DEVELOPMENT, QUALITY ASSESSMENT AND CLINICAL EFFICACY OF 'FUNCTIONAL FOOD SUPPLEMENT' (FFS) FOR LIFE STYLE DISEASE MANAGEMENT

by

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(2011-24-101)

THESIS

Submitted in partial fulfillment of the requirement for the degree of

DOCTORATE OF PHILOSOPHY IN HOME SCIENCE

(Food Science and Nutrition)

Faculty of Agriculture

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KERALA, INDIA

2014

DECLARATION

I, hereby declare that this thesis entitled **"Development, quality assessment and clinical efficacy of 'Functional Food Supplement' (FFS) for life style disease management"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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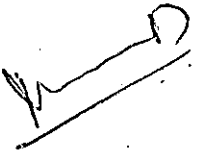

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ACKNOWLEDGEMENT

"When you want something, all the universe conspires in helping you to achieve it."

— Paulo Coelho, the Alchemist

Though only my name appears on the cover of this dissertation, a great many people have contributed to its production. I owe my gratitude to all those people who have made this dissertation possible and because of whom my graduate experience has been one that I will cherish forever.

Thank God for the wisdom and perseverance that he has been bestowed upon me during this research project, and indeed, throughout my life.

My deepest gratitude is to my major advisor, Dr. Mary Ukkuru, P, Professor. I have been amazingly fortunate to have an advisor who gave me the freedom to explore on my own and at the same time the guidance to recover when my steps faltered. Madam taught me how to question thoughts and express ideas. Her patience and support helped me overcome many crisis situations and finish this dissertation. Your advice on both research as well as on my career have been priceless. I hope that one day I would become as good an advisor to my students as mam has been to me.

My utmost and sincere thanks to Dr. P. V. Nandini, Professor and Head for her whole hearted co-operation, critical scrutiny of manuscript and help during the course of the study and period of investigation.

I am grateful to Dr. C. Nirmala, Assoc. Prof., for her encouragement and practical advice. I am also thankful to

her for reading my reports; commenting on my views and helping me understand and enrich my ideas.

I accost my sincere thanks to other committee members, Dr. Sudharmai Devi. C. R, Professor for her immense guidance and help in the field of analytical works; Dr. Meena Kumari. K. S, Professor and Head for her immense help, constructive suggestions and timely support; Dr. Vijaya Raghava Kumar, Professor and Head for his keen interest, constructive suggestions and co- operation. . I also thank them all for letting my defense be an enjoyable moment, and for the brilliant comments and suggestions.

My heartfelt thanks to Dr. Suma Diwakar, Dr. Prasanna Kumari, Dr. Soffi Cheriyan and Dr. Rari John for their encouragement, insightful comments, and hard questions rendered throughout my research endeavour.

My deep sense of gratitude goes to Mr. C. E. Ajith Kumar, programmer, Department of Agriculture Extension for executing the statistical analysis of my research data.

I also extend my acknowledgement to Manju Chechi and Shibu Chettan of the Department of Home Science for the help rendered to me during the course of study.

I would like to acknowledge Mr. Koteswara Reddy, Ph.D Scholar, Pondicherry University; Dr. Ashish Kumar Singh, Senior Scientist, Dairy Technology Division National Dairy Research Institute Karnal and Mr. Tomy, M. J, Research Associate, Kannur Unniversity without whose support and help my analytical works would not be complete.

I am also thankful to all the respondents of my research for giving me their full support, cooperation and confidence in proceeding with the investigation.

Many friends (Krishnendu, Krishnasree, Stephy, Saima, Midhila, Anju, Sreedevi, Poornima and my other juniors) have helped me stay sane through these difficult years. Their support and care helped me overcome setbacks and stay focused on my study. I greatly value their friendship and I deeply appreciate their belief in me.

Most importantly, none of this would have been possible without the love and patience of my family. Words cannot express how grateful I am to my mother, father, sister, mother-in law and father-in-law, for all of the sacrifices that you've made on my behalf. Your prayer for me was what sustained me thus far. At the end I would like express appreciation to my beloved husband Mr. Sajith Kumar, Cameraman, Asianet who spent sleepless nights and was always my support in the moments when there was no one to answer my queries.

Finally, I appreciate the financial support from UGC and KAU that has funded the research discussed in this thesis.

At last I thank all those who extended help and support to me during the course of my work.

Krishnaja. U

DEDICATED TO MY FAMILY

CONTENTS

S. No	Contents	Page No.
1	INTRODUCTION	01
2	REVIEW OF LITERATURE	05
3	MATERIALS AND METHODS	29
4	RESULTS	65
5	DISCUSSION	170
6	SUMMARY	229
7	REFERENCE	244
	ABSTRACT	297
	APPENDIX	300

LIST OF TABLES

Table No	Title	Page No.
1	Ratio of the constituents for various combinations	33
2	Fermentation techniques involved in preparation of FFS II	36
3	Analytical procedures for macronutrients	38
4	Analytical procedures for minerals	41
5	Analytical procedures for vitamins	42
6	Analytical procedures for phytochemicals	43
7	Bulk density, Dispersibilty & Rehydration ratio of FFS I	67
8	Gelatinization time and Temperature of FFS I	68
9	Processing loss and Yield ratio of FFS I	68
10	Sensory Evaluation Scores of FFS I (POWDER)	70
11	Sensory Evaluation Scores of Cooked FFS I (PORRIDGE)	72
12	Hedonic Scale Ratings of FFS I (PORRIDGE)	73
13	Bulk density, Dispersibilty & Rehydration ratio of FFS II	75
14	Gelatinization time and Temperature of FFS II	76
15	Processing loss and Yield ratio of FFS II	77
16	Sensory Evaluation Scores of FFS I (POWDER)	78
17	Sensory Evaluation Scores of Cooked FFS II (PORRIDGE)	79
18	Hedonic Scale Ratings of FFS II (PORRIDGE)	80
19	Rank table of functional Properties of FFS II	81
20	Rank table of sensory evaluation of cooked FFS II	82
21	Rank table of sensory evaluation of FFS II (POWDER)	83
22	Proximate compositions of FFS I & II	87

23	Vitamin compositions of FFS I & II	91
24	Mineral compositions of FFS I & II	92
25	Phytochemical contents of FFS I & II	94
26	Amino acid profile of FFS I & II	97
27	Essential Amino acid (EAA) score of FFS I & II in comparison with reference protein (egg)	99
28	Total Antioxidant Capacity (TAC) (µg / g) of FFS I & II	103
29	DPPH free radical scavenging activity of FFS I & II in comparison with standard	105
30	Observed and expected DPPH scavenging activity of FFS I & II	106
31	Nutritional and antioxidant quality indices of FFS I & II	108
32	Functional properties of FFS I & II	109
33	Pasting properties of FFS I & II	111
34	Textural properties of FFS I & II	113
35	Colour attributes of FFS I & II	115
36	Particle size of FFS I	117
37	Particle size of FFS II	119
38	Moisture (%) of stored FFS I & II	123
39	Peroxide value (meq/kg) of stored FFS I & II	124
40	Microbial loads of stored FFS I (cfu/g)	126
41	Cost of production of FFS I	128
42	Sensory evaluation scores of recipes standardized from FFS I & II	130
43	Nutrient content of one portion (20 g) of FFS I & II	132
44	Socio-economic profile of the respondents	134
45	Food use frequency table of the respondents	137

46	Anthropometric measurements of the respondents	139
47	Fasting Blood Sugar (mg/dl) of subjects supplemented with FFS I & II	143
48	Mean FBS (mg/dl) values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	144
49	Post Prandial Blood Sugar (PPBS) (mg/dl) of subjects supplemented with FFS I & II	145
50	Mean PPBS (mg/dl) values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	146
51	Mean rate of change (%) FBS values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	147
52	Mean rate of change (%) PPBS values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	147
53	Total Cholesterol (TC) levels (mg/dl) of subjects supplemented with FFS I & II	148
54	Mean TC values (mg/dl) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	149
55	Low density Lipoprotein (LDL) levels (mg/dl) of subjects supplemented with FFS I & II	150
56	Mean LDL values (mg/dl) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	151
57	Very Low density Lipoprotein (VLDL) levels (mg/dl) of subjects supplemented with FFS I & II	152
58	VLDL values (mg/dl) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	153
59	Triglycerides (TG) levels (mg/dl) of subjects supplemented with FFS I & II	154
60	Mean TG values (mg/dl) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	155

61	High Density Lipoprotein (HDL) levels (mg/dl) of subjects supplemented with FFS I & II	156
62	Mean HDL values (mg/dl) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	157
63	Mean rate of change (%) total cholesterol values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	158
64	Mean rate of change (%) LDL values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	158
65	Mean rate of change (%) VLDL values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	159
66	Mean rate of change (%) TG values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	159
67	Mean rate of change (%) HDL values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	160
68	Systolic /diastolic blood pressure levels (mm Hg) of subjects supplemented with FFS I & II	161
69	Mean Systolic blood pressure values (mm Hg) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	162
70	Mean diastolic blood pressure values (mm Hg) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	163
71	Mean rate of change (%) systolic blood pressure values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	164
72	Mean rate of change (%) diastolic blood pressure values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90	165
73	Mean Glucose Tolerance Test (GTT) values of the respondents	167

LIST OF FIGURES

Fig No.	Title	Between pages
1	Nutritional and antioxidant indices of FFS I & II	108 - 109
2	EAA & DPPH Indices of FFS I & II	108 - 109
3	Bulk density (g/cm^3) of FFS I	172 - 173
4	Processing loss & Yield ratio of FFS I	172 - 173
5	Sensory evaluation and Hedonic scale ratings of cooked FFS I	173 - 174
6	Rank Charts of Functional Properties of FFS II	173 - 174
7	Rank Charts of Sensory Evaluation of Cooked FFS II	173 - 174
8	Energy (kcal) content of FFS I & II	183 - 184
9	Carbohydrates & protein content of FFS I & II	183 - 184
10	Fiber, fat, ash & β-glucan content of FFS I & II	183 - 184
11	β-carotene content of FFS I & II	184 – 185
12	Vitamin E content of FFS I & II	184 - 185
13	B complex vitamins of FFS I & II	184 - 185
14	Macro minerals of FFS I & II	186 - 187
15	Micro minerals of FFS I & II	186 – 187
16	Trace minerals of FFS I & II	186 – 187
17	Amino acid profile of FFS I & II	192 – 193
18	Dose inhibition curve and IC_{50} values FFS I by fitting regression equation	197 – 198
19	Dose inhibition curve and IC_{50} values FFS II by fitting regression equation	197 – 198
20	Dose inhibition curve and IC_{50} values standard (Ascorbic acid) by fitting regression equation	197 – 198

21	Comparison of dose inhibition curve and IC_{50} values FFS I & II with standard by fitting regression equation	197 - 198
22	Change in viscosity of FFS I & II depending on time and temperature	206 – 207
23	Change in moisture (%) of FFS I & II during storage study	215 – 216
24	Overall acceptability scores of recipes standardized from FFS I & II	215 – 216
25	Effect of supplementation of FFS I & II on mean FBS of subjects	222- 223
26	Effect of supplementation of FFS I & II on mean PPBS of subjects	222 – 223
27	Effect of supplementation of FFS I & II on mean Total Cholesterol of subjects	224 – 225
28	Effect of supplementation of FFS I & II on mean HDL of subjects	224 – 225
29	Comparison between mean Glucose Tolerance Test (GTT) of FFS I, II & Glucose (Standard)	227 – 228
30	Glycemic Index (GI) of FFS I & II	227 – 228
31	Glycemic load of FFS I & II	227 – 228

LIST OF FLOWCHARTS

Flowchart No.	Title	Page No.
1	Processing technique I	34
2	Processing technique II	35

LIST OF PLATES

Plate No.	Title	Between pages
1	Constituents of FFS	29 - 30
2	Processing of constituents for the development of FFS	30 - 31
3	Steps in standardizing FFS I	37 - 38
4	Steps in standardizing FFS II	37 - 38
5	Recipes standardized from FFS I	129 - 130
6	Recipes standardized from FFS II	129 - 130
7	Respondents of supplementation study	142 - 143

LIST OF APPENDICES

S. No	Title	Appendix No.
1	Score card for organoleptic qualities of recipes from FFS I & II	I
2	Questionnaire to elicit information on dietary pattern, lifestyle, health and disease of the subjects selected for the study	II
3	Glucose Tolerance Test (GTT)	III
4	Dietary habits of the respondents	IV
5	Food frequency table of the respondents	V
6	Health habits and physical exercises of the respondents	VI

LIST OF ABBREVIATIONS

FFS	Functional Food Supplement
LSD	Lifestyle diseases
CVD	Cardio Vascular Diseases
NCD	Non Communicable Diseases
CHD	Coronary Heart Disease
MS	Metabolic Syndrome
cfu/g	colony forming unit per gram
et al	and others
Fig	figure
g	gram
g/100g	gram per 100 gram
meq/g	milli equivalents per gram
mg	milligram
min	minute
RVU	Relative Value Unit
BP	Blood Pressure
GTT	Glucose Tolerance Test
GI	Glycemic Index
GL	Glycemic Load

Introduction

1. INTRODUCTION

Diabetes, cardiovascular diseases, obesity, and other lifestyle diseases are affecting the health and well-being of hundreds of millions of people not only in the developed world but also the millions of people in developing countries (IOA, 2008).

Lifestyle diseases (LSD) are a group of diseases that share similar risk factors because of exposure to unhealthy diets, smoking, lack of exercise, and stress, culminating in high mortality rates among populations (WHO, 2010).

As of 2012, about half of all adult population have one or more chronic health conditions such as heart disease, stroke, cancer, diabetes, obesity, and arthritis. One of four adults has two or more chronic health conditions (Ward *et al.,* 2014). Seven of the top 10 causes of death in 2010 were chronic diseases (Centres for Disease Control and Prevention, 2013a). Two of these chronic diseases—heart disease and cancer together accounted for nearly 48 per cent of all deaths worldwide. Diabetes is the leading cause of kidney failure, lower limb amputations, and new cases of blindness among adults (Centres for Disease Control and Prevention, 2013b).

Misra *et al.* (2011) stated that, Indians are prone to lifestyle diseases at a higher pace when compared to the Westerners. Thankappan *et al.* (2010) observed that Kerala is emerging as the 'capital' of lifestyle diseases of India with the prevalence of hypertension, diabetes, obesity and other risk factors for heart disease reaching levels comparable to those in Western Countries.

India has already passed the early stages of a chronic disease and injury epidemic; in view of the implications for future disease burden and the demographic transition that is in progress in India, the rate at which effective prevention and control is implemented should be substantially increased (Patel *et al.,* 2011).

Rapid economic growth, globalization, urbanization, rural–urban migration and aggressive marketing are all leading to a dramatic shift in the diet and living behaviours of individuals, families and communities (Hawkes, 2005 and Reddy *et al.*, 2006).

The evolution of the human diet over the past 10,000 years to our current modern pattern of intake has resulted in profound changes in dietary behaviour. Shifts have occurred from diets rich in fruits and vegetables, lean meats, and seafood to processed foods high in sodium and transfat and low in fiber. These dietary changes along with sedentary lifestyle have resulted in an increase in obesity and chronic disease, including cardiovascular disease (CVD), diabetes, and cancer (Stephanie *et al.*, 2009).

Medical and scientific evidence documents the importance of proper diet and benefits of nutritional supplements for health maintenance and prevention of lifestyle diseases (Wagner, 2002). Many studies have investigated the health benefits of various functional food ingredients, including omega-3 fatty acids, polyphenols, fiber, and plant sterols. These bioactive compounds may help to prevent and reduce incidence of chronic diseases, which in turn could lead to health cost savings (Shahidi, 2010).

The concept of functional food use is increasingly felt, and has become the popular choice among consumers as they are less expensive, beneficial and a more natural alternative (Rao *et al.*, 2011). An ever-increasing number of consumers are concerned with maintaining the quality of life by using the best effective alternative natural products like functional foods (Ernst and Young; FICCI, 2009).

The tenet "Let food be thy medicine and medicine be thy food," espoused by Hippocrates nearly 2,500 years ago, has received renewed interest at present. There has been an explosion of consumer interest in the health enhancing role of specific

foods, so-called functional foods (Hasler and Clare, 2005). Clearly, all foods are functional, as they provide taste, aroma, or nutritive value.

During the past decade, functional foods and nutraceuticals have emerged as a major consumer-driven trend, serving the desire of aging populations to exercise greater control over health, delay aging, prevent disease and enhance well-being and performance. This trend is expected to continue, and the need for an interest in scientific information on all aspects of functional foods will continue to be vital to the advancement of this emerging sector (Howard and Kritchevsky, 2007).

The science of functional foods and nutraceuticals is at the confluence of two major factors in our society — food and health. T he link between diet and disease has now been quite widely accepted, not only at the institutional level by organizations but also by a large portion of the populace (Shi *et al.,* 2002). In recent years, there appears to have been a growing desire by individuals to play a greater role in their own health and well-being rather than rely strictly on conventional medical practice (Kalra, 2003). As a result, there has been a burgeoning market for a wide range of dietary supplements and nutraceutical products that are perceived by the consuming public to be beneficial in the maintenance of their health and in the prevention of diseases (Trueman, 2010).

Functional foods and nutraceuticals provide an opportunity to improve the health, reduce health care costs and support economic development in rural communities (Dilip, 2010). According to market statistics, the global functional foods and nutraceuticals market is growing at a rate that is outpacing the traditional processed food market (Schieber and Lopes-Lutz, 2011).

In 2013, "ingredients added for special health benefits" and "higher in nutrients", were the top two attributes that made a food product good for health and wellness, according to one research group (Hartman, 2013). The combination of

nutritional benefits, indulgence, and culinary/gourmet excitement is the key to consumers' decisions to try new healthy food products (IRI, 2014).

Developing a processed product with good sensory qualities and prolonged shelf life would bring benefit to consumers in preparation as well as health promotion. This will also allow exploring more marketing niches in the countries (Kordylas, 2000).

There is a need to make new products from indigenous raw materials having nutritional value which open up new channels for domestic and export market. Hence research in this field should be focused to develop nutrient packed food supplements from locally available resources (Pai, 2007).

There is a hoard of food resources in our state rich in bioactive compounds that accord valuable health benefits and therapeutic properties. However, incomplete knowledge about the importance of these natural ingredients and its combination in the management of lifestyle diseases has lead to the improper utilization of these materials in the daily diet of the population.

Thus, keeping in mind the above factors that demand for the use of functional foods from natural ingredients, the present study entitled "Development, quality assessment and clinical efficacy of 'Functional Food Supplement' (FFS) for life style disease management" was carried out with the following objectives:

- To develop a 'Functional Food Supplement' (FFS), from natural resources for the therapeutic management of life style diseases.
- Quality evaluation of the developed product (FFS).
- Validation of its health benefits and therapeutic properties through clinical trials thereby promoting it for commercialization

Review of Literature

2. REVIEW OF LITERATURE

The literature reviewed on the present study entitled "Development, quality assessment and clinical efficacy of 'Functional Food Supplement' (FFS) for life style disease management" is presented below:

2.1. Lifestyle diseases
2.2. Definition and significance of functional foods
2.3. Role of foods rich in bioactive compounds for the management of lifestyle diseases
2.4. Market trends in functional foods
2.5. Opportunities and challenges in functional foods

2.1. LIFESTYLE DISEASES

People are predisposed to various diseases based on their way of living and occupational habits that are preventable, and can be lowered with changes in diet, lifestyle, and environment (Lichtenstein *et al.*, 2000).

Pollen and Michael (2001) viewed that lifestyle diseases are diseases that appear to increase in frequency as countries become more industrialized and people live longer. They are Alzheimer's disease, Atherosclerosis, asthma, cancer, chronic liver disease, Chronic Obstructive Pulmonary Disease, Type 2 diabetes, Heart disease, metabolic syndrome, Crohn's disease nephritis or chronic renal failure, osteoporosis, stroke, depression and obesity.

Sandvik *et al.* (2000) reported that lifestyle diseases have their onset later in an individual's life and need a longer lifespan in order to become the cause of death. Riebel (2003) opined that lifestyle diseases have peculiar "follow others" pattern which becomes more complicated due to ignorance, especially in families where

elders have addiction and unhealthy habits. The youngsters growing up in such circumstances have "role model phenomenon" and adopt the unhealthy lifestyle making them prone to a multitude of lifestyle diseases.

2.1.1. Prevalence and Causes of Lifestyle Diseases

Lichtenstein *et al.* (2000) opined that lifestyle diseases characterize those diseases whose occurrence is primarily based on daily habits of people and are a result of an inappropriate relationship of people with their environment.

Qiao (2003) and Teoh *et al.* (2007) viewed that Indians are succumbing to diabetes, high blood pressure and heart attacks 5–10 years earlier than their Western counterparts. Scientific data also show that socio-economically disadvantaged sections of the population are now the dominant victims of CVD and its risk factors (Rastogi *et al.*, 2004).

Chattopadhayay and Agnihotram (2005) reported that although Kerala has the highest (82.8 per cent) prevalence rate of coronary artery disease in patients above 60 years, states in North West India like Punjab have even higher prevalence in younger age group.

Thankappan *et al.* (2010) observed that the overall prevalence of diabetes in Kerala is about 16.2 per cent — 50 per cent higher than in the US. High blood pressure is present in 32 per cent. Close to 57 per cent people studied had abnormal levels of cholesterol, while 39.5 per cent had low HDL cholesterol — again, comparable to the rates seen in America.

Kearney *et al.* (2005); Reddy *et al.* (2006) and Mohan *et al.* (2007) estimated that by 2020, CVD will be the largest cause of disability and death in India. The country already has more than 40.9 million people with diabetes and more than 118

million people with hypertension, which is expected to increase to 69.9 and 213 million respectively, by 2025 unless urgent preventive steps are taken.

WHO (2005) estimated that India lost 9 billion dollars in national income from premature deaths due to heart disease, stroke and diabetes in 2005, and is likely to lose 237 billion dollars by 2015. There is also preliminary evidence that the burden of CVD in rural areas is increasing (Joshi *et al.*, 2006).

Gupta (2008) mentioned that epidemiological studies performed in last 50 years have revealed that there is a significant rise in prevalence of coronary artery disease in urban as well as in rural Indian population and Coronary Artery Disease (CAD) has been predicted to assume epidemic proportions in India by the year 2015.

WHO (2011) stated that cardiovascular diseases rank first among NCDs and contribute to 48 per cent of deaths throughout the world. Indian Heart Watch study released in connection with World Congress of Cardiology stated that among Indians, high cholesterol was found in one – quarter of all men and women and diabetes was reported in 34 per cent men and 37 per cent women. Thus India has the dubious distinction of being as the coronary and diabetes capital of the world (Rajeev *et al.*, 2012).

Diabetes is the third most common and significant chronic endocrine disorder affecting millions of people worldwide (Collene *et al.*, 2005). The occurrence of diabetes has rapidly increased, in both developed and developing countries, due to increased life span, obesity and faulty dietary habits. India is one of the leading countries with high number of people with diabetes mellitus and it is estimated that around 57 million peoples will be suffering from diabetes mellitus by the year 2025 (Aravind *et al.*, 2005).

Anjana *et al.* (2011) opined that diabetes prevalence in India indicates that the epidemic is progressing rapidly across the nation, reaching a total of 62.4 million and 77.2 million reaching the threshold in 2011.

The Assocham report (2009) stated that around 52 per cent of corporate employees are afflicted to lifestyle diseases especially of eating habits while 24 per cent suffer from chronic diseases whereas 18 per cent have acute ailment.

Rawat (2009) said that 26 per cent of corporate employees suffer from obesity and 18 per cent from depression which is part of life style disease. High blood pressure and diabetes are the third and fourth largest disease with a share of 12 per cent and 10 per cent respectively as suffered among the corporate employees.

The report by (Sabu *et al.*, 2009) says that the increasingly demanding schedules and high stress levels are leading to sleep disorders in individual lives. Loss of sleep has wide ranging effects including daytime fatigue, physical discomfort, psychological stress, performance deterioration, low pain threshold and increased absenteeism.

Misra *et al.* (2011) studied that Delhi ranked first afflicted to life-style diseases followed by Mumbai (second), Ahemdabad (third) Chandigarh (fourth), Hyderabad (fifth), Pune (sixth) and Chennai (seventh).

Vimala *et al.* (2009) reported that in a study conducted in Trivandrum city of Kerala out of a total of 482 individuals (212 males and 270 females) only 11.4 per cent of study participants had blood pressure (BP) in the normal range and all others had either hypertension or prehypertension.

Vasan *et al.* (2011) studied that individuals with pre hypertension have two-fold higher risk of mortality associated with stroke and coronary artery disease when

compared with normotensives (individuals with BP less than 120/80 mm Hg). In addition, pre hypertensives are at higher risk of developing hypertension and CVD in their later lives.

Co-existent CVD risks factors like dyslipidaemia, raised blood sugar levels and higher body weight are common among pre hypertensives (Greenlund, 2011). Ray *et al.* (2011) in their study found a high prevalence of prehypertension (79.8 per cent), lipid abnormalities (67 per cent) and overweight/obesity (29.9 per cent) among Indian military subjects.

Ray *et al.* (2011) noted that lipid abnormalities especially low levels of high density lipoprotein (HDL) were observed in high proportion of the study subjects from central India also showing subnormal HDL levels in 50 per cent of the participants. They also noticed high cholesterol and triglyceride levels among 21.9 and 14.1 per cent respectively, though 92.1 per cent of the cohort reported moderate to heavy physical activity. Use of high fat diet (73.1 per cent reported daily use of ghee or butter) might have been a reason for adverse lipid abnormalities in this population.

Deepa *et al.* (2007) revealed that overweight/obesity seen among 29 physically active, relatively young adult populations is also thought provoking. Abdominal obesity and visceral adiposity are the key determinants of insulin resistance, an important component of metabolic syndrome (MS) – the major CVD risk factor in all populations.

McGill *et al.* (2008) viewed that metabolic syndrome (MS) is a cluster of CVD and diabetes risk factors including elevated waist circumference, blood pressure, triglycerides, cholesterol and fasting glucose levels. The presence of three or more of the risk factors increases a person's risk of developing diabetes and CVD later. Prevalence of MS has reached epidemic proportions in India in recent years.

Even with lower BMI, Asians have higher visceral adiposity than Caucasian populations. For this reason, the international task force of World Health Organization (WHO, 2005) has set lower cut-off BMI values for Asians to define overweight and obesity (more than 23 and 25 kg/m^2 respectively) .

Misra *et al.* (2011) studied that prevalence of overweight and obesity are increasing in India in recent years even though undernutrition continues to be an important public health issue even in the 21st century. Despite the availability of a few therapeutic agents, the management of obesity is still mainly non-pharmacological (Pappachan *et al.,* 2011). Physical activity and dietary modifications are the cornerstones of management of overweight and obesity.

Kamble *et al.* (2010) viewed that management of dyslipidaemia is primarily through dietary modifications and lifestyle changes like increasing physical activity. Restrictions on food products to encourage the population to adopt healthy dietary practices are undesirable in the Indian context, unlike in the developed countries.

Sharma (2010) studied that while two-third of working women suffer from lifestyle diseases, 53 per cent of them skip meals and go for junk food due to work pressure and deadlines. According to Assocham report (2009), 68 per cent of working women were found to be afflicted with lifestyle ailments such as obesity, depression, chronic backache, diabetes and hypertension.

Sugathan *et al.* (2008) opined that it is strange that for NCDs, lifestyle diseases are noticed to be the major culprit for the high mortality and morbidity rates in Kerala where paradoxically the other health indicators such as high expectancy, very low fertility rate, high literacy, a reasonably good health care system both in government and private sector, match closely with the developed countries.

The main factors contributing to the lifestyle diseases include bad food habits, physical inactivity, wrong body posture, and disturbed biological clock. Rapidly changing disease patterns throughout the world are closely linked to changing lifestyles, which include intake of diets rich in sugars, widespread use of tobacco, and increased consumption of alcohol (WHO, 2005).

Bagchi *et al.* (2004) opined that since the start of the industrial age, lifestyles of human beings have dramatically changed. Increasing work and living speed, longer work schedules, and various psychological pressures have pushed people into various fast-eating cultures with more instant and tasty meals, but decreased quantity and quality in nutrients. These problems have led to an increased incidence of diabetes, obesity, various cancers and vascular diseases, physiological problems, as well as other degenerative diseases.

The relationship between the major modifiable risk factors and the main lifestyle diseases is similar in all regions of the world. Current socio-economic, cultural, political, and environmental determinants that have a significant influence on lifestyle diseases are urbanisation, globalisation, and population ageing (WHO, 2005 and 2008).

Krisela and Albertino (2006) stated that chronic diseases usually emerge in middle age after long exposure to an unhealthy lifestyle involving tobacco use, a lack of regular physical activity, and consumption of diets rich in highly saturated fats, sugars, and salt, typified by "fast foods."

Adverse dietary changes in the population, sedentary activity, increasing tobacco use with consequent changes in the CVD risk factors and other are accruing at great speed and at earlier stages in India than other countries (Reddy *et al.*, 2006).

Krisela and Albertino (2006) were of the opinion that lifestyle results in higher levels of risk factors, such as hypertension, dyslipidemia, diabetes, and obesity that act independently and synergistically.

The reports of (WHO, 2008) suggested that a large percentage of LSD are preventable by changing modifiable and intermediate risk factors. Poor diet and physical inactivity directly account for 4.8 million deaths each year accounting to over 14 million deaths a year.

The study reports of Pierdomenico *et al.* (2009) reviewed that persistent hypertension is one of the risk factors for stroke, myocardial infarction, heart failure and arterial aneurysm, and is a leading cause of chronic kidney failure.

2.2. DEFINITION AND SIGNIFICANCE OF FUNCTIONAL FOODS

Many definitions exist worldwide for functional foods, but there is no official or commonly accepted definition. One view is that any food is indeed functional because it provides nutrients and has a physiological effect.

The definition of functional food was given by: International Life Sciences Institute of North America (ILSI) (Fong *et al.*, 1990); Food and Nutrition Board of the National Academy of Science (US), (Food and Nutrition Board, 1994); International Food Information Council (IFIC) (Miller *et al.*, 1994); Health Canada (1998); The *Nutrition Business* (http://www.nibr.novartis.com); Taiz and Zeiger, 1998); FUFOSE (The European Commission Concerted Action on Functional Food Science in Europe) (Diplock *et al.*, 1999); Institute of Food Technologists (IFT) (MacAulay *et al.*, 2005); American Dietetic Association (ADA, 2005); Food and Agricultural Organization FAO (2007); Food Safety and Standards Act 2006 (FSSA) etc. The most relevant ones are as follows:

International Food Information Council (IFIC), functional foods are "foods or dietary components that may provide a health benefit beyond basic nutrition" (Miller *et al.*, 1994).

