm$ 23.27	227.30 $\pm$ 16.5	215.46 $\pm$ 15.56	216.62 $\pm$ 29.22		213.31 $\pm$ 16.81	204.14 $\pm$ 32.45	
	t value	0.554	1.352	0.026	1.810		1.757	1.984	
TG	Pre	169.59 $\pm$ 48.49	70.64 $\pm$ 48.99	158.09 $\pm$ 14.62	184.34 $\pm$ 43.56		167.58 $\pm$ 59.28	175.38 $\pm$ 39.57	
	Post	168.74 $\pm$ 48.10	180.50 $\pm$ 48.00	155.94 $\pm$ 38.68	173.81 $\pm$ 53.62		155.39 $\pm$ 52.54	163.42 $\pm$ 27.98	
	t value	1.447	1.915	0.170	0.725		2.472 *	3.169 **	
HDL-C	Pre	45.65 $\pm$ 1.29	42.78 $\pm$ 5.30	43.03 $\pm$ 4.07	42.55 $\pm$ 4.39		42.02 $\pm$ 5.27	39.46 $\pm$ 5.97	
	Post	45.58 $\pm$ 1.94	42.06 $\pm$ 5.46	43.42 $\pm$ 4.45	42.75 $\pm$ 5.15		42.08 $\pm$ 5.47	41.50 $\pm$ 4.58	
	t value	0.190	0.895	0.457	0.280		0.023	1.361	
VLDL-C	Pre	33.91 $\pm$ 9.69	34.12 $\pm$ 9.79	31.61 $\pm$ 2.92	36.86 $\pm$ 8.71		33.51 $\pm$ 11.85	35.07 $\pm$ 7.91	
	Post	34.74 $\pm$ 9.62	35.10 $\pm$ 10.33	31.18 $\pm$ 7.73	34.76 $\pm$ 10.72		31.07 $\pm$ 10.50	32.68 $\pm$ 5.59	
	t value	1.447	1.915	0.170	0.725		2.472 *	3.169 **	

**Note**

- \*Significant from the baseline value at  $p < 0.05$
- \*\* Significant from the baseline value at  $p < 0.01$
- a,b,c- the non identical letters in any two columns within a row denote a significant difference at a minimum of 5% level.
- NS - Non Significant.

Table 5.3.2.12: Impact of Probiotic and Synbiotic Supplementation on the Atherogenic Indices of Elderly Subjects Based on the Initial BMI Values (Mean± SD, mg/dl)

Parameters	Group I			Group II		Group III		
	BMI<23 N=7	BMI≥23 N=13		BMI<23 N=12	BMI≥23 N=8	BMI<23 N=14	BMI≥23 N=12	
LDL/HDL	Pre	3.11 ± 0.52	3.48 ± 0.85	3.30 ± 0.43	3.57 ± 0.62	3.84 ± 1.05	3.91 ± 1.00	
	Post	3.18 ± 0.61	3.53 ± 0.67	3.26 ± 0.42	3.34 ± 1.20	3.21 ± 1.01	3.40 ± 0.63	
	t value	0.855	0.332	0.265	0.881	1.480	2.066 *	
TG/HDL	Pre	3.72 ± 1.02	4.00 ± 1.21	3.70 ± 0.49	4.33 ± 0.91	4.05 ± 1.57	4.55 ± 1.41	
	Post	3.87 ± 0.67	4.43 ± 1.63	3.62 ± 0.96	4.04 ± 1.00	3.83 ± 1.67	3.97 ± 0.99	
	t value	0.484	1.626	0.275	0.794	0.659	2.341 *	
TC/LDL	Pre	1.58 ± 0.16	1.54 ± 0.15	1.53 ± 0.07	1.54 ± 0.13	1.53 ± 0.21	1.50 ± 0.11	
	Post	1.60 ± 0.20	1.52 ± 0.10	1.53 ± 0.10	1.60 ± 0.21	1.58 ± 0.14	1.54 ± 0.10	
	t value	0.940	0.739	0.129	1.115	0.687	1.271	
TC/HDL	Pre	4.86 ± 0.38	5.28 ± 0.87	5.04 ± 0.46	5.43 ± 0.63	5.70 ± 1.07	5.82 ± 1.09	
	Post	5.00 ± 0.49	5.33 ± 0.82	4.98 ± 0.38	5.15 ± 1.15	4.98 ± 1.26	5.20 ± 0.69	
	t value	1.495	0.323	0.436	1.191	1.603	2.250	

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant.

Table 5.3.2.13: Impact of Probiotic and Synbiotic Supplementation on the Lipid Profile of Elderly Subjects Based on the Initial BP Values (Mean± SD, mg/dl)

Parameters	Group I		Group II		Group II	
	Normotensives N= 13	Hypertensives N= 7	Normotensives N= 7	Hypertensives N= 13	Normotensives N= 9	Hypertensives N= 17
TC	Pre	219.45 ±12.10	228.13 ± 29.36	221.29 ± 13.99	225.63 ± 23.59	235.53 ± 28.90
	Post	219.36 ± 15.55	230.83 ± 28.32	217.49 ± 13.74	215.45 ± 28.78	205.67 ± 14.18
	t value	0.022	1.840	1.112	1.370	2.336 *
TG	Pre	177.72 ± 51.13	155.54 ± 33.75	172.79 ± 43.36	174.41 ± 34.62	154.90 ± 47.04
	Post	179.32 ± 52.25	156.56 ± 42.48	166.09 ± 36.44	166.97 ± 54.52	146.50 ± 34.33
	t value	0.124	0.104	0.344	0.637	1.574
HDL-C	Pre	44.47 ± 4.68	42.51 ± 4.16	42.02 ± 3.83	43.13 ± 4.44	39.38 ± 5.16
	Post	44.32 ± 3.80	41.39 ± 6.12	43.20 ± 4.65	42.92 ± 5.02	39.75 ± 3.79
	t value	0.391	0.812 *	1.174	0.329	0.228
VLDL-C	Pre	35.54 ± 10.22	31.10 ± 7.75	34.55 ± 8.67	34.88 ± 6.92	30.98 ± 9.40
	Post	35.86 ± 10.45	31.31 ± 8.49	33.21 ± 7.28	33.39 ± 10.90	29.30 ± 6.86
	t value	0.124	0.104	0.344	0.637	1.575
LDL-C	Pre	139.42 ± 22.94	154.50 ± 27.86	144.70 ± 12.37	147.61 ± 22.41	165.17 ± 34.10
	Post	139.17 ± 20.07	158.11 ± 27.03	141.06 ± 11.48	139.12 ± 35.16	136.6 ± 16.88
	t value	0.061	2.339	1.006	0.944	2.126

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant.

Table 5.3.2.14: Impact of Probiotic and Synbiotic Supplementation on the Atherogenic Indices of Elderly Subjects Based on the Initial BP Values (Mean± SD, mg/dl)

Parameters		Group I		Group II		Group III	
		Normotensives N= 13	Hypertensives N= 7	Normotensives N= 7	Hypertensives N= 13	Normotensives N= 9	Hypertensives N= 17
LDL/HDL	Pre	3.19 ±0.76	3.66 ± 0.72	3.461 ± 0.64	3.46 ± 0.39	3.68 ± 0.98	4.25 ± 0.99
	Post	3.16 ±0.56	3.86 ± 0.65	3.32 ± 1.15	3.30 ± 0.48	3.23 ± 0.89	3.48 ± 0.67
	t value	0.233	1.333	0.576	0.344	1.577	1.908
TG/HDL	Pre	4.02 ±1.18	3.70 ± 1.07	4.05 ± 0.71	4.13 ± 1.05	4.47 ± 1.39	4.04 ± 1.68
	Post	4.45 ±1.57	3.84 ± 1.07	3.88 ± 1.05	3.87 ± 0.92	4.02 ± 1.43	3.69 ± 0.82
	t value	1.447	1.000	0.662	1.973	1.717	1.034
TC/LDL	Pre	1.59 ±0.16	1.48 ± 0.09	1.54 ± 0.12	1.53 ± 0.08	1.55 ± 0.17	1.44 ± 0.14
	Post	1.59 ±0.15	1.47 ± 0.07	1.59 ± 0.21	1.54 ± 0.08	1.58 ± 0.12	1.51 ± 0.11
	t value	0.114	1.082	0.995	0.504	0.526	1.597
TC/HDL	Pre	4.99 ±0.73	5.40 ± 0.79	5.27 ± 0.67	5.28 ± 0.42	5.61 ± 1.11	6.06 ± 0.98
	Post	4.98 ±0.57	5.65 ± 0.84	5.09 ± 1.08	5.08 ± 0.55	5.03 ± 1.12	5.22 ± 0.67
	t value	0.113	1.179	0.797	0.378	1.829	1.962

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant.

**Effect of probiotic and synbiotic fermented milk supplementation on lipid profile and atherogenic indices of older adult participants in relation to both BMI and hypertension as a complication**

Since BMI and hypertension can have an influence on the response to supplementation, the data on lipid profile was looked in relation to both BMI and hypertension (Table 5.3.2.15).

Table 5.3.2.16 shows the atherogenic indices of the participants who were hypertensive and had a BMI >23. Probiotic fermented milk supplementation did not have significant impact on lipid profile of older adult participants who were overweight and hypertensive. However significant reduction in TC and LDL -C was observed in group III participants having both BMI and hypertension as a complication.

However the decreasing trend in TC and LDL-C irrespective of the initial levels in both the experimental groups, may call for multiple strategies to bring about the beneficial effects.

**Gut micro flora of participants before and after supplementation of probiotic and synbiotic fermented milk:**

As Seen in Table 5.3.2.17, analysis of gut micro flora revealed that significant changes occurred for various bacterial counts in the stool samples of older adult participants before and after the study period.