Institute of Food Technologists (IFT) defined functional foods as, foods and food components that provide a health benefit beyond basic nutrition (for the intended population), including conventional foods, fortified, enriched or enhanced foods, and dietary supplements. They provide essential nutrients often beyond quantities necessary for normal maintenance, growth, and development, and/or other biologically active components that impart health benefits or desirable physiological effects (MacAulay *et al.*, 2005).

According to American Dietetic Association (ADA, 2005) functional foods are foods that have health benefits beyond the nutrients they contain.

According to FAO (2007), Functional foods are similar in appearance to conventional foods; the former being consumed as part of the normal diet. In contrast to conventional foods, functional foods, however, have demonstrated physiological benefits and can reduce the risk of chronic disease beyond basic nutritional functions, including maintenance of gut health when food is being cooked or prepared using "scientific intelligence" with or without knowledge of how or why it is being used, the food is called "functional food". Thus, functional food provides the body with the required amount of vitamins, fats, proteins, carbohydrates, *etc.*, needed for its healthy survival.

The FSSA defines foods for special dietary uses/functional foods/ nutraceuticals/health supplements as: "foods which are specially processed or formulated to satisfy particular dietary requirements which exist because of a particular physical or physiological condition or specific diseases and disorder wherein the composition must differ significantly from the composition of ordinary

foods of comparable nature does not claim to cure or mitigate any specific disease, disorder or condition (except for certain health benefit or such promotion claims) as may be permitted by the regulations made under FSSA" (Palthur *et al.,* 2009).

Berger and Shenkin (2006) viewed that these functional or medicinal foods and phytonutrients or phytomedicines play positive roles in maintaining well being, enhancing health, and modulating immune function to prevent specific diseases. They also hold great promise in clinical therapy due to their potential to reduce side effects associated with chemotherapy or radiotherapy and significant advantages in reducing the health care cost.

Ramaa *et al.* (2006) opined that the raised demands for health care have dramatically increased the cost of medical care. Now, more and more people realize that a healthy body is more important than money or work in their lives. Therefore, people have tried to achieve a better quality of life by eating more vegetables, fruits, and other plant foods, taking dietary supplements or nutraceuticals.

Wildman and Kelley (2007) reviewed that the principal reasons for the growth of the functional food market are current population and health trends. People can optimize the health-promoting capabilities of their diet by way of supplementation and by consuming foods that have been formulated or fortified to include health-promoting factors.

2.3. ROLE OF FOODS RICH IN BIOACTIVE COMPOUNDS FOR THE MANAGEMENT OF LIFESTYLE DISEASES

Bioactive compounds in foods that have or appear to have significant health potentials are: Carotenoids, Phenolic compounds (flavonoids, phytoestrogens, phenolic acids), Phytosterols and Phytostanols, Tocotrienols, Organosulfur compounds (allium compounds and glucosinolates), and Nondigestible carbohydrates

(dietary fiber and prebiotics) etc. They are validated to posses glaring nutritional and therapeutic roles (Rodriguez *et al.*, 2006).

Saikia and Deka (2011) stated that natural products such as cereals are likely to form the basis of nutraceuticals, as the nutrients in the cereals where proved for its potential for reducing the risk of coronary heart disease, reducing tumour incidence, cancer risk, lowering blood pressure, reducing the rate of cholesterol and fat absorption, delaying gastrointestinal emptying and providing gastrointestinal health.

Liu *et al.* (2007) reported that the consumption of whole grains has been associated with reduced risk of some cancers and cardiovascular disease as well as type 2 diabetes. Lunasin is a novel cancer preventive, anti-inflammatory and cholesterol- reducing peptide originally isolated from later found in cereals (barley, rye, wheat, triticale) (Nakurte *et al.*, 2013).

VanDam *et al.* (2006) were of the opinion that whole grains are a rich source of magnesium that acts as a co-factor for more than 300 enzymes, including enzymes involved in the body's use of glucose and insulin secretion.

Bell *et al.* (2001) observed that among the food grains, **oat** is the most concentrated source of β-glucan, a soluble non-starch polysaccharide known to reduce risk of coronary heart disease. Various components such as phytates, phenolics, vitamins and minerals, which confer other physiological benefits, are also present.

Oat was the first specific food allowed to have a health claim under the US Nutrition Labelling and Education Act (Hasler, 1998). FDA allowed the claim "soluble fiber from oatmeal, as part of a low saturated fat, low cholesterol diet, may reduce the risk of heart disease." FDA has acknowledged that β–glucan is the main active ingredient responsible for this health claim (Oomah and Mazza, 2000).

Ripsin *et al.* (2002) summarized that about three gm per day of soluble fiber from oat products can achieve a clinically relevant serum cholesterol-lowering effect, and that the reduction is greater in individuals with higher initial blood cholesterol levels.

Annapurna (2011) viewed that **barley** (*Hordeum vulgare*) is a good old grain with many health benefits like weight reduction, decreasing blood pressure, blood cholesterol, blood glucose in Type 2 diabetes and preventing colon cancer. It contains both soluble and insoluble fiber, protein, vitamins B and E, minerals selenium, magnesium and iron, copper, flavonoids and anthocynins.

AbuMweis (2010) reported that barley and β-glucan isolated from barley lowered total and low-density lipoprotein (LDL) cholesterol concentrations by 0.30mol/l and 0.27mmol/l respectively, compared with control.

Barley, as a whole grain or as an extract, can serve as a fat replacer in food products and can provide a useful addition to menus to control plasma glucose responses (Behall *et al.*, 2002, 2004).

Hallfrisch *et al.* (2003) studied that plasma total cholesterol and triglycerides decreased significantly in men with moderate and high beta-glucan intakes from barley and total cholesterol and LDL cholesterol decreased in post-menopausal women. In studies comparing the response of plasma cholesterol and triglycerides to diets rich in oats or barley, barley appeared to be more effective in lowering plasma cholesterol than oats, perhaps because of its higher beta-glucan content.

According to the investigation reports of Siebenhandl- Ehn *et al.* (2011) hulless barley is a good source of β-glucan, arabinoxylans, phenolics, flavonoids, anthocyanins, vitamin E, lutein and zeaxanthin; lutein and zeaxanthin act together with other bioactive compounds against cancer effects. Tsangpa with barley flour is

regarded as a white medicine, one of the main reasons why heart disease and colon cancers occur at a lower rate in Tibet than that of expected (Nyima *et al.*, 2012).

Lakshmi and Sumathi (2002) and Kang *et al.* (2008) reported that **Finger millet (Ragi, *Eleusine coracana*)** provides highest level of calcium, antioxidants, phytochemicals, which; helps to control blood glucose levels in diabetic patients very efficiently.

Wadikar *et al.* (2007) found that finger millet has gained importance because of its functional components, such as slowly digestible starch and resistant starch. Desai *et al.* (2009) recommended ragi as an ideal food for diabetic individuals due to its low sugar content and slow release of glucose/sugar in the body.

Darmadi-Blackberry *et al.* (2004) in their study investigated that, legumes are also complex foods rich in soluble fibers and polyphenols, as well as folic acid, a B vitamin that reduces blood homocysteine concentrations, a risk factor for CVD.

A large incident case-control study in Costa Rica by Kabagambe *et al.* (2005) concluded that the consumption of one serving of legumes daily was associated with a 40 per cent lower risk of myocardial infarction. Interestingly, legumes were the only food group predictive of survival among five long-lived elderly cohorts in Japan, Sweden, Greece, and Australia.

Evidence from experimental research indicates that cholesterol-lowering effects of legumes are probably due to the combined effects of several bioactive components, such as protein, soluble and insoluble fibres, and phytosterols (Martins *et al.*, 2005).

Soybean has been cultivated and consumed as food in Asia for over 5000 years. However scientific interest on its health benefits started much later. It is not

only a source of high quality proteins but also of phytosterols, saponins, phenolic acids, phytic acid and isoflavones (Messina and Barnes, 2001).

The Food and Drug Administration of the U.S. approved a health claim for soy protein in reducing the risk of heart disease (Department of Health and Human Services, 1999).

Kris-Etherton *et al.* (2002) reported that several classes of anticarcinogenic phytochemicals have been identified in soybeans, of which the isoflavones genistein and daidzein are noteworthy because soybeans are the only significant dietary sources of these compounds.

Population studies (Nagata *et al.*, 2002) showed that countries consuming diets high in soy products have the lowest rates of CVD. An inverse association between soy food product consumption and cholesterol level has been observed in Japanese men and women.

Erdman and Potter (2003) reviewed that soybean has been known to have a protective role in women's health, particularly the alleviation of menopausal symptoms and promotion of bone health. A clinical study found that daily intake of 40 g isolated soy protein (ISP), containing 90 mg total isoflavones, significantly increased both bone mineral content and density in the lumbar spine after 6 months in postmenopausal women.

Anderson *et al.* (2005) showed that daily consumption of 47 g soy protein resulted in significant decreases in total cholesterol (9 per cent), LDL cholesterol (13 per cent), and triglycerides (11 per cent) and an increase in HDL cholesterol (2 per cent).

World Cancer Research Fund (2007) in the human ecological observations supported a cancer-protective effect of soybeans. Vegetarians and population groups (e.g. Japanese women) who often consume relatively greater amounts of soy products, have a lower risk of certain cancers, including breast cancer.

Nestel *et al.* (2007); Hodgson *et al.* (2008) reported that the well-documented physiological effect of soybean is its cholesterol-lowering effect. Investigations on the specific components responsible for this effect of soybean have focused on the isoflavones.

Crouse *et al.* (2008) showed that naturally occurring isoflavones (62 mg) isolated with soy protein reduced the plasma concentrations of total and LDL cholesterol, without affecting concentrations of triacilglycerols or HDL cholesterol, in mildly hypercholesterolemic individuals.

Animal studies indicate that the cardioprotective effect of soybeans goes beyond cholesterol-lowering (Potter, 2008), such as decreases of atherosclerotic lesion and thrombus formation and in atherosclerotic plaque.

Rao *et al.* (2001) reported that **Plantain** or **banana** (Musa ABA), a widely grown fruit throughout the world, is the best source of potassium, an essential mineral for maintaining normal blood pressure and heart function. Bananas contain chemicals that inhibit the angiotensin converting enzyme, which acts to constrict blood vessels and raise blood pressure

Kanazawa and Sakakibara (2000); Alothman *et al.* (2009) reported that bananas are rich in phenolic compounds and flavanoids, and are rich in dopamine, an antioxidant. Astringent taste of unripe banana is due to phenolic compounds.

Lehmann and Robin (2007) found that carbohydrates in banana are resistant starch type and non starch polysaccharides, which show low glycemic index or low digestibility and are helpful for diabetics.

Anwar *et al.* (2007) revealed that **Moringa** has different types of biological activities like; antitumor, antiepileptic, anti-inflammatory, antidiabetics, antibacterial, antiulcer, anti spasmodic, antipyretic, antihypertensive, antioxidants, epatoprotective, cholesterol lowering, diuretic, cardiac and fungal activity.

John and Chellappa (2005) proved that incorporation of moringa leaf powder at 8g per day for a period of 14 days has shown marked reduction in the mean fasting and postprandial plasma glucose levels. Moringa leaf powder can be strongly recommended in the daily diet of NIDDM subjects for the effective management of diabetes.

Kumar and Pari (2003) reported that, the moringa leaf extract was found to enhance the recovery from hepatic damage induced by antitubercular drugs. It has potential for cancer chemoprevention and can be claimed as a therapeutic target for cancer (Sreelatha *et al.*, 2011).

According to Tahiliani and Kar (2000), the aqueous moringa leaf extracts regulate thyroid hormone and can be used to treat hyperthyroidism. Moringa leaf may be applicable as a prophylactic or therapeutic anti-HSV (Herpes Simplex Virus type1) (Lipipun *et al.*, 2003).

Manzi *et al.* (2001) and Mattila *et al.* (2001) reviewed that **edible mushrooms** have higher protein contents and minerals and contain less fat but are rich in B vitamins, vitamin D, vitamin K and sometimes vitamins A and C.

Bobek *et al.* (2005) studied that mushrooms are not only sources of nutrients but also have been reported as therapeutic foods, useful in preventing diseases such as hypertension, diabetes, hypercholesterolemia and cancer.

Manzi *et al.* (2001) reported that the functional characteristics of oyster mushrooms are mainly due to the presence of dietary fiber and in particular chitin and beta glucans. Studies have also shown that certain mushrooms species have antitumor, antiviral, antithrombotic and immunomodulating properties.

Oyster mushrooms have shown that they serve as repositories of B-vitamins such as niacin, flavin and pyridoxine (Solomko and Eliseeva, 1988) organic acids such as ascorbate, shikimate, malate and fumarate; carbohydrates such as glucans; monoterpenoid and diterpenoid lipids; proteins such as hydrophobins and trace elements such as selenium (Dikeman *et al.*, 2005; Valentao *et al.*, 2005).

Cui (2002) and Acharya *et al.* (2005) investigated that the substances in oyster mushroom have been found through several *in vitro* and *in vivo* studies to be responsible for the antimicrobial, antioxidant, and antitumor, antihypertensive and antiaging potentials of edible mushrooms. The antioxidant property of mushroom is due to its phenolic, terpenoids and polysaccharide polypeptide contents. These bioactive compounds mediate biological activities including stimulation of interleukin-12 production, nitric oxide syntheses activation, free radical scavenging and iron chelating properties.

Jaziya (2011) reported that incorporation of 5 gm dried oyster mushroom (*Pleurotus florida*) powder in the daily diet, reduced blood glucose and blood lipid levels. This might be attributed to the beta glucan content and other phytochemicals of oyster mushroom. Similar reports were given by Anju (2013) on *Calocybe indica.*

Mushrooms have been used widely since ancient times as foods and medicinal as well as functional purposes. The antitumor effects of mushrooms included to

breast cancer, colon cancer, gastic cancer, prostate cancer, pancreatic cancer, cervical and ovarian as well as endomentrial cancer (Roupas *et al.,* 2012), those are primarily due to biopolymers (Lemieszek *et al.,* 2013).

2.4. MARKET TRENDS IN FUNCTIONAL FOODS

Fuelled by consumers who are more conscious about overall health and healthy eating, the global nutraceuticals market, including functional food, functional beverages and dietary supplements, is on track for continued steady growth through 2017, according to a new report from Research and Markets (IndustryARC, 2013).

According to Kotilainen *et al.* (2006), the global market size of functional food was estimated to be approximately US$30 to US$60 billion which represents 1-3 per cent of the total food market. Revenue generated in 2007 by the functional food and natural health product sector in Canada was approximately $3.7 billion (Cinnamon, 2007).

Between 2003 and 2010, the global functional food and drink market increased 1.5 times, with a CAGR of 14 per cent, reaching $24.2 billion USD in 2010. Between 2010 and 2014, Leatherhead (2011) forecasts a total global market growth of 22.8 per cent, to reach $29.8 billion by 2014.

According to the "Nutraceuticals Product Market: Global Market Size, Segment and Country Analysis and Forecasts (2007-2017)" report, functional foods remained the fastest-growing segment of North America nutraceutical market at 6.5 per cent CAGR during 2007-2011. Omega fatty acid fortified food segment, Protein and peptide supplements and non-herbal segment of dietary supplement market is expected to have a steady growth (NRA, 2014).

The North America and Asia Pacific nutraceutical market is expected to have a market share of 39.2 per cent and 30.4 per cent in 2017 (HealthFocus, 2012). Japan is the largest consumer of nutraceuticals, while China is second largest consumers of nutraceutical product as people are more conscious about their food habits and they have the largest population in the world (Nutraceuticals Product Market: 2007-2017).

IndustryARC (2013) estimated the global functional food market revenue for the year 2013 to be around $175 billion. With an annual average growth rate of about 15 per cent the global market for functional food is forecast to exceed $230 billion by 2015 (Mintel, 2013).

Leatherhead (2011) had reported that functional foods have its presence right form cereals, grains, nuts, vegetables, fruits, dairy products, confectionery items and snacks to non alcoholic beverages. More than 75 per cent of US functional food market is dominated by global key players such as Coca Cola Co., Dean Foods, General Mills Inc., Kellogg Co., Kraft Foods, Nestle S.A. and Pepsi Co. Functional food fastest growth is being recorded in energy drinks, healthy snacks and breakfast products that include cereals and grains (Jacobsen, 2014).

DMI (2014) reported that with the introduction of probiotic drinks, Enhanced Water and Calorie Burning drinks, functional beverage market has surged owing to the health conscious needs of consumers. The leading countries in the consumption of nutraceuticals are USA, Canada, Japan, China, Brazil, UK, Russia, Mexico and India (http://www.researchandmarkets.com/research/dvlqfb/functional_foods).

IFIC (2013) stated that dairy accounts for the largest share of functional foods, followed by bakery/cereals, beverages, and fats and oils. In terms of CAGR, however, bakery/cereals are leading (18 per cent), followed neck and neck by fats and oils (14 per cent) and dairy (14 per cent).

BCC Research (2011) reported that there is growing demand for functional foods, especially in developed economies due to increasing awareness towards health benefits of functional foods and increase in disposable incomes. This growth is mainly driven by the continuously growing demand for energy drinks and fortified dairy products (Sloan, 2014).

Reports of IRI (2014) stated that less/reduced calories and sugar-free were the most frequent health claims touted by the best-selling new better-for-you foods/drinks in 2013. The United States is the world's largest functional food market with sales of $43.9 billion in 2012, +6.9 per cent over 2011 (NBJ, 2013).

MSI (2012a) reported that Six in 10 U.S. adults consume specially formulated functional foods/beverages at least occasionally. Yogurt for digestive health and cereal for heart health are the most-consumed items, followed by cholesterol-lowering butter/margarine, cholesterol-lowering orange juice, and shakes/bars to reduce hunger, orange juice for joint health, immune-boosting dairy beverages, and medicinal teas.

One in five adults cut back on their use of supplements because they are eating so many fortified foods; 88 per cent regularly consume vitamin- or mineral-fortified foods (MSI, 2012b). In 2013, China had the highest expenditure on health and wellness retail products, followed by Brazil, the United States, Russia, and Mexico. Globally, general well-being, weight management, digestive health, energy boosting, and endurance were the top five positioning for health/wellness in 2013; energy boosting, food intolerance, general well-being, digestive health, and beauty from within were the fastest-growing (Euromonitor, 2014).

Global Nutraceutical market (2011) opined that functional foods and Functional beverages are relatively nascent markets in India primarily due to a burgeoning middle class that relies on traditional practices such as Ayurveda. The

functional food market in India is expected to have moderate growth compared to dietary supplement. The functional food and beverage market in India is expected to have 70.74 per cent growth compared to the dietary supplement in 2017 (Nielsen, 2013).

New analysis from Frost and Sullivan (http://www.chemicals.frost.com), the Indian Nutraceutical market, finds that the market earned revenues of $1480 Million in 2011 and could grow to $2731 Million in 2016 at a CAGR of 13.0 per cent. Dietary supplements were the largest category accounting for 64 per cent of the nutraceuticals market, driven primarily by the pharmaceutical sector in the form of Vitamin and Mineral supplements.

2.5. OPPORTUNITIES AND CHALLENGES IN FUNCTIONAL FOODS

Kotilainen *et al.* (2006) were of the opinion that functional foods have entered the global markets with a considerable force in the past decade and rapidly gained market share than that for organic foods. Thus, in addition to the health benefits, functional foods present new economic opportunities.

Poonia and Dabur (2009) reported that functional foods sell at higher prices and contain larger profit margins than conventional foods. This makes the food sector attractive for the players of food supply chains including marketing, storage and transportation.

Hasler *et al.* (2002) opined that retail prices of functional foods are generally higher ranging between 30 to 500 percent above the comparable conventional foods. Moreover, demand for functional foods within the developing countries is growing, presenting a lucrative opportunity to develop domestic markets.

Kotilainen *et al.* (2006) suggested that functional foods can be an opportunity for economic growth for many developing countries endowed with rich biodiversity and traditional knowledge of the health effects of certain indigenous plant species.

Sharma (2005) viewed that besides opportunities for the diversified and high-value production, farming for the functional foods industry can benefit the primary producers and rural communities in various ways. Opportunities exist for innovative dairy beverages targeting functional food trends.

According to William *et al.* (2006) a clear regulatory system for production, sales, certification, and advertising of functional foods, together with consistent enforcement are critical factors in building consumer trust in functional foods. Developing institutions like food research centres, advisory services for producers, educators in food sector marketing and management, and authorities approving health claims for functional foods are essential.

Verschuren (2002) viewed that the development and marketing of functional foods require significant research efforts because most markets require scientific evidence and proof of functionality. This research requires time, financing, and skilled labour, especially for products destined for export markets. Innovation and research capacity is required to screen local biodiversity to uncover potential new sources for functional foods (William *et al.*, 2006).

Johnson and Williamson (2008) suggested that based on absorption, tissue distribution and functional consequences of absorption which are complex and not fully understood and prediction of their bioavailability is problematic especially in lipid soluble phytochemicals.

During formulation of functional foods, some bioactive substances present in foods are either lost or become less available when exposed to heat during processing

become less stable. Bioactive substances like vitamins are destroyed due to heat during processing (Verschuren, 2002). Some bioactive substances during processing are found to cause undesirable flavour, taste and rancidity. Dietary fibres/ prebiotics cause bitterness during processing.

Sharma (2005) reported that shelf life, flavour and physical stability are other challenges. During manufacturing of phytosterols are insoluble in water and difficulty in incorporation in low fat beverages arises.

Lesmes and McClements (2009) viewed that the functionality and bioavailability of bioactive compounds are strongly affected and determined by their chemical properties, in terms of solubilisation and depolymerisation. Processing of the food material dramatically affects the bioavailability of nutrients and phytochemicals, as do the environmental conditions during its passage through the gastrointestinal tract.

Lattanzio *et al.* (2009) viewed that the influence of heat and mass transfer in food processing affects food microstructures. The complexity of food matrix determines both food texture and also the release of functional components.

Faulks and Southon (2008) reported that the understandings of the development of novel products with added health beneficial value, absorption rate, metabolism and bioavailability of bioactive compounds within the human organism are crucial.

Jones and Jew (2007) concluded that current developments in the area of functional food have already demonstrated that the bioavailability of bioactive ingredients can be improved by the selection and protection method for bioactive ingredients.

Carratù and Sanzini (2005) reported that the bioavailability of phytochemicals can be influenced by intrinsic factors in food and/or in human, in general the substances are little adsorbed, largely metabolized and rapidly eliminated.

Hedges and Lister (2008) were of the opinion that a number of factors combine to determine the levels of both core nutrients and other phytochemicals in a food. These include the variety/cultivar of the plant, their agronomy, soils, cultivation protocols, degree of ripeness at harvest, and processing practices.

Frankel (2003) reported that processing is expected to affect the content, activity and availability of bioactive compound. Nott *et al.* (2000) viewed that according to the desired products the method of adding bioactive ingredients specifically to food items and so is creating or re-creating the desired nutritional value of the product, the modern way of food processing aims at preserving native bioactive ingredients in the raw food as much as possible.

Zhong *et al.* (2004) demonstrated that through ohmic heating and microwave technology, a suitable food product could be manufactured with the same level of safety as by production with conventional heat treatment processes, but with improved organoleptic properties.

Palzer (2009) suggested that the novel technologies differ from the traditional food processing methods and have certain advantages in their capacity to prevent the inactivation of bioactive ingredients.

Chen *et al.* (2006) opined that in order to provide a greater amount and variety of functional foods, beside the traditional natural products, food manufacturing companies are working continuously on the development of novel products which include delivery of the protected bioactive ingredients to their target site and release under certain trigger factors.

Materials & Methods

3. MATERIALS AND METHODS

Several measures used to correct the imbalances of life style degenerative diseases, do not become healthy alternatives as besides being expensive, produce wide spectrum of adverse effects (Hasegava and Mogi, 2002).

There is a wide range of food stuffs that exerts promotive action for counteracting these adverse effects but at present is not used in our daily diet due to ignorance or oversight. Incorporating a food supplement along with medicines creates a more favorable option for the patients in the prevention and management of lifestyle diseases.

Developing a functional food supplement using available food items that are rich in bioactive compounds will have the twin ability of nourishment and therapeutic action. A combination of natural food items serves a better purpose than a single ingredient. Putting together the essential ingredients in a nut shell package also covers the defect of planning a cumbersome menu which becomes practically inapplicable.

Thus the present study entitled "**Development, quality assessment and clinical efficacy of 'Functional Food Supplement' (FFS) for life style disease management**" was conducted in three experiments as follows:

3.1. Development of 'Functional Food Supplement' (FFS)

3.2. Quality evaluation of the identified FFSs

3.3. Evaluation of clinical efficacy of the identified FFS

3.1. DEVELOPMENT OF 'FUNCTIONAL FOOD SUPPLEMENT' (FFS)

Scientific evidences validate the therapeutic importance of various food substances in the management of various diseases. Natural food resources apart from

Plate: 01 Constituents of FFS

providing nutrients are found to have other benefits viz. health promoting factors, chemo preventive effects, cholesterol lowering properties, blood sugar level control, insulin resistance, immunomodulation, blood pressure control, weight management etc. However in this study the hypoglycemic, hypocholestremic and hypotensive effects of the selected food materials were the main focus. Food substances containing bioactive compounds, which has got greater health benefits but are less utilized or consumed in the daily diet was judiciously selected for the formulation of FFS.

Annapurna (2011) viewed that **barley** (*Hordeum vulgare*) is a good old grain with many health benefits like weight reduction, decreasing blood pressure, blood cholesterol, blood glucose in Type 2 diabetes and preventing colon cancer.

Lakshmi and Sumathi, (2002); Kang *et al.* (2008) reported that **Finger millet (Ragi, *Eleusine coracana*)** provides highest level of calcium, antioxidants, phytochemicals, which; helps to control blood glucose levels in diabetic patients very efficiently. Desai *et al.* (2009) recommended ragi as an ideal food for diabetic individuals due to its low sugar content and slow release of glucose/sugar in the body.

Numerous epidemiological studies have demonstrated an association between the consumption of soybean (*Glycine max*) and improved health, particularly as a reduced risk for cancers or diseases, such as breast cancer, cardiovascular disease, and atherosclerosis (Dai *et al.*, 2002; Jenkins *et al.*, 2002; Yamamoto *et al.*, 2003).

Rao *et al.* (2001) reported that banana is the best source of potassium, an essential mineral for maintaining normal blood pressure and heart function. Bananas contain chemicals that inhibit the angiotensin converting enzyme, which acts to constrict blood vessels and raise blood pressure.

Plate: 02 Processing of constituents for the development of FFS

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Anwar *et al.* (2007) revealed that Moringa has different types of biological activities like; antitumor, antiepileptic, anti-inflammatory, antidiabetics, antibacterial, antiulcer, anti spasmodic, antipyretic, antihypertensive, antioxidants, hepatoprotective, cholesterol lowering, diuretic, cardiac and fungal activity.

Oyster mushrooms have shown that they serve as repositories of B-vitamins such as niacin, flavin and pyridoxine (Solomko and Eliseeva, 1988) organic acids; carbohydrates such as the -glucans; lipids; proteins and trace elements such as selenium (Dikeman *et al.*, 2005; Valentao *et al.*, 2005) which are responsible for the antimicrobial, antioxidant, and antitumor, antihypertensive and antiaging potentials of edible mushrooms.

Thus based on the support from the previous scientific investigations, the constituents for the FFS was chosen to contain barley, ragi and banana (Rasakadhali / Njalipoovan - mature, unripe) as base materials along with defatted soy flour, drumstick leaves powder and mushroom powder as other constituents in different proportions for obtaining maximum health benefits.

3.1.1. Selection and Procurement of the Constituents for the FFS

Selection of the various constituents for the formulation of the FFS was based on their nutritional qualities like low calorie, low fat, adequate carbohydrates, sufficient protein, high fiber and adequate micronutrients suitable for the management of lifestyle diseases and also on their sensory qualities. The materials for the study were procured locally and processed in the laboratory. Processing of each of the constituents for the development of FFS is given in Plate No: 02.

3.1.2. Standardization of Functional Food Supplement (FFS)

Dehydration and fermentation were the two processing techniques applied to standardize FFS.

In the first processing technique (I), the constituents were dried individually in a cabinet drier, powdered mechanically, sieved thrice to obtain a fine powder and then blended into different proportions to formulate the FFS. The processing steps involved in each of the constituents of technique I, is given in Flow chart – 01.

In the second processing technique (II), fermentation followed by dehydration was the processing techniques applied to standardize FFS. Ragi was cleaned, washed, allowed to soak in triple the amount of water for 12 hrs, drained excess water, kept covered in a muslin cloth, allowed to germinate for 24hrs, cabinet dried for four hours, then milled in a laboratory mill to obtain fine powder and packed. The other constituents were dried individually, powdered mechanically and then packed separately to formulate the FFS. The processing steps involved in each of the constituents of technique II, is given in Flow chart – 02.

3.1.3. Optimization of the Constituents for the FFS

Proportions of various ingredients were optimized by trial and error method. Approximate proportion of ingredients for formulating the supplement is as follows:

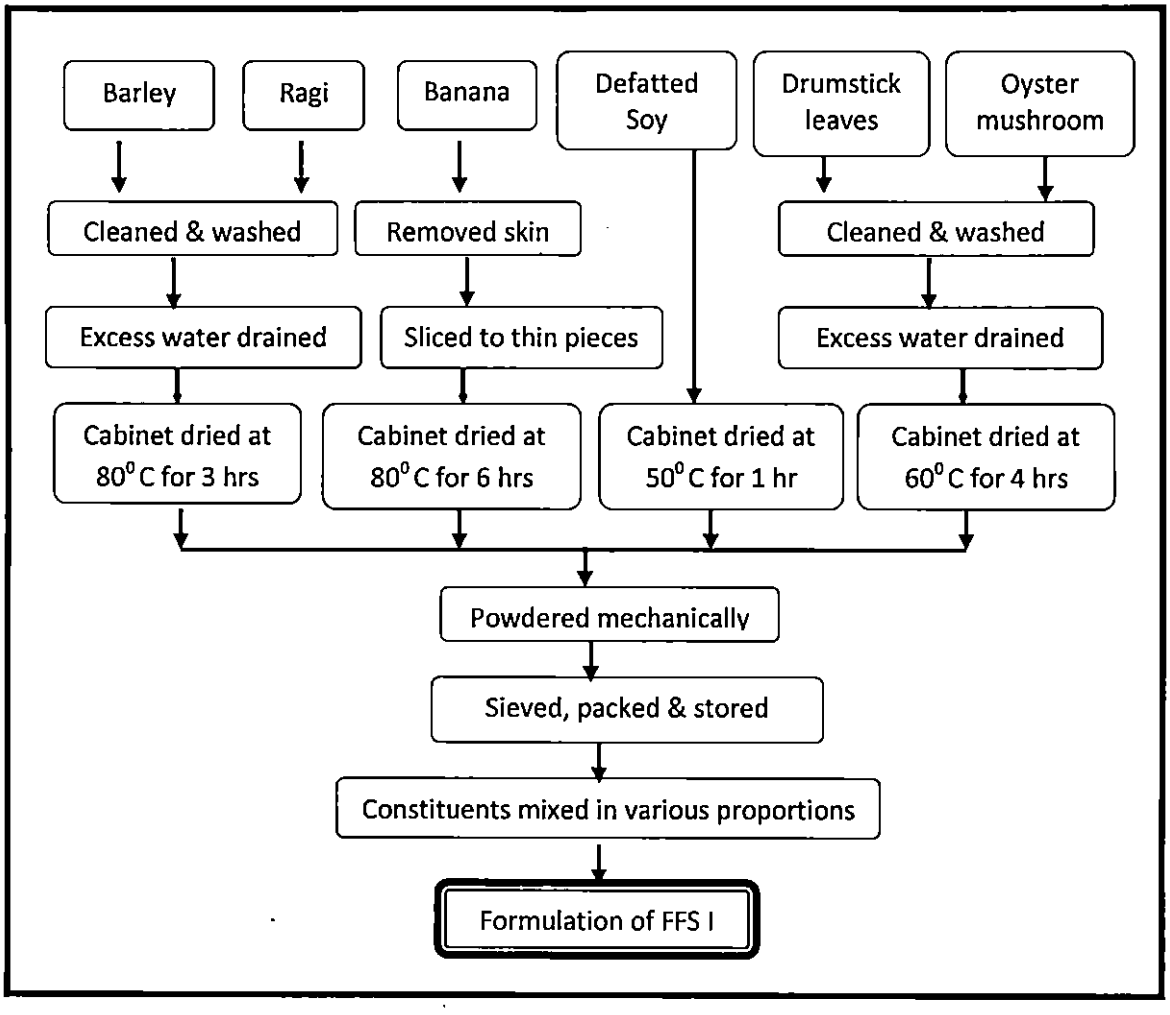
Barley	-	20-40%
Ragi	-	20-30%
Banana powder	-	20-40%
Defatted soy flour	-	15-20%
Drumstick leaves powder	-	0-10%
Mushroom powder	-	0-10%

Different proportions of each of the ingredients were tried and their nutritive values were computed. The ratio of each ingredient i.e. Barley: Ragi: Banana powder: Defatted Soy Flour: Drumstick leaves powder: Mushroom powder of the various combinations is represented as B:R:Bp:DSF:DLp:Mp in Table: 01.

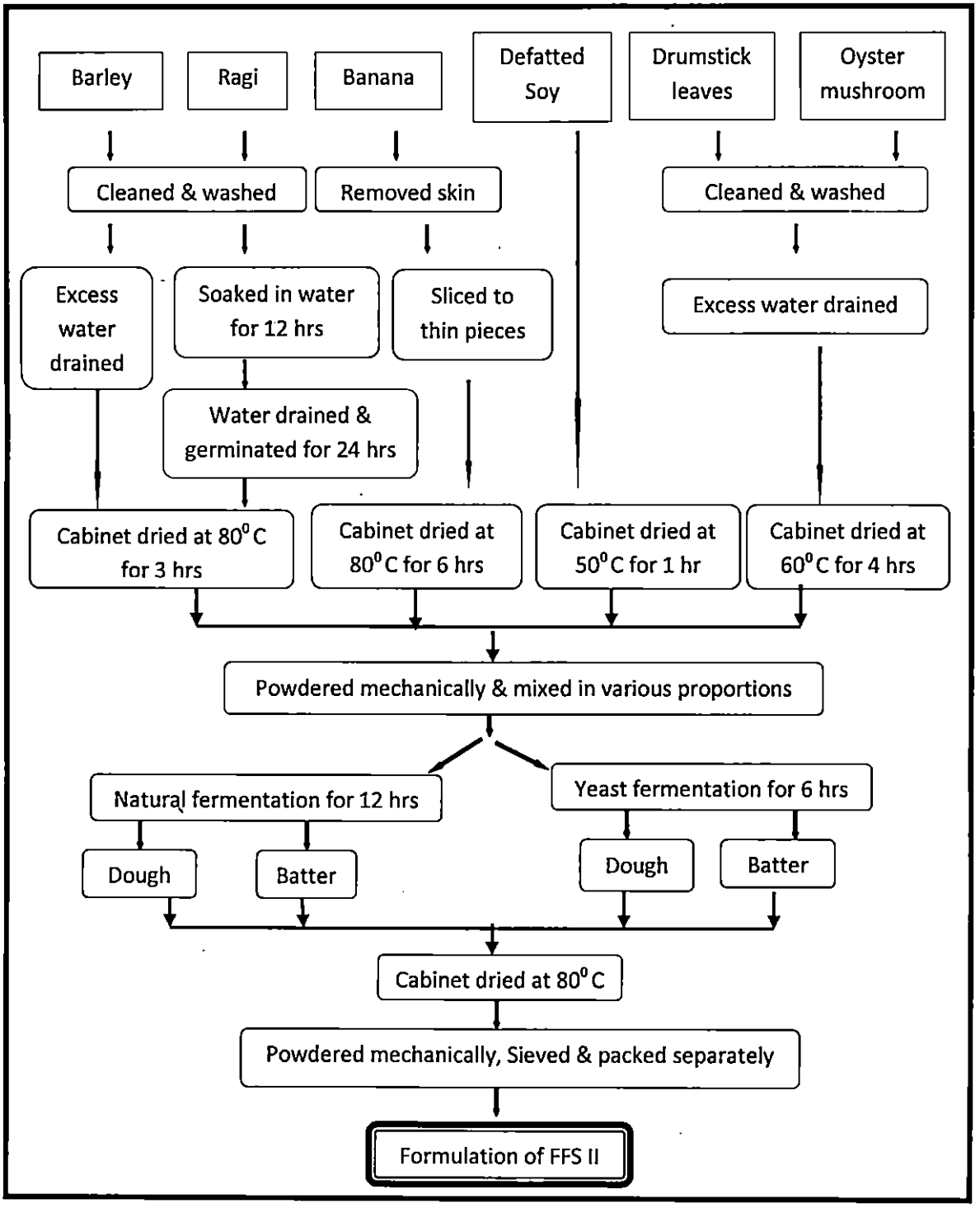
Table 01. Ratio of the constituents for various combinations

S. No	Combination	B: R: Bp: DSF: DLp: Mp
1	I	3.0:2.0:1.5:2.5:0.5:0.5
2	II	2.5:2.5:2.0:2.0: 0.5:0.5
3	III	4.0:2.0:1.5:1.5: 0.5:0.5
4	IV	2.5:2.0:1.5:3.0: 0.5:0.5
5	V	2.0:2.0:1.5:3.5: 0.5:0.5
6	VI	3.5:2.0: 1.5:2.0:0.5:0.5
7	VII	2.0:2.0:1.0:4.0: 0.5:0.5
8	VIII	2.0:3.0:1.0:3.0: 0.5:0.5
9	IX	2.5:2.0:2.0:2.5: 0.5:0.5
10	X	3.0:2.5:1.5:2.0:0.5:0.5
11	XI	3.0:2.0:2.0:2.5:0:0.5
12	XII	3.0:2.0:2.0:2.5:0.25:0.25
13	XIII	3.25:2.0:2.0:2.5:0:0.25
14	XIV	3.5:2.0:1.5:2.5:0.25:0.25
15	XV	3.25:2.0:1.75:2.5:0.25:0.25
16	XVI	3.5:1.5:2.0:2.5:0.25:0.25
17	XVII	3.25:1.75:2.0:2.5:0.25:0.25
18	XVIII	3.0:2.0:2.0:2.5:0.5:0
19	XIX	3.25:2.0:2.0:2.5:0.25:0
20	XX	3.25:2.0:2.0:2.5:0:0.25

Flow chart: 01 Processing technique I



Flow chart: 02 Processing technique II



From the various proportions tried, the most suitable four combinations were screened and identified based on parameters mentioned in selection criteria and also on sensory qualities. The four combinations selected from the dehydration technique were named as FFS DT_1, DT_2, DT_3, and DT_4 respectively. The best identified FFS was taken as FFS I.

Similarly, from the various proportions tried, the best combination was selected based on the computed nutrient contents suitable for lifestyle diseases. This combination was further subjected to four different fermentation techniques (Table No: 02) and named as FFS FT_1, FT_2, FT_3, and FT_4 respectively. Among the four techniques, the most suitable FFS was finally screened and identified as FFS II based on parameters mentioned in selection criteria and also on sensory qualities.

Table 02. Fermentation techniques involved in preparation of FFS II

FFS	Ingredients	Form	Fermentation technique	Time of fermentation (hrs)	Drying Temperature* and Time
FT_1	Selected combination + water	Dough	Natural	6 hrs	4 hrs
FT_2	Selected combination + water	Batter	Natural	6 hrs	6 hrs
FT_3	Selected combination + water + yeast (5g for 1kg FFS)	Dough	Yeast	12 hrs	4hrs
FT_4	Selected combination + water + yeast (5g for 1kg FFS)	Batter	Yeast	12 hrs	6hrs

* All the four FFS were dried in a cabinet drier at 80 ° C

Plate No: .03 and 04 gives the steps involved in the standardization of the identified FFS I & II.

The best identified combinations from each technique (I & II) were investigated in depth for the nutrient content, phytochemical properties, functional qualities, storage stability and clinical efficacy.

3.2. QUALITY EVALUATION OF THE IDENTIFIED FFSs

The quality of the FFS (I and II), was evaluated on nutrient content, functional properties, phytochemical content, storage stability and feasibility of incorporation into various food preparations/products.

3.2.1 Nutrient Analysis

The following nutrients were determined in triplicate by using standard procedures.

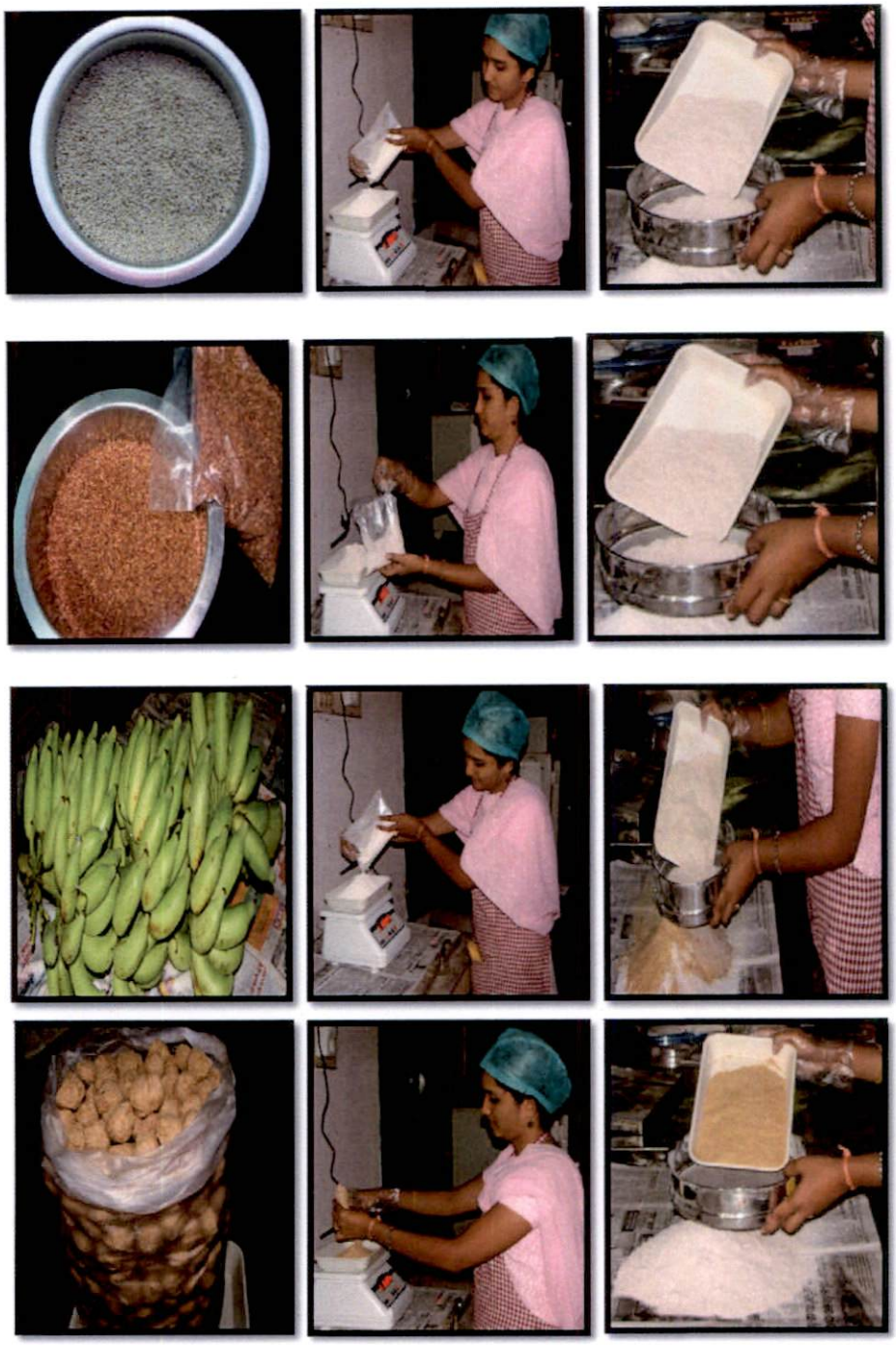
3.2.1.1. Proximate Analysis

Energy is essential for rest, activity, growth and maintenance of sound health (Sheng *et al.*, 2010).

Protein is one of the most important nutrients required by the body to carry out a wide range of functions essential for the maintenance of life. Proteins are essential component of tissues and cells of the body (Gopalan *et al.*, 2009).

Fats, as long as they come from the right sources can be part of a healthy diet. A balanced diet deriving approximately one-third of its total calories from monounsaturated and polyunsaturated fats has many health benefits (Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, 2002).

Plate: 03 Steps in standardizing FFS I



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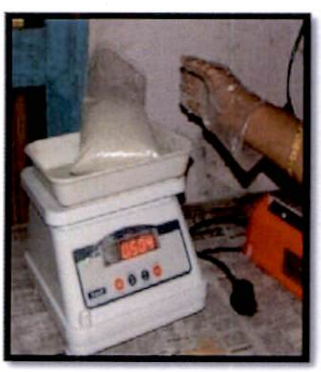
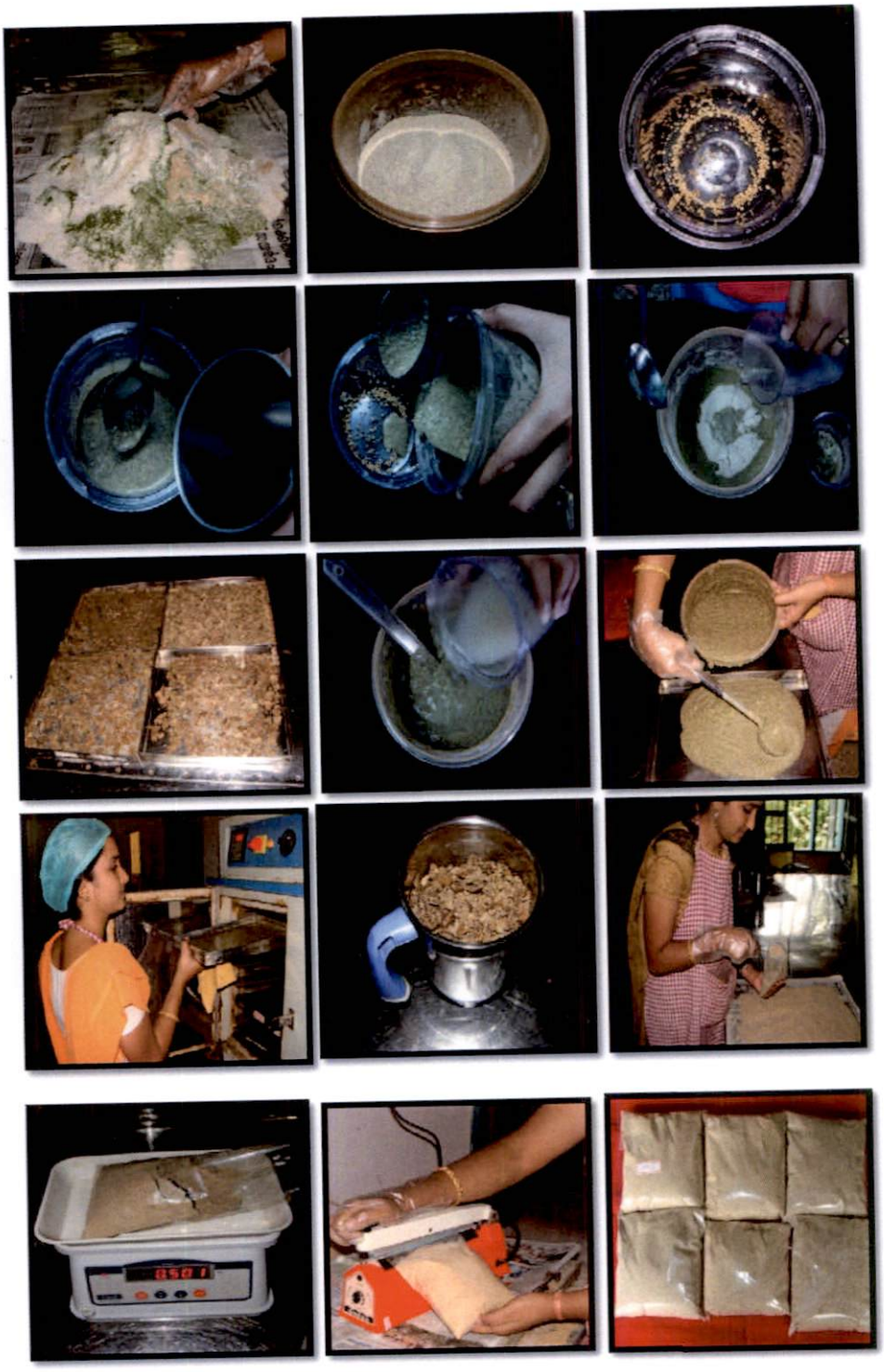



Plate: 04 Steps in standardizing FFS II



Fibre is found only in plant foods,like whole-grain breads and cereals, beans and peas, and other vegetables and fruits. Eating a variety of fibre-containing plant foods is important for proper bowel function and can reduce symptoms of chronic constipation, diverticular disease, and hemorrhoids, as well as lower the risk of heart disease and some cancers.

Table 03. Analytical procedures for macronutrients

Nutrients	Method
Energy	Association of Official Analytical Chemists (AOAC, 2005)
Protein	Association of Official Analytical Chemists (AOAC, 2005)
Moisture	Association of Official Analytical Chemists (AOAC, 2005)
Total ash	Association of Official Analytical Chemists (AOAC, 2005)
Total fat	Sadasivam and Manickam (1992)
Crude Fibre	Sadasivam and Manickam (1992)
Amino acids	Association of Official Analytical Chemists (AOAC, 2005) – HPLC method
Amino acid score	Gopalan *et al.*, 2004

Evaluation of Protein Quality of FFS I & II

According to Ghosh and Chakravarthy (1990), the quality of protenacious food depends on its amino acid composition in relation to the protein content and digestibility. Amino acid composition may also serve as a good relative measure to compare mushroom with other food stuffs of established nutritive value.

In the present investigation, the quality of FFS I & II was analyzed in detail by estimating amino acid content of the test protein through HPLC method. Total of fifteen amino acid including seven essential amino acids (except tryptophan) and seven non essential amino acids were determined. Based on the amino acid content, Amino acid score (AAS), essential amino acid index (EAA index), nutritional index (NI) were also computed.

Essential amino acid index is the ratio of essential amino acid contained in a food to the essential amino acid content in reference protein (Ghosh and Chakravarthy, 1990). In the present investigation, EAA computation was done following the method suggested by Oser (1995). Amino acid score also coined as chemical score is considered as second alternative to the animal feeding studies for the determination of nutritional value. It is based on the amount of limiting amino acid present in the test protein in relation to its presence in reference protein. Amino acid score was calculated using the following formula.

$$\text{Amino acid score} = \frac{\text{mg of amino acid/ g test protein}}{\text{mg of amino acid/ g reference protein}} \times 100$$

In the present investigation the EAA index was computed using the formula given below

$$\text{EAA index} = \frac{\text{Geometric mean of amino acid in the FFS}}{\text{Geometric mean of amino acid in the reference protein}} \times 100$$

Nutritional index (NI) based on the protein quality was computed using the formula presented by Crisan and Sanda (1978).

$$\text{Nutritional index (NI)} = \frac{\text{EAA index} \times \% \text{ protein}}{100}$$

3.2.1.2. Minerals and Vitamins

Even the most complex multivitamin cannot hope to mimic the content of a healthful diet that includes a wide variety of unprocessed foods since normal human exposure to vitamins and minerals interacts in complex ways with each other and there is individual variation in metabolizing these nutrients. Additionally, the components of single vitamin or multivitamin supplements may vary substantially from what is found in whole foods, which could alter biological impact (Balk *et al.*, 2006).

Oxidative damage by free radicals and other reactive species is ubiquitous and there are numerous, interacting biochemical mechanisms by which vitamins and/or minerals might protect against these effects, thus reducing both CVD and cancer risk (Grundy, 2005).

Fat-soluble antioxidant vitamins such as vitamin E circulate principally in lipoproteins, especially LDLs. Oxidized LDL is highly atherogenic and vitamin E protects against this oxidation. To maintain vitamin E in its antioxidant or reduced state, however, circulating, water-soluble antioxidants such as vitamin C are required (Rashid *et al.*, 2003).

Natural, enzymatic antioxidants (e.g., superoxide dismutase, glutathione peroxidase) catalyze the reactions that suppress free radicals and peroxide and contain copper, zinc, and manganese as integral parts of their structure, providing a rationale for supplementing with minerals (Hill *et al.*, 2005).

Several B vitamins (folate, B_6, and B_{12}) are important in homocysteine metabolism. Vitamin E (gamma-tocopherol), zinc, and vitamin A are thought to

inhibit inflammation, another presumed protective mechanism provided by vitamins and minerals. In addition, other effects of vitamins relevant to other chronic diseases, such as enhanced immunity (vitamins A, C, and E and zinc and calcium) or stimulation of collagen synthesis (vitamin C) (Wing and Phelan, 2005).

Table 04. Analytical procedures for minerals

Minerals	Method
Potassium	(AOAC, 2005) Standard flame emission photometer
Sodium	(AOAC, 2005) Spectronic 20
Phosphorus	(AOAC, 2005) Spectronic 20
Calcium	Titrimetry
Magnesium	Titrimetry
Iron	(AOAC, 2005) Atomic Absorption Spectrophotometer
Zinc	(AOAC, 2005) Atomic Absorption Spectrophotometer
Copper	(AOAC, 2005) Atomic Absorption Spectrophotometer
Manganese	(AOAC, 2005) Atomic Absorption Spectrophotometer
Selenium	(AOAC, 2005) Atomic Absorption Spectrophotometer

3.2.1.3. Vitamins

Table 05. Analytical procedures for vitamins

Vitamins	Method
ß- carotene	Colorimetric method -Sadasivam and Manickam (1992)
Thiamine	Flurimetric method -Sadasivam and Manickam (1992)
Ribloflavin	Flurimetric method -Sadasivam and Manickam (1992)
Niacin	Colorimetric method -Sadasivam and Manickam (1992)
Folic acid	AOAC (2005) - HPLC
Vitamin C	Sadasivam and Manickam (1992)
Vitamin E	AOAC (2005) -HPLC

3.2.1.4. Phytochemicals

Phytochemicals are promoted for the prevention and treatment of many health conditions, including cancer, heart disease, diabetes, and high blood pressure. There is some evidence that certain phytochemicals may help prevent the formation of potential carcinogens (substances that cause cancer), block the action of carcinogens on their target organs or tissue, or act on cells to suppress cancer development (Premier, 2010).

Table 06. Analytical procedures for phytochemicals

Phytochemicals	Method
Flavonoid	Bohm and Kocipai- Abyazan (1974)
Poly phenols	Sadasivam and Manickam (1992)
Tannin	Sadasivam and Manickam (1992)
Saponin	Obdoni and Ochuko (2001)
Alkaloid	Harborne method (1973)
Oxalates	Colorimetric method (AOAC, 2005)

Determination of β-glucans

β -glucans are polysaccharides which occur as a principal component of the cellular walls of some microorganisms, such as yeast and mushrooms, and also cereals such as oats and barley. These substances stimulate the immune system, modulating humoral and cellular immunity, and thereby have beneficial effect in fighting infections (bacterial, viral, fungal and parasitic). β -glucans also exhibit hypocholesterolemic and anticoagulant properties. Recently, they have been demonstrated to be anti-cytotoxic, antimutagenic and anti-tumorogenic (Zekovic *et al.,* 2005).

The enzymatic method of b-glucan determination is based on the procedure of McCleary and Glennie-Holmes (1985) and a subsequent, more-streamlined procedure developed by McCleary and Codd (1991). More recently, the streamlined procedure was developed into official methods of analysis (AACC, 1995; AOAC Int., 2000). In

this study, the standard Megazyme procedure (Megazyme, 2001; http://www.megazyme.com) was carried out.

Determination of Total Antioxidant Capacity

Antioxidants plays an important role in the chronic disease prevention by arresting oxidative damage caused by reactive oxygen species (ROS) to vital bio molecules such as DNA, lipids, and proteins. The cellular damage caused by ROS has been implicated in the development of many disease states, such as cancer, diabetes, and cardiovascular disease, atherosclerosis, and neurodegenerative diseases. Because of their potential harmful effects, excessive ROS must be promptly eliminated from the cells by this variety of antioxidant defence mechanisms (Hollman, 2001).

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* (1999). The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. The sample was extracted using various solvents like petroleum ether, Chloroform – water mixture, Acetic acid, Acetone, Ethanol, Aqueous Ethanol, n Butanol, Benzene, Ethyl acetate, Diethyl ether and Hot water. 0.3 ml of extract was combined with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95^0 C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. The respective solvent (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Diphenyl Picryl Hydrazyl (DPPH) Free Radical Scavenging Activity

DPPH free radical scavenging assay was measured using DPPH free radical test, by employing the method of Wong *et al.* (2000). The different concentrations of each of the extracts were prepared in methanol and were added to 3ml of 0.1mM methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30 min at room temperature in the dark. Changes in absorbance of samples were measured at 517 nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid served as the standard. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula,

$$\% \text{ Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A_0 is the absorbance of the control
A_1 is the absorbance of test samples.

All the tests were performed in triplicates and the results are reported as IC50, which is the amount of antioxidant necessary to decrease the initial DPPH concentration by 50 per cent.

3.2.2. Functional Qualities

Food designers need to understand the properties of foods when they are designing new dishes, to make sure they match the product profile.

The functional properties for the final four combinations from each of the FFS techniques were carried out to identify the best suitable combination. The functional qualities studied were:

3.2.2.1. Bulk Density

50 g of sample was put into a 100 ml measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density (g cm^{-3}) was calculated as weight of sample [g] divided by volume of sample after tapping (cm^3) (Okaka and Potter, 1979).

3.2.2.2. Dispersibility

Dispersibility in water, which indicates the ability to reconstitute, was determined by the method of Kulkani *et al.* (1991). 10 g of powder sample were weighed into a 100 ml measuring cylinder. Distilled water was added up to 100 ml volume. The sample was vigorously stirred and allowed to settle for 3 hours. The volume of the solution was recorded and subtracted from 100 to give a difference that is taken as percentage dispersibility.

3.2.2.3. Rehydration Ratio

About 10g of the sample was mixed with 100 ml of distilled water and stirred for 5 minutes. The contents were filtered using a filter paper. The rehydrated sample was weighed (Ranganna, 1995).

$$\text{Rehydration Ratio} = \frac{\text{Weight of sample (g)}}{\text{Drained weight of sample (g)}}$$

3.2.2.4. Gelatinization Time and Temperature

10g of the sample was slowly stirred into 100 ml of water. Then it was cooked over direct heat. Finally, heated to boiling until the mixture appears thick. Once it

started thickening, stop heating and noted the temperature and time immediately (Swaminathan, 1999).

3.2.2.5. Processing Loss

$$\text{Processing loss} = \frac{\text{Initial weight – final weight after drying (g)}}{\text{Initial weight (g)}}$$

3.2.2.6. Yield Ratio

$$\text{Yield Ratio} = \frac{\text{Weight of FFS (g)}}{\text{Weight of ingredients (g)}}$$

The best identified combinations in each technique, i.e. FFS I and II were further subjected to analysis of other functional properties as follows:

3.2.2.7. Swelling capacity

Swelling pattern of the powder indicates the level of crystalline packaging of the starch granules present in the sample. Swelling capacity was determined by the method described by Lalude (2006) with modification for small samples. One gram of the flour sample was mixed with 10 ml of distilled water in a pre-weighed centrifuge tube and heated at 80^0 C for 30 min. This was continually shaken during the heating period. After heating, the suspension was centrifuged at 1000×g for 15 min. The supernatant was decanted and the weight of the paste was taken.

The swelling power was calculated as:

Swelling power (ml/g) = weight of the paste / weight of dry flour

3.2.2.8. Water Absorption Capacity

Water absorption capacity gives an indication of the amount of water available for gelatinization. Lower absorption capacity is desirable for making thinner gruels. It was determined according to method of Sathe and Salunkhe (1981) with slight modifications. One gram of the sample was added to 10 ml distilled water in a weighed centrifuge tube. The tube was agitated well by mixing for about 5 min and allowed to stand in room temperature for 15 min before being centrifuged at 5,000×g for 30 min. The mixture was decanted and the clear supernatant discarded. Adhering drops of water were carefully siphoned as much as quantitatively possible by allowing the tube to be inverted over absorbent paper and the tube was reweighed. Water absorption capacity was expressed as the weight of water bound by 100 g dry powder.