Table 5.3.2.15: Elderly Subjects with BMI >23 and Hypertension as a Complication (Mean  $\pm$  SD, mg/dl)

Parameters	Group I			Group II			Group III		
	Normotensives BMI<23 N=14	Hypertensives BMI $\geq$ 23 N=6		Normotensives BMI<23 N=14	Hypertensives BMI $\geq$ 23 N=6		Normotensives BMI<23 N=18	Hypertensives BMI $\geq$ 23 N=8	
TC	Pre	219.26 $\pm$ 15.46	230.02 $\pm$ 31.69	219.27 $\pm$ 9.43	226.19 $\pm$ 23.68		220.83 $\pm$ 22.67	229.28 $\pm$ 24.65	
	Post	219.97 $\pm$ 14.95	232.48 $\pm$ 30.65	217.28 $\pm$ 10.89	215.68 $\pm$ 27.05		203.19 $\pm$ 37.87	212.01 $\pm$ 17.78	
	t value	0.059	1.439	0.300	1.617		0.822	2.157 *	
TG	Pre	159.10 $\pm$ 41.18	174.61 $\pm$ 50.48	156.85 $\pm$ 8.97	181.12 $\pm$ 41.88		166.52 $\pm$ 39.46	183.60 $\pm$ 66.89	
	Post	159.37 $\pm$ 45.82	176.49 $\pm$ 51.31	152.30 $\pm$ 30.30	172.81 $\pm$ 53.55		156.86 $\pm$ 29.28	166.14 $\pm$ 60.84	
	t value	0.023	0.158	0.296	0.646		3.062 **	3.588 **	
HDL-C	Pre	44.57 $\pm$ 4.51	41.95 $\pm$ 4.05	44.70 $\pm$ 3.15	41.90 $\pm$ 4.36		41.54 $\pm$ 6.29	40.25 $\pm$ 5.39	
	Post	44.53 $\pm$ 3.74	40.41 $\pm$ 5.07	45.15 $\pm$ 3.37	42.11 $\pm$ 6.09		42.73 $\pm$ 5.92	40.35 $\pm$ 4.49	
	t value	0.1132	0.991	0.425	0.313		0.436	0.834	
VLDL-C	Pre	31.82 $\pm$ 8.23	34.92 $\pm$ 10.0	31.37 $\pm$ 1.79	36.22 $\pm$ 8.37		33.30 $\pm$	36.72 $\pm$ 13.37	
	Post	31.87 $\pm$ 9.16	35.29 $\pm$ 10.26	30.46 $\pm$ 6.06	34.56 $\pm$ 10.71		31.72 $\pm$	33.72 $\pm$ 12.16	
	t value	0.023	0.158	0.296	0.646		3.062 **	3.588 **	
LDL-C	Pre	139.75 $\pm$ 22.07	156.24 $\pm$ 30.10	143.19 $\pm$ 12.40	148.05 $\pm$ 21.69		141.56 $\pm$ 32.29	155.72 $\pm$ 28.51	
	Post	139.63 $\pm$ 19.36	160.19 $\pm$ 28.99	141.86 $\pm$ 19.80	139.00 $\pm$ 32.41		127.22 $\pm$ 33.02	134.28 $\pm$ 17.91	
	t value	0.031	2.213	0.171	1.184		0.463	1.996	

## Note

- \* Significant from the baseline value at  $p < 0.05$
- \*\* Significant from the baseline value at  $p < 0.01$
- NS - Non Significant.

Table 5.3.2.16: Impact of Probiotic and Synbiotic Supplementation on the Atherogenic Indices of Elderly Subjects with Bmi >23 and Hypertension as a Complication (Mean± SD, mg/dl)

Parameters	Group I			Group II			Group III		
	Normotensives BMI<23 N= 14	Hypertensives BMI≥23 N= 6		Normotensives BMI<23 N= 14	Hypertensives BMI≥23 N= 6		Normotensives BMI<23 N= 18	Hypertensives BMI≥23 N= 8	
LDL/HDL	Pre	3.19 ± 0.73		3.22 ± 0.45	0.56 ± 0.58		3.72 ± 1.05	3.95 ± 1.01	
	Post	3.16 ± 0.53		3.13 ± 0.31	3.39 ± 1.12		3.09 ± 1.11	3.42 ± 0.66	
	t value	0.276		0.456	0.799		1.055	2.04 *	
TG/HDL	Pre	3.94 ± 1.17		3.51 ± 0.25	4.32 ± 0.87		4.51 ± 1.76	4.24 ± 1.38	
	Post	4.34 ± 1.56		3.40 ± 0.75	4.08 ± 1.03		4.10 ± 2.01	3.82 ± 0.78	
	t value	1.443		0.343	0.758		1.560	2.016 *	
TC/LDL	Pre	1.59 ± 0.16		1.53 ± 0.07	1.54 ± 0.12		1.59 ± 0.23	1.49 ± 0.12	
	Post	1.59 ± 0.15		1.54 ± 0.09	1.59 ± 0.20		1.62 ± 0.14	1.53 ± 0.10	
	t value	0.68		0.160	1.091		0.338	1.473	
TC/HDL	Pre	4.97 ± 0.70		4.93 ± 0.48	5.42 ± 0.58		5.69 ± 1.13	5.80 ± 1.07	
	Post	4.96 ± 0.55		4.81 ± 0.23	5.20 ± 1.07		4.91 ± 1.45	5.18 ± 0.72	
	t value	0.152		0.693	1.090		1.339	2.185 *	

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant.

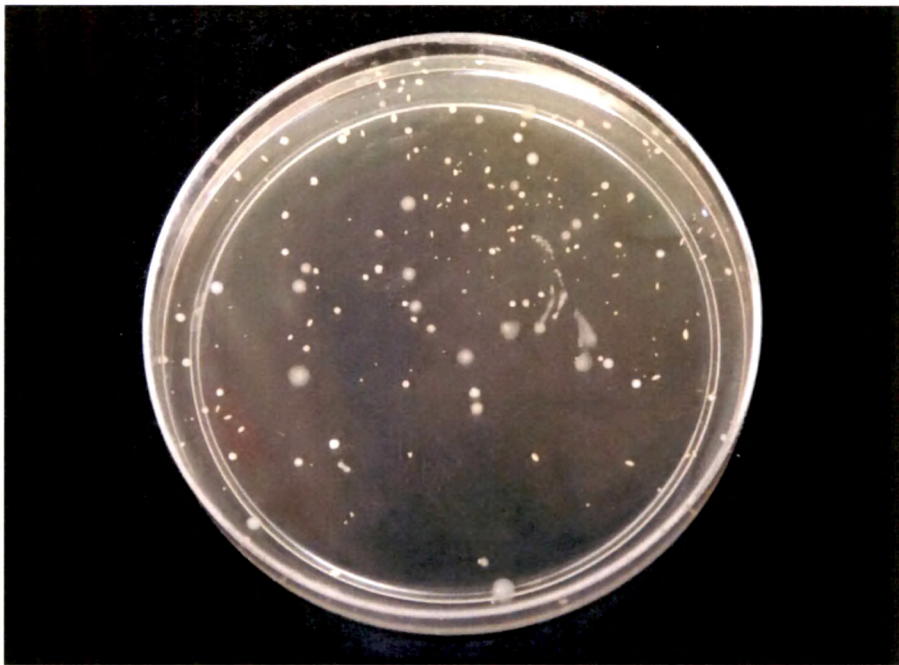
Table 5.3.2.17: Gut Microflora of Older Adults Before and After Supplementation of Probiotic and Synbiotic Fermented Milk  
(Mean± SD, mg/dl)

Gut microflora		Group I (n=20)	Group II (n=20)	Group III (n=26)	Anova
<i>E. coli</i>	Pre	6.40±0.84	6.66±1.2	6.51±0.88	1.072
	Post	6.47±0.53 <sup>a</sup>	6.28±0.96 <sup>a</sup>	5.88±0.2 <sup>b</sup>	3.516*
	't' value	0.312	1.996*	5.762***	
	% change	1.09	5.8	9.80	
<i>Lactic acid bacteria</i>	Pre	6.54±0.93	6.43±1.5	6.90±0.79	1.006
	Post	6.35±0.40 <sup>a</sup>	6.86±0.54 <sup>a</sup>	7.65±0.18 <sup>b</sup>	4.518*
	't' value	1.932	2.700**	4.435***	
	% change	2.90	6.6	9.18	
<i>Bifidobacterium</i>	Pre	8.17±0.78	7.90±2.0	7.82±0.66	2.089
	Post	8.02±0.81 <sup>a</sup>	8.23±1.1 <sup>a</sup>	8.58±0.47 <sup>b</sup>	6.318*
	't' value	1.367	3.553**	6.227***	
	% change	1.80	4.17	9.71	

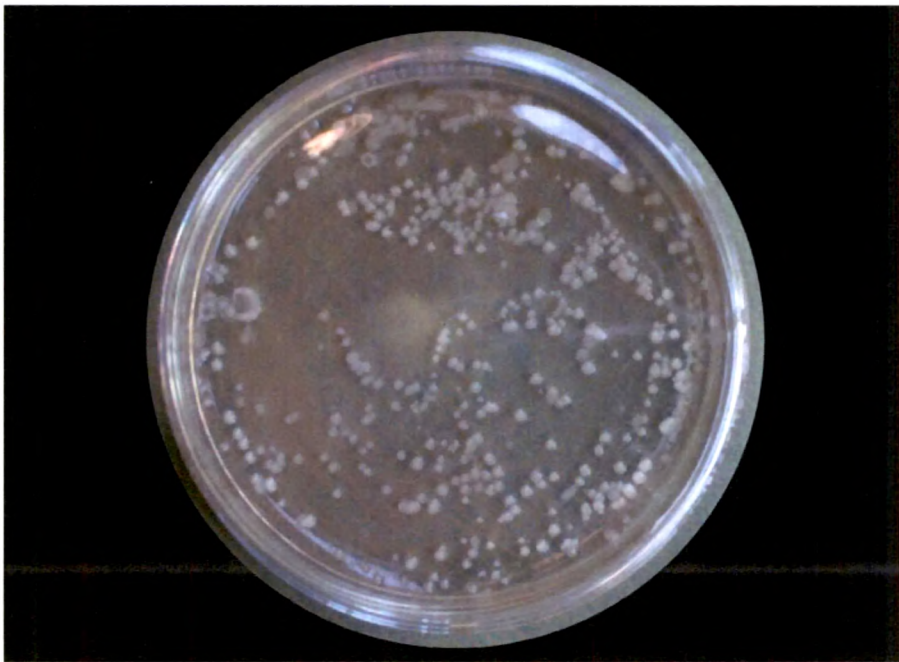
Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- a,b,c- the non identical letters in any two columns within a row denote a significant difference at a minimum of 5% level.
- NS - Non Significant.