3.2.2.9. Water Absorption Index (WAI)

The water absorption index (WAI) measures the volume occupied by the starch granule after swelling in excess of water. Water absorption index (WAI) was measured using the technique according to Anderson *et al.* (1969). 2.5 g of the sample was suspended in 30 ml of distilled water in a 100 ml centrifuge tube at room temperature for 30 minutes with gently intermittent stirring, and then centrifuged at 3000 rpm for 10 minutes. The supernatant was poured into a petri dish and the remaining gel was weighed. WAI is the weight of gel obtained after removal of the supernatant and is expressed as the ratio of the weight of obtained gel per gram of sample (g/g).

3.2.2.10. Water Solubility Index (WSI)

Water solubility index (WSI) determines the amount of free molecules leached out from the starch granule in addition to excess water. It was measured using the technique of Anderson *et al.* (1969). The WSI was calculated from the weight of dry solids recovered by evaporating the supernatant from the water absorption index (WAI) which was dried in a hot air oven at 110°C. The WSI is the weight of dry soluble solids in the supernatant, is expressed as a percentage of the original weight of sample (g/g).

3.2.3. Physico - Chemical Properties

The physico chemical properties of the samples like pasting properties, textural properties, colour, particle size etc were analysed using various instruments.

3.2.3.1. Pasting Properties

A Rapid Visco Analyser (RVA, Newport Scientific Pty. Ltd., Warriewood, Australia) was used to measure the apparent viscosity of samples as a function of temperature. Three grams of sample, adjusted to 14 per cent water on a wet basis, were added to 25 g of distilled water. The samples were initially kept at 25 °C for 4 minutes, then heated to 95 °C at a constant heating rate of 14 °C/minute, held at this temperature for 3 minutes, cooled down to 25 °C in 5 minutes at the same rate, and finally held at 25 °C for an additional 4 minutes. The paste viscosity responses were: cold viscosity (CV, defined as the peak viscosity in the beginning of the RVA curve at 25 °C), peak viscosity (PV, maximum viscosity during heating), breakdown (BD, difference between the peak viscosity and the lowest viscosity after heating ramp) and setback (SB, difference between the maximum viscosity during cooling and the lowest viscosity after the heating ramp).

3.2.3.2. Textural Properties

Texture is an important attribute in that it affects processing and handling, influences habits, and affects shelf-life and consumer acceptance of products. Texture analysis is the mechanical testing of food, cosmetics, pharmaceuticals, adhesives and other consumer products in order to measure their physical properties. Because of its adaptability, texture analysis has become commonplace in many industries to measure a specific or range of characteristics or properties relating to the way a material behaves, breaks, flows, sticks, bends, etc. (Hoover *et al.*, 2003).

Major manufacturers routinely apply texture analysis techniques both in new product development and as part of quality control in all stages of manufacture. It is a cost-effective method to determine the effects of raw material quality or the adjustment of formulation or processing variables on end product acceptability. Where problematic textural issues occur during storage or transportation, texture analysis can provide a useful assessment. It may also prove to be an effective means of comparison with competitive products, or where claims substantiation is necessary to take a technical pro-active stance in market (Lambo *et al.*, 2004).

The textural properties of the FFS I & II, like: hardness, cohesiveness, adhesiveness, gumminess, springiness, chewiness and stringiness were analyzed using Stable Microsystem TA HD PLUS, Model No. 5192.

3.2.3.3. Colour Attributes

Surface color of FFS I & II was measured using a HunterLab D25-2 Color Difference Meter (Hunter Associates, Inc., Reston, VA). L*(lightness), a*(redness) and b*(yellowness) were evaluated and recorded. The parameters determined were L* (L* = 0 [black] and L* = 100 [white]), a* (−a* = greenness and +a* = redness) and b* (−b* = blueness and +b* = yellowness).

3.2.3.4. Particle Size

The particle size of food products especially flours is one of the most important characteristics, which may influence other physicochemical properties such as swelling power; paste clarity, and water-binding capacity (Singh *et al.*, 2003).

Particle size of FFS I & II were analyzed using Zetasizer Ver. 6.12, Malvern Instruments Ltd, Serial Number: MAL1043157.

3.2.4. Storage Studies

Assessment of shelf life quality is important since it determines the suitability of a particular ingredient for the product development. Shelf life is the recommendation of time that products can be stored, during which the defined quality of a specified proportion of the goods remains acceptable under expected conditions of distribution, storage and display (Azanha and Faria, 2005).

To observe the keeping quality, the FFS (I & II) were stored separately in heat sealed laminated polythene packs and stored at ambient conditions. Moisture, peroxide value (Sadasivam and Manickam, 1992) and microbial contaminations were analyzed initially and monthly up to a period of six months. 50 g of FFS I & II were stored separately in 30 laminated pouches each, at the rate of 5 pouches for each FFS to be picked randomly every month. The values were analyzed in triplicates. The randomly picked 3 packets every month was used for all the analysis of moisture, peroxide value and microbial contaminations.

Total Microbial Growth

The stored product samples were assessed for the presence of various micro organisms viz. bacteria, actinomycetes, fungus and coliforms. The serial dilution of samples followed by pour plating was employed to estimate the population of viable

micro - organisms in the FFS I & II (Johnson and Curl, 1973). The procedure adopted for serial dilution was as follows. One ml from each sample was taken in a test tube containing 9ml sterile water, making the dilution of 10^{-1}. From this one ml of the dilution was further transferred into test tube containing sterile water, so that dilution becomes 10^{-2}. Likewise further dilutions of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} were made.

Nutrient agar (NA), Potato Dextrose Agar (PDA), Eiosin Methylene Blue (EMB) and Ken knight's reagent (KEN) medium were used for culturing of bacteria, fungi, coliforms and actinomycetes respectively. One ml of the suspension from each dilution was transferred on to plates followed by the respective media. The whole procedure was done aseptically in a laminar air flow chamber. Plates were kept for incubation at 28°C. Colonies appearing in the plates were recorded after two days in the case of bacteria and after four days for fungi and yeast. The microbial load of the samples was then expressed as cfu/g of the flour or mixes.

The number of dilutions to be made was standardized for FFS I & II and finally fixed up to 10^{-3} for both the samples.

3.2.5. Cost of Production

Cost of production of FFS I & II was worked out in terms of product yield and cost per kilogram of the products.

3.2.6. Suitability of Developing Various Food Preparations/Products Using FFS (I & II)

Sensory quality evaluation plays an important role in acceptability study of a new product (Jellinick, 1985). Quality is the ultimate criteria of the desirability of any product. The overall quality of a food depends on the nutritional and other

hidden attributes and sensory quality as assessed by means of human sense organs (Manay and Shadakshraswamy, 1998).

Sensory evaluation is considered to be an important analytical tool in a present day competitive corporate environment. Measuring the sensory properties and determining the importance of these properties as a basis for predicting acceptance by the consumer represents major accomplishments for sensory evaluation. Scientific methods of sensory analysis of foods are becoming increasingly important in assessing the acceptability of food products (Swaminathan, 1987). The evaluation is based on primary criteria, that is, sense of sight, smell, taste etc.

The sensory acceptance of the final four combinations from each of the FFS techniques was carried out to identify the best suitable combination using a five point scale and hedonic scale ratings.

Mudambi and Rajagopal (2000) were of the opinion that a person who eats a good breakfast performs better at work and is likely to eat less during the day. They also have expressed that those who have good breakfasts have a greater work output and are more alert. It is very important to plan breakfasts with foods, which are sufficient both in quantity and quality. About $1/4^{th}$ to $1/3^{rd}$ of the day's food allowance should be taken as breakfast.

Suitability of incorporating the FFS (I & II) into various preparations/products was tried out and subject to organoleptic evaluation for the sensory characteristics like colour and appearance, flavour, texture, taste and overall acceptability using a panel of trained members. The score card used for the evaluation of developed product is given in Appendix I.

Cahill *et al.* (2013) studied that adults who skipped breakfast were associated with excess body weight, hypertension, insulin resistance, and elevated fasting lipid

concentrations. Men who regularly skip breakfast are at 27 percent higher risk of suffering a heart attack or fatal coronary disease, compared to those who eat a morning meal daily; while women who skipped breakfast even once a week were 20 percent more likely to develop type 2 diabetes than those who ate a meal every morning.

Keeping the importance of breakfast in the management of lifestyle diseases, commonly consumed breakfast items like Idli, Dosa, Chappathi, Noolputtu, Puttu and porridge were standardized in the laboratory by the incorporation of FFS I & II. The qualities were assessed using a score card on a five point scale.

Portion sizing was fixed based on a pilot study done in the laboratory mainly focusing on the sensory acceptance on the level of incorporation of FFS I & II separately in different recipes. Substitution levels from 10 per cent to 50 per cent were initially carried out in various recipes to study the best level of acceptance.

Feasibility of substitution of the FFS (I & II) in the Food Exchange List, especially in the breakfast items was computed based on their nutrient contents. Carbohydrate exchange, protein exchange and fat exchange of FFS I & II were calculated using the available data.

3.3. EVALUATION OF CLINICAL EFFICACY OF FFS (I & II)

The clinical efficacy of the FFS (I & II) was ascertained through case studies.

3.3.1. Selection of Area

The study was conducted in the Elamkulam Panchayath, Malappuram district of Kerala, selecting respondents from a list comprising of 250 members. Clinical efficacy of FFS (I & II) was determined in the three disease conditions viz.

Hyperglycemia, Hypertension and Hyperlipidemia which is highly prevalent in our state.

3.3.2. Selection criteria

From the list of 250 members, people in the early stages of diseases like hyperglycemia, hyperlipidemia and hypertension were screened. Subjects who were not on medication were again scrutinized. Willingness of participation of the subjects throughout the period of study was confirmed. The final list of the subjects for the study was in the age group of 40-55 yrs, same gender and without any other complications. A reserve list was also maintained to overcome the problem of dropouts in the study.

3.3.3. Selection of Respondents

Five subjects each for three disease conditions and two different FFS developed were selected for the case studies. FFS I & II developed was supplemented in the breakfast of subjects for a period of three months. On the whole, thirty subjects were finally selected for the conduct of the study.

3.3.4. Conduct of Study

After the selection process, preliminary information regarding their socio-economic profile, health status, dietary and life style pattern and nutritional status were collected through a suitably structured questionnaire (Appendix II).

3.3.4.1. Socio-economic Profile

The socio economic profile of the subjects such as socioeconomic status, religion and family background in general has a very distinct part to play in

determining attitude and food consumption, health and behavioural pattern of the individual (Arrora, 1991).

In order to elicit information on socio-economic profile of the respondents details regarding age of the subjects, family income, type and size of the family, religion, educational status, money spent on food and health care etc. were collected using the questionnaire.

- **Age**

It refers to the number of calendar years completed by the respondents at the time of interview. This variable measured directly by asking the respondent the number of years he/she completed at the time of investigation (Sindhudevi, 1994).

- **Family Income**

Monthly family income from all sources was taken into account for measuring this variable.

- **Family size**

In the present study family size was measured by taking into consideration the specific number of members in the family of the respondents.

- **Educational status**

It is defined as the formal education attained by the respondent (Jayalekshmi, 2001).

3.3.4.2. Dietary Habits and Meal Pattern

Dietary pattern have been used to identify typical combinations of food which is associated with disease risk. According to Swaminathan (1993), through diet

surveys, information on nutrient intake level, source of nutrients, food consumption pattern and preferences of the subjects could be collected. Food habits of the respondents were collected in order to understand whether diet has any influence on their disease condition. In the dietary habits, details on number of main meals and snacks per day; their preference of Non Vegetarian foods, individual food plans if any, etc. were inquired.

Meal pattern of the subjects, inclusion of fruits in the daily diet, food frequency, type of oil used for cooking, habit of watching television while eating, etc were also noted.

Food frequency method was done to assess how often specific foods are eaten which are important contributors to the intake of energy and other nutrients. The data was collected using food frequency table. The total score for each food group used by the subject as well as preference scores of the subject for different items were calculated separately using the formula suggested by Raeburn *et al.* (1971).

$$\frac{R_1S_1 + R_2S_2 + R_3S_3 + \ldots\ldots\ldots\ldots + R_nS_n}{100} = \frac{\sum R_iS_i}{100}$$

Where,

$S_1 = n = 6$, $S_2 = n\text{-}1 = 5$, $S_n = 1$

S_i = scale rating given for frequency of use of food item ($i = 1, 2, 3...n$)

R_i = percentage of respondents placed under each frequency group ($i = 1, 2, 3...n$)

$$\text{Percentage of total score for each food group} = \frac{\text{Mean score}}{n} \times 100$$

Where, n = 6 for 6 fold classification.

3.3.4.3. Medical and Health Status

Details on the medical history of the subjects, family history of diseases, use of medications and morbidity status of the respondents were estimated. Home remedies if any used for management of the diseases, personal habits like consumption of alcohol, smoking, and exercise pattern etc. were also collected.

3.3.4.4. Nutritional Status

According to Kamath (1986) nutritional status is defined as the state of health enjoyed as a result of nutrition. It is one of the critical indicators of health, therefore regular nutritional assessment is important to maintain the health of respondents (Mourya and Jaya, 1997).

Nutritional status of the selected respondents was assessed through anthropometry. Anthropometry provides the single most universally applicable, inexpensive technique for assessing the size, proportions and composition of the human body. Anthropometry has been accepted as an important tool for the assessment of nutritional status (Vijayaraghavan, 1987).

Anthropometric measurements relevant to the study include height, weight, BMI and Waist – Hip ratio.

- **Height**

Height or the total length apart from nutritional and environmental factors is influenced by hereditary factors. The extend of height deficit in relation to age as

compared to regional standards is regarded as a measure of the duration of malnutrition (Gopaldas and Sheshadri, 1987).

To determine height, an anthropometric rod was used. The rod was fixed vertically on a smooth wall, perpendicular to the ground taking care to see that the floor was smooth.

The subjects were asked to remove their slippers and to stand with feet paralleled and heels, buttocks, shoulder and back of head touching the wall. The head was held comfortably erect; the arms hanging close by the side. A smooth, thin ruler was held on the top of the head in the centre crushing the hair at angles to the wall and the height read off from the lower edge of the ruler to the nearest 0.1 cms. An average of the three measurements was taken as final measurement of height of the respondents.

- **Weight**

Weight is the measurement of body mass (Rao and Vijayaraghavan, 1986). According to Kaul and Nyamongo (1990) a change in body weight may be the result of changes in the health of an individual, changes in food consumed or even changes in one's physical activity.

For weighing, platform weighing balance was used as it is portable and is convenient to use in the field. The weighing scale was checked periodically for accuracy. The scale was adjusted to zero before each measurement. The subjects having minimum clothing were asked to stand on the platform of the scale, without touching anything and looking straight ahead. The weight was recorded to the nearest of 0.5 Kg. Each reading was taken thrice to ensure correctness of the measurement.

❖ **Body Mass Index (BMI)**

BMI is expressed as the ratio of weight to height square i.e. Weight (Kg) / Height (m^2) was used as a good parameter to grade chronic energy deficiency (James *et al.,* 1988).

$$BMI = \frac{Weight(Kg)}{Height(m^2)}$$

❖ **Waist-Hip ratio**

The Waist Hip Ratio (WHR) reflects the proportion of body fat located intradominally as opposed to that in the subcutaneous region (Lean *et al.,* 1995). Waist was measured using a measuring tape above the umbilicus meaning the narrowest circumference and hip was measured in the broadest area of hip. After documenting the waist and hip measurements of the respondents their WHR was calculated by dividing the circumference of the waist by the circumference of the hip (Chadha *et al.,* 1995).

3.3.4.5. Other Details

Details on stress level, frequency of health checkups and blood profile monitoring and also opinion on dietary modifications of the respondents were also documented using the questionnaire.

3.3.5. Diet Counseling

Nutrition education is a process by which beliefs, attitudes, environmental influences and understandings about food lead to practices that are scientifically sound, practical and consistent with individual needs. Individual counselling was imparted to the selected respondents under case study regarding the dietary regime to

be followed for the specific disease condition. Explanations on need for special diet, foods to be restricted for disease condition, dietary modifications to be followed, method of incorporation of the supplement in the diet were also given. To avoid monotony, which is common when consuming the same food daily, different recipes in which the supplement can be incorporated were demonstrated.

3.3.6. Impact Evaluation of the Supplements (FFS I & II)

The amount of FFS (I & II) to be supplemented to the respondents was optimized based on the portion sizing and sensory attributes of the products. The optimized quantities of the FFS I & II to be supplemented for each day to the subjects were packed into polythene pouches for saving them the inconvenience of measuring the supplements daily and also to confirm the right amount of intake.

Any laboratory investigation should reach the community to make the research more meaningful and finally the society has to be benefited. The transition from laboratory to the domestic table is something of a quantum leap by any stretch of the imagination (Lawless and Meiselman, 1992). Impact of the supplementation of FFS (I & II) on the subjects was monitored initially (before), intermittently (after 45 days) and finally (after) the conduct of the study. Frequent follow ups were done to understand the difficulties if any faced by the subjects in incorporating the supplements in their daily diet and to also to confirm there is no plate waste.

3.3.7. Monitoring Indicators

Clinical parameters like Fasting Blood Sugar, Post Prandial Blood Sugar, Glycemic Index, blood pressure and lipid profile, general health and morbidity of the subjects was monitored before and after the conduct of the study.

Blood profiles were monitored by using standard procedures followed in the clinical laboratories. General health and morbidity was ascertained with the help of a medical practitioner.

3.3.7.1. Glycemic Index (G.I)

3.3.7.1.a) Conduct of Glucose Tolerance Test (GTT) of the respondents:

For the conduct of GTT, the subjects were asked to fast overnight. Venous blood samples were collected for determining their fasting blood glucose level. In order to get the glucose tolerance, the subsequent blood glucose was recorded at 0 minutes, 30 minutes, 60 minutes, 90 minutes and 120 minutes. Procedure followed is appended (Appendix III).

3.3.7.1. b) Computation of Glycemic Index

Glycemic index of the test foods (FFS I & II) was calculated as per the method suggested by Jenkins (1982).

Each subject was given 50g of glucose and portions of test food (FFS I & II) containing 50g available carbohydrate on separate occasions after an overnight fasting. A gap of one week's time was given in between the testing of glucose, FFS I & FFS II respectively.

Venous blood samples were collected before the administration of glucose, FFS I & II to access the fasting blood sugar level of the subjects. After the administration of test meal, blood glucose level of the subjects were analysed independently at half hour interval up to two hours (1/2 hr, 1 hr, 11/2 hr and 2 hr).

The response of the reference food mainly glucose and also that of the test food administered on the subjects were plotted, against time 't'.

The area under curve (AUC) thus obtained was found out from test food as well as for reference food (glucose). The Glycemic Index (GI) of test food is computed as the ration of AUC of test food and AUC of reference food.

$$G.I. = \frac{AUC(Test\ food)}{AUC(reference\ food)} x\,100$$

Using the glycemic index, glycemic load (G.L.) was also calculated. The formula used for finding out the glycemic load was

$$G.L. = \frac{G.I.X\ Available\ CHOH\ in\ the\ portion\ size}{100}$$

3.4. STATISTICAL ANALYSIS

In order to obtain meaningful results, the data generated was subjected to the following statistical analysis:

Mean values of triplicate determinations were reported with their standard deviations. Analyses of Variance (ANOVA) and Student's't' test were achieved to calculate significant differences in the treatment means, and the mean separations were achieved by Duncan's Multiple Range Test (DMRT). Regression analysis was the other statistical tool used.

Selection index was developed to compare and identify the most suitable supplements among FFS I & II. This index is the weighted average of observed values across all variables in a group, with weights as the inverse of the variance of observation across samples. Then the product index is the average over the samples of the product.

The weighted average is used to calculate the average value of a particular set of numbers with different levels of relevance. The relevance of each number is called its weight. The weights should be represented as a percentage of the total relevancy. Therefore, all weights should be equal to 100 per cent, or 1.

$$\bar{x} = \frac{\sum_{i=0}^{n-1} W_i x_i}{\sum_{i=0}^{n-1} W_i}$$

Where, X = weighted mean variable

W_i = allocated weighted value

X_i = observed value

Results

4. RESULTS

The results of the present study entitled "**Development, quality assessment and clinical efficacy of 'Functional Food Supplement' (FFS) for life style disease management**" are detailed in this chapter under the following headings:

4.1. Development of 'Functional Food Supplement' (FFS)

4.2. Quality evaluation of the identified FFSs

4.3. Evaluation of clinical efficacy of the identified FFSs

4.1. DEVELOPMENT OF 'FUNCTIONAL FOOD SUPPLEMENT' (FFS)

4.1.1. Development of FFS I – Dehydration Technique

Search for the best nutritious food to have a healthy and disease free long life has always been both a desire and incessant effort of humankind ever since the evolution of *Homosapiens*. It is now well known that there exists a strong connection between what we eat and our health. A better understanding of the same has indeed led to the development of alternate mode of healthcare through the right choice of food and nutrition (Pushpangadan *et al.*, 2013).

Focusing on the main objective of the study, to develop a nutraceutical mix using available food items that are rich in bioactive compounds and which will have the twin ability of nourishment and therapeutic action; the constituents for the FFS contained barley, ragi and banana, defatted soy flour, drumstick leaves powder and mushroom powder in different proportions. Dehydration and fermentation were the two processing techniques applied to standardize Functional Food Supplement (FFS). Proportions were optimized based on their nutritional and health promoting properties.

Among the twenty combinations worked out (Table No: 01) as described in the methodology, different levels of screening was done based on their nutritional qualities viz. low calories, low fat, adequate carbohydrates, sufficient protein, high fiber and required micronutrients suitable for the management of lifestyle diseases. Besides, sensory quality of the developed products was another criterion for the selection of best treatment.

Based on the above descriptions, the four combinations (combination X, XI, XII and XIV) were selected under dehydration technique of FFS. These four combinations were subjected for further in depth investigations on functional properties and sensory evaluation in order to select the best suitable combination. In the following descriptions the combinations X, XI, XII and XIV will be represented as FFS DT_1, DT_2, DT_3, and DT_4 respectively.

4.1.1.1. Functional Properties

The functional qualities help in the quality assessment and acceptability of any product. The functional qualities of the FFS developed involving the dehydration technique is given in the following tables.

Table 07. Bulk density, Dispersibilty and Rehydration ratio of FFS I

S. no	FFS	Bulk density (g/cm^3)	Dispersibilty (%)	Rehydration ratio
1	DT_1	0.82 ^{a}	9.33	0.26 ^{a}
2	DT_2	0.78^b	8.0	0.25^{ab}
3	DT_3	0.75 ^{c}	8.67	0.25^{bc}
4	DT_4	0.75 ^{c}	7.33	0.23^c
	± S.E.M	0.003	0.577	0.002
	CD	0.0109	-	0.0054

Values are means of triplicates.
S.E.M: standard error of the mean.
Values with the same superscript letter in a row are not significantly different at 5%.
** ($p < 0.01$).

From the above table it is clear that the bulk density ranged between 0.75 to 0.82 g/ cm^3 and the combination DT_3 and DT_4 are comparatively less than the other two but is on par with each other. The bulk density is a reflection of the load the flour samples can carry, if allowed to rest directly on one another. The density of processed products dictate the characteristics of its container or package, besides it influences the amount and strength of packaging material, texture or mouth feel. With respect to dispersibility, Table 07 showed that there was no significant variation among the combinations.

The rehydration ratio shows that combination DT_1 (0.26) had better rehydration and combination DT_4 (0.23) was less rehydratable. Lesser the rehydration ratio and dispersibility better the suitability of the combination for supplementation for management of lifestyle diseases. The variations among the four treatments were significant even at 1 per cent. However DT_2 and DT_3 were on par with each other.

Table 08. Gelatinization time and temperature of FFS I

S. no	FFS	Gelatinization time (min)	Gelatinization temperature (° C)
1	DT_1	1.25 [a]	92.7[b]
2	DT_2	1.26 [a]	96.0[a]
3	DT_3	1.05[b]	88.7[c]
4	DT_4	0.48 [c]	85.3[d]
	± S.E.M	0.007	0.408
	CD	0.217	0.331

Values are means of triplicates.
S.E.M: standard error of the mean.
Values with the same superscript letter in a row are not significantly different at 5%.
** ($p < 0.01$)

The gelatinization time varied from 0.48 to 1.26 min. The combination DT_4 took the least time to gelatinize (0.48 min) while combination DT_2 had the highest gelatinization time of 1.26 min. Also combination DT_4 had the lowest gelatinization temperature (85.3° C) while combination DT_2 had the highest gelatinization temperature (96° C).

Table 09. Processing loss and Yield ratio of FFS I

S. no	FFS	Processing Loss	Yield ratio
1	DT_1	0.56[a]	0.44[c]
2	DT_2	0.54[b]	0.46[b]
3	DT_3	0.48[c]	0.52[a]
4	DT_4	0.48[c]	0.52[a]
	± S.E.M	0.004	0.004
	CD value	0.0133	0.0133

Values are means ± SD of triplicates.
S.E.M: standard error of the mean.
Values with the same superscript letter in a row are not significantly different at 5%.
** ($p < 0.01$)

The processing loss will affect the qualitative and quantitative characters of food. Processing loss of combination DT_3 (0.48) and DT_4 (0.52) were the lowest while greater processing loss was noted in combination DT_1 (0.56).

The yield ratio was highest for combination DT_3 (0.52) and DT_4 (0.52) while combination DT_1 had the lowest yield ratio of 0.44. Lesser the processing loss greater will be the yield ratio. These correlations are clear from the above tables.

From the above tables and explanations it was evident that FFS DT_4 proved better in most of the functional qualities. In order to confirm the above findings, further studies on sensory qualities were evaluated.

4.1.1.2. Sensory Evaluation of FFS – Dehydration Technique

Sensory analysis is a scientific discipline that applies principles of experimental design and statistical analysis to the use of human senses viz. sight, smell, taste, touch and hearing for the purposes of evaluating consumer products (IFT, 2005). The sensory parameters such as colour & appearance, flavour, texture, taste, and overall acceptability of any food product depends on the extent of oxidation of fats and oils in the food due to the formation of peroxides, aldehydes and ketones (Gupta, 2005).

Although sensory evaluation of foods is the most important quality assessment, taste evaluations are not practical for routine quality control. It is always preferable to have a quantitative method for which rejection points may be established by sensory means (Jonnalagadda *et al.*, 2001). The discipline requires panels of human assessors, on whom the products are tested, and recording the responses made by them. When food is assessed by human sensory organs, the evaluation is said to be sensory analysis (Simi, 2002). Numerical scoring is used to

evaluate particular characteristics of one or more samples indicating the rating as excellent, very good, good, fair and poor (Manay and Swamy, 2000).

Sensory evaluation of the FFS powders and cooked FFS in the form of porridge was carried out. 15 g of the FFS added to 100 ml of boiling water and cooked for 3 min with a pinch of salt and continuous stirring was given for evaluation.

Table 10. Sensory evaluation scores of FFS I (POWDER)

FFS	Colour and Appearance	Texture	Taste	Flavour	Overall Acceptability
DT_1	3.7	3.2	3.2^{cd}	2.8	3.4^{bc}
DT_2	3.9	3.5	3.3^{bc}	3.2	3.8^{ab}
DT_3	3.5	3.6	3.0^{d}	3.2	3.2^{c}
DT_4	4.3	3.7	4.0^{a}	3.7	4.0^{a}
± S.E.M	0.23	0.17	0.23	0.23	0.19
CD	-	-	0.657	-	0.558

Values are means of determinations with 10 panelists.

S.E.M: standard error of the mean.

Values with same superscript letter in a column are not significantly different at 5%.

* ($p < 0.05$)

Colour and Appearance

Colour is one of the important visual attribute that has been used to judge the overall quality of foods for a very long time. If the colour is unattractive, a potential consumer may not be impressed by any other attributes. The first impression of food

is usually visual and a major part of willingness to accept a food depends on its appearance. The colour and appearance of four combinations of FFS powder developed did not exhibit any difference though they were in a score ranging between 3.5 (DT_3) to 4.3 (DT_4).

Texture

Texture constitutes a physical property of food stuffs apprehended by the eye, skin and muscle senses located in the mouth. The texture of FFS in powder form did not show any significant differences in scores. The scores were in between 3.2 for DT_1 and 3.7 for DT_4.

Taste

The taste is the major attribute which determine the acceptability of a food. Taste is the sensation produced when a substance in the mouth reacts chemically with receptors of taste buds. Maximum score for taste was noticed in FFS DT_4 (4.0), followed by DT_2 (3.3), DT_1 (3.2) and DT_3 (3.0) and also the scores varied significantly at 5 per cent.

Flavour

Odour preference is generated by stimulation of sensory cells by specific volatile compounds present in foods. The flavour of FFS did not differ in scores. DT_1 obtained the least scores of 2.8. DT_2 and DT_3 received a score of 3.2 each. Maximum score was for DT_4 (3.7).

The overall acceptability score found that combination DT_4 (4.0) was the most preferred, followed by combination DT_2 (3.8) and DT_1 (3.4) respectively. Combination DT_3 (3.2) was the least preferred. Since combination DT_2 lacked drumstick leaves powder the colour was more over similar to that of combination

DT_4 and equally acceptable. The higher percentages of mushroom powder in combination DT_1 scored least for flavour. Taste and overall acceptability of the four combinations of FFS were significantly different at 5 per cent.

Table 11. Sensory evaluation scores of cooked FFS I (PORRIDGE)

FFS	Colour and appearance	Texture	Taste	Flavour	Overall Acceptability
DT_1	3.1	3.5	2.8^{d}	3.0	3.2^{c}
DT_2	3.4	3.7	3.5^{bc}	3.2	3.6^{bc}
DT_3	3.0	3.6	2.9^{cd}	3.1	3.1^{d}
DT_4	3.9	4.0	4.0^{a}	3.7	4.2^{a}
± S.E.M	0.25	0.27	0.23	0.22	0.21
CD	-	-	0.658	-	0.614

Values are means of determinations with 10 panelists.

S.E.M: standard error of the mean.

Values with same superscript letter in a column are not significantly different at 5%.

* ($p < 0.01$)

There were no significant differences in colour, appearance, texture and flavour of the porridge. However, taste and overall acceptability showed variation at 1 per cent. DT_4 scored the maximum (4.0) while DT_1 scored the least (2.8) in the case of taste.