Typical shiny off white colonies of *Bifidobacteria* in stool sample of institutionalized elderly subject (code no. 16; dilution= 10<sup>8</sup>)



**Plate 5.1: *Bifidobacterium* counts before supplementation of Synbiotic fermented milk**



**Plate 5.2: *Bifidobacterium* counts after supplementation of Synbiotic fermented milk**

Typical buttery colonies of *Lactic acid bacteria* in stool sample of institutionalized elderly subject (code no 43; dilution=  $10^6$ )

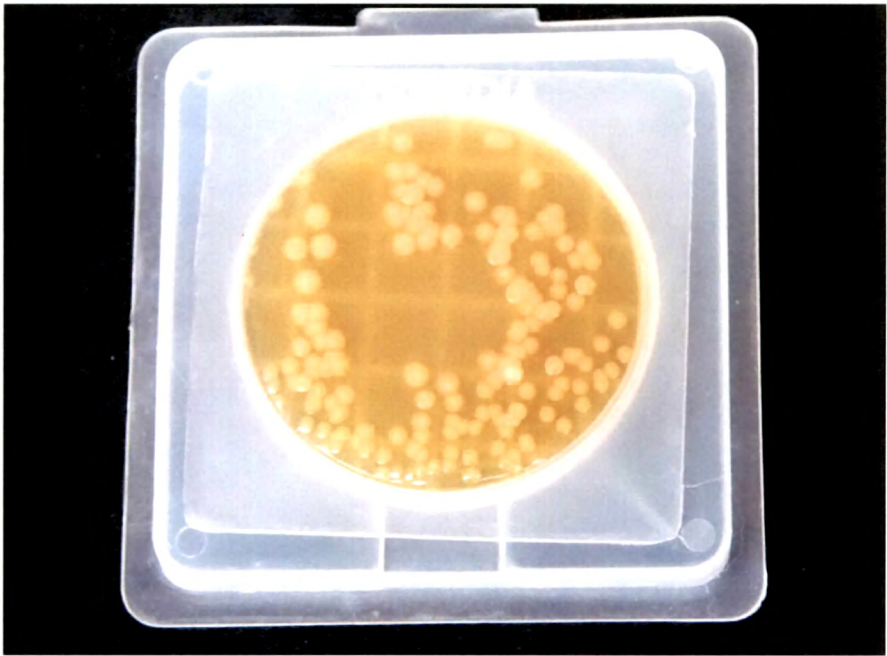


Plate 5.3: *Lactic acid bacteria* counts before supplementation of Synbiotic fermented milk

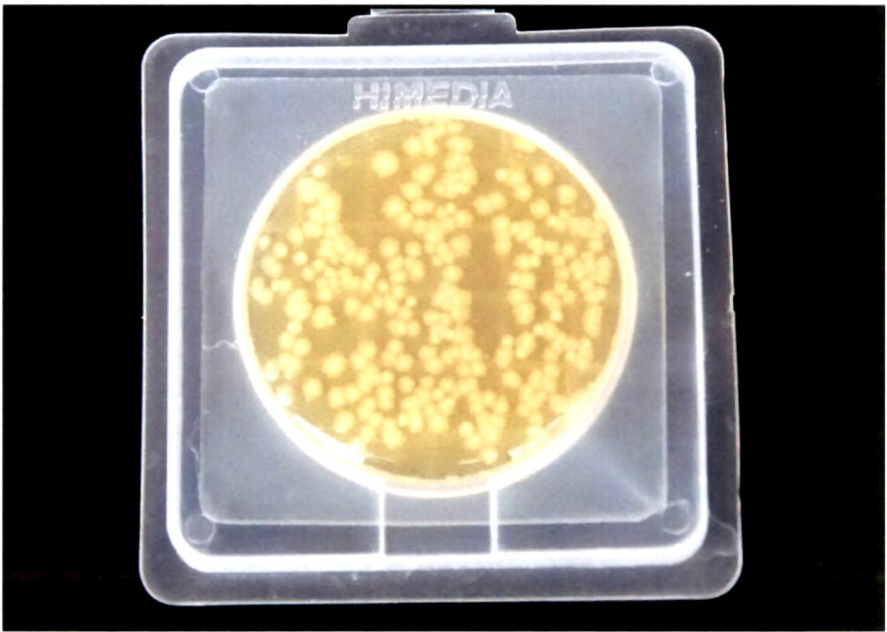


Plate 5.4: *Lactic acid bacteria* counts after supplementation of Synbiotic fermented milk



Typical dark red colonies of *E. coli* in stool sample of institutionalized elderly subject (code no 31; dilution=  $10^6$ )

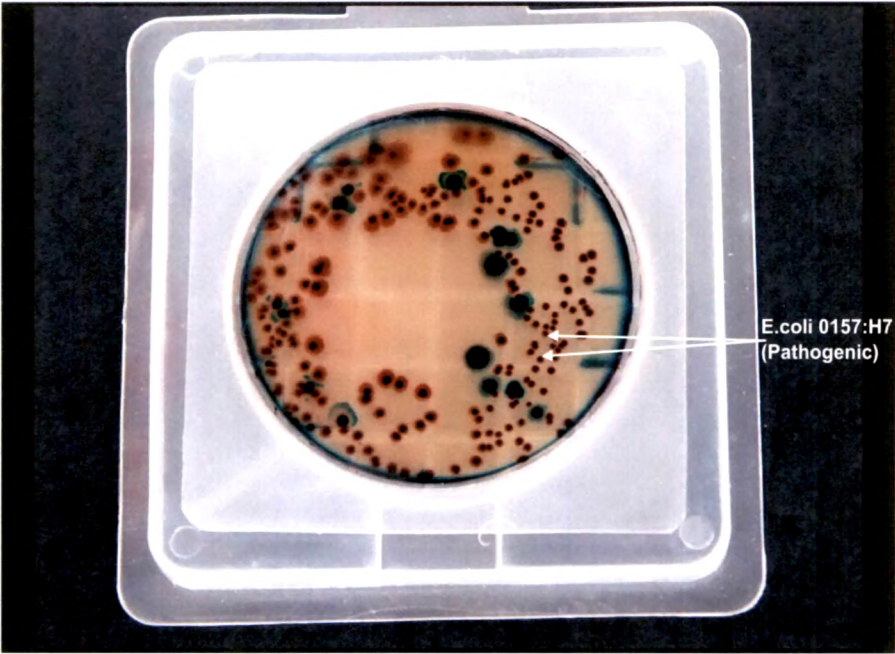


Plate 5.5: *E. coli* counts before supplementation of Synbiotic fermented milk

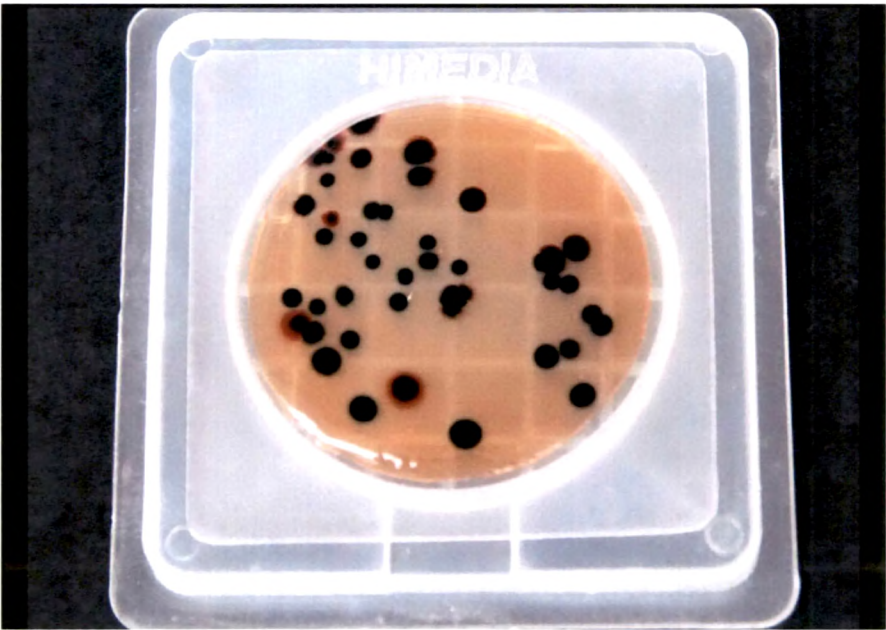


Plate 5.6: *E. coli* counts after supplementation of Synbiotic fermented milk

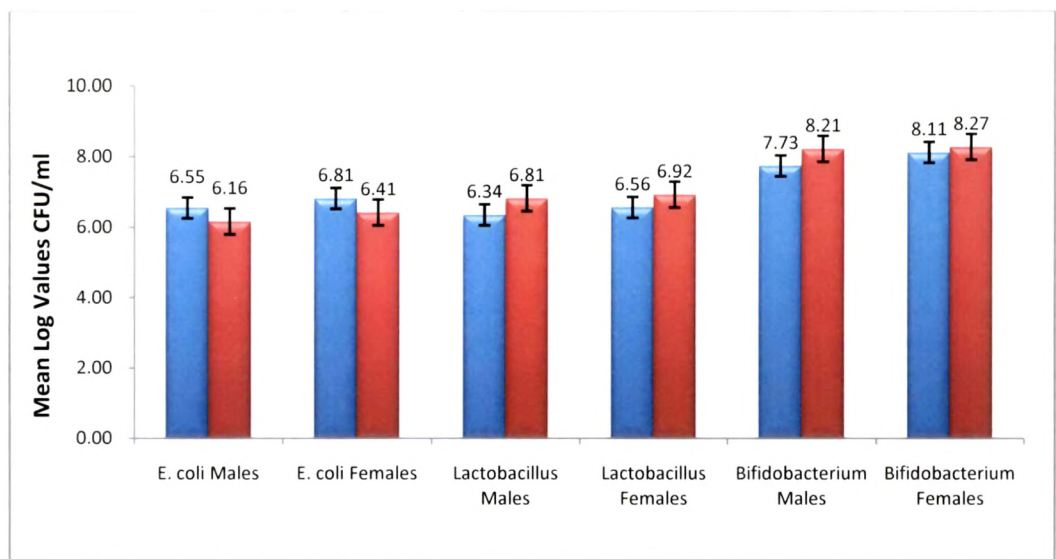


Fig. 5.3.2.4 Gut Microflora of Male and Female Elderly Subjects Before and After Probiotic Fermented Milk Supplementation

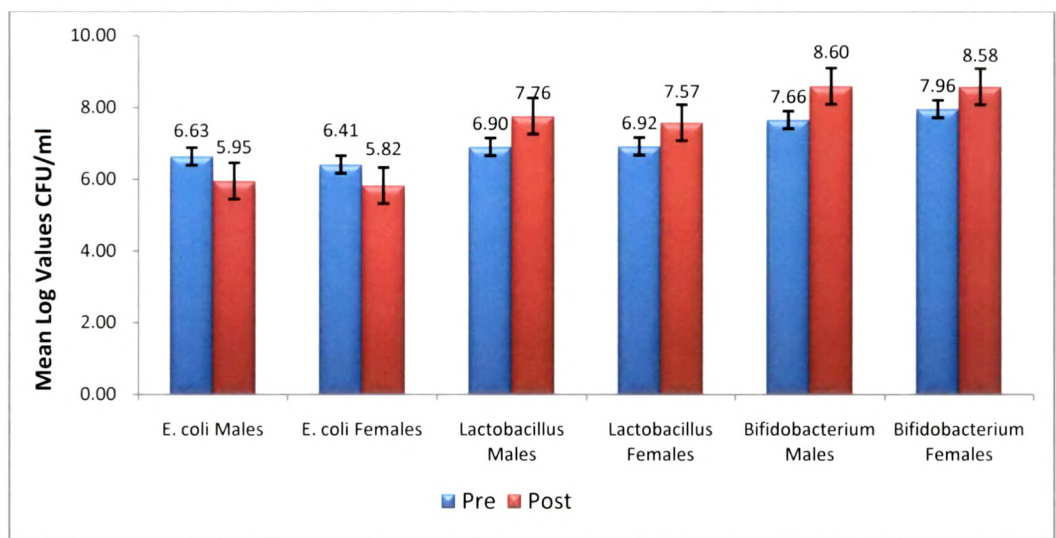


Fig. 5.3.2.5: Gut Microflora of Male and Female Elderly Subjects Before and After Synbiotic Fermented Milk Supplementation

The mean log values in CFU/g (wet weight) increased significantly for *bifidobacteria* (from 7.90 to 8.23) and *lactobacilli* (6.43 to 6.86) in group II participants whereas the increase was higher ( $p<0.001$ ) in synbiotic supplemented group for *bifidobacteria* (from 7.82 to 8.58) and *lactobacilli* (6.92 to 7.65). Decrease in mean log counts of *E.coli* was observed from 6.66 to 6.28 and from 6.51 to 5.88 in probiotic and synbiotic group respectively (Table 5.3.2.17).

**Table 5.3.2.18: Gut Microflora of Male and Female Older Adults Before and After Supplementation of Probiotic and Synbiotic Fermented Milk (Mean $\pm$  SD, mg/dl)**

Parameters	Group I		Group II		Group III	
	Males (n=10)	Females (n=10)	Males (n=11)	Females (n=11)	Males (n=11)	Females (n=15)
<i>E.coli</i>	Pre	6.21 $\pm$ 0.96	6.60 $\pm$ 0.68	6.54 $\pm$ 1.06	6.81 $\pm$ 0.68	6.40 $\pm$ 0.81
	Post	6.24 $\pm$ 0.44	6.69 $\pm$ 0.52	6.16 $\pm$ 0.95	6.41 $\pm$ 1.01	5.82 $\pm$ 0.71
	t value	0.097 ns	0.403	0.881 ns	0.965	1.829
<i>Lactobacilli</i>	Pre	6.62 $\pm$ 0.34	6.55 $\pm$ 0.56	6.34 $\pm$ 0.67	6.55 $\pm$ 0.48	6.91 $\pm$ 0.77
	Post	6.36 $\pm$ 0.42	6.35 $\pm$ 0.94	6.81 $\pm$ 0.54	6.91 $\pm$ 0.64	7.75 $\pm$ 0.50
	t value	1.444 ns	1.095	2.337 *	1.441	3.052 **
<i>Bifidobacteria</i>	Pre	8.10 $\pm$ 0.43	8.24 $\pm$ 0.91	7.73 $\pm$ 2.22	8.11 $\pm$ 1.90	7.51 $\pm$ 1.15
	Post	7.92 $\pm$ 0.60	8.12 $\pm$ 0.97	8.21 $\pm$ 1.24	8.26 $\pm$ 0.82	8.59 $\pm$ 0.52
	t value	1.720 ns	0.354	0.881	0.322	3.044 **

**Note**

- \*Significant from the baseline value at  $p < 0.05$
- \*\* Significant from the baseline value at  $p < 0.01$
- NS - Non Significant.



There was a better establishment of beneficial microorganisms in the gut of the male participants compared to female participants in both the supplemented groups (Table 5.3.2.18 and Figures 5.3.2.4 -5.3.2.5). A significant increase in establishment of *Lactobacilli* was observed in male participants of group II and group III whereas the subjects supplemented with synbiotic fermented milk shown significantly higher establishment of *bifidobacteria* and significant reduction in *E.coli* only in the male participant.

#### **Identification of bacterial species:**

The bacterial species identified in stool samples of elderly subjects were *B. bifidum*, *B. longum*, *B. brevis*, *L. acidophilus*, *L. casei*, *L. bulgaricus* and *L. leishmanii*.

#### **Association between gut microflora and life style factors after supplementation of probiotic and synbiotic fermented milk**

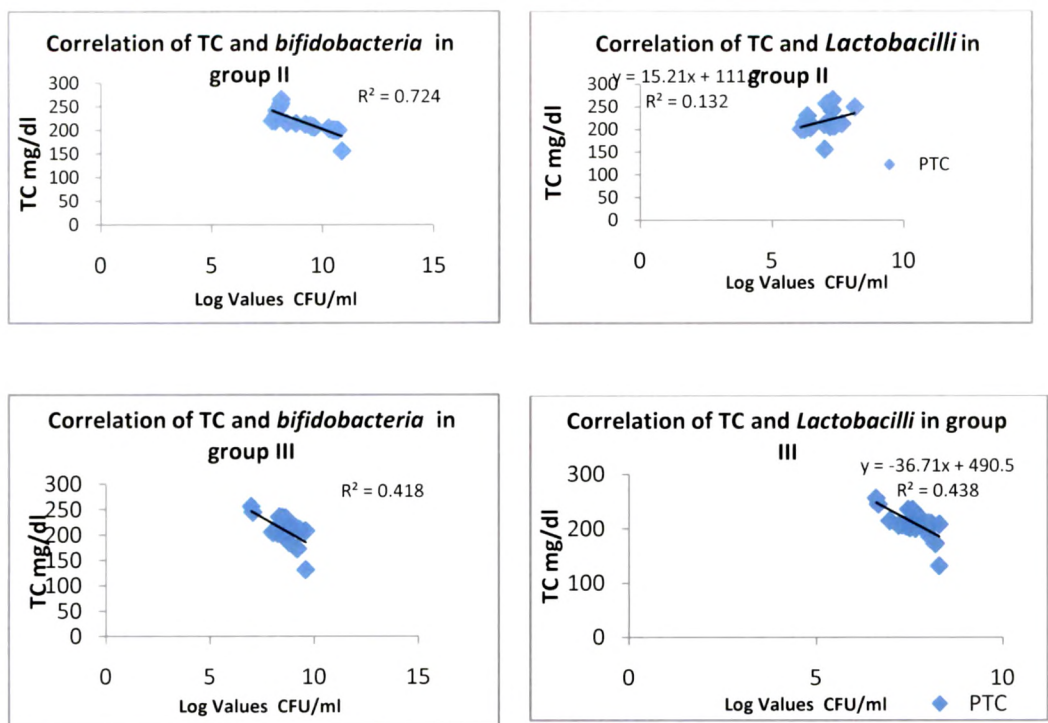
As seen in Table 5.3.2.19, supplementation with probiotic and synbiotic fermented milk resulted in a negative correlation between TC, LDL-C, TC: HDL-C and beneficial microorganisms i.e. *bifidobacteria* and *lactobacilli*. A positive correlation was observed between establishment of *E.coli* and atherogenic parameters whereas establishment of *bifidobacteria*, *lactobacilli* and *E.coli* could be significantly correlated with TG, VLDL, and TG: HDL and FBS in group III participants only (Fig. 5.3.2.6-5.3.2.8)

#### **Effect of probiotic and synbiotic fermented milk supplementation on Gut microflora of the participants in relation to their initial TC and TG values**

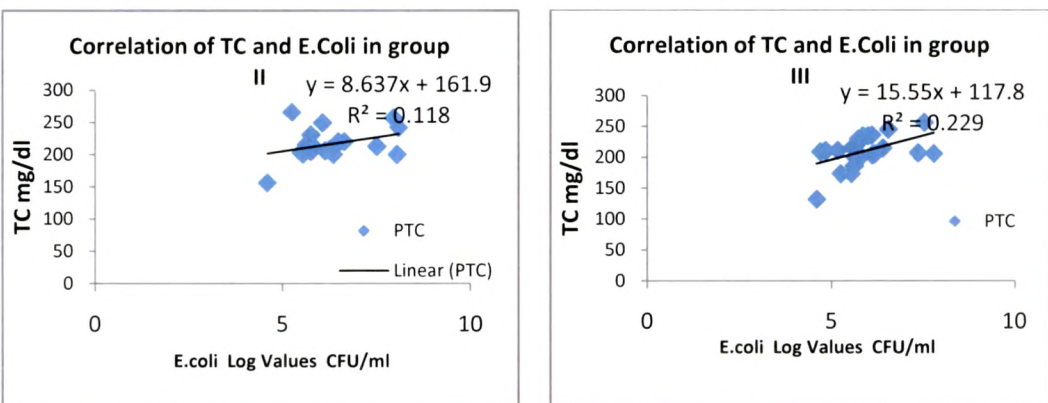
As seen in Table 5.3.2.20 and 5.3.2.21, when the gut microflora was studied with respect to the initial TC values and TG values, there was a increase in mean log counts of *lactobacilli* ( $p < 0.05$ ), *bifidobacteria* and significant decrease in mean log counts of *E.Coli* ( $p < 0.05$ ) in older adult participants of group II and group III with TC > 210 mg/dl. Total cholesterol has an independent effect on the establishment of beneficial microflora in the gut in both the supplemented groups.