The overall acceptability score of FFS porridge showed that combination DT_4 (4.2) was the most preferred followed by combination DT_2 (3.6) and DT_1 (3.2) respectively. Combination DT_3 (3.1) was the least preferred. The higher percentage of mushroom powder in combination DT_1 scored least for flavour and taste but was

not significantly different from the other at 1 per cent. The observations showed that the scores were similar for FFS powder and porridge.

Table 12. Hedonic scale ratings of FFS I (PORRIDGE)

S. no	FFS	Hedonic Scale Ratings
1	DT_1	6.1^{d}
2	DT_2	6.7^{bc}
3	DT_3	6.3^{cd}
4	DT_4	7.4^{a}
	± S.E.M	0.244
	F value	**
	CD value	0.695

Values are means of determinations with 10 panelists (1-9 point scale)

S.E.M: standard error of the mean.

Means with different superscript letters in the same column are significantly different at 5%.

** ($p < 0.01$)

Table 12 elicited that combination DT_4 scored the highest hedonic scale rating (7.4) followed by combination DT_2 (6.7) and DT_3 (6.3). Combination DT_1 (6.1) was the least liked.

With respect to functional qualities and sensory evaluation, combination DT_4 proved to be the best suitable combination in the dehydration technique of development of FFS. Indepth analysis of FFS combination DT_4, which would be denoted as FFS I in the further detailing was carried out.

4.1.2. Development of FFS II – Fermentation Technique

Fermented foods with both its prebiotic and probiotic effect is finding a greater role in the field of nutraceuticals and functional foods. Hence the second method of processing was considered as fermentation.

As in the case of FFS I, in the fermentation technique which was adopted as the second method, twenty combinations were worked out (Table 01) and different levels of screening was done based on their nutritional qualities and also on their sensory qualities to choose the best combination of FFS. Combination XIV was identified for further treatment. This combination was further subjected to four different fermentation techniques (Table 02) and named as FFS FT_1, FT_2, FT_3, and FT_4 respectively.

Fermented foods with their microbial activity play an essential role in conferring the required stability, safety and sensory properties to the product. On the nutritional side, fermentation helps in degradation of anti-nutritional factors and increases mineral bio-availability, protein digestibility of tannin-rich cereals, and degradation of flatulence-causing oligosaccharides. Reduction of viscosity of starchy porridges is reached by addition of germinated cereal grains; this enables the preparation of porridges of increased nutrient density (Stanton *et al.*, 2005).

4.1.2.1. Functional Properties of FFS II

Quality assessment and acceptability of any product can be studied with the help of functional qualities. The functional qualities of the FFS developed involving the fermentation technique is given in the following tables.

Table 13. Bulk density, Dispersibilty and Rehydration ratio of FFS II

S. no	FFS	Bulk density (g/cm^3)	Dispersibilty (%)	Rehydration ratio
1	FT_1	0.89	1.33	0.26^{d}
2	FT_2	0.88	1.33	0.28^{a}
3	FT_3	0.88	2.67	0.27^{bc}
4	FT_4	0.89	2.67	0.27^{c}
	± S.E.M	0.009	0.471	0.003
	CD value	-	-	0.01

Values are means.

S.E.M: standard error of the mean.

Values with the same superscript letter in a row are not significantly different at 5%.

** ($p < 0.01$)

The functional properties viz. bulk density and dispersibility did not have any significant difference among the four treatments.

Rehydration ratio was highest for the treatment FT_2 (0.28) followed by FT_3 (0.27) and FT_4 (0.27). Treatment FT_2 had the least rehydration ratio (0.26) and there was significant differences among the values at 1 per cent level.

Table 14. Gelatinization time and temperature of FFS II

S. no	FFS	Gelatinization time (min)	Gelatinization temperature (° C)
1	FT_1	2.53^a	90.33^b
2	FT_2	1.53^b	94.0^a
3	FT_3	1.25^c	87.33^d
4	FT_4	1.16^d	88.33^c
	± S.E.M	0.012	0.204
	CD value	0.04	0.63

Values are means of triplicates.

S.E.M: standard error of the mean.

Values with the same superscript letter in a row are not significantly different at 5%.

** ($p < 0.01$)

The gelatinization time was highest (2.53 min) for FT_1 and lowest (1.16 min) for FT_4. This makes treatment FT_4 to be a suitable combination as it is less time consuming on cooking. On considering the gelatinization temperature, treatment FT_2 had the highest (94.0° C) while FT_3 (87.33° C) had the least and all the four treatments were significantly different from each other at 1 per cent level.

Table 15. Processing loss and Yield ratio of FFS II

S. no	FFS	Processing Loss	Yield ratio
1	FT_1	0.46^{d}	0.54^{a}
2	FT_2	0.48^{bc}	0.50^{d}
3	FT_3	0.49^{a}	0.51^{c}
4	FT_4	0.48^{c}	0.52^{b}
	± S.E.M	0.002	0.002
	CD value	0.006	0.006

Values are means of triplicates.

S.E.M: standard error of the mean.

Values with the same superscript letter in a row are not significantly different at 5%.

** ($p < 0.01$)

Treatment FT_3 (0.49) had the highest processing loss followed by FT_2 (0.48) and FT_4 (0.48) and minimal processing loss was noted in treatment FT_2 which was also significantly different. On the other hand, treatment FT_1 (0.54) had the highest yield ratio and FT_2 (0.50) had the least value. Based on the processing loss and yield ratio treatment FT_1 can be considered as the suitable treatment as the processing loss was minimal and the yield ratio maximum. Sensory evaluation and hedonic scale ratings were carried out to substantiate the selection of best combination.

Table 16. Sensory evaluation scores of FFS II (POWDER)

FFS	Colour and Appearance	Texture	Taste	Flavour	Overall Acceptability
FT_1	3.4	3.2	2.8	2.9	3.0
FT_2	3.1	3.2	2.4	3.1	2.8
FT_3	3.6	3.5	2.8	3.1	3.4
FT_4	3.3	3.4	2.9	3.1	3.4
± S.E.M	0.26	0.22	0.23	0.25	0.27
CD value	-	-	-	-	-

Values are means of triplicates.

S.E.M: standard error of the mean.

The above table elucidated that there was no significant difference in the color and appearance, texture, taste, flavor and overall acceptability among the FFS powders of the four treatments of fermentation.

It was difficult to conclude on the best treatment technique from the above findings. Hence sensory evaluation of the cooked form of the four treatments of FFS were proceeded to select the suitable treatment.

Table 17. Sensory evaluation scores of cooked FFS II (PORRIDGE)

FFS	Colour and Appearance	Texture	Taste	Flavour	Overall Acceptability
FT_1	3.2	3.2	2.7	2.6	3.0
FT_2	3.2	3.1	2.4	2.7	2.7
FT_3	3.1	3.2	2.7	3.2	2.9
FT_4	3.1	3.4	2.8	2.8	3.0
± S.E.M	0.28	0.24	0.28	0.28	0.49
CD value	-	-	-	-	-

Values are means of triplicates.

S.E.M: standard error of the mean.

Similar to that of sensory evaluation scores of FFS powder (Table 17) developed using four different fermentation techniques there was no significant difference in the color and appearance, texture, taste, flavor and overall acceptability among the cooked FFS of the four treatments also.

Hence hedonic scale ratings of the cooked form of the four treatments of FFS II was proceeded to select the suitable treatment.

Table 18. Hedonic scale ratings of FFS II (PORRIDGE)

S. no	FFS	Hedonic Scale Ratings
1	FT_1	6.3
2	FT_2	5.9
3	FT_3	6.7
4	FT_4	6.8
	± S.E.M	0.49
	CD value	-

Values are means of determinations with 10 panelists (1-9 point scale)

S.E.M: standard error of the mean.

The hedonic scale ratings of the four treatments of the cooked FFS also did not differ significantly from each other. Considering the similarity of the products, ranking was done incorporating all the values of functional qualities, sensory evaluation (powder and porridge) and also hedonic scale ratings of cooked FFS.

Ranking of functional qualities was given based on the positive and negative characters for selecting the treatments. Dispersibility, rehydration ratio, yield ratio and sensory scores were considered as the positive characters, as increase in these characters would favour the developed product. On the other hand, bulk density, gelatinization time and temperature and processing loss were given negative scores, as decrease in these qualities will be suitable for the product. The most suitable value scored 1 and the least scored 4. The rank table is as follows:

Table 19. Rank table of functional properties of FFS II

Functional Properties	FT_1	FT_2	FT_3	FT_4
Bulk density (g/cm^3)	3.5	1.5	1.5	3.5
Dispersibilty (%)	3.5	3.5	1.5	1.5
Rehydration ratio	4	1	2.5	2.5
Gelatinization time (min)	4	3	2	1
Gelatinization temperature (^{o} C)	3	4	1	2
Processing Loss	1	2.5	4	2.5
Yield ratio	1	2.5	4	2.5
Total rank	**20**	**19.5**	**15.5**	***14.5***

From the above table it is clear that treatment FT_4 (14.5) with its least scores is the most suitable treatment, followed by treatment FT_3 (15.5), FT_2 (19.5) and FT_1 (20).

Table 20. Rank table of sensory evaluation of cooked FFS II

Sensory qualities	FT_1	FT_2	FT_3	FT_4
Colour and appearance	1.5	1.5	3.5	3.5
Texture	2.5	4	2.5	1
Taste	2.5	4	2.5	1
Flavour	4	3	1	2
Overall Acceptability	1.5	4	3	1.5
Hedonic scale ratings	3	4	2	1
Total rank	**15**	**20.5**	**14.5**	***10***

Similarly, from the above table it is clear that treatment FT_4 (10) with its least scores is the most suitable treatment, followed by treatment FT_3 (14.5), FT_1 (15) and FT_2 (20.5). Based on functional qualities, sensory evaluation and hedonic scale ratings of the four treatments of FFS, treatment FT_4 seems to be the best treatment.

Table 21. Rank table of sensory evaluation of FFS II (POWDER)

Sensory qualities	FT_1	FT_2	FT_3	FT_4
Color and appearance	2	4	1	3
Texture	3.5	3.5	1	2
Taste	2.5	4	2.5	1
Flavour	4	2	2	2
Overall Acceptability	3	4	2	1
Total rank	**15**	**17.5**	***8.5***	**9**

Unlike the other two rank tables, the above table shows that treatment FT_3 (8) with its least scores is the most suitable treatment, followed by treatment FT_4 (9), FT_1 (15) and FT_2 (17.5).

But it could be noted that there is no much differences in the ranks of treatment FT_3 (8.5) and treatment FT_4 (9.0). So based on the other characters, treatment FT_4 was selected for further in depth analysis. FFS FT_4 will be denoted as FFS II in the further descriptions.

The best identified combinations from each technique (I & II) were further investigated indepth for the nutrient content, phytochemical properties, functional qualities, storage stability and clinical efficacy.

The **salient features** of the study are: The functional quality assessment of the four combinations of FFS I (dehydration technique) showed the bulk density ranged between 0.75 to 0.82 g/ cm^3 and the combination DT_3 and DT_4 are

comparatively less than the other two but was on par with each other. Combination DT_1 (9.33 per cent) had high dispersibility followed by combination DT_3 (8.67 per cent), DT_2 (8.0 per cent) and DT_4 (7.33 per cent) respectively. The rehydration ratio showed that combination DT_1 (0.26) had better rehydration and combination DT_4 (0.23) was less rehydratable.

The gelatinization time varied from 0.48 to 1.26 min. The combination DT_4 took the least time to gelatinize (0.48 min) while combination DT_2 had the highest gelatinization time of 1.26 min. Also combination DT_4 had the lowest gelatinization temperature (85.3° C) while combination DT_2 had the highest gelatinization temperature (96° C). Processing loss of combination DT_3 (0.48) and DT_4 (0.52) were the lowest while greater processing loss was noted in combination DT_1 (0.56). The yield ratio was highest for combination DT_3 (0.52) and DT_4 (0.52) while combination DT_1 had the lowest yield ratio of 0.44.

The sensory evaluation scores of the four combinations of FFS I powder showed that, the colour and appearance of four combinations of FFS powder developed did not exhibit any difference though they were in a score ranging between 3.5 (DT_3) to 4.3 (DT_4). The texture of FFS in powder form did not show any significant differences in scores. The scores were in between 3.2 for DT_1 and 3.7 for DT_4. Maximum score for taste was noticed in FFS DT_4 (4.0), followed by DT_2 (3.3), DT_1 (3.2) and DT_3 (3.0) and also the scores varied significantly at 5 per cent. The flavour of FFS did not differ in scores. DT_1 obtained the least scores of 2.8. DT_2 and DT_3 received a score of 3.2 each. Maximum score was for DT4 (3.7). The overall acceptability score found that combination DT_4 (4.0) was the most preferred, followed by combination DT_2 (3.8) and DT_1 (3.4) respectively. Combination DT_3 (3.2) was the least preferred.

There were no significant differences in colour and appearances, texture and flavour of the porridge. However, taste and overall acceptability showed variation at 1 per cent. DT_4 scored the maximum (4.0) while DT_1 scored the least (2.8) in the case of taste.

The overall acceptability score of FFS I porridge of different combinations showed that combination DT_4 (4.2) was the most preferred followed by combination DT_2 (3.6) and DT_1 (3.2) respectively. Combination DT_3 (3.1) was the least preferred. Combination DT_4 scored the highest hedonic scale rating (7.4) followed by combination DT_2 (6.7) and DT_3 (6.3). Combination DT_1 (6.1) was the least liked.

In the case of FFS II (fermentation technique), the functional attributes of the different treatments showed that, bulk density and dispersibility did not have any significant difference among the four treatments. Rehydration ratio was highest for the treatment FT_2 (0.28) followed by FT_3 (0.27) and FT_4 (0.27). Treatment FT_2 had the least rehydration ratio (0.26) and there was a significant difference among the values at 1 per cent level.

The gelatinization time was highest (2.53 min) for FT_1 and lowest (1.16 min) for FT_4. On considering the gelatinization temperature, treatment FT_2 had the highest (94.0° C) while FT_3 (87.33° C) had the least and all the four treatments were significantly different from each other at 1 per cent level. Treatment FT_3 (0.49) had the highest processing loss followed by FT_2 (0.48) & FT_4 (0.48) and minimal processing loss was noted in treatment FT_2 which was also significantly different. On the other hand, treatment FT_1 (0.54) had the highest yield ratio and FT_2 (0.50) had the least value.

The sensory evaluation scores and hedonic rating of the FFS II powder and porridge showed there was no significant difference in the colour and appearance, texture, taste, flavour and overall acceptability among the four treatments of fermentation. The rank table which was used to select the best combination among the four treatments showed that, F T_4 with its functional property score of (14.5), sensory evaluation score of porridge of (10) and powder (9.0) was most acceptable.

4.2. QUALITY EVALUATION OF THE IDENTIFIED FUNCTIONAL FOOD SUPPLEMENTS (FFS)

Quality is a very important parameter for judging the edible nature of any food product (Sharma, 2006). It is the ultimate criterion for the desirability of any food product. It has been variously defined as "the quality characteristics of food that is acceptable to consumers including external factors such as appearance, texture, and flavour, and internal standards such as physical, chemical, and microbial attributes". The requirements necessary to satisfy the needs and expectations of the consumer, including food safety and the totality of characteristics of an entity that bears on its ability to satisfy, stated and implied needs (Peri, 2006).

The quality of the FFS (I and II), was evaluated on nutrient content, functional properties, phytochemical content, storage stability and feasibility of incorporation into various food preparations/products.

4.2.1. Nutrient Analysis of FFS I & II

The following nutrients were determined in triplicate by using standard procedures.

4.2.1.1. Proximate Analysis of FFS I & II

Man needs a wide range of nutrients to perform various functions in the body and to lead a healthy life. The nutrients include proteins, fat, carbohydrate, vitamins and minerals. These nutrients are chemical substances which are present in the food we eat daily. Most foods contain almost all the nutrients in various proportions some foods being rich in certain nutrients.

Protein, fat and carbohydrates are referred to as proximate principles. In plant foods, fibres (dietary fibre) which are indigestible complex molecules also contribute to the bulk and have some useful function in the digestive tract. Vitamins and minerals do not supply energy but they play an important role in the

regulation of the metabolic activity in the body and help in the utilization of the proximate principles (Gopalan *et al.*, 2004).

The proximate compositions of the identified FFS I and II are given in the table 22. Students' t test was carried out to understand the variation in the treatment techniques of FFS.

Table 22. Proximate compositions of FFS I & II

NUTRIENTS (per 100 g)	FFS I	FFS II	t values
Energy (kcal)	384	378	6.73**
Carbohydrates (g)	60.5	58	1.99
Protein (g)	21.4	16.5	7.90**
Crude Fibre (g)	4.0	3.3	2.86*
Fat (g)	1.88	1.56	5.56**
Moisture (%)	10.95	10.8	1.19
Ash (g)	3.0	3.2	0.39
β-glucan (g)	1.60	1.68	0.84

Stanton *et al.* (2005) reported that, fermented foods play an essential role in conferring the required stability, safety and sensory properties to the product. Fermentation helps in degradation of anti-nutritional factors and increases mineral bio-availability, protein digestibility of tannin-rich cereals, and degradation of flatulence-causing oligosaccharides.

The energy content of FFS I was 384 kcal while that of FFS II was 378kcal and the reduction was highly significant at 1 per cent level.

The carbohydrate content of FFS I was 60.5 g against 58 g of FFS II. Carbohydrates are a class of energy yielding substances. It includes starches, digestible carbohydrates, non digestible or unavailable carbohydrates or fibre. The amount of carbohydrates and available insulin may be the most important factor influencing glycemic response after eating and should be considered when developing the eating plan.

Monitoring of carbohydrate intake, either by carbohydrate counting or through experience based estimation, remains a key strategy in achieving glycemic control in diabetic individuals. For good health, carbohydrate intake from vegetables, fruits, whole grains, legumes, and dairy products should be advised over intake from other carbohydrate sources, especially those that contain added fats, sugars, or sodium (Alison *et al.*, 2014).

Protein is one of the most important nutrients required by the body to carry out a wide range of functions essential for the maintenance of life (Gopalan *et al.*, 2009). Protein can increase insulin response without increasing plasma glucose concentrations in subjects with lifestyle diseases (American Diabetes Association, 2004).

Fats are the most concentrated source of energy (calories) in the diet, providing nine calories per gram compared to four calories per gram for either protein or carbohydrates. Dietary fats make up 25–35 per cent of a person's total daily calories. The minimum value protects against energy and nutrient deficiencies, elevated triglyceride levels, and lowers HDL-C levels while the upper limit helps curb saturated fat intake and excess energy consumption (US Department of Health and Human Services and US Department of Agriculture, 2005).

The protein and fat contents of the developed food supplements were 21.4 g and 1.88 g in FFS I as against 16.5 g and 1.56 g respectively in FFS II. The above table describes that, similar to that of energy, changes were noted in the protein and fat contents of FFS I & II. That is there was significant reduction in the above nutrients present in FFS II.

Fibre is the indigestible portion of food derived from plants. Fibres can act by changing the nature of the contents of the gastrointestinal tract and by changing how other nutrients and chemicals are absorbed. Consuming a high-fibre diet (50 g fiber/ day) reduces glycemia in subjects with type I diabetes and glycemia, hyperinsulinemia, and lipemia in subjects with type II diabetes (Franz *et al.*, 2002). The variation in the fibre contents of FFS I (4.0 g) and II (3.33 g) were significant at 5 per cent level only.

β -glucans exhibit hypocholesterolemic, anticoagulant properties, anti-cytotoxic, antimutagenic and anti-tumorogenic properties (Zekovic *et al.*, 2005). From table 22, it is evident that both FFS I & II contain sufficient amounts of β – glucans. Though FFS II is found to contain higher amounts of β –glucans (1.68 g) compared to FFS I (1.60 g), statistically the difference is insignificant.

Moisture content of the food material is an important factor as it affects the physical and chemical aspects of food which relates with the freshness and stability of the food. The moisture content of both the FFS denotes that, they can be categorized under non-perishable foods, since they contain only 10.95 per cent and 10.8 per cent of moisture respectively. This provides a better opportunity for longer shelf life of the FFSs.

It could be noted that the t values given in the above table reveals that there is no significant differences in the moisture, ash and carbohydrate contents of both the FFS.

Total minerals or ash content is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of

specific inorganic components present within a food, such as Ca, Na, K, Cl etc. There was no significant variation between ash contents of FFS I (3.0) and II (3.2).

Although numerous studies have attempted to identify the optimal mix of macronutrients for the lifestyle disease management diet, it is unlikely that one such combination of macronutrients exists. The best mix of carbohydrate, protein, and fat appears to vary depending on intra individual variations (Walti *et al.*, 2003).

The Daily Recommended Intake (DRI) report (2002) recommends that, to meet the body's daily nutritional needs while minimizing risk for chronic diseases, healthy adults should consume 45–65 per cent of total energy from carbohydrate, 20–35 per cent from fat, and 10–35 per cent from protein. It must be clearly recognized that regardless of the macronutrient mix, total caloric intake must be appropriate to weight management goals (Institute of Medicine, 2002).

From the above table it could be noted that fermentation has brought about significant changes in the nutrient contents of FFS II when compared to FFS I.

4.2.1.2. Vitamin Status of Functional Food Supplements I & II

Table 23. Vitamin compositions of FFS I & II

VITAMINS (per 100 g)	FFS I	FFS II	t values
β-carotene (µg)	1948	2910	26.93**
Vitamin E (µg)	3.35	3.0	3.66*
Vitamin C (mg)	13.1	8.73	9.01**
Thiamine (mg)	0 .8	1.63	11.0**
Riboflavin (mg)	0.77	1.3	6.27**
Niacin (mg)	1.88	2.68	10.12**
Folic acid (mg)	29.56	40.0	23.88**

The above table presents that there was significant variation in the almost all the vitamin contents of FFS I & II at 1 per cent level. It was observed that the FFS II on fermentation had produced a remarkable increment in the β-carotene (2910 µg), thiamine (1.63 mg), riboflavin (1.3 mg), niacin (2.68 mg) and folic acid (40.0 mg) levels. Whereas, in FFS I β-carotene (1948 µg), thiamine (0.8 mg), riboflavin (0.77 mg), niacin (1.88 mg) and folic acid (29.56 mg) had reduced considerably. However changes in vitamin E (3.35 µg) of FFS I to that of FFS II (3.0 µg) content were significant only at 5 per cent level. On the other hand, FFS II had a comparatively lower Vitamin C (8.73 mg) content than FFS I (13.1 mg), which might be brought about by the double drying process involved in FFS II development.

4.2.1.3. Mineral Status of Functional Food Supplements I & II

Table 24. Mineral compositions of FFS I & II

MINERALS (mg/100g)	FFS I	FFS II	t values
Macro minerals			
Potassium	497	425.4	20.35**
Sodium	498	475	7.38**
Calcium	472	458	4.05**
Magnesium	467	378	27.91**
Phosphorus	141	109	13.98**
Micro minerals			
Iron	9.2	8.5	2.70*
Copper	7.6	3.6	8.09**
Zinc	8.64	2.88	12.75**
Manganese	2.38	2.12	5.09**
Trace minerals			
Selenium (µg)	0.67	0.73	18.13**

Lichtenstein *et al.* (2006) had reported that, available evidence is inadequate to recommend folate and other B vitamin supplements as a means to reduce CVD risk. However folate intake and to a lesser extent intake of vitamins B6 and B12 are inversely associated with blood homocysteine levels. Increased blood levels of homocysteine are associated with an increased risk of CVD.

The study clearly indicated that there were significant variations in the mineral contents of FFS I & II on fermentation. The mineral contents viz. copper, zinc, potassium, sodium, calcium, magnesium, phosphorus and manganese of FFS I reduced from 7.6 mg/100g to 3.6 mg/100g, 8.64 mg/100g to 2.88 mg/100g, 497 mg/100g to 425.4 mg/100g, 498 mg/100g to 475 mg/100g, 472 mg/100g to 458 mg/100g, 467 mg/100g to 378 mg/100g, 141 mg/100g to 109 mg/100g and 2.38 mg/100g to 2.12 mg/100g respectively in FFS II at 1 per cent significance level. However, selenium which is an antioxidant was found to be significantly higher in FFS II (0.73 µg) than FFS I (0.67 µg). The decrease in mineral contents may be due to leaching out during cooking processes. Similar results were reported by Mubarak (2005), in his study on nutritional composition and anti nutritional factors of mung bean seeds as affected by some processing techniques practiced traditionally at house hold levels.

Uncontrolled diabetes is often associated with micronutrient deficiencies (Mooradian, 2009). Individuals with diabetes should be aware of the importance of acquiring daily vitamin and mineral requirements from natural food sources and a balanced diet. Health care providers should focus on nutrition counselling rather than micronutrient supplementation in order to reach metabolic control of their patients (Kligler, 2004). Research including long-term trials is needed to assess the safety and potentially beneficial role of chromium, magnesium, and antioxidant supplements and other complementary therapies in the management of type 2 diabetes (Guerrero-Romero and Rodriguez-Moran, 2005).

Chromium, potassium, magnesium, and possibly zinc deficiency may aggravate carbohydrate intolerance. Serum levels can readily detect the need for potassium or magnesium replacement, but detecting deficiency of zinc or chromium is more difficult (Mooradian *et al.*, 2009). Data from recent small studies indicate that chromium supplementation may have a role in the management of glucose intolerance, gestational diabetes mellitus (GDM), and corticosteroid-induced diabetes (Cefalu and Hu, 2008; Althuis *et al.*, 2009).

4.2.1.4. Phytochemical Contents of Functional Food Supplements I & II

Table 25. Phytochemical contents of FFS I & II

Constituents (per 100 g)	FFS I	FFS II	t values
Flavonoids (%)	4.6	1.23	142.79**
Poly phenols (mg)	73.25	48.25	19.61**
Tannin (Tannic Acid Equivallence, mg)	10.09	5.77	23.15**
Saponin (%)	3.64	1.28	14.55**
Alkaloids (%)	0.8	0.2	47.38**
Oxalates (mg)	5.32	1.92	42.79**
Phytates (mg)	155	93.5	26.73**

The phytochemicals are found to possess therapeutic functions like antioxidant and reducing properties. Flavonoids and sulfur-containing compounds are classes of compounds found in fruits and vegetables that may be important in reducing the risk of atherosclerosis. Within these categories are multiple possible compounds, most of which are not well characterized and whose modes of action has to be established (Howard and Kritchevsky, 2007).

Flavonoids are most commonly known for their antioxidant activity. They are modifiers which modify the body's reactions to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobical and anticancer activity (Balch and Balch, 2000; Ekam and Ebong, 2007), and may be useful in therapeutic roles (Jisika *et al.*, 2000). The flavonoid contents of FFS I

was 4.6 per cent while FFS II was found to contain 1.23 per cent which was highly significant at 5 per cent level. Fermentation technique involved in the processing of FFSs might have contributed to the reduction of flavonoids in FFS II.

Alkaloids are organic compounds that contain nitrogen, and are physiologically active with sedative and analgesic properties. They are used in relieving pains, anxiety and depression (Jisika *et al.*, 2000). Alkaloids are toxic due to their stimulatory effects, leading to excitation of cells and neurological dysfunction (Obochi, 2006; Ekam and Ebong, 2007). Thus it is scientifically documented that the alkaloid content of the FFS has therapeutic roles. The alkaloid content of FFS I was (0.8 per cent) against 0.2 per cent for FFS II.

Yang *et al.* (2001) reviewed the inhibition of carcinogenesis by dietary polyphenolic compounds and questioned the link between the antioxidative and anticarcinogenic properties, asserting that polyphenols may inhibit carcinogenesis by affecting the molecular events in the initiation, promotion and progression stages of cancer. The polyphenol content of FFS I was 73.25 mg whereas fermentation reduced its content to 48.25 mg in FFS II.

From the above table, it was noted that the tannin contents of FFS I and II were 10.09 mg and 5.77 mg respectively. Tannins (tannic acids) and saponins are responsible for the antibacterial activity of the plant seed extracts (Gloor, 1997).

Saponins are used in veterinary vaccines as adjuvant (e.g. foot-and-mouth disease vaccines) helping to enhance immune response. They are also mild detergents and can be used commercially as well as for research (Balch and Balch, 2000). They can also be used in intracellular histo chemistry staining to allow antibody access to intracellular proteins (Balch and Balch, 2000).

Saponins are used in laboratory applications to treat live cells in order to facilitate peptide or reagents such as antibodies entering cells instead of the

detergents (Balch and Balch, 2000). In this study, the saponin contents of FFS I was 3.64 per cent compared to 1.28 per cent in FFS II.

Too much of soluble oxalate in the body prevents the absorption of soluble calcium ions as the oxalate bonds the calcium ions to form insoluble calcium oxalate complex. The oxalate content of FFS I and II were 5.32 mg and 1.92 mg respectively. On the other hand, people suffering from coronary heart disease are encouraged to consume moderately oxalate rich foods as it helps to reduce blood cholesterol (Savage, 2000).

The results indicated that the phytochemicals viz. polyphenols, alkaloids, saponins, oxalates, tannins and phytates analyzed in FFS I & II follows a similar pattern of reduction on fermentation as that of flavonoids.

4.2.1.5. Amino Acid profile of Functional Food Supplements I & II

Table 26, Amino acid profile of FFS I & II

Amino acids (nmoles/ml)	FFS I	FFS II
Essential amino acids		
Methionine	17.2	17.9
Valine	42.6	34.7
Phenylalanine	45.0	39.7
Histidine	**16.0**	**3.9**
Threonine	40.9	31.5
Isoleucine	34.1	24.9
Leucine	52.3	60.3
Lysine	42.9	35.6
TEAA	**291**	**248.5**
Non Essential amino acids		
Alanine	22.2	15.3
Aspartic acid	48.6	32.0
Glutamic acid	***132.9***	78.3
Serine	51.1	69.1
Glycine	88.5	***107.6***
Arginine	97.1	96.3
Tyrosine	27.2	24.0
TNEAA	**467.6**	**414.4**

According to Ghosh and Chakravarthy (1990), the quality of protenacious food depends on its amino acid composition in relation to the protein content and digestibility. Amino acid composition may also serve as a good relative measure to compare the developed FFSs with other food stuffs of established nutritive value.

From the above mean table it can be identified that Glutamic acid (132.9 nmoles/ml) is the highest amino acid content in FFS I followed by arginine 97.1 nmoles/ml, Glycine (88.5 nmoles/ml), Leucine (52.9 nmoles/ml), Serine (51.6 nmoles/ml), Aspartic acid (48.6 nmoles/ml), Phenylalanine (45.0 nmoles/ml), Lysine (42.9 nmoles/ml), Valine (42.6 nmoles/ml), Threonine (40.9 nmoles/ml), IsoLeucine (34.9 nmoles/ml), Tyrosine (27.2 nmoles/ml), Alanine (22.2 nmoles/ml) and Methionine (17.2 nmoles/ml). On the other hand FFS II elicited high amounts of Glycine (107.6 nmoles/ml), followed by Arginine (96.3 nmoles/ml), Glutamic acid (78.3 nmoles/ml), Serine (69.1 nmoles/ml), Leucine (60.3 nmoles/ml), Phenylalanine (39.7 nmoles/ml), Lysine (35.6 nmoles/ml), Valine (34.7 nmoles/ml), Aspartic acid (32.0 nmoles/ml), Threonine (31.5 nmoles/ml), IsoLeucine (24.9 nmoles/ml), Tyrosine (24.0 nmoles/ml), Methionine (17.9 nmoles/ml) and Alanine (15.3 nmoles/ml). Histidine is the most limiting amino acid in both FFS I (16.0 nmoles/ml) and II (3.9 nmoles/ml). These changes may be brought about due to the breaking down of other nutrients like carbohydrate to synthesis amino acids that the germinating seeds needed for its biochemical activities and growth.

The Total Essential Amino acid (TEAA) content of FFS I & II were, 291 and 248.5 nmoles/ml. The Total Non Essential Amino acid (TNEAA) content of FFS I & II were, 467.6 and 414.4 nmoles/ml.

Scientific studies have documented that the protein content of sprouted or germinated plant increased due to degradation of nutrients like carbohydrate and fat in the synthesizing of protein (Enujiugha *et al.*, 2003; Fasasi, 2009).

Consumption of cereals alone without complement with other protein-based foods like legumes may not adequately meet the nutritional needs of the consumers.

Table 27. Essential Amino acid (EAA) score of FFS I & II in comparison with reference protein (egg)

Essential Amino acid score	FFS I	FFS II	Egg
Histidine	165	40	150
Threonine	153	117	320
Methionine	122	127	210
Valine	111	90	450
Phenylalanine	206	182	360
Isoleucine	109	80	410
Leucine	132	152	520
Lysine	142	118	440
EAA Index	42.57	31.26	
Nutritional Index (NI) %	9.12	5.16	
Most limiting amino acid	***Iso leucine***	***Histidine***	
Amino acid sequence	**Iso leucine, Valine, Methionine**	**Histidine, Iso leucine, Valine**	

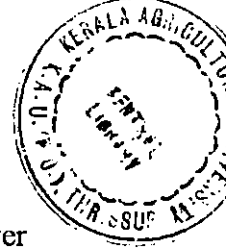
The above table presents that in FFS I Isoleucine (109) is the most limiting amino acid, whereas in FFS II it is Histidine (40). Phenylalanine is the most

abundant amino acid in both FFS I (206) & II (102). FFS I follows an amino acid sequence of Iso leucine, Valine, Methionine while in FFS II it is Histidine, Iso leucine, Valine. FFS I have a higher EAA index and Nutritional index % of 42.57 and 9.12 respectively when compared to FFS II (31.26) and 5.16 respectively.

Oser (1999) pointed out that a protein-based food material is of good nutritional quality when its biological values (BV) is high (70 to 100 per cent) and also when the essential amino acid index (EAAI) is above 90 per cent and to be useful as food when the values is around 80 per cent. It is well documented that during the processing techniques the lipids, carbohydrates and storage proteins within the seed are broken down in order to obtain the energy and amino acids necessary for the micro-organisms' and plant's development (Ziegler, 2001).

The **salient findings** of the nutrient analysis showed that, there was a reduction in the energy content of FFS II (378 kcal) from that of FFS I (384 kcal). The protein and fat contents were 21.4 g and 1.88 g for FFS I as against 16.5g and 1.56 g respectively for FFS II. The variation in the fibre contents of FFS I (4.0 g) and II (3.33 g) were significant at 5 per cent level only. Though FFS II was found to contain higher amounts of β –glucans (1.68 g) compared to FFS I (1.60 g), statistically the difference is insignificant. The moisture content of both the FFS denotes that, they can be categorized under non-perishable foods, since they contain only 10.95 per cent and 10.8 percent of moisture. The carbohydrate content of FFS I was 60.5 g against 58 g of FFS II which was not significantly different.

The vitamin content analysis carried out in the present investigation showed that, FFS II on fermentation had produced a remarkable increase in the β-carotene (2910 μg), thiamine (1.63 mg), riboflavin (1.3 mg), niacin (2.68 mg) and folic acid (40.0 mg) levels. Whereas in FFS I vitamins viz. β-carotene (1948 μg), thiamine (0.8 mg), riboflavin (0.77 mg), niacin (1.88 mg) and folic acid (29.56 mg) reduced considerably. Changes in vitamin E (3.35 μg) of FFS I to that of FFS



II (3.0 µg) were noted. On the other hand, FFS II had a comparatively lower Vitamin C (8.73 mg) content than FFS I (13.1 mg).

In the case of mineral composition, the mineral contents like iron, copper, zinc, potassium, sodium, calcium, magnesium, phosphorus and manganese of FFS I reduced from 9.2 mg/100g to 8.5 mg/100g, 7.6 mg/100g to 3.6 mg/100g, 8.64 mg/100g to 2.88 mg/100g, 497 mg/100g to 425.4 mg/100g, 498 mg/100g to 475 mg/100g, 472 mg/100g to 458 mg/100g, 467 mg/100g to 378 mg/100g, 141 mg/100g to 109 mg/100g and 2.38 mg/100g to 2.12 mg/100g respectively in FFS II. However, selenium was found to be significantly higher in FFS II (0.73 µg) than FFS I (0.67 µg).

The results of phytochemical analysis proved that, there was reduction in flavanoids from 4.6 per cent (FFS I) to 1.23 per cent (FFS II) is highly significant. The alkaloid content of FFS I was (0.8 per cent) against 0.2 per cent for FFS II. The polyphenol content of FFS I was 73.25 mg whereas fermentation reduced its content to 48.25 mg in FFS II. The tannin contents of FFS I and II were 10.09 mg and 5.77 mg respectively. The oxalate contents of FFS I and II were 5.32 mg and 1.92 mg respectively.

Amino acid profiling of FFS I & II depicted that, Glutamic acid (132.9 nmoles/ml) is the highest amino acid content in FFS I followed by arginine 97.1 nmoles/ml, Glycine (88.5 nmoles/ml), Leucine (52.9 nmoles/ml), Serine (51.6 nmoles/ml) etc. On the other hand FFS II elicited high amounts of Glycine (107.6 nmoles/ml), followed by Arginine (96.3 nmoles/ml), Glutamic acid (78.3 nmoles/ml), Serine (69.1 nmoles/ml), Leucine (60.3 nmoles/ml) etc. Histidine is the most limiting amino acid in both FFS I (16.0 nmoles/ml) and II (3.9 nmoles/ml).

The Total Essential Amino acid (TEAA) content of FFS I & II were, 291 nmoles/ml and 248.5 nmoles/ml. Whereas, the Total Non Essential Amino acid (TNEAA) content of FFS I & II were, 467.6 nmoles/ml and 414.4 nmoles/ml

respectively. In FFS I Isoleucine (109) is the most limiting amino acid, whereas in FFS II it is Histidine (40). Phenylalanine is the most abundant amino acid in both FFS I (206) and II (102). FFS I follows an amino acid sequence of Iso leucine, Valine, Methionine while in FFS II it was Histidine, Iso leucine, Valine. FFS I have a higher EAA index and Nutritional index percentage of 42.57 and 9.12 respectively when compared to FFS II (31.26) and 5.16 respectively.

4.2.1.6. Total Antioxidant Capacity (TAC) of Functional Food Supplements I & II

The recent changes in lifestyle or environmental factors such as pollution, radiation, cigarette smoke and herbicides can generate free radicals in the human body. This can cause further oxidative stress responsible for DNA, protein, membrane damage and contribute to current non-communicable diseases (NCDs). Although antioxidant defence and repair systems are available in humans and other organisms to protect them against oxidative damage, these systems are insufficient totally prevent the damage (Mau *et al.*, 2002).

The consumption of dietary antioxidants will help to prevent free radical damage. According to Olajire and Azeez (2011), antioxidants have the ability to scavenge free radicals by inhibiting the initiation step or interrupting the propagation step of oxidation of lipid and as preventive antioxidants which slow the rate of oxidation by several actions.

There are many epidemiological studies which suggest that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of cardiovascular diseases, stroke and certain types of cancer in which polyphenol is linked to the antioxidant properties (Jagadish *et al.*, 2009).

Table 28. Total Antioxidant Capacity (TAC) (µg / g) of FFS I & II

SOLVENTS	FSS I	FFS II	Mean
Petroleum ether	0.84	0.62	0.728
$CHCl_3$:H_20	0.95	0.66	0.803
Acetic acid	0.68	0.51	0.594
Acetone	0.80	0.60	0.698
Ethanol	0.98	0.83	0.901
Aqueous Ethanol	1.09	0.82	0.953
n butanol	0.74	0.53	0.633
Benzene	0.75	0.55	0.648
Ethyl acetate	0.95	0.66	0.803
Diethyl ether	0.61	0.49	0.55
Hot water	0.57	0.41	0.488
Mean	0.811	0.607	

$F_{(10,66)}$ Solvent	345.21 **
Product	1869.89 **
Solvent * product	13.33 **
CD - solvent	0.022
CD – product	0.009
CD – solvent * product	0.023

Different solvent media were used to extract the total antioxidant activity of the developed FFS I and II. Results indicated that the total antioxidant activity of FFS I was 0.811 µg / g and that of FFS II was 0.607 µg / g. The total antioxidant activity (1.09 µg / g) was highest when aqueous ethanol was used. But on the other hand, for FFS II (0.83 µg / g) the maximum antioxidant activity was derived while using absolute ethanol as solvent extraction medium. However it could be noted that there was not much variation in the activity levels of aqueous ethanol (0.82 µg / g) and absolute ethanol in the case of FFS II. In both FFS I (0.57 µg / g) & II (0.41 µg / g), hot water extraction produced the least antioxidant activity.

For FFS I the total antioxidant activity of different solvents followed the pattern of aqueous ethanol> ethanol> $CHCl_3$:H20> ethyl acetate > petroleum ether > acetone > benzene > n butanol > acetic acid > diethyl ether > hot water. On the other hand FFS II followed the pattern of ethanol> aqueous ethanol> CHCl3:H20> ethyl acetate > petroleum ether > acetone > benzene > n butanol > acetic acid > diethyl ether > hot water. This shows that both FFS I & II follow a similar trend in the antioxidant activity levels on using different solvent extraction media.

The amount of the antioxidant components that can be extracted from a plant material is mainly affected by the vigour of the extraction procedure, which may probably vary from sample to sample. Amongst other contributing factors, efficiency of the extracting solvent to dissolve endogenous compounds might also be very important (Sultana *et al.,* 2007). The polarity and solubility of the solvent and the compound also has a role in this activity.

Since lifestyle diseases are a state of increased oxidative stress, there has been interest in antioxidant therapy. Although antioxidant supplements are not recommended, food sources rich in antioxidants, principally from a variety of plant derived foods such as fruits, vegetables, whole grains, and vegetable oils have been recommended.

4.2.1.7. DPPH Scavenging Activity of Functional Food Supplements I & II

During oxidative stress and exposure to radiation, excessive free radicals are produced that are known to cause damage to the bio molecules. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron that is responsible for the absorbance at 540 nm and also for the visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance.

Table 29. DPPH free radical scavenging activity of FFS I & II in comparison with standard

Concentration (µg/ml)	FFS I		FFS II		Standard (Ascorbic acid)	
	% inhibition	IC 50 value (µg/ml)	% inhibition	IC 50 value (µg/ml)	% inhibition	IC 50 value (µg/ml)
100	11.67	585.35	10.67	639.00	15	477.86
200	21.67		19.67		27.5	
400	33.0		31.33		48.5	
600	53.67		49.33		61.5	
800	68.33		64.67		77	
1000	80.0		72.33		89.5	
F value: 394.645**						
CD-values (i=2 to n); (j=1 to i-1)						
13.2434						
14.8065 14.8065						

*Each value is presented as mean.

**The IC50 was obtained by linear regression equations

From the table presented above, it was evident that the IC 50 value of FFS I (585.35) and II (639.00) was closer to the IC 50 values of standard ascorbic acid (477.86) which is a potent antioxidant though there is significant variation at 1 per cent. Though it was evident that there was variation in the % inhibition of FFS I & II, when compared to standard, their potency of inhibition is still higher. Thus it could be concluded that, both FFS I & II possesses greater antioxidant properties through their inhibitory effects.

Table 30. Observed and expected DPPH scavenging activity of FFS I & II

DPPH	FFS I (% inhibition)		FFS II (% inhibition)		Standard (Ascorbic acid) (% inhibition)	
Concentration (µg/ml)	Observed value	Expected value	Observed value	Expected value	Observed value	Expected value
100	11.67	12.58	10.67	11.84	15	19.21
200	21.67	20.29	19.67	18.92	27.5	27.36
400	33	35.71	31.33	33.08	48.5	43.66
600	53.67	51.13	49.33	47.24	61.5	59.96
800	68.33	66.55	64.67	61.40	77	76.26
1000	80	81.97	72.33	75.56	89.5	92.56

The values for IC 50 were obtained by fitting the regression equation; $Y = 4.870 + 0.077x$, $Y = 4.759 + 0.077x$ and $Y = 11.055 + 0.082x$ for FFS I, II and standard ascorbic acid respectively.

From the above figures it could be noted that the observed values and the expected values are almost on the same line when fitted a linear regression

equation. In all the three observations, it could be noted that the R^2 values of FFS I, II & standard (0.994, 0.990 and 0.987 respectively) are almost 1. This shows the validity of the data analyzed.

The **salient findings** of the quality assessment of FFS I & II based on the total antioxidant activity showed that, FFS I (1.09 µg / g) had the highest total antioxidant activity when aqueous ethanol was used. For FFS II (0.83 µg / g) the maximum antioxidant activity was derived while using absolute ethanol. However it could be noted that there was not much variation in the activity levels of aqueous ethanol (0.82 µg /g) and absolute ethanol in the case of FFS II. In both FFS I (0.57 µg / g) & II (0.41 µg /g), hot water extraction produced the least antioxidant activity.

Similarly, DPPH scavenging activity of FFS I & II proved that they had higher levels of inhibitory effects. Though there was significant variation between the IC 50 values of FFS I (585.35) & II (639) and also in comparison with standard ascorbic acid (477.86), their potency were found to be higher. Also, regression analysis showed that, the expected values were in line with the observed values in all the three cases.

4.2.1.8. Selection Index for FFS I & II

As described earlier in chapter 3.4 of the materials and methods, an index was developed to determine the best functional food supplements based on the parameters viz. macronutrient composition, fibre, β –glucan, vitamin composition, phytochemical, minerals composition, Essential Amino Acid (EAA) index, Total Antioxidant Capacity (TAC) and DPPH free radical scavenging properties of the two developed FFSs. The details of which are given below:

Table 31. Nutritional and antioxidant quality indices of FFS I & II

Selection criteria	FFS I index	FFS II Index	Selected product
Macronutrients	2.355	2.020	**I**
Fibre + β –glucan	2.448	2.263	**I**
Vitamins	2.240	2.400	**II**
Phytochemicals	2.230	1.975	**I**
Minerals	2.891	2.640	**I**
EAA Index	42.57	31.26	**I**
Total Antioxidant capacity	0.811	0.607	**I**
DPPH free radical scavenging activity	585.35	639	**I**
All	2.57	2.41	**I**

From the above table it could be concluded that, based on the above mentioned characters of the developed functional food supplements, FFS I was found to be the best in terms of the nutritional properties. Though the selection index of vitamins scored higher for FFS II, in terms of all the other characters, FFS I was found to obtain the highest scores.

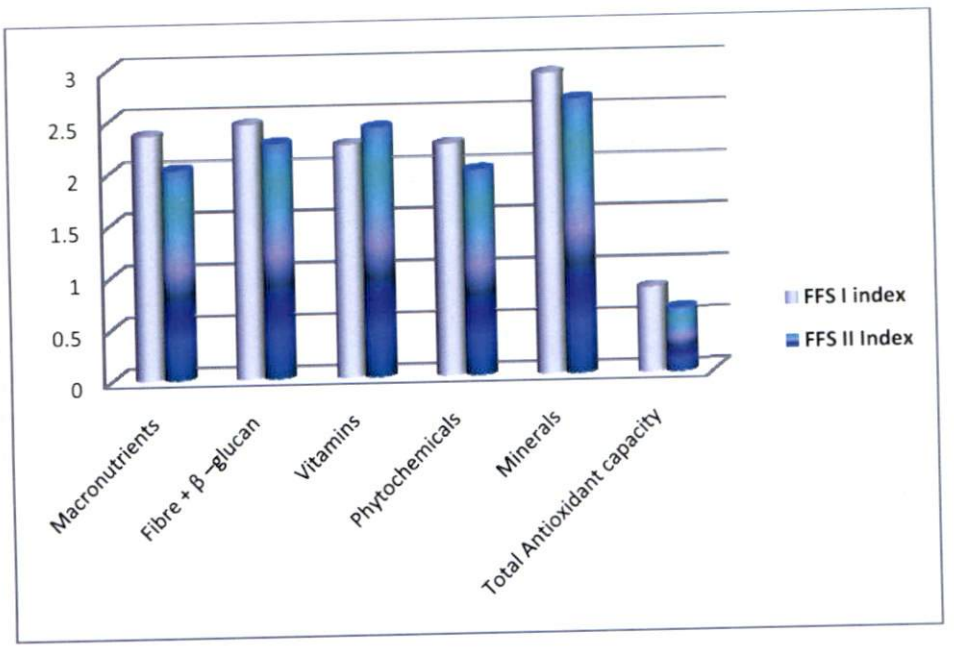


Fig: 01 Nutritional & antioxidant indices of FFS I & II

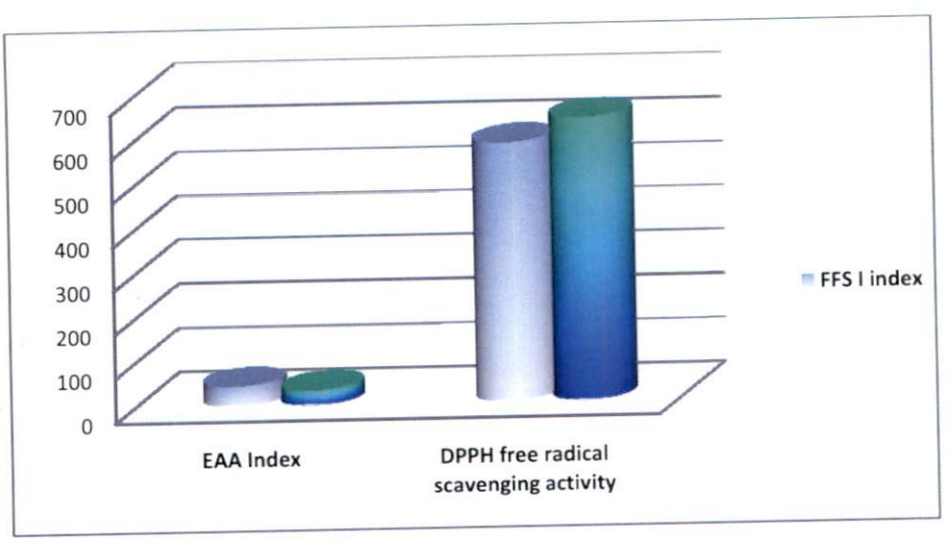


Fig: 02 EAA & DPPH Indices of FFS I & II

4.2.2. Assessment of Functional Qualities of FFS I & II

Table 32. Functional properties of FFS I & II

Functional properties	FSS I	FFS II	t values
Bulk density (g/cm^3)	0.75	0.90	38.62**
Dispersibility (%)	7.25	2.75	6.65**
Rehydration ratio	0.23	0.27	15.0**
Gelatinization time (min)	0.48	1.16	57.77**
Gelatinization temperature (^{0} C)	85.25	88.25	8.49**
Processing loss (g)	0.48	0.48	1
Yield ratio	0.52	0.52	1
Swelling capacity (g/g)	7.04	6.66	30.60**
Water Absorption Capacity (WAC) (g/g)	1.44	1.59	15.58**
Water Absorption Index (WAI)	2.68	2.65	1.16
Water Solubility Index (WSI)	0.13	0.14	3.27**

The functional properties determine the application and use of food material for various food products. It can be noted that there is significant differences in the functional qualities of FFS I and II.

The above table portrays that the functional characters like bulk density (0.90 g/cm^3), rehydration ratio (0.27), gelatinization time (1.16 min) and temperature (88.25^0 C), Water Absorption Capacity (WAC) (1.59) and Water

Solubility Index (0.14) significantly increased in the fermentation technique of FFS II on comparison with FFS I, which was recorded as: bulk density (0.75 g/cm^3), rehydration ratio (0.23), gelatinization time (0.48 min) and temperature (85.25^0 C), Water Absorption Capacity (WAC) (1.44 g/g) and Water Solubility Index (0.13). On the other hand, dispersibility (2.75 per cent), swelling capacity (6.66 g/g) and Water Absorption Index, WAI (2.65) decreased significantly in FFS II when compared to FFS I values like dispersibility (7.25 per cent), swelling capacity (7.04 g/g) and Water Absorption Index, WAI (2.68). However, there were no differences noted in the processing loss and yield ratio of the two FFS.

Bulk density is generally affected by the particle size and density of the flour and it is very important in determining the packaging requirement, material handling and application in wet processing in the food industry (Karuna *et al.*, 1996).

The bulk density value is of importance in packaging (Snow, 2004). The lower loose bulk density implies that less quantity of the food samples would be packaged in constant volume thereby ensuring an economical packaging. However, the packaged bulk densities would ensure more quantities of the food samples being packaged, but less economical. Nutritionally, loose bulk density promotes easy digestibility of food products, particularly among children with immature digestive system (Osundahunsi and Aworh, 2002).

Dispersibility in water, which indicates the ability to reconstitute, showed that FFS II had much lower values when compared to FFS I. Swelling power is an indication of the water absorption index of the granules during heating (Loos *et al.*, 1981). Swelling capacity decreased with increasing fermentation period. This is comparable to the results of the present study.

WAI is affected by cooking temperature. Anderson (1982) reported that cooking temperature increased WAI for several grains. Results of this study agree with those findings. It may be suggested that WAI is mainly influenced by the

affinity of the flour particles for water because finer particles form a greater gel matrix with water trapped into it.

WSI correlated negatively with temperature. Low cooking temperatures stimulated the migration of certain solutes which can be leached out on contact with water; this behaviour led to high WSI values of FFS II.

4.2.3. Assessment of Physico – Chemical Properties of FFS I & II

4.2.3.1. Pasting Properties of Functional Food Supplements I & II

Table 33. Pasting properties of FFS I & II

Pasting properties	FSS I	FFS II	t values
Pasting temp (^{0}C)	84.45	Not detected	-
Pasting point (cP)	59.25	Not detected	-
Peak viscosity (cP)	1190.75	223.75	2735.09**
Hold viscosity (cP)	983.75	220	1595.42**
Final viscosity (cP)	1958.75	453.75	4256.78**
Break down (cP)	206.75	4	811**
Set back (cP)	975	234	1815.07**

The physico – chemical properties like pasting properties, textural properties, colour attributes, particle size etc are important characteristic features in analysing the ability of a product to withstand market value. They are also an indication of nutrients present in the product, which directly or indirectly affect

the above mentioned properties. This in turn may affect the acceptability of the product in the market. It must also be noted that, it is the role of the nutrients present in the product which produces the desired therapeutic effects. Thus physico – chemical properties are the combined effect of the nutrient content of the product as well as market value.

The results of the pasting properties of the FFS I & II projected that several changes may occur upon heating a starch-water system, including enormous swelling, increased viscosity, translucency and solubility and loss of anisotropy (birefringence). These changes are due to gelatinization of the starches present in the developed functional food supplement.

For FFS I, the pasting temperature (84.45^0 C) and pasting point (59.25 cP) were notable. However, these properties could not be detected for FFS II.

The pasting temperature is one of the pasting properties which provide an indication of the minimum temperature required for sample cooking, energy costs involved and other components stability (Shimelis *et al.*, 2006).

The peak viscosity which is the ability of starch to swell freely before their physical breakdown ranged between 1190.75 cP for FFS I and 223.75 cP for FFS II. The peak viscosity indicates the water binding capacity of starch. The relatively high peak viscosity exhibited by FFS I is indicative that the starch may be suitable for products requiring high gel strength and elasticity.

FFS II had the lowest (4.0 RVU) breakdown viscosity when compared to FFS I (206.75 RVU), hence the higher the breakdown in viscosity, the lower the ability of the sample to withstand heating and shear stress during cooking (Adebowale *et al.*, 2005). Hence, FFS I might be able to withstand heating and shear stress.

The final viscosity, which is the change in the viscosity after holding cooked starch, ranged between 1958.75 RVU for FFS I and 453.75 RVU for FFS

II. Final viscosity is used to define the particular quality of starch and indicate the stability of the cooked paste in actual use; it also indicates the ability to form various paste or gel after cooling and less stability of starch paste commonly accompanied with high value of breakdown.

The setback value of the FFS I & II were 975 and 234 RVU, respectively. The higher the setback value, the lower the retrogradation during cooling of the products made from the flour.

4.2.3.2. Assessment of Textural Properties of Functional Food Supplements I & II

Table 34. Textural properties of FFS I & II

Textural properties	FSS I	FFS II
Hardness (N)	9.06 ± 0.36	Nd
Cohesiveness	0.499 ± 0.04	Nd
Adhesiveness (Ns)	-37.515 ± 4.91	Nd
Gumminess (N)	4.79 ± 0.99	Nd
Springiness (s)	5.4 ± 0.21	Nd
Chewiness (Ns)	25.86 ± 1.7	Nd
Resilence	6.77 ± 0.05	Nd

Nd: Not detected.

Texture analysis is the mechanical testing of food, cosmetics, pharmaceuticals, adhesives and other consumer products in order to measure their physical properties. Problematic textural issues occurring during storage or

transportation can be overcome by texture analysis. It may also prove to be an effective means of comparison with competitive products, or where claims substantiation is necessary to take a technical pro-active stance in market. In this context texture analysis can serve as an effective tool for assessment (Lambo *et al.*, 2004).

FIRMNESS/HARDNESS/SOFTNESS is the textural properties that are generally on the same property spectrum. A soft product is one that displays a slight resistance to deformation, a firm product describes one that is moderately resistant to deformation and hardness describes a product which displays substantial resistance to deformation. FFS I scored 9.06 on the hardness scale.

COHESIVENESS is the tendency of a product to cohere or stick together. The intermolecular attractions by which the elements of a body or mass of material are held together determine its cohesiveness. It is related to the internal stickiness of a product and is usually determined by measurement of the amount of force to remove an item from the product mass. FFS I showed a cohesiveness value of 0.499.

STICKINESS/ADHESIVENESS is a major problem in the food industry, especially in the baking and confectionery industries, where it can cause considerable difficulty during processing by causing interruptions in production, waste and contamination of machinery. Sticking of food to packaging materials is generally regarded as undesirable resulting in possible packaging material damage, product loss and disfigurement of the product surface. It is commonly the textural property possessed by confectionery products, cooked pasta products, raw bakery products, pharmaceutical patches and more obviously – adhesives. Adhesiveness value of FFS I (- 37.515) indicated that it is less sticky in nature and the granules stick less to each other.

SPRINGINESS is a measurement of elastic recovery. It is commonly the textural property possessed by baked goods such as cake or bread but also

possessed by novel confectionery products and pharmaceutical materials. From the above table it could be noted that the springiness value of FFS I is 5.4.

TOUGHNESS - whilst Hardness/Firmness is commonly the textural property possessed by most products. The word 'toughness' or 'chewiness' may often be substituted for a textural property more associated with the product. The chewiness range of FFS I showed 25.86, which denotes much less chewy products could be developed using it.

Texture profile analysis showed very low values for FFS II which could not be detected in hardness, cohesiveness, elasticity, gumminess etc., of the fermented flour paste. The altered textural properties were attributed to greater starch granule stability due to short amylose-like fragments formed by enzymatic hydrolysis of amylopectin (Schwartz, 2006).

4.2.3.3. Colour Attributes of Functional Food Supplements I & II

Table 35. Colour attributes of FFS I & II

Type	L	a	b
Control	93.98	-0.97	0.53
FFS I	73.72	1.71	15.33
FFS II	64.77	3.50	22.00

L: lightness; a: redness; b: yellowness

Colour of the product is an important criterion which determines the marketing potential of any product.

The mean L* value ranged between 73.72 for FFS I and 64.77 for FFS II which had the lowest L* value. This indicates that a substantial level of colour change had occurred during drying that yielded dark brown powder, particularly in the FFS II. The presence of glucose, fructose and protein, an extension of Maillard reaction had occurred within FFS II. In addition, certain enzymes such

as polyphenol oxidase may be present that could contribute a certain stage of enzymatic browning that took place during drying. There is some evidence that a* values of FFS I (1.71) were lower than those of FFS II (3.50). However, as a result of the browning reactions during drying, FFS II (22.0) exhibited higher b* values than FFS I (15.33).

A low value for chroma and a high value for lightness are desired for the product to meet the consumer preference. In terms of product colour (Table 27), it was observed that FFS I had a high value of whiteness (L = 73.72) and a low value of chroma (a = 1.71). Thus, in this study colour of FFS I can meet consumer preference due to the highest whiteness and low chroma values compared to FFS II.

4.2.3.4. Particle Size of Functional Food Supplements I & II

Powders and particles are used in a wide variety of fields for a wide range of objectives and applications. Measuring the particle size distribution is essential for stabilizing or improving the characteristics, performance, or quality of powders or particles. The mouth, tooth, and tongue feel and other characteristics of bread, cakes, pasta, etc. depend on the particle size distribution. Also, controlling the particle size distribution in food products is important to ensure consistent quality. Advancements in research are bringing about an awareness of the importance of food particle size/shape and its significance on palatability, digestion, bioavailability, and metabolism along with handling, packaging, storage and transport of food stocks (http://www.chemicalprocessing.com).