#### **Effect of probiotic and synbiotic fermented milk supplementation on the gut microflora of older adult participants in relation to their initial BMI**

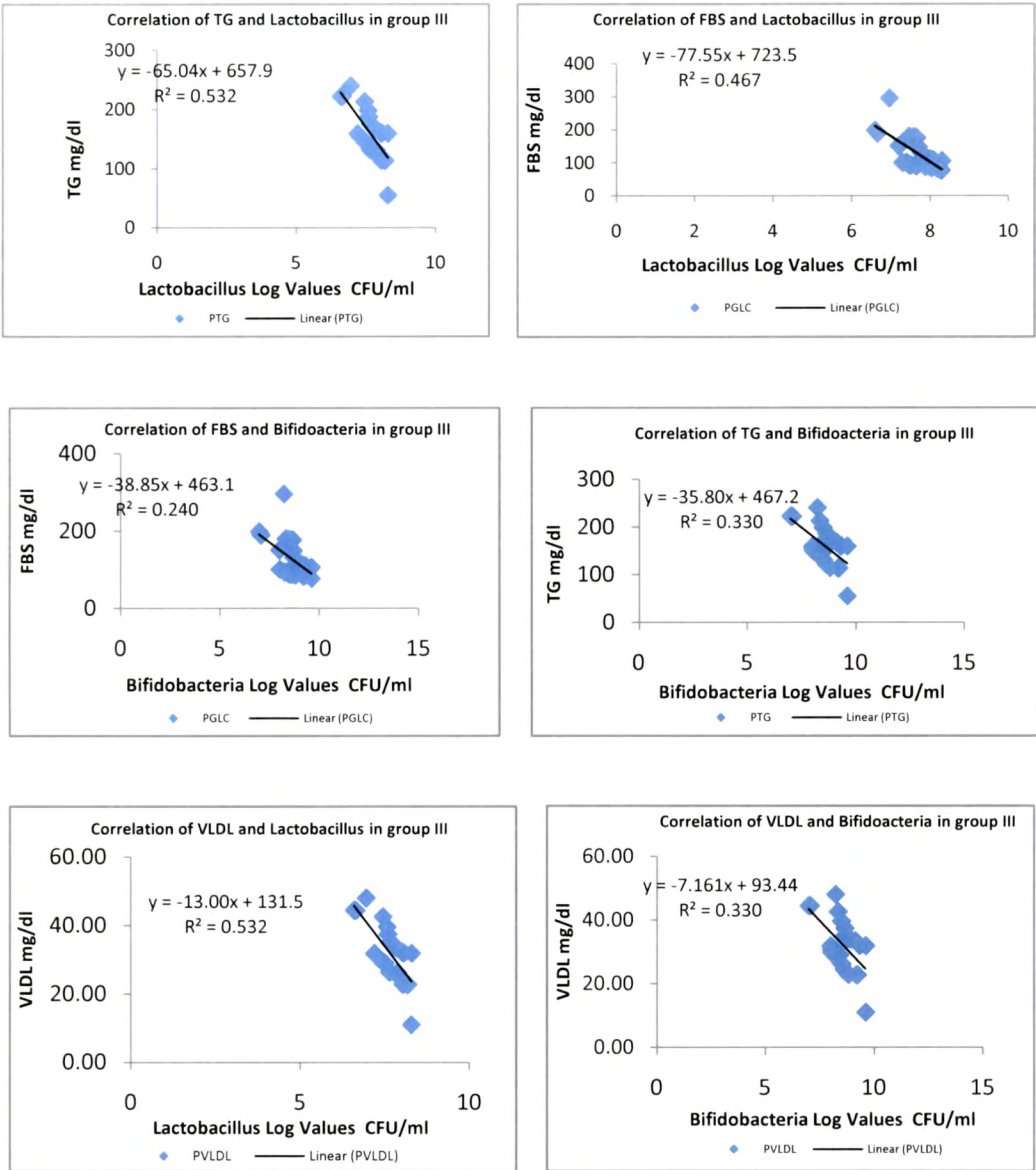
When the gut microflora of the older adult participants was studied with respect to their initial BMI values as seen in Table 5.3.2.22, there was a increase in mean log.



**Fig. 5.3.2.6 Correlation of Total Cholesterol and Beneficial Microorganisms (Bifidobacteria and Lactobacilli) After Probiotic and Synbiotic Fermented Milk Supplementation**



**Fig. 5.3.2.7 Correlation of Total Cholesterol and E.Coli After Probiotic and Synbiotic Fermented Milk Supplementation**



**Fig. 5.3.2.8 Correlation of Biochemical Parameters and Beneficial Microorganisms (Bifidobacteria and Lactobacilli) After Synbiotic Fermented Milk Supplementation**

Table 5.3.2.19: Association of Biochemical Parameters and Gut Microflora Before and after Probiotic and Symbiotic Fermented Milk Supplementation

Parameters	Group II			Group II			Group III			Group III		
	(Before supplementation)			(After supplementation)			(Before supplementation)			(After supplementation)		
	E.coli	Lactobacillus	Bifidobacteria	E.coli	Lactobacillus	Bifidobacteria	E.coli	Lactobacillus	Bifidobacteria	E.coli	Lactobacillus	Bifidobacteria
TC	0.293	-0.155	-0.359	0.344	0.364	0.224	0.269	-0.152	-0.345	0.479*	-0.662*	-0.603*
HDL	-0.201	0.024	0.154	-0.079	-0.108	-0.333	0.228	0.466	0.427	-0.210	0.237	0.273*
TG	0.357	-0.133	-0.265	0.204	0.066	-0.112	0.228	-0.294	0.302	0.487	-0.729*	-0.575*
LDL	-0.054	-0.063	-0.332	0.248	0.320	0.297	0.098	-0.345	0.515*	0.456*	-0.594*	-0.591*
VLDL	0.357	-0.133	-0.265	0.204	0.066	-0.112	0.228	-0.294	-0.302	0.487	-0.729*	-0.575*
LDL/HDL	-0.158	-0.034	-0.248	0.225	0.272	0.423	0.058	-0.472	-0.573*	0.374	-0.506*	-0.517*
TG/HDL	0.017	0.032	0.222	0.368	0.061	-0.026	0.059	0.002	0.031	0.469	-0.707*	-0.599*
TC/LDL	0.122	0.072	0.419*	-0.075	-0.184	-0.320	0.028	0.411	0.503*	-0.250	0.197*	0.326
TC/HDL	0.061	0.007	0.329	0.226	0.280	0.257	0.200	0.553*	0.577*	0.407	-0.567*	-0.550*
FBS	0.133	-0.236	-0.130	-0.419	0.097	-0.352	0.019	-0.080	-0.134	0.446	0.446*	-0.490
BMI	0.207	0.048	0.078	-0.260	-0.194	-0.050	0.348	0.207	0.026	-0.192	0.086*	0.133
BP systolic	0.055	-0.043	-0.094	0.458	0.355	0.354	0.112	-0.110	-0.134	0.213	-0.284*	-0.209
BP diastolic	0.217	-0.295	-0.259	0.267	0.267	0.351	0.207	-0.124	-0.186	0.108	-0.156*	-0.069

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant.

Table 5.3.2.20: Impact of Probiotic and Synbiotic Supplementation on Gut Microflora of Older Adults Based on the Initial TC Values  
(Mean± SD, mg/dl)

Parameters	Group I			Group II			Group III		
	TC<210 N=11	TC≥210 N=9		TC<210 N=11	TC≥210 N=9		TC<210 N=14	TC≥210 N=12	
<i>E.coli</i>	Pre	6.60 ± 0.91	6.24 ± 0.77	6.20 ± 0.65	7.04 ± 0.92		6.48 ± 0.88	6.53 ± 0.77	
	Post	6.30 ± 0.34	6.661 ± 0.62	6.08 ± 0.86	6.51 ± 1.06		6.27 ± 0.84	5.54 ± 0.53	
	t value	1.004	1.619	0.736	2.758 *		0.565	5.359 ***	
<i>Lactobacilli</i>	Pre	6.56 ± 0.47	6.52 ± 0.46	6.50 ± 0.61	6.36 ± 0.59		7.00 ± 0.92	6.82 ± 0.62	
	Post	6.38 ± 0.85	6.33 ± 0.62	6.58 ± 0.46	7.14 ± 0.54		7.27 ± 0.31	7.55 ± 0.53	
	t value	0.892	1.657	0.469	4.334 **		3.268	3.592 ***	
<i>Bifidobacteria</i>	Pre	8.01 ± 0.69	8.36 ± 0.71	8.27 ± 2.52	7.59 ± 1.6		7.69 ± 1.14	7.80 ± 0.97	
	Post	8.06 ± 0.86	7.97 ± 0.74	8.44 ± 1.09	8.06 ± 1.02		8.43 ± 0.64	8.72 ± 0.64	
	t value	0.230	1.30	0.234	0.987		2.278	2.716 **	

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant

Table 5.3.2.21: Impact of Probiotic and Synbiotic Supplementation on the Gut Microflora of Elderly Subjects Based on the Initial TG Values (Mean± SD, mg/dl)

Parameters	Group I			Group II			Group III		
	TG<150 N=12	TG≥150 N=8		TG<150 N=12	TG≥150 N=8		TG<150 N=9	TG≥150 N=17	
E.coli	Pre	6.59 ± 0.81	6.13 ± 0.84	6.63 ± 0.93	6.80 ± 0.85		6.39 ± 0.67	6.57 ± 0.89	
	Post	6.56 ± 0.34	6.33 ± 0.72	6.46 ± 0.92	5.53 ± 0.80		5.75 ± 0.98	5.04 ± 0.66	
	t value	0.087	0.787	0.546	1.819		1.802	2.297	
Lactobacilli	Pre	6.65 ± 0.43	6.46 ± 0.47	6.58 ± 0.37	6.42 ± 0.64		6.99 ± 0.89	6.75 ± 0.56	
	Post	6.520±0.53	6.26 ± 0.81	6.75 ± 0.47	6.88 ± 0.61		7.72 ± 0.47	7.52 ± 0.41	
	t value	1.233	1.268	0.871	2.609		3.400	4.455 **	
Bifidobacteria	Pre	8.24 ± 0.30	8.12 ± 0.89	8.30 ± 1.96	6.68 ± 0.15		8.11 ± 0.87	7.07 ± 1.01	
	Post	8.27 ± 0.43	7.85 ± 0.94	8.31 ± 1.08	7.91 ± 0.97		8.51 ± 0.73	8.72 ± 0.24	
	t value	0.171	0.970	0.246	1.420		1.734	4.121 **	

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant

Table 5.3.2.22: Impact of Probiotic and Synbiotic Supplementation on the Gut Microflora of Elderly Subjects Based on the Initial BMI Values (Mean± SD, mg/dl)

Parameters	Group I		Group II		Group III	
	BMI<23 N=7	BMI≥23 N=13	BMI<23 N=12	BMI≥23 N=8	BMI<23 N=14	BMI≥23 N=12
<i>E.coli</i>	Pre	6.37 ± 1.14	6.42 ± 1.14	6.73 ± 0.74	6.38 ± 1	6.62 ± 0.58
	Post	6.34 ± 0.18	6.54 ± 0.18	6.36 ± 1.01	6.14 ± 0.92	5.86 ± 0.35
	t value	0.078 <sup>ns</sup>	0.536 <sup>ns</sup>	0.63	1.366	5.011 <sup>***</sup>
	%change	0.47	1.86	3.92	8.76	11.48
<i>Lactobacilli</i>	Pre	6.79 ± 0.29	6.40 ± 0.41	6.37 ± 0.69	6.48 ± 0.54	6.66 ± 0.7
	Post	6.71 ± 0.37	6.16 ± 0.78	6.73 ± 0.5	6.94 ± 0.62	7.81 ± 0.29
	t value	0.456 <sup>ns</sup>	1.773 <sup>ns</sup>	1.468	2.236 *	5.920 <sup>***</sup>
	%change	1.17	3.75	5.65	7.09	17.26
<i>Bifidobacteria</i>	Pre	7.93 ± 0.41	8.30 ± 0.41	8.03 ± 1.88	7.62 ± 2.36	7.69 ± 0.5
	Post	7.99 ± 0.78	8.04 ± 0.78	8.52 ± 0.81	8.04 ± 1.17	8.65 ± 1.5
	t value	0.196 <sup>ns</sup>	1.172 <sup>ns</sup>	1.090	1.137	2.37*
	%change	0.75	3.13	5.97	5.51	12.48

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant

Table 5.3.2.23: Impact of Probiotic and Synbiotic Supplementation on the Gut microflora of Elderly Subjects Based on the Initial BP Values (Mean± SD, mg/dl)

Parameters	Group I			Group II			Group III		
	Normotensives N=13	Hypertensives N=7		Normotensives N=7	Hypertensives N=13		Normotensives N=9	Hypertensives N=17	
<i>E.coli</i>	Pre	6.33 ± 0.38	6.44 ± 1.01	6.65 ± 1.00	6.69 ± 0.74		6.64 ± 0.89	6.25 ± 0.61	
	Post	6.34 ± 0.49	6.53 ± 0.54	6.36 ± 1.18	6.10 ± 0.29		6.02 ± 0.77	5.61 ± 0.74	
	t value	0.040	0.339	0.687	1.632		2.026 *	2.908 *	
<i>Lactobacilli</i>	Pre	6.56 ± 0.31	6.50 ± 0.63	6.60 ± 0.36	6.34 ± 0.68		6.99 ± 0.55	6.86 ± 0.90	
	Post	6.47 ± 0.32	6.14 ± 1.14	6.99 ± 0.70	6.78 ± 0.51		7.40 ± 0.52	7.79 ± 0.35	
	t value	0.905	1.498	1.303	2.378 *		2.105 *	4.704 ***	
<i>Bifidobacteria</i>	Pre	8.05 ± 0.60	8.39 ± 0.86	8.05 ± 2.33	7.62 ± 1.47		7.83 ± 1.03	7.59 ± 1.07	
	Post	8.00 ± 0.61	8.05 ± 0.67	8.28 ± 1.05	8.14 ± 1.10		8.40 ± 0.68	8.93 ± 0.40	
	t value	0.375	0.733	0.493	1.174		2.066 *	3.458 **	

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant



counts of the *bifidobacteria* and lactic acid bacteria and decreasing trend in the *E.Coli* counts in older adults of probiotic and synbiotic ( $p<0.05$ ) fermented milk supplemented groups based on their initial BMI levels ( $BMI>23$ ). No significant changes were observed in the control group. BMI has an independent effect on the reduction in *E.coli* and establishment of beneficial microflora in the synbiotic fermented milk

#### **Effect of probiotic and synbiotic fermented milk supplementation on the gut microflora of older adult participants with hypertension as a complication**

As observed in Table 5.3.2.23, there was increase in mean log values of *Bifidobacteria* (6.82%) and *lactobacilli* (6.94%) in older adult participants having initial blood pressure levels above 140/90 compared to 5.90% and 2.85% increase in normotensive participants of probiotic fermented milk supplemented group ( $P<0.05$ ). There was significant increase in mean counts of lactic acid bacteria ( $p<0.001$ ), *bifidobacteria* ( $p<0.05$ ) and decrease in mean counts of *E.Coli* ( $p<0.05$ ) in participants of synbiotic fermented milk supplemented group based on initial blood pressure values (Table 5.3.2.23). However hypertension does not have any independent effect on the rise in beneficial microflora in the gut in both the supplemented groups.

#### **Effect of probiotic and synbiotic fermented milk supplementation on gut microflora of older adult participants in relation to both BMI and hypertension as a complication**

As observed in Table 5.3.2.24, there was increase in mean log values of *Bifidobacteria*, *lactobacilli* ( $p<0.05$ ) in older adult participants in probiotic fermented milk supplemented group with BMI and hypertension as a complication. There was significant increase in mean log counts of *bifidobacteria*, *lactic acid bacteria* and decrease in mean counts of *E.coli* was observed in participants with BMI and hypertension as a complication in synbiotic ( $P<0.001$ ) fermented milk supplemented group.

Subjects with two complications such as BMI and hypertension did not exhibit an independent effect on the establishment of *lactobacilli* and *E.coli* in both the groups whereas an independent effect was observed in the establishment of *bifidobacteria*.

Table 5.3.2.24: Impact of Probiotic and Synbiotic Supplementation on the Gut Microflora of Older Adult Participants Based with BMI >23 and Hypertension as a Complication (Mean± SD, mg/dl)

Parameters	Group I			Group II			Group III		
	Normotensives BMI<23 N=14	Hypertensives BMI ≥23 N=6		Normotensives BMI<23 N=14	Hypertensives BMI ≥23 N=6		Normotensives BMI<23 N=18	Hypertensives BMI ≥23 N=8	
<i>E.coli</i>	Pre	6.33 ± 0.42	6.44 ± 0.97	6.44 ± 0.042	6.76 ± 1.03		6.64 ± 1.12	6.45 ± 0.66	
	Post	6.31 ± 0.53	6.54 ± 0.52	6.16 ± 1.05	6.32 ± 0.95		6.31 ± 1.05	5.681 ± 0.54	
	t value	0.076	0.393	0.645	1.128		2.297 *	5.038 ***	
<i>Lactobacilli</i>	Pre	6.59 ± 0.32	6.42 ± 0.70	6.5 ± 0.5	6.27 ± 0.79		7.05 ± 0.7	6.58 ± 0.91	
	Post	6.53 ± 0.39	6.94 ± 1.11	6.84 ± 0.62	6.88 ± 0.48		7.56 ± 0.47	7.86 ± 0.33	
	t value	0.615	1.919	1.737	2.518		3.23 **	3.400 **	
<i>Bifidobacteria</i>	Pre	8.01 ± 0.60	8.55 ± 0.83	8.09 ± 1.79	7.45 ± 2.67		7.92 ± 1.09	7.68 ± 1.03	
	Post	8.08 ± 0.89	7.87 ± 0.52	8.07 ± 1.16	8.62 ± 0.64		8.44 ± 0.4	8.65 ± 0.73	
	t value	0.415	1.773	0.061	1.116		1.739	3.473 **	

Note

- \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant

### **Nutrient intake of the participants before and after supplementation of probiotic and synbiotic supplemented group:**

Table 5.3.2.25 shows the mean nutrient intake of institutionalized males and females before and after supplementation. The mean energy intake of the male participants before supplementation in probiotic group was 1571 kcal and in synbiotic group was 1559 kcal and after supplementation the mean energy intake was 1629 kcal and 1539 kcal in probiotics and synbiotic supplemented groups respectively. Whereas the mean energy intake of female participants before supplementation in probiotic group was 1250 kcal and synbiotic group was 1260 kcal and after supplementation was 1290 kcal and 1245 kcal /day respectively. There was a significant increase in energy and calcium intake in the probiotic supplemented group males and females. A decrease was observed for energy intake compared to a significant increase in calcium and fibre intake in synbiotic supplemented group.

### **Hematological indices of elderly subjects before and after supplementation of probiotic and synbiotic group:**

As seen in Table 5.3.2.26, there was 0.95% and 1.55% rise in hemoglobin levels in group II and group III ( $P<0.01$ ) after supplementation of probiotic and synbiotic fermented milk. There is a significant increase in the mean count of red blood cells from  $4.31\pm0.38$  to  $4.73\pm0.38$  and  $4.34\pm0.54$  to  $4.70\pm0.48$  g/dl; mean corpuscular volume from  $83.54\pm7.49$  to  $92.12\pm10.56$  fl and  $81.66\pm8.45$  to  $89.93\pm7.84$  fl; Mean Cell Hemoglobin from  $26.40\pm3.03$  to  $31.62\pm4.07$  pg and  $25.13\pm3.38$  to  $30.45\pm3.21$  pg in probiotic and synbiotic supplemented group respectively. There is a significant decrease in the mean count of lymphocytes from  $34.88\pm6$  % to  $29.62\pm7.01$ %; eosinophils from  $3.38\pm1.10$  to  $2.38\pm1.02$  % and monocytes from  $1.62\pm0.70$  to  $1.23\pm0.43$  % in synbiotic supplemented group compared to a non significant decrease in probiotic group. Association of hemoglobin and WBC levels to infectious morbidity before and after supplementation in elderly subjects revealed a significant positive correlation between infections like cold, spells of sneezing, pneumonia and fever with counts of white blood cells. A significant negative association was observed between non infectious morbidity such as fatigue and breathlessness with hemoglobin levels before and after supplementation of probiotic and synbiotic fermented milk (Table 5.3.2.27-5.3.2.28).

Table 5.3.2.25: Nutrient Intake of Male and Female Older Adults Before and After Supplementation of Probiotic and Synbiotic Fermented Milk (Mean± SD, mg/dl)

Parameters	Group I		Group II		Group III	
	Males (n=10)	Females (n=10)	Males (n=11)	Females (n=11)	Males (n=11)	Females (n=15)
Energy	Pre	1545 ± 215	1279 ± 165	1250 ± 128.22	1559 ± 124.34	1260 ± 110
	Post	1558 ± 214	1285 ± 148	1571 ± 141	1290 ± 96.18	1245 ± 112
	t value	0.431 ns	0.881 ns	2.621 **	1.999 **	3.982 **
Proteins	Pre	42.77 ± 6.38	34.13 ± 2.72	42.94 ± 4.85	32.94 ± 7.33	33.11 ± 4.63
	Post	42.93 ± 6.10	34.06 ± 5.34	47.18 ± 5.34	36.17 ± 6.97	33.02 ± 5.58
	t value	0.235 ns	0.045 ns	2.494	5.375 **	0.087 ns
Calcium	Pre	408.41 ± 56.84	373.69 ± 42.69	401.52 ± 69.42	386.52 ± 37.97	380.89 ± 45.85
	Post	403.54 ± 48.16	375.94 ± 47.36	486.43 ± 101.88	467.99 ± 71.82	466.61 ± 44.93
	t value	0.785 ns	0.191 ns	4.630 ***	5.068 **	6.000 ***
Fibre	Pre	5.83 ± 1.27	5.62 ± 1.38	5.89 ± 1.65	5.31 ± 1.11	5.89 ± 1.05
	Post	5.75 ± 1.32	5.64 ± 1.58	5.98 ± 1.09	5.85 ± 1.07	20.41 ± 1.93
	t value	0.272 ns	0.021 ns	0.252 ns	1.000 ns	25.84 ***
Fe	Pre	11.85 ± 2.61	10.74 ± 2.13	12.43 ± 1.79	11.03 ± 2.36	11.80 ± 1.13
	Post	11.93 ± 2.68	10.84 ± 2.65	12.45 ± 2.34	11.17 ± 2.26	11.65 ± 1.11
	t value	0.831 ns	0.324 ns	0.041	1.000 ns	1.629 ns
Vit.C	Pre	31.25 ± 12.45	31.04 ± 16.74	32.17 ± 12.60	32.72 ± 6.26	31.60 ± 11.59
	Post	31.80 ± 12.91	31.44 ± 18.12	32.59 ± 12.04	33.11 ± 4.98	31.96 ± 9.14
	t value	0.473 ns	0.164 ns	0.165	1.414 ns	0.273 ns
Fats	Pre	44.35 ± 5.19	45.16 ± 8.02	44.45 ± 6.92	42.36 ± 6.28	42.14 ± 4.71
	Post	44.49 ± 7.40	45.36 ± 6.69	46.71 ± 7.83	44.69 ± 6.31	42.06 ± 6.13
	t value	0.105 ns	0.176 ns	1.463	2.062	0.118 ns