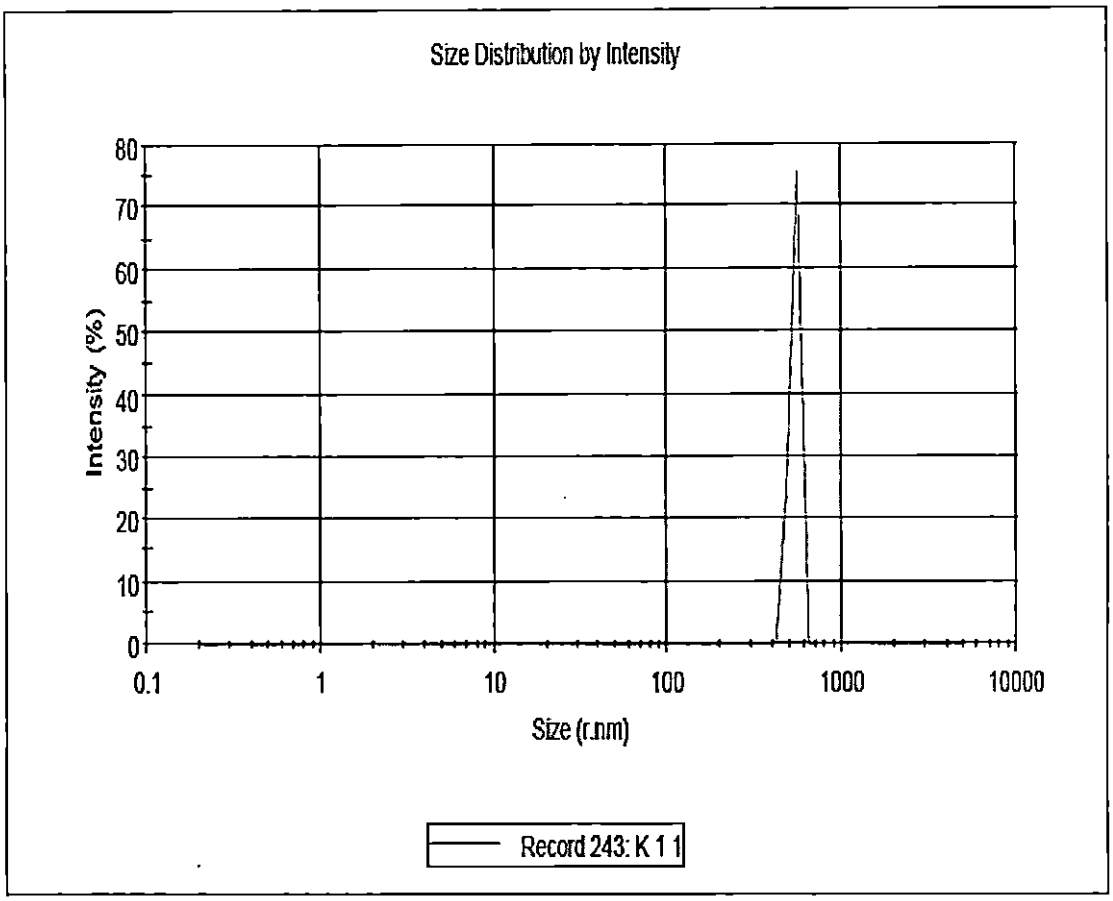
Table 36. Particle size of FFS I

System

Temperature (°C):	25.0	Duration Used (s):	60
Count Rate (kcps):	233.9	Measurement Position (mm):	1.25
Cell Description:	Disposable sizing cuvette	Attenuator:	6

RESULTS

			Size (r. nm)	% intensity	Width (r.nm)
Z – Average (r. nm)	2525	Peak 1	534.7	100.0	32.51
PdI	0.127	Peak 2	0.000	0.0	0.000
Intercept	0.934	Peak 3	0.000	0.0	0.000



Data given in the table shows the particle size (Z average, nm), polydispersity index and mean intensity peak (nm) for 100 nm standard particles. Measurements were made on triplicate sample preparations with six replicate measurements per sample preparation. At an appropriate dilution, FFS I showed a single sharp peak with low polydispersity index (0.127) and attenuator of 6. The largest particle was in the size of 534.7. From the graph it can be concluded that the particles of FFS I ranged between 400 to 650 r. nm. The maximum width of the particles of FFS I was 32.51 r. nm.

Table 37. Particle size of FFS II

System

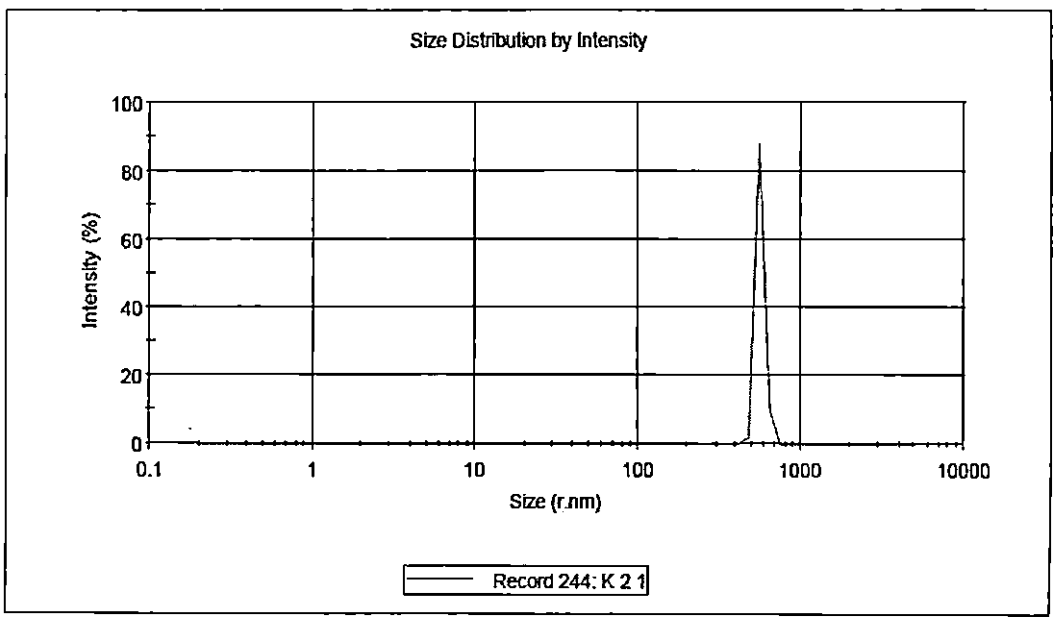
Temperature (°C): 25.0 Duration Used (s): 60

Count Rate (kcps): 256.4 Measurement Position (mm): 1.25

Cell Description: Disposable sizing cuvette Attenuator: 6

Results

			Size (r. nm)	% intensity	Width (r. nm)
Z – Average (r. nm)	2377	Peak 1	560.6	100.0	29.07
PdI	0.526	Peak 2	0.000	0.0	0.000
Intercept	0.984	Peak 3	0.000	0.0	0.000



At an appropriate dilution, FFS II also showed a single sharp peak with high polydispersity index (0.526) and attenuator of 6. The largest particle was in the size of 560.6 r. nm. From the graph it can be concluded that the particles of FFS II ranged between 400 to 800 r. nm. The maximum width of the particles of FFS II was 29.07 r. nm.

From the derived values it could be inferred that the particle size for FFS I with the largest particle was in the size of 534.7 in comparison to FFS II which had a particle size of 560.6 r. nm. The particles of FFS I ranged between 400 to 650 r. nm while for FFS II it ranged between 400 to 800 r. nm. The maximum width of the particles of FFS I was 32.51 r. nm and 29.07 r. nm for FFS II.

The **salient findings** of the functional properties and physico – chemical properties depicted that, the functional characters like bulk density (0.90 g/cm^3), rehydration ratio (0.27), gelatinization time (1.16 min) and temperature (88.25^0 C), Water Absorption Capacity (WAC) (1.59) and Water Solubility Index (0.14) significantly increased in the fermentation technique of FFS II on comparison with FFS I, which had the following values like bulk density (0.75 g/cm^3), rehydration ratio (0.23), gelatinization time (0.48 min) and temperature (85.25^0 C), Water Absorption Capacity (WAC) (1.44) and Water Solubility Index (0.13). On the other hand, dispersibility (2.75 per cent), swelling capacity (6.66) and Water Absorption Index, WAI (2.65) decreased significantly in FFS II when compared to FFS I values like dispersibility (7.25 per cent), swelling capacity (7.04) and Water Absorption Index, WAI (2.68). However, there were no differences noted in the processing loss and yield ratio of the two FFS.

With regard to pasting properties, for FFS I, the pasting temperature (84.45^0 C) and pasting point (59.25 cP) were notable. However, these properties could not be detected for FFS II. The peak viscosity which is the ability of starch to swell freely before their physical breakdown ranged between 1190.75 cP for FFS I and 223.75 cP for FFS II. FFS II had the lowest (4.0 RVU) breakdown viscosity when compared to FFS I (206.75 RVU). The final viscosity, which is

the change in the viscosity after holding cooked starch, ranged between 1958.75 RVU for FFS I and 453.75 RVU for FFS II. The setback value of the FFS I & II were 975 and 234 RVU, respectively.

Texture profile analysis showed very low values for FFS II which could not be detected in hardness, cohesiveness, elasticity, gumminess etc., of the fermented flour paste. FFS I scored 9.06 on the hardness scale. FFS I showed a cohesiveness value of 0.499. Adhesiveness value of FFS I was (- 37.515). The springiness and gumminess values of FFS I was 5.4 and 4.79 respectively. The chewiness range of FFS I showed 25.86.

The colour attributes when analyzed portrayed that, the mean L* value ranged between 73.72 for FFS I and 64.77 for FFS II which had the lowest L* value. a* values of FFS I (1.71) were lower than those of FFS II (3.50). However, as a result of the browning reactions during drying, FFS II (22.0) exhibited higher b* values than FFS I (15.33). A low value for chroma and a high value for lightness are desired for the product to meet the consumer preference.

Comparing the particle size, for FFS I, the largest particle was in the size of 534.7 in comparison to FFS II which had a particle size of 560.6 r. nm. The particles of FFS I ranged between 400 to 650 r. nm while for FFS II it ranged between 400 to 800 r. nm. The maximum width of the particles of FFS I was 32.51 r. nm and 29.07 r. nm for FFS II.

4.2.4. Shelf life Quality Assessment of FFS I & II

Assessment of shelf life quality is important since it determines the suitability of a particular ingredient for the product development. Shelf life is the recommendation of time that products can be stored, during which the defined quality of a specified proportion of the goods remains acceptable under expected conditions of distribution, storage and display (Azanha and Faria, 2005).

Product quality depends on exposure to light and heat, transmission of gases (including humidity), mechanical stresses, and contamination by things such as micro-organisms. Product quality is often influenced by concentration of a chemical compound, a microbiological index, or moisture content (Gyesley, 2003).

The shelf life quality of FFS developed were analysed by assessing the moisture content, peroxide value and microbial growth initially and up to a period of six months. Storage studies will give a clear picture of ambient conditions of storage, quality of packing materials and other details regarding the product requirement.

4.2.4.1. Moisture Content of Stored FFS I & II

Moisture content is one of the most commonly measured properties of food materials. Knowledge of the moisture content is often necessary to predict the behaviour of foods during processing. For estimating the moisture content the developed products were packed in laminated pouches, sealed air tight and stored at ambient conditions. The moisture content was recorded periodically up to 6 months and the data is shown in Table no. 38.

Table 38. Moisture (%) of stored FFS I & II

Period of study	FFS I	FFS II
Initial	10.83	10.68
1st month	10.93	10.68
2nd month	10.98	10.75
3rd month	10.98	10.78
4th month	11.13	10.83
5th month	11.15	10.95
6th month	11.3	10.97
F value (Product) - 203.04 ** ,	CD- 0.044	
F value (Months)- 27.60 **	CD- 0.017	
F value (Product * Months) - 3.408 *	CD- 0.101	

For FFS I the moisture percentage increased from 10.83 per cent from the initial recording to 11.3 per cent after a period of six months. Similarly, in FFS II the initial moisture percentages recorded were 10.68 which gradually increased to 10.97 per cent in the end of the period of the study.

The statistical analysis through ANOVA revealed that, there was significant variation in the moisture content of both FFS I & II over a period of 6 months of storage. Also the F values pointed out that, even though the variation in each month seems minor, the differences were significant at 1 per cent level in both the FFSs.

4.2.4.2. Assessment of Peroxide Value of Stored FFS I & II

Peroxide value gives an indication about the extent of peroxidation taking place in stored food materials. The acceptability of a food product depends on the extent to which deterioration has occurred and oxidative rancidity is a major cause of food deterioration. This in turn represents a major cause of loss of nutritional quality as well as cause of concern for food safety, as the oxidized fats in a very high dosage have been shown to have toxic effects (Sen and Sen, 2009). The peroxide value was recorded for a period of 6 months similar to that of moisture.

Table 39. Peroxide value (meq/kg) of stored FFS I & II

FFS	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
I	0	0	0	0	0	0.22	0.23
II	0	0	0	0	0	0	0.21

The peroxide content was not observed for both FFS I & II for the first four months of the study. The above mean table elicited that the FFS I reported peroxide contents in the 5th month (0.22 meq/kg) and 6th month (0.23 meq/kg). On the other hand, FFS II elucidated peroxide contents (0.21 meq/kg) only in the final (6th) month of the storage studies. This can be correlated with the lesser content of fat in FFS II when compared to FFS I. However, it could be noted that the peroxide contents in the FFS I & II were much minimal than the permitted limits. This shows that, both FFS I and II can be stored for a period of six months without any discriminate changes, thereby giving them a higher shelf life and marketable values.

4.2.4.3. Assessment of Total Microbial Population of FFS I & II

Microbial population in developed food products is important as it determines the quality and safety of food products. The microbiological safety of food is achieved by as far as possible ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication (Beckers, 1988). Food products that have been subjected to an adequate heat-treatment during processing are free of vegetative pathogens. So it is regarded as safe. Microbial analyses of stored products were done to ascertain the shelf life of the products. The products were stored at ambient condition for 3 months. The microbial evaluation was done initially and at 30 days interval up to 3 months. The growth of bacteria, fungi, actinomycetes and E-coli were determined using Nutrient Agar (NA), Potato Dextrose Agar with Rose Bengal (PDARB), Ken Knight's Agar (KEN) and Eosin Methylene Blue (EMB). This was done by serial dilution of the samples followed by pour plating techniques suggested by Johnson and Curl (1972).

Processed foods which are stored and consumed after a period of storage require certain microbial criteria to be employed to ensure their quality and safety. Many organisms causing food borne illness may grow significant effect on the quality of final product. According to Shankaran (2000) several factors such as raw material quality, storage temperature, storage containers, processing methods, the environment in which it is processing etc will affect the microbiological quality of processed foods. Since processed foods provide ample scope for contamination with spoilage and pathogenic microorganisms, the microbial quality was assessed.

Table 40. Microbial loads of stored FFS I (cfu/g)

Microbes	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
Bacteria	Nil	Nil	Nil	Nil	Nil	$1x10^{-1}$	$2x10^{-1}$
Actinomycetes	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Fungi	Nil	Nil	Nil	Nil	Nil	Nil	$1x10^{-1}$
Pathogens	Nil	Nil	Nil	Nil	Nil	Nil	Nil

It was evident from the above table that during the six months of storage period no actinomycetes were found to be appeared in the developed products i.e. FFS I & II. But bacterial colonies were observed from the 5th month (1×10^{-1} cfu/g) and 6th month (2×10^{-2} cfu/g). Simultaneously, fungi were detected in the 6th month (1×10^{-1} cfu/g) of the storage studies. Even though bacteria and fungi were detected, it was present only in negligible levels. No pathogenic organisms could be detected in the FFS I. It must also be brought into the consideration that, among the replicate samples analyzed, bacterial colonies and fungus was notable only in one sample randomly selected for analysis. So it could be concluded that, it might be due to cross contamination produced in any one lot of the FFS I during bulk production.

However, FFS II was found to be sterile and free of microbial contamination even after the 6th month of the storage studies. This might be brought about by the double drying technique involved in the processing of FFS II. The above findings prove that both FFS I & II are safe and stable up to a period of six months.

It was also noted that, even after six months of storage study, both FFS I & II had scored high scores for acceptability studies. This further emphasises that

both FFS I & II could be stored even after six months at ambient temperatures without any changes. This further increase the scope of marketability of the FFSs in terms of sensory attributes also.

The following are the **salient findings** of the storage studies. For FFS I the moisture percentage increased from 10.83 per cent from the initial to 11.3 per cent and in FFS II the initial moisture percentages recorded were 10.68 per cent which gradually increased to 10.97 per cent in the end of the period of the study.

The peroxide content was not observed for both FFS I & II for the first four months of the study. FFS I reported peroxide contents in the 5^{th} month (0.22 meq/kg) and 6^{th} month (0.23 meq/kg). On the other hand, FFS II elucidated peroxide contents (0.21 meq/kg) only in the final (6^{th}) month of the storage studies.

It was evident that during the six months of storage period no actinomycetes were found in the developed products i.e. FFS I & II. But bacterial colonies were observed from the 5^{th} month (1×10^{-1} cfu/g) and 6^{th} month (2×10^{-2} cfu/g). Simultaneously, fungi were detected in the 6^{th} month (1×10^{-1} cfu/g) of the storage studies. Even though bacteria and fungus was detected, it was present only in permissible limits. No pathogenic organisms could be detected in the FFS I. However, FFS II was found to be sterile and free of microbial contamination even after the 6^{th} month of the storage studies.

Even after six months of storage study, both FFS I & II had scored high scores for acceptability. This further emphasises the increased the scope of marketability of the FFSs in terms of sensory attributes also.

4.2.5. Cost of Production of Functional Food Supplements I & II

Cost of production of FFS I & II was worked out in terms of product yield and cost per kilogram of the products.

Table 41. Cost of production of FFS I

Ingredients	Quantity (gm) (per Kg of FFS I)	Rate (Rs) / kg	Quantity required accounting processing loss (gm)	Amount (Rs.)
Barley	350	80	370	29.60
Ragi	200	35	210	7.35
Banana	250	60	850	51.00
Defatted soy	150	80	170	13.60
Drumstick leaves powder	25	0	83	0.00
Oyster mushroom powder	25	300	357	70.00
Labour charges and other over head charges				28.00
			Total	199.55 (200.00)

The above table showed that the cost of one kilo gram of FFS I was Rs. 200/-. Similarly, the cost of FFS II was found to be Rs. 202/- accounting the amount of yeast required and considering the electrical charges involved in double dehydration of FFS II. Thus the cost of one Kg of both FFS I & II can be rounded off to Rs. 200/-. When calculated the cost of one portion size (20 g) of FFS I & II each, it could be noted that the cost was Rs. 4.0/- only. Subjects of any class would not hesitate to spend an amount of Rs. 4.0/- per day considering their health rather than drugs which not only produces higher side effects but are costly also.

On the health point of view, the rates of both FFS I & II were admissible when compared to the commercial products and also the large amount spent on medications which are proven to produce adverse effects in long run. So both FFS I & II are better products and economically viable when scaled against health.

4.2.6. Suitability of Developing Various Food Preparations/Products Using FFS (I & II)

Suitability of incorporating the FFS (I & II) into various preparations/products was tried out and subject to organoleptic evaluation for the sensory characteristics like colour and appearance, flavour, texture, taste and overall acceptability using a panel of trained members.

Organoleptic evaluation has been defined as a scientific method used to evoke, measure, analyze and interpret those responses to products as perceived through the sense of sight, smell, touch, taste and hearing (Stonel and Sidel, 2002).

Planning a proper breakfast plays an important role in the management of lifestyle diseases. Commonly consumed breakfast items like Idli, Dosa, Chappathi, Noolputtu, Puttu and porridge were standardized in the laboratory by the incorporation of FFS I & II (Plate 05 and 06). The acceptability of the standardized recipes by the incorporation of FFS I & II were assessed by a panel of trained members using a score card on a five point scale.

The effect of the product and their combined effects were ascertained using ANOVA in CRD. A 2 * 2 – first being the product (2 levels), second being the recipe (6 levels) ANOVA model was tried and the results of the mean table is given below.

The sensory evaluation was done by taking judges' ratings in a five point scale with 5 for excellent and 1 for poor. However in this case, not only the

Plate: 05 Recipes standardized from FFS I

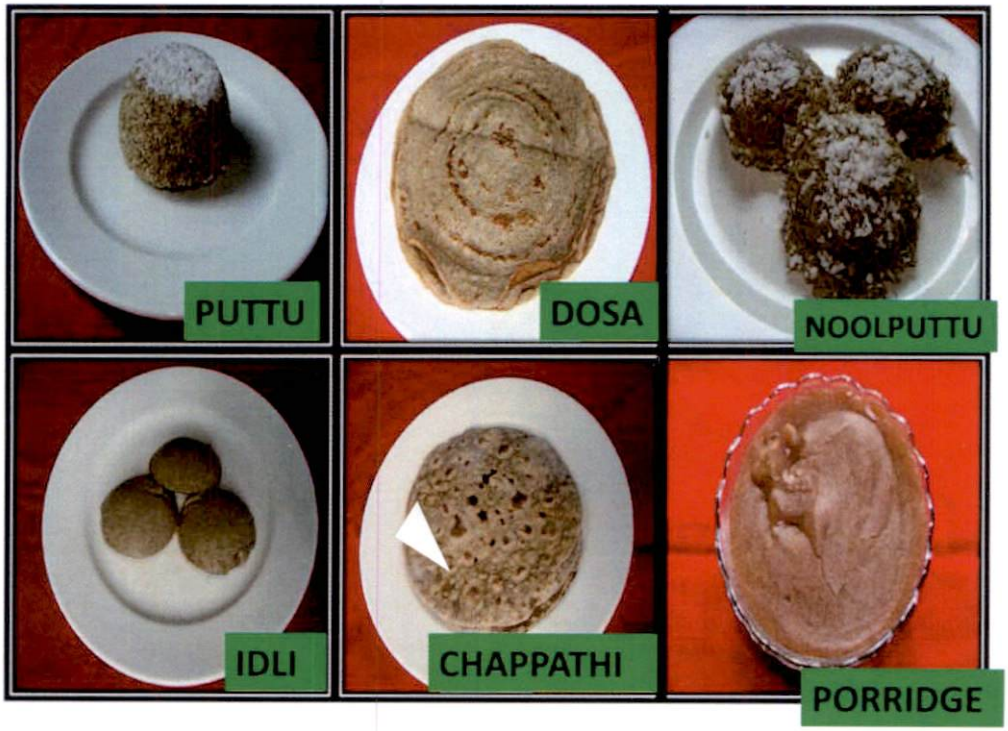


Plate: 06 Recipes standardized from FFS II

individual variation of recipes and product is very important but their combined effect was also very much relevant. Therefore, instead of a non parameteric ANOVA, usual ANOVA was carried out.

Table 42. Sensory evaluation scores of recipes standardized from FFS I & II

Recipes	Colour and appearance		Texture		Taste		Flavour		Overall Acceptability	
	FFS I	FFS II	FFS I	FFS II	FFS I	FFS II	FFS I	FFS II	FFS I	FFS II
Idli	4.1	3.7	4.0	3.6	4.0	3.7	3.9	3.6	3.9	3.7
Dosa	4.1	3.6	3.9	3.7	4.0	3.8	3.7	3.7	3.8	3.7
Puttu	4.1	4.2	4.2	4.0	4.3	4.1	4.1	3.9	4.0	4.0
Noolputtu	4.1	3.6	3.7	3.6	3.8	3.7	3.7	3.5	3.8	3.4
Chappathi	4.4	4.1	4.5	4.3	4.2	4.0	4.1	4.1	4.2	4.1
Porridge	3.7	3.8	3.6	3.6	3.5	3.2	3.4	3.5	3.5	3.6
CD value	-	-	0.45	0.45	0.36	0.36	-	-	-	-

Values are means of determinations with 10 panelists.

* ($p < 0.01$)

The above table revealed that, there were no significance differences in colour and appearance, flavor and overall acceptability of the recipes developed from FFS I at 1 per cent. But it could be observed that, in the aspects of texture and taste, there were significant variations among the recipes. Among the recipes, puttu (4.3) got the maximum score for taste, followed by chappathi (4.2), idli (4.0) and dosa (4.0) which were on par with each other, and then noolputtu (3.8) and finally porridge (3.5) scored the least.

Similar to that of FFS I, the values for FFS II also revealed that, there was no significance differences in color and appearance, flavour and overall acceptability of the recipes developed from FFS II. But it could be observed that, in the aspects of texture and taste, there were significant variations among the recipes at 1 per cent level. Among the recipes, puttu (4.1) got the maximum score for taste, followed by chappathi (4.0), dosa (3.8), idli (3.7) and noolputtu (3.7) which were on par with each other, and then finally porridge (3.2) scored the least.

From the above table, it might appear that the recipes from FFS II scored less comparatively to that of FFS I. But statistical studies proved that the differences between FFS I & II in the case of colour and appearance, flavour and overall acceptability of the recipes were insignificant.

Finally the ANOVA tables also proved that, in the case of texture there was significant variation among the recipes of both FFS I & II, which could be noted from the F values and CD values. Concomitantly, in the aspects of taste, the statistical analysis showed that there was significant variation among each of the recipes and also among the products i.e. FFS I & II. The F values (4.21*) showed that, there was significant variation among FFS I & II in the case of recipe standardization at 5 per cent level. On comparing the taste of each of the recipes from FFS I & II, the variation was significant at 1 per cent level (F – 5.35**).

From the above findings it could be concluded that, both FFS I & II were equally acceptable in whichever form of recipes might be supplemented.

Portion sizing was fixed based on a pilot study done in the laboratory mainly focusing on the sensory acceptance on the level of incorporation of FFS I & II separately in different recipes. Substitution levels from 10 per cent to 50 per cent were initially carried out in various recipes to study the best level of acceptance. Finally it was concluded that, 20 per cent of incorporation for both FFS I & II was the best acceptable level for almost all the recipes standardized in

the laboratory. Thus 20 gm was fixed as the portion size for both FFS I & II. It is less than one portion of cereals and millet in the exchange list (NIN, 2004).

Table 43. Nutrient content of one portion (20 g) of FFS I & II

Nutrients	FFS I	FFS II
Energy (Kcal)	76.8	75.6
Carbohydrates (g)	12.0	11.6
Protein (g)	4.2	3.4
Fat (g)	0.4	0.32

20 gm of the FFSs (i.e. one part) were supplemented with two parts of the basic ingredients for recipe formulation. 20 gm of FFS I was found to contain, 76.8 Kcal of energy, 12 g of carbohydrates, 4.2 g of protein and 0.4 g of fat. Concomitantly, 20 gm of FFS II was found to contain, 75.6 Kcal of energy, 11.6 g of carbohydrates, 3.4 g of protein and 0.32 g of fat. The laboratory studies proved that, the recipes developed in this manner were sufficient in quantity for the controlled diet pattern (in this case breakfast) of subjects with lifestyle diseases. This would help the subjects with lifestyle diseases to have a control over their blood profiles.

Feasibility of substitution of the FFS (I & II) in the Food Exchange List, especially in the breakfast items was computed based on their nutrient contents. From the nutrient content analysis table, it was evident that FFS I contains, 384 kcal energy, 60.5 g of carbohydrates, 21 g of protein and 1.9 g of fat per 100 g. Similarly, FFS II contains 378 kcal of energy, 58 g of carbohydrates, 16.5 g of protein and 1.6 g of fat per 100 g. Keeping the above points in mind, FFS I & II could be used in the cereal, pulse and other exchange lists. This would help to avoid monotony of diets for the subjects with lifestyle diseases.

4.3. EVALUATION OF CLINICAL EFFICACY OF FFS (I & II)

The clinical efficacy of the FFS (I & II) was ascertained through case studies.

The study was conducted in the Elamkulam Panchayath, Malappuram district of Kerala, selecting respondents from a list comprising of 250 members. Clinical efficacy of FFS (I & II) was determined in the three disease conditions viz. Hyperglycemia, Hypertension and Hyperlipidemia which is highly prevalent in our state.

From the list of 250 members, people in the early stages of diseases like hyperglycemia, hyperlipidemia and hypertension were screened. Subjects who were not on medication were again scrutinized. Willingness of participation of the subjects throughout the period of study was confirmed. The final list of the subjects for the study was in the age group of 40-55 yrs, same gender and without any other complications.

Five subjects each for three disease conditions and two different FFS developed were selected for the case studies. FFS I & II developed was supplemented in the breakfast of subjects for a period of three months. After the selection process, preliminary information regarding their socio-economic profile, health status, dietary and life style pattern and nutritional status were collected through a suitably structured questionnaire the details of which are given below.

4.3.1. General Information of the Respondents

Table 44. Socio-economic profiles of the respondents

S. no	General information	Number/Percent of respondents		
		Hyperglycaemic	Hypercholesterolemic	Hypertensive
1	**Age (years)**			
	40-50	05 (50)	02 (20)	05 (50)
	51-55	05 (50)	08 (80)	05 (50)
	Total	10 (100)	10 (100)	10 (100)
2	**Sex**			
	Male	0 (0)	10 (100)	05 (50)
	Female	10 (100)	0 (0)	05 (50)
	Total	10 (100)	10 (100)	10 (100)
3	**Religion**			
	Hindu	06 (60)	09 (90)	08 (80)
	Muslim	04 (40)	01 (10)	02 (20)
	Christian	0 (0)	0 (0)	0 (0)
	Total	10 (100)	10 (100)	10 (100)
4	**Occupation**			
	Employed	07 (70)	10 (100)	10 (100)
	Unemployed	03 (30)	0 (0)	0 (0)
	Total	10 (100)	10 (100)	10 (100)
5	**Family income**			
	< 25,000	04 (40)	05 (50)	05 (50)
	25,000-50,000	05 (50)	04 (40)	04 (40)
	>50, 000	01 (10)	01 (10)	01 (10)
	Total	10 (100)	10 (100)	10 (100)

6	**Income spend on food**			
	Below 10%	05 (50	07 (70)	07 (70)
	Above 10%	05 (50)	03 (30)	03 (30)
	Total	10 (100)	10 (100)	10 (100)

* Numbers in parenthesis indicates percentage of respondents

From the above table it could be noted that, maximum effort was taken to group the subjects based on their socio-economic profile. This was done to avoid any variation or influence brought about by the differences in the socio-economic background of the subjects on the supplementation studies. Based on each of the disease condition of the subjects, i.e. hyperglycemia, hypercholesterolemia and hypertension, the subjects were selected according to the socio-economic profile from the main list.

Socio economic profiles like, age, sex, religion etc were considered carefully to group the respondents for the study. The family size of the subjects included 4 to 5 members; major part of the respondents (16) had a degree and was government employees. Eight subjects had their educational qualification of plus two and the rest tenth standard.