- Note : \*Significant from the baseline value at p<0.05
- \*\* Significant from the baseline value at p<0.01
- NS - Non Significant

Table 5.3.2.26: Impact of Fermented Milk supplementation on hematology of older Adult Participants (Mean± SD, mg/dl)

Parameters		Group I (n=20)	Group II (n=20)	Group III (n=26)	Annova
Hb	Pre	11.50±1.81	12.45±1.31	11.88±1.48	
	Post	11.45±1.75	12.57±1.41	12.05±1.38	
	T value	0.486	2.164*	3.034**	3.215*
T-RBC	Pre	4.67±0.53	4.31±0.38	4.34±0.54	
	Post	4.29±0.46	4.73±0.38	4.70±0.48	
	T value	2.900**	5.080***	3.716**	3.585*
PCV	Pre	36.82±4.11	39.00±4.36	38.72±4.85	
	Post	35.81±4.74	39.31±4.62	42.70±20.95	
	T value	1.511	.430	1.017	1.434
MCV	Pre	83.98±11.53	83.54±7.49	81.66±8.45	
	Post	79.25±9.05	92.12±10.56	89.93±7.84	
	T value	1.709	4.623***	4.985***	.417
MCH	Pre	27.83±4.49	26.40±3.03	25.13±3.38	
	Post	24.70±4.14	31.62±4.07	30.45±3.21	
	T value	3.099**	7.072***	7.904***	3.084
TLC	Pre	7370.00±1207.48	7875.00±2102.84	8946.15±5160.63	
	Post	8175.00±2709.61	7195.00±1613.91	8110.00±4247.77	
	T value	1.240	2.622*	1.627	.613
Polymorphs	Pre	63.05±4.40	63.85±5.42	64.65±6.79	
	Post	65.60±7.27	61.75±5.60	58.96±13.31	
	T value	1.692	1.345	1.854	2.603
Lymphocytes	Pre	32.75±5.06	33.60±6.35	34.88±6.45	
	Post	30.05±6.91	31.55±4.89	29.62±7.01	
	T value	1.865	1.261	4.407***	.543
Eosinophils	Pre	2.75±1.37	3.60±0.94	3.38±1.10	
	Post	3.05±1.05	3.15±1.38	2.38±1.02	
	T value	.730	2.015	3.759**	3.074
Monocytes	Pre	1.30±0.47	1.80±0.52	1.62±0.70	
	Post	1.60±0.50	1.35±0.58	1.23±0.43	
	T value	2.349	2.651*	2.301*	3.088

Note : \*Significant from the baseline value at p<0.05 , \*\* Significant from the baseline value at p<0.01 , NS - Non Significant

**Table 5.3.2.27: Correlations of hemoglobin and WBC levels to infectious morbidity before and after supplementation in elderly subjects**

Infectious morbidity	HB	PHB	WBC	PWBC
Cold	.170	.127	.101	.235*
Spells of sneezing	.170	.038	.234*	.324**
Brochnits	-.022	-.006	-.083	.023
Pneumonia	-.130	-.020	.594***	.498***
Malaria	-.062	-.069	-.072	-.033
Fever	.053	.071	.258*	.330**
Infections	-.041	-.126	.061	.012

**Table 5.3.2.28: Correlations of hemoglobin and WBC levels to non-infectious morbidity before and after supplementation in elderly subjects**

Non-Infectious morbidity	HB	PHB	WBC	PWBC
diarrhea	.189	.139	-.128	.128
constipation	-.139	-.097	-.086	-.147
Acidity	.084	.150	.114	.114
Loss of appetite	.059	-.007	-.025	.089
Fatigue	-.296**	-.242*	-.087	-.126
Stomachache	-.148	-.231*	-.116	-.086
Ulcer	.013	.077	-.037	-.046
Asthma	-.062	-.069	-.072	-.033
Breathlessness	.003	-.032	-.038	.082

#### **Fermented milk consumption pattern of the older adult participants before and after supplementation of the fermented milk**

Majority of older adult participants (81.8%) preferred fermented milk in their diets.

When the diets of institutionalized older adult participants were supplemented with probiotic and synbiotic fermented milk it was found that almost all participants preferred fermented milk at the end of study period however the difference was not significant as seen in Table 5.3.2.29

From Table 5.3.2.30, it can be seen that those individuals who preferred fermented milk in their diets had occasional to once a week consumption of fermented milk. This may be due to financial constrains and the lack of supply of fermented milk on daily basis in the institution.

**Table 5.3.2.29: Preference for curd by elderly subjects before and after supplementation of curd**

Treatment	Percent subjects							
	Grp I (n=20)		Grp II (n=20)		Grp III (n=26)		Total (n=66)	
	YES	NO	YES	NO	YES	NO	YES	NO
Before supplementation	65(13)	35(7)	90(18)	10(2)	88.5(23)	11.5(3)	81.8(54)	18.2(12)
$\chi^2$ value	5.474 <sup>NS</sup>							
After supplementation	65(13)	35(7)	100(20)	0(0)	100(26)	0(0)	89.4(59)	10.6(7)
$\chi^2$ value	18.010***							

NS = Non Significant ; Values in Parentheses indicate no. of subjects

**Table 5.3.2.30: Frequency of curd consumption by elderly subjects**

Subjects	Consumption pattern			
	Daily	Alternate day	weekly	Occasionally
Total( n=66)	5(1)	0(0)	4.5(3)	93.9(62)
Group I ( n=20)	5(1)	0(0)	0(0)	95(19)
Group II ( n=20)	0(0)	0(0)	10(2)	90(18)
Group III ( n=26)	0(0)	0(0)	3.8(1)	96.2(25)

NS = Non Significant ; Values in Parentheses indicate no.of subjects

Table 5.3.2.31 and 5.3.2.32 depicts percent subjects avoiding fermented milk in diseased condition and the major reasons for avoiding fermented milk by older adult participants respectively. Cough and cold was the major reason for avoiding fermented milk followed by acidity and open wounds.

**Table 5.3.2.31: Percent elderly subjects avoiding curd in diseased conditions in all groups before and after supplementation**

Treatment	Percent subjects							
	Total (n=66)		Grp I (n=20)		Grp II(n=20)		Grp III(n=26)	
	YES	NO	YES	NO	YES	NO	YES	NO
Before supplementation	50(33)	50(33)	(13)	(7)	50(10)	50(10)	(10)	(16)
$\chi^2$ value	3.185 <sup>NS</sup>							
After supplementation	30.3(20)	69.7(46)	15.4(4)	84.6(22)	15.4(4)	84.6(22)	20(4)	80(16)
$\chi^2$ value	12.096 <sup>**</sup>							

NS = Non Significant ; Values in Parentheses indicate no.of subjects

**Table 5.3.2.32 : Major reasons for avoiding curd by elderly subjects**

Subjects	Reasons				
	Cough & Cold	Acidity	Asthma	Open Wound	Lactose intolerance
Group I ( n=13)	76.9(10)	23.1(3)	30.8(4)	100(13)	0(0)
Group II ( n=10)	90.0(9)	0(0)	0(0)	60(6)	10(1)
Group III ( n=10)	60(6)	0(0)	40(4)	80(8)	0(0)

NS = Non Significant ; Values in Parentheses indicate no.of subjects

### **Mental health assessment before and after supplementation of probiotic and synbiotic fermented milk**

Information on mental health status of the older adults was gathered using geriatric depression inventory scale. As seen in Table 5.3.2.33, 65% and 65.9% participants were normal at the baseline as compared to 80 % and 76.9% after supplementation in probiotic and synbiotic groups respectively.

### **Disease burden of the participants before and after supplementation of probiotic and synbiotic fermented milk:**

Using exhaustive checklist method information regarding disease profile was collected from the participants and parameters like major health problems pertaining to various health systems of the body and minor illnesses were determined before and after supplementation of probiotic and synbiotic fermented milk.



**Table 5.3.2.33: Percent Participants Reporting Depression Levels Before and After Supplementation Probiotic and Synbiotic Fermented Milk**

Depression levels	Group I (n=20)		Group II (n=20)		Group III (n=26)	
	N and % Pre	N and % Post	N and % Pre	N and % Post	N and % Pre-	N and % Post
Normal	7(35)	7(35)	13(65)	16(80)	17(65.4)	20(76.9)
Mild depression	11(55)	11(55)	6(30)	4(20)	3 (11.5)	1(3.8)
Moderate depression	1(5)	1(5)	1 (5)	0(0)	4(15.4)	4(15.4)
Severe Depression	1 (5)	1 (5)	0(0)	0(0)	2 (7.7)	1(3.8)

(Figures in parenthesis denote percentages)

It is clearly evident from Table 5.3.2.34, that 100% of the participants in both experimental groups had oral cavity problems. Supplementation resulted in decrease in the number of participants reporting gastrointestinal problems and respiratory problems, locomotor and problems of central nervous system with greater reduction seen in the synbiotic supplemented group than the probiotic supplemented group.