4.3.2. Dietary Habits of the Respondents

The details collected on the dietary habits (Appendix IV) of the subjects revealed that, all the respondents were non-vegetarians. Most of the subjects 100 per cent of hyperglycemic, 90 per cent of hypercholesterimic and 100 per cent of hypertensive were having three main meals. 60 per cent, 80 per cent and 70 per cent respectively in each group had one snack per day and had a diet plan of their own. Majority of the subjects had their food especially breakfast and dinner in front of the television (40 per cent, 80 per cent and 60 per cent respectively in each group) and also used coconut oil as their main medium of cooking (50 percent, 60 per cent and 60 per cent respectively in each group).

The 24-hour dietary recall of the subjects, helped in getting a clear picture about their meal pattern. The collected data showed that, only 10 subjects were including fruits in their daily diet. Others took fruits only when the recipe demanded, or when the fruits like jackfruit, mango, papaya, banana and other were seasonally and when available. Almost all the subjects consumed fish once a day, especially in the lunch. It was evident that, the subjects belonging to the Muslim community consumed red meat mostly. Even though the subjects with diabetes (pre-diabetes) consumed tea or coffee without sugar, their meals or snacks involved fried foods and foods of dense calories. Also intake of oil through snacks and other fried foods was high in most of the subjects.

The details regarding the eating pattern of the respondents were collected using a food frequency table (Appendix V).

Table 45. Food use frequency table of the respondents

Food items	Frequency
Whole cereals	91.5
Pulses	100
Fruits	86
Vegetables	100
Green leafy vegetable	74.8
Milk & milk products	100
Chicken	49.6
Mutton	19.3
Beef	13
Pork	2.16
Fish	86.3
Egg	63.3
Fried foods	100
Baked foods	100
Processed foods	100
Ghee/dalda	36

From the above table on food use frequency of the subjects, it could be observed that, their eating pattern was varied and inconsistent. It also depicts a picture of unhealthy eating pattern, which might be the baseline reason for their morbidity conditions.

Though the subjects scored higher scores like 91.5 for whole cereals, 100 for pulses, vegetables, milk & milk products, 86.6 for fish, 86 for fruits and 74.5 for green leafy vegetables, the very high score of 100 for fried, baked and

processed foods which are some of the foods which may lead to lifestyle diseases indicate unhealthy eating pattern.

Individual counseling was imparted to the selected respondents under case study regarding the dietary regime to be followed for the specific disease condition. Explanations on need for special diet, foods to be restricted for disease condition, dietary modifications to be followed, method of incorporation of the supplement in the diet were also given.

4.3.3. Health Information

4.3.3.1. Morbidity Pattern of the Selected Subjects

Details on health status revealed that most of the respondents had a combination of diseases. The general trend in the disease condition proved to be higher among males. Most of the lifestyle diseases prevalent among the subjects were due to hereditary reasons. The duration of the diseases prevalent showed duration of less than one year of onset. The commonly reported other diseases included thyroid problems, migraine, osteoporosis and sinusitis.

A very few of the respondents were following home remedies like, consuming fenugreek, gooseberries and some other green leafy vegetables for reducing blood sugar; drinking water after boiling it with coconut shell and chillies (small) for reducing cholesterol; drinking drumstick leaves and bark juice for controlling blood pressure.

4.3.3.2. Health Habits of the Subjects

The details regarding the health habits of the respondents are appended (Appendix VI). 80 per cent of hypercholesterolemic and hypertensive subjects each smoked and 80 per cent and 70 per cent subjects consumed alcohol. Even though subjects explained smoking and alcohol consumption, it was very occasional or rare.

Physical activity and exercises were practiced by most of the subjects. Two subjects did not do any regular exercises. Lack of time was the reason given for not doing any exercises. This showed comparatively higher chances of lifestyle disease prevalence among those subjects. Walking was the main type of aerobic exercise practised. A few of the subjects even practised yoga.

Nearly half of the women respondents had assistance for household work daily as they were employed. A very few of the employed women did have any assistance. Most of the women respondents considered household chores as their exercise. Almost all the respondents were sedentary workers.

4.3.4. Nutritional Status of the Selected Respondents

Nutritional status of the selected respondents was assessed through anthropometry. Anthropometry provides the single most universally applicable, inexpensive technique for assessing the size, proportions and composition of the human body. Anthropometry has been accepted as an important tool for the assessment of nutritional status (Vijayaraghavan, 1987).

Details of the anthropometric measurements of the subjects relevant to the study like height, weight, BMI and Waist – Hip ratio are given below.

Table 46. Anthropometric measurements of the respondents

Subjects	Height (cm)	Weight (Kg)	BMI	Waist-hip ratio
Hyperglycaemic subjects				
1	159	63	24.92	0.8
2	160	60	23.44	0.75
3	157	62	25.15	0.85
4	158	65	26.04	0.85
5	158	68	27.24	0.9
6	161	67	25.85	0.8

7	155	62	25.81	0.8
8	160	63	24.61	0.8
9	162	64	24.39	0.85
10	158	65	26.04	0.85
Hypercholeserimic subjects				
1	172	78	26.37	1.05
2	173	80	26.73	1.05
3	170	72	24.91	1
4	171	74	25.31	1.1
5	172	75	25.35	1.05
6	174	78	25.76	1.1
7	173	75	25.06	1
8	170	73	25.26	1.2
9	169	70	24.51	0.9
10	172	75	25.35	1
Hypertensive subjects				
1	173	80	26.73	1.05
2	170	72	24.91	1
3	171	74	25.31	1
4	172	75	25.35	1.05
5	174	78	25.76	1.1
6	160	60	23.44	0.75
7	157	62	25.15	0.85
8	158	65	26.04	0.85
9	158	68	27.24	0.9
10	161	67	25.85	0.8

Having weight more than the ideal weight for age and height is a risk factor for development of many diseases and it would also exacerbate the

symptoms of osteoarthritis. The weight is a continuous variable, reflecting the body mass of an individual in light clothing; it is used for calculating BMI.

Height is another key variable required for calculation of body mass index (BMI). Height is a continuous variable measured with the individual standing on a firm levelled surface, without wearing any foot wear, and stand with feet together, with heels, calves, buttocks, dorsal spine and head in same plane.

BMI (Body mass index) is a valid indicator for finding out whether the body weight of an individual is appropriate for the height of the individual. It is calculated from height and weight measurements as body weight per meter^2. Worldwide researches have shown that there is a strong association between BMI and health risk. The excess of adipose tissue in the adults is associated with excess morbidity and mortality from a large number of health conditions like diabetes, hypertension, hypercholesterolemia, carcinomas of colon and breast, gall bladder stones and osteoarthritis. On the other hand low BMI is an indicator of risk to health, often being associated with tobacco, alcohol use and drug addiction.

The waist circumference is one of the sensitive indicators for abdominal obesity. Abdominal obesity has got a stronger association with coronary heart diseases as compared to BMI. The waist measurement is taken at the level of midpoint between the inferior margin of the rib and crest of ileum in the mid auxiliary plane, using a non-stretchable measuring tape, without clothing. A cut-off level of 102 cm in males and 88 cm in females have been recommended for developed countries (ATP3 Guidelines), however lower cut-off levels are appropriate for Indians- 90 cm in males and 80 cm in females (The Asia Pacific Guidelines).

From the above the table, it was vivid that only eight out of the thirty subjects had normal BMI, i.e. less than 25. All the other subjects falls under grade I obesity, since they had a BMI of 25 – 29.9. Also the waist – hip ratio denotes upper body obesity in most of the subjects. Even for the subjects with

normal BMI, the waist – hip ratio was on the higher side, denoting the predominant distribution of fat in the upper part of the body.

The data collected also visualized that, almost all the subjects were feeling stressed at minute difficulties. Yoga and exercise helped them to reduce to some extent. 50 per cent of the subjects had health checkups once in a year, while 30 per cent had it occasionally or when demanded by the health professionals and 20 per cent did not follow any fixed schedule or did not have health checkups. Similar trend was followed by the respondents in the case of blood profile monitoring also. Only 40 to 45 per cent of the subjects were on the importance of dietary modifications, but almost all were eager to understand the need of dietary changes in the control and management of lifestyle diseases.

4.3.5. Impact Evaluation of the Supplements (FFS I & II)

The amount of FFS (I & II) to be supplemented to the respondents was optimized based on the portion sizing and sensory attributes of the products. The optimized quantities (20 gm/day) of the FFS I & II to be supplemented for each day to the subjects were packed into polythene pouches for saving them the inconvenience of measuring the supplements daily and also to confirm the right amount of intake.

Impact of the supplementation of FFS (I & II) on the subjects was monitored initially (before), intermittently (after 45 days) and finally (after) the conduct of the study. Frequent follow ups were done to understand the difficulties if any faced by the subjects in incorporating the supplements in their daily diet and to also to confirm there was no plate waste.

Clinical parameters like Fasting Blood Sugar, Post Prandial Blood Sugar, Glycemic Index, blood pressure and lipid profile, general health and morbidity of the subjects was monitored before, in between and after the conduct of the study.

Plate: 07 Respondents of supplementation study

Blood profiles were monitored by using standard procedures followed in the clinical laboratories. General health and morbidity was ascertained with the help of a medical practitioner.

4.3.5.1. Impact Evaluation of FFS I & II on Hyperglycemic Subjects

Blood parameters like Fasting Blood Sugar (FBS) and Post Prandial Blood Sugar of the subjects was monitored before, in between and after the conduct of the study to understand the impact of supplementation of FFS I & II. Five subjects each for FFS I & II had undergone the study. Thus a total of ten subjects with hyperglycemia were supplemented with FFS I & II accordingly.

The details of the Fasting Blood Sugar (FBS) of the subjects during the conduct of the study are given below.

Table 47. Fasting Blood Sugar (mg/dl) of subjects supplemented with FFS I & II

Subjects	FBS (mg/dl)			Subjects	FBS (mg/dl)		
	Days				Days		
	0 (initial)	45	90		0 (initial)	45	90
HG I - A	125	120	110	HG II - A	135	130	100
HG I - B	130	120	105	HG II - B	135	125	110
HG I - C	135	125	105	HG II - C	130	120	105
HG I - D	130	125	100	HG II - D	125	120	105
HG I - E	125	115	100	HG II - E	130	125	110

In the above table HG – I, was the group with the hyperglycemia subjects, supplemented with FFS I, while table HG – II, was the group with the

hyperglycemia subjects, supplemented with FFS II. Subjects A to E are five respondents in each group.

To study the impact of supplementation of FFS I & II, in the blood profiles of the subjects and also to analyze the variation produced by FFS I & II, ANOVA in split plot was carried out. Since the observations corresponding to one day will naturally be dependent on its value corresponding to a previous day; instead of doing ANOVA in 2 x 2 models, ANOVA in this case was done in split plot fashion with days in the sub plot and product (FFS I & II) in the main plot. The details of which are as follows:

Table 48. Mean FBS (mg/dl) values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	FBS (mg/dl)			
	Day 0	**Day 45**	**Day 90**	**Product mean**
FFS I	129	121	104	118
FFS II	131	124	106	120
Mean -Days	130	122.5	105	
F value (Product) – NS F value (Days) – 117.92 ** CD- Days. 3.54				

From the above ANOVA table it was observed that, there was significant variation at 1 per cent level in the FBS levels of subjects supplemented with FFS I & II over a period of 90 days. The mean initial (Day 0) FBS values for the subjects supplemented with FFS I & II were 129 mg/dl and 131 mg/dl respectively. By the end of supplementation on day 90, the values significantly reduced to 104 mg/dl and 106 mg/dl for FFS I & II respectively.

But on the other hand, it could also be noted that, there was no differences among FFS I & II in the level of variation in FBS of the subjects. This concludes that both FFS I & II are equally effective in producing favorable results.

It can also be noted that, in both the cases of FFS I & II, the final values matched the FBS values of normal subjects or those who are under control.

Table 49. Post Prandial Blood Sugar (PPBS) (mg/dl) of subjects supplemented with FFS I & II

Subjects	PPBS (mg/dl)			Subjects	PPBS (mg/dl)		
	Days				Days		
	0 (initial)	45	90		0 (initial)	45	90
HG I - A	200	190	160	HG II - A	215	200	180
HG I - B	210	190	165	HG II - B	210	195	175
HG I - C	210	185	160	HG II - C	210	200	175
HG I - D	200	185	150	HG II - D	210	195	170
HG I - E	200	180	170	HG II - E	215	205	170

It can be noted from the above table that, in both the cases of FFS I & II, the final values decreased considerably over a period of 90 days. It was also observed that, the variation was notable only after the 45th day in most of the cases.

Table 50. Mean PPBS (mg/dl) values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	PPBS (mg/dl)			
	Day 0	**Day 45**	**Day 90**	**Product mean**
FFS I	204	186	161	183.667
FFS II	212	199	174	195
Mean - Days.	208	192.5	167.5	
F Product - 32.11 **		CD- Product. 4.612		
F Days - 196.51 **		CD- Days. 4.371		

From the above ANOVA table it was observed that, there was significance variation at 1 per cent level in the PPBS levels of subjects supplemented with FFS I & II over a period of 90 days. The mean initial (Day 0) PPBS values for the subjects supplemented with FFS I & II were 204 mg/dl and 212 mg/dl respectively. By the end of supplementation on day 90, the values significantly reduced to 161 mg/dl and 174 mg/dl for FFS I & II respectively.

It was also evident that, there was significant variation among the FFS I & II in reducing the PPBS of the subjects. On a whole, FFS I was found to be more effective in reducing the PPBS of the subjects when compared to FFS II.

To overcome the doubts of initial blood values of the subjects affecting the over the period changes on supplementation, ANOVA was carried out to understand the rate of change (%) per day over the previous time and the results are as follows:

Table 51. Mean rate of change (%) FBS values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	Day 0 - 45	Day 45 - 90	Product mean
FFS I	1.1	1.26	1.18
FFS II	1.074	1.24	1.157
Mean-Days.	1.087	1.25	
F – days - 80.138 **		**CD – days** - 0.042	
F – products - NS			

Table 52. Mean rate of change (%) PPBS values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	Day 0 - 45	Day 45 - 90	Product mean
FFS I	0.5	0.73	0.615
FFS II	0.384	0.612	0.498
Mean - Days.	0.442	0.671	
F – Products	73.404 **	**CD–Products**	0.031
F – days	90.647 **	**CD – days**	0.056

Similar to that of the mean PPBS levels of the subjects, the mean rate of change (%) of PPBS also denotes there was significant variation in the initial and final levels. Also, the variation between the FFSs in reducing the PPBS was significant at 1 per cent level. Thus portraying FFS I to be better than FFS II in altering the PPBS levels of the subjects.

From the above findings, it can be seen that even the rate of change (%) of both FBS and PPBS is concomitant to that of the values of the subjects over a period of 90 days. This helps to overcome the fact that, the changes may be due to the variation in the initial values of the subjects.

4.3.5.2. Impact Evaluation of FFS I & II on Hypercholesterolemic Subjects

Blood parameters like Total Cholesterol (TC), Low density Lipoprotein (LDL), Very Low density Lipoprotein (VLDL), High density Lipoprotein (HDL) and Triglycerides (TG) of the subjects was monitored before, in between and after the conduct of the study to understand the impact of supplementation of FFS I & II. Five subjects each for FFS I & II had undergone the study. Thus a total of ten subjects with hypercholesterolemia were supplemented with FFS I & II accordingly.

The details of the Total Cholesterol of the subjects during the conduct of the study are given below.

Table 53. Total Cholesterol (TC) levels (mg/dl) of subjects supplemented with FFS I & II

Subjects	Total Cholesterol (mg/dl)			Subjects	Total Cholesterol (mg/dl)		
	Days				Days		
	0 (initial)	45	90		0 (initial)	45	90
HC I - A	245	230	200	HC II - A	242	225	198
HC I - B	240	230	195	HC II - B	240	220	185
HC I - C	240	225	190	HC II - C	245	215	200
HC I - D	235	210	185	HC II - D	235	210	190
HC I - E	240	220	180	HC II - E	240	230	190

In the above table HC – I, is the group with the hypercholesterimic subjects, supplemented with FFS I, while table HC – II, is the group with the hypercholesterimic subjects, supplemented with FFS II. Subjects A to E are five respondents in each group.

It can be noted from the above table that, in both the cases of FFS I & II, the final values i.e. on 90th day of supplementation, decreased considerably over a period of 90 days. It was also observable that, the variation was notable only after the 45th day in most of the cases, similar to that of blood glucose levels of the subjects under study.

Table 54. Mean TC values (mg/dl) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	Total cholesterol values (mg/dl)			
	Day 0	**Day 45**	**Day 90**	**Product Mean**
FFS I	240	223	190	217.667
FFS II	240.4	220	192.6	217.667
Mean –Days	240.2	221.5	191.3	
F – Days - 252.94 ** **CD- Days. -** 4.651 **F – Product -** NS				

From the above ANOVA table it is observable that, there was significance variation at 1 per cent level in the total cholesterol levels of subjects supplemented with FFS I & II over a period of 90 days, showing differences over the period of days. It was notable that, the mean initial TC values of the subjects supplemented with FFS I & II were 240 mg/dl for each, which had reduced to 190 mg/dl and 193 mg/dl respectively. However, the mean reduction of both FFS I & II denotes

that there is no variation among the products in the ability to decrease total cholesterol.

But on the other hand, it could also be noted that, there was no differences among the subjects of FFS I & II group in the level of variation in total cholesterol. This concludes that both FFS I & II are equally effective in producing favorable results in the case of total cholesterol.

It can also be noted that, in both the cases of FFS I & II, the final values matched the total cholesterol values of normal subjects or those who are under control.

Table 55. Low density Lipoprotein (LDL) levels (mg/dl) of subjects supplemented with FFS I & II

Subjects	LDL (mg/dl)			Subjects	LDL (mg/dl)		
	Days				Days		
	0 (initial)	45	90		0 (initial)	45	90
HC I - A	160	155	150	HC II - A	159	152	148
HC I - B	155	152	150	HC II - B	149	148	143
HC I - C	158	150	145	HC II - C	161	158	153
HC I - D	150	145	142	HC II - D	155	152	145
HC I - E	153	145	143	HC II - E	160	155	152

It can be noted from the above table that, in both the cases of FFS I & II, the final values i.e. on 90th day of supplementation, decreased considerably over a period of 90 days. It was also observed that, the variation was notable only after the 45th day in most of the cases, similar to that of blood glucose and total cholesterol levels of the subjects under study. It can also be noted that, in both the

cases of FFS I & II, the final values matched the values of normal subjects or those who are under control. But when compared to total cholesterol levels of the subjects under study, variation in the LDL levels were less evident.

Table 56. Mean LDL values (mg/dl) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	LDL values (mg/dl)			
	Day 0	**Day 45**	**Day 90**	**Product Mean**
FFS I	155.2	149.4	146	150.2
FFS II	156.8	153	148.2	152.667
Mean-Days.	156	151.2	147.1	
F – Days - 88.502 ** **CD - Days.** - 1.420				
F – Products - **NS**				

From the afore mentioned ANOVA table it was evident that, there was significance variation at 1 per cent level in the LDL levels of subjects supplemented with FFS I & II over a period of 90 days, showing differences over the period of days. It could be observed that, the mean initial LDL values of the subjects supplemented with FFS & II were 155 mg/dl and 157 mg/dl respectively. By the end of 90 days of the study, the values had decreased to 146 mg/dl and 148 mg/dl for FFS I & II respectively.

However, it could also be noted that, there was no differences among the subjects of FFS I & II groups in the level of variation in LDL. This concludes that both FFS I & II are equally effective in producing favorable results in the case of LDL, similar to that of FBS and total cholesterol.

Table 57. Very Low density Lipoprotein (VLDL) levels (mg/dl) of subjects supplemented with FFS I & II

Subjects	VLDL (mg/dl)			Subjects	VLDL (mg/dl)		
	Days				Days		
	0 (initial)	45	90		0 (initial)	45	90
HC I - A	40	39	37	HC II - A	45	42	40
HC I - B	38	38	35	HC II - B	40	39	37
HC I - C	42	40	38	HC II - C	45	43	40
HC I - D	38	37	35	HC II - D	38	35	35
HC I - E	40	38	35	HC II - E	42	40	36

It can be noted from the above table that, in both the cases of FFS I & II, the final values i.e. on 90th day of supplementation, decreased considerably over a period of 90 days. It was also observed that, the variation was notable only after the 45th day in most of the cases, similar to that of blood glucose and total cholesterol levels of the subjects under study.

It can also be noted that, in both the cases of FFS I & II, the final values for each subjects in both the cases matched the values of normal subjects or those who are under control. But when compared to total cholesterol levels of the subjects under study, variation in the VLDL levels is not that prominent.

Table 58. Mean VLDL values (mg/dl) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	VLDL values (mg/dl)			
	Day 0	**Day 45**	**Day 90**	**Product Mean**
FFS I	39.6	38.4	36	38
FFS II	42	39.8	37.6	39.8
Mean -Days.	40.8	39.1	36.8	
F – Days - 74.387 **		**CD - Days.** - 0.698		
F – Products - **NS**				

From the afore mentioned ANOVA table it was evident that, there was significance variation at 1 per cent level in the VLDL levels of subjects supplemented with FFS I & II over a period of 90 days, showing differences over the period of days. It could be observed that, the mean initial VLDL values of the subjects supplemented with FFS & II were 40 (39.6) mg/dl and 42 mg/dl respectively. By the end of 90 days of the study, the values had decreased to 36 mg/dl and 38 (37.6) mg/dl for FFS I & II respectively.

However, it could also be noted that, there was no differences among the subjects of FFS I & II groups in the level of variation in VLDL, declaring no variation in the products' ability to reduce VLDL levels of subjects. This concludes that both FFS I & II are equally effective in producing favorable results in the case of VLDL, similar to that of FBS and other lipid profiles.

Table 59. Triglycerides (TG) levels (mg/dl) of subjects supplemented with FFS I & II

Subjects	TG (mg/dl)			Subjects	TG (mg/dl)		
	Days				Days		
	0 (initial)	45	90		0 (initial)	45	90
HC I - A	190	185	178	HC II - A	185	180	172
HC I - B	182	175	170	HC II - B	180	175	168
HC I - C	185	180	170	HC II - C	190	185	178
HC I - D	175	172	160	HC II - D	170	165	158
HC I - E	180	175	164	HC II - E	185	180	172

It can be noted from the above table that, in both the cases of FFS I & II, the final values i.e. on 90th day of supplementation, decreased considerably over a period of 90 days. It was also observed that, the variation was notable only after the 45th day in most of the cases, similar to that of blood glucose and other lipid profiles of the subjects under study.

It can also be noted that, in both the cases of FFS I & II, the final values matched the values of normal subjects or those who are under control. Rate of variation in the TG levels of the subjects were comparable to that of the total cholesterol levels and was prominent, unlike other lipid parameters.

Table 60. Mean TG values (mg/dl) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	TG values (mg/dl)			
	Day 0	**Day 45**	**Day 90**	**Product mean**
FFS I	182.4	177.4	168.4	176.067
FFS II	182	177	169.6	176.2
Mean -Days.	182.2	177.2	169	
F – Days - 364.410 ** **CD - Days.** - 1.047 **F – Products** - **NS**				

Through statistical analysis of the data using ANOVA, it was evident that, there was significance variation at 1 per cent level in the TG levels of subjects supplemented with FFS I & II over a period of 90 days, showing differences over the period of days being significant. Conversely, it could also be noted that, there was no differences among the subjects of FFS I & II groups in the level of variation in TG, declaring no variation in the products' ability to reduce TG levels of subjects.

When subjects were supplemented with FFS I & II, the mean initial TG levels which were 182 (182.4) mg/dl and 182 mg/dl respectively, gradually declined to 168 (168.4) mg/dl and 170 (169.6) mg/dl respectively by the 90th day. Since there was no much variation noted among the mean of the products, the analysis shows that there was no changes among the FFS I & II in its therapeutic effect.

This concludes that both FFS I & II are equally effective in producing favorable results in the case of TG, similar to that of FBS and other lipid profiles.

Table 61. High Density Lipoprotein (HDL) levels (mg/dl) of subjects supplemented with FFS I & II

Subjects	HDL (mg/dl)			Subjects	HDL (mg/dl)		
	Days				Days		
	0 (initial)	45	90		0 (initial)	45	90
HC I - A	39	40	42	HC II - A	37	37	40
HC I - B	39	40	42	HC II - B	38	40	45
HC I - C	42	43	48	HC II - C	42	42	49
HC I - D	42	43	48	HC II - D	42	42	49
HC I - E	40	42	45	HC II - E	40	42	45

It can be noted from the above table that, in both the cases of FFS I & II, the final values i.e. on 90th day of supplementation, increased considerably over a period of 90 days. It was also observed that, the variation was notable only after the 45th day in most of the cases, similar to that of blood glucose and other lipid profiles of the subjects under study.

It can also be noted that, in both the cases of FFS I & II, the final values matched the values of normal subjects or those who are under control. Rate of variation in the HDL levels of the subjects were not comparable to that of the total cholesterol levels and was not prominent, similar to that of other lipid parameters.

Table 62. Mean HDL values (mg/dl) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	HDL values (mg/dl)			
	Day 0	**Day 45**	**Day 90**	**Product mean**
FFS I	40.4	41.6	45	42.333
FFS II	39.8	40.6	45.6	42
Mean -Days.	40.1	41.1	45.3	
F – Days - 69.207 **		**CD - Days.** - 0.994		
F – Products - NS.				

From the statistical analysis of the data using ANOVA, it was evident that, there was significant variation at 1 per cent level in the HDL levels of subjects supplemented with FFS I & II over a period of 90 days, showing differences over the period of days being evidently significant. Conversely, it could also be noted that, there is no differences among the subjects of FFS I & II groups in the level of variation in HDL, declaring no variation in the products' ability to increase HDL levels of subjects.

On supplementation with FFS I & II to subjects with hypercholesterolemia, the mean initial HDL levels which were 40 (40.4) mg/dl and 40 (39.8) mg/dl respectively steadily increased to 45 mg/dl and 46 (45.6) mg/dl by the end of the study. From the mean values of the products given in the table it could be concluded that both FFS I & II are equally effective in producing favorable results in the case of HDL, similar to that of FBS and other lipid profiles.

Similar to that of subjects with hyperglycemia, to clear the problem that the initial blood values of the subjects might affect over the period changes on supplementation, ANOVA was carried out to understand the rate of change (%) per day over the previous time and the results are as follows:

Table 63. Mean rate of change (%) total cholesterol values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	Day 0 - 45	Day 45 - 90	Product mean
FFS I	1 0.1600	0.464	0.312
FFS II	0.19	0.442	0.316
Mean -Days.	0.175	0.453	
F – Days	131.546 **	**CD – days**	0.056
F – products	NS		

Table 64. Mean rate of change (%) LDL values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	Day 0 - 45	Day 45 - 90	Product mean
FFS I	0.838	0.87	0.854
FFS II	0.808	0.85	0.829
Mean -Days.	0.823	0.86	
F – Days	50.617 **	**F – products** - NS	
CD – days	0.012		

Table 65. Mean rate of change (%) VLDL values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	Day 0 - 45	Day 45 - 90	Product mean
FFS I	1.866	1.89	1.878
FFS II	1.854	1.874	1.864
Mean -Days.	1.86	1.882	
F – Days	35.444 **	**F – products** - NS	
CD – days	0.009		

Table 66. Mean rate of change (%) TG values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	Day 0 - 45	Day 45 - 90	Product mean
FFS I	0.58	0.664	0.622
FFS II	0.586	0.656	0.621
Mean -Days.	0.583	0.66	
F – Days	204.339 **	**F – products** - NS	
CD – days	0.012		

Table 67. Mean rate of change (%) HDL values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	Day 0 - 45	Day 45 - 90	Product mean
FFS I	1.836	1.806	1.821
FFS II	1.846	1.802	1.824
Mean -Days.	1.841	1.804	
F – Days	43.066 **	**F – products** - NS	
CD – days	0.013		

From the above details, it can be concluded that even the rate of change (%) from initial (0 days) to intermittent day (45th day) and to the final day (90th day) of the lipid profiles, which was inclusive of total cholesterol, LDL, VLDL, triglycerides and HDL are concomitant to that of the individual and mean values of the subjects supplemented with FFS I & II over a period of 90 days. This helps to overcome the fact that, the changes may be due to the variation in the initial values of the subjects or due to individual variations.

The above findings further help to substantiate the facts that, there were no significant variations among the FFS I & II in altering the lipid profiles of the subjects. But both FFS I & II were equally potential to alter the lipid profiles of the subjects over a period of 90 days. This strengthens the therapeutic effect of both FFS I & II equally.

4.3.5.3. Impact Evaluation of FFS I & II on Hypertensive Subjects

Blood parameters like systolic and diastolic blood pressure of the subjects were monitored before, in between and after the conduct of the study to understand the impact of supplementation of FFS I & II. Five subjects each for FFS I & II had undergone the study. Thus a total of ten subjects with hypertension were supplemented with FFS I & II accordingly.

The details of the systolic blood pressure of the subjects during the conduct of the study are given below.

Table 68. Systolic /diastolic blood pressure levels (mm Hg) of subjects supplemented with FFS I & II

Subjects	Blood pressure mm Hg			Subjects	Blood pressure mm Hg		
	Days				Days		
	0 (initial)	45	90		0 (initial)	45	90
HT I - A	160/100	145/95	135/85	**HT II - A**	150/100	145/98	140/95
HT I - B	160/100	140/95	130/80	**HT II - B**	160/100	156/95	145/85
HT I - C	150/90	140/85	130/85	**HT II - C**	155/95	151/95	142/85
HT I - D	150/95	140/90	120/90	**HT II - D**	145/95	140/90	130/85
HT I - E	150/100	150/90	120/85	**HT II - E**	145/95	141/92	130/80

In the above table HT – I, is the group with the hypertensive subjects, supplemented with FFS I, while table HT – II, is the group with the hypertensive

subjects, supplemented with FFS II. Subjects A to E are five respondents in each group.

It can be noted from the above table that, in both the cases of FFS I & II, the final values i.e. on 90th day of supplementation, decreased considerably over a period of 90 days. It was also observed that, the variation was notable only after the 45th day in most of the cases, similar to that of blood glucose levels of the subjects under study. However, rate of variation in the blood pressure levels of the subjects were not comparable to that of the total cholesterol and blood glucose levels and was not prominent, similar to that of other lipid parameters.

Table