There was decrease in the episodes of acidity, constipation, indigestion, gas formation, and stomachaches being more prominent in group II than group III. After supplementation of probiotic and synbiotic fermented milk for a period of 6 weeks no participant reported problem of constipation whereas 65% to 15%; 76% to 26% participants reported reduction in acidity respectively (significant at  $p < 0.05$ ). There was reduction in participants reporting reversal from episodes of flatulence from 25 % to 0% and 26% to 10% in both the groups respectively. Regarding respiratory problems there was decrease in percent participants reporting occurrence of cold from 30% to 0%; 38% to 20%, spells of sneezing from 15% to 5%; 31% to 15%, breathlessness from 15% to 5%; 19% to 5% in probiotic and synbiotic fermented milk supplemented groups.

**Table 5.3.2.34: Percent Participants Reporting Disease Burden Before and After Supplementation Probiotic and Synbiotic Fermented Milk**

Disease burden	Group I (n=20)		Group II (n=20)		Group III (n=26)	
	Pre N and %	Post N and %	Pre N and %	Post N and %	Pre N and %	Post N and %
Oral cavity problems	20 (100)	20 (100)	18(90)	18 (90)	20(100)	20 (100)
Gastro intestinal problems	17 (85)	18(90)	15 (75)	14(70)	22(84.6)	8 (40)
Respiratory problems	13(65)	15(75)	8(40)	5(25)	16 (61.5)	7 (35)
Cardiovascular problems	12(60)	12(60)	11 (55)	11(55)	15(57.7)	15(57.7)
Locomotor problems	17 (85)	17 (85)	9(45)	8 (40)	16 (61.5)	12(60)
Neurological problems	10 (50)	15(75)	8 (40)	2(10)	13 (50)	9(45)
Miscellaneous problem	11 (55)	12(60)	10 (50)	2 (10)	12 (46.2)	7(35)

(Figures in parenthesis denote percentages)

## DISCUSSION

This study demonstrated that supplementation of probiotic and synbiotic fermented milk resulted in significant reduction in biochemical parameters such as TC, FBS whereas a significant reduction in TG and LDL levels was seen only in synbiotic fermented milk supplemented participants. Consistent with these improvements there was significant increase in mean log counts of beneficial microorganisms in group II and group III participants.

Several studies have shown that when only probiotics were administered in daily diets for 4 to 8 weeks, reducing trend in serum cholesterol were observed (Anderson et al 1999, Bukowska et al, Agerholm Larsen et al 2000, Agerbaek et al 1995, Richelsen et al 1996, Bertolami et al 1999, Andersson et al 1995, St onge et al 2000). However meta analysis shows an inconsistent trend in the LDL-C affected by intake of probiotics (de Roos et al 1999, Massey LK 1984, Lin et al 1989, Schaafsma et al 1998, Bertolami et al 1999). In the present study even six weeks of probiotic fermented milk did not bring about a significant change in LDL-C and HDL-C values whereas the synbiotic fermented milk brought about a significant reduction was also observed in LDL-C, TG and LDL/HDL ratio. This indicates there could be an independent effect of inulin which was added in the diets of group III participants. Similar results have been reported by many investigators (Yamashita et al 1984, Brighenti et al 1999, Canzi et al 2000, Jackson et al 1999, Kawase et al 2000, Hidaka et al 1986).

With regards to hypocholesterolemic effect of prebiotics, several mechanisms have been proposed wherein gut fermentation of inulin leads to production of SCFA mostly acetate, propionate and butyrate which are almost completely absorbed along the digestive tract and reaches the portal vein. When acetate enters the hepatocyte it is activated mainly by cytosolic acetyl coenzyme A synthetase 2 and then enters cholesterologenesis and lipogenesis pathway. Conversely, propionate is a competitive inhibitor of the protein controlling the entrance of acetate in to the liver cells. The production of high concentration of propionate through fermentation has been proposed as a mechanism to explain reduction in serum and hepatic cholesterol (Levart et al 1994, Demigne et al 1995, Jenkins et al 1998, Chang and Lai 2000,

Lopez et al 2001, Delzenne and Kok 2001). This explains a possibility of greater reduction of cholesterol in the synbiotic supplemented group in the present study. In a randomized crossover trial in participants with modest hyperlipidemia, showed significantly lower total and LDL concentrations during the inulin (Raftiline) phase was seen compared with the placebo phase, but the authors reported no effects on HDL cholesterol or serum triglyceride concentrations (Davidson et al 1998). A number of factors may have contributed to the positive findings for effects of inulin or synbiotic supplemented group on TC levels in this study. The reduction in the lipid parameters as a result of increased beneficial microorganisms is seen more in male participants compared to female older adults. Such findings have been reported when other functional foods were supplemented for improving the lipemic and glycemic profile of hypercholesterolemic subjects.

A higher reduction in FBS in synbiotic group compared to probiotic group may be attributed to the additional effect of inulin as a prebiotic. A recent animal study has shown attenuation of both insulin and glucose concentrations following long-term neosugar (OFS) feeding in rats. The effects were attributed to the actions of OFS on secretion of the gut hormones glucose-dependent insulintropic polypeptide and glucagon-like peptide 1 (Hata et al 1996). A study carried out in non-insulin-dependent diabetic participants (Yamashita et al 1984) demonstrated a significant lowering of blood glucose levels with OFS, however, very little is known about the effects of fructans, especially inulin, in normal healthy adults.

As a result of intervention, a reduction in the mean BMI was seen in group II and group III participants, with a significant reduction in the synbiotic fermented milk supplemented group. Past studies involving animal models mainly rats have shown that ingestion of inulin-type fructans could regulate body weight via the promotion of endogenous glucagon-like peptide-1 (GLP-1) in the gut. GLP-1 is a key hormone released from enteroendocrine-L cells in response to nutrient ingestion and is the key modulator of food intake by promoting satiety (Delzenne et al 2007). This consequently reduces the intake of food which leads to a decrease in body weight and BMI. Most of the studies involving the promotion of satiety by fructans via increased

production of GLP-1 were performed in animal models and little information is available on human participants. However, this remains a possible mechanism of fructans in promoting satiety. Piche et al (2003) had previously demonstrated that ingestion of 6.6 g of oligofructose three times a day for seven days increased the release GLP-1 in nine participants. Studies have shown that ingestion of 16g/day of oligofructose for two weeks decreased the total energy intake by 5% and increased satiety following breakfast and dinner in human participants (Piche et al 2003).

A shift in 20% of synbiotic fermented milk supplemented participants was observed for hypertension from stage I hypertension to pre hypertension. Preliminary evidence indicates that synbiotic or their fermented products may also play a role in blood pressure control, with animal and clinical studies documenting antihypertensive effects of synbiotic consumption (Namakmura et al 1995). Various mechanisms have been postulated to explain the ability of prebiotics to reduce the risk of hypertension. One of the possible mechanisms is via the lowering of blood lipid and cholesterol. Previous studies have demonstrated that intensive reduction of cholesterol may be beneficial in the treatment of patients with isolated systolic hypertension (Ferrier et al 2002). Gallaher et al. 2002 proposed that fiber could bind with bile acids and reduce solubilisation of cholesterol leading to a cholesterol lowering effect. The reduction of total cholesterol regulates the receptors of low density lipoprotein (LDL) and thus increases the clearance of LDL cholesterol (Aller et al 2004). This overall cholesterol lowering effect could reduce the stiffness of large arteries and thus could potentially reduce blood pressure (Ferrier et al 2002). In another study, Lairon et al. (2005) suggested that the reduction of obesity upon consumption of prebiotics such as fiber could prevent the elevation of blood pressure. Generally, overweight and obese individuals are at a higher risk of hypertension than those with a healthy body mass index (BMI) (Rahmouni et al 2005).

Obesity has also been commonly associated with the over activity of the sympathetic nervous system where long-term sympatho-activation could raise arterial pressure by causing peripheral vasoconstriction and by increasing renal tubular sodium reabsorption (Rahmouni et al 2005). Therefore, decreasing obesity due to

consumption of synbiotic fermented milk may have helped to reduce the risk of hypertension. Additionally, inulin has also been reported to reduce the risk of hypertension by improving the absorption of mineral such as calcium in the gastrointestinal tract (Streppel et al 2005). Past studies have shown promising evidence on the correlation of dietary calcium and hypertension. Allender et al (1996) conducted a meta analysis of randomized clinical trials on the correlation of dietary calcium and blood pressure and had found that median intake of calcium by 1 g/day could significantly decrease the systolic blood pressure by 1 mm Hg to 2 mm Hg. Diets high in calcium have been found to reduce peripheral vascular resistance and blood pressure leading to a reduced risk of hypertension (Zernel et al 2005). Increased calcium intake in the present study due to intake of fermented milk could have served as an additional factor for reducing hypertension.

In this study, the synbiotics and probiotics have shown to modify the composition of intestinal *bifidobacterial* populations in the healthy older adults, demonstrating that it has potential to be of particular benefit to individuals with more unbalanced gut ecosystems.

The fecal *bifidobacterial* levels observed in the present study prior to supplementation were within the range of those reported previously in older adults (Mutai et al 1987, Mitukosa T 1990) and supplementation of probiotic and synbiotic fermented milk significantly increased beneficial microorganisms and decreased the log counts of *E.coli*. Kleesen et al in 1997 investigated the relative impact of lactose and inulin on the gut microbiota composition of 25 constipated older adults in a parallel double-blinded feeding study. The authors reported that numbers of fecal *bifidobacteria* increased significantly in the inulin-fed participant along with a concomitant reduction in the numbers of *enterococci* and the *enterobacteria*. Bartosch et al (2005) examined the impact of a synbiotic containing *Bifidobacterium bifidum* and *B. lactis* and the inulin-type fructan-based prebiotic, Synergy on the gut microbiota composition of 18 older adults in a placebo-controlled, double blind, parallel feeding study and observed significantly higher numbers of *bifidobacteria* and *lactobacilli* in the stool samples of the synbiotic-fed participants compared with the placebo group. The EU-funded project Crownalife has investigated the impact of inulin-type fructans (in a synbiotic

preparation) on the composition of the older adults gut microbiota and a similar increase in fecal *bifidobacteria* was observed.

Intestinal propagation of *bifidobacteria* is not only important for preventing bacterial diseases such as *E. coli* scours in infants but for improving the health of adults, particularly the older adults (Bengmark S 1998). The possible mechanism may be the production of acetic acid and lactic acid produced from break down of prebiotics lowering the intestinal pH and thereby inhibiting excessive growth of unfavorable bacteria such as *Escherichia coli* and *Clostridium perfringens*.

The high reduction in disease burden, especially with respect to gastrointestinal disease was observed in the probiotic fermented milk supplemented participants. Normalization of stool frequency and reduction in flatulence is reported by several investigators in subjects supplemented with probiotic organisms (Niedzielin et al 2000). Higher reduction in respiratory disorders in group II older adults may be attributed to improved immune functions after dietary probiotic supplementation (Gill et al 2004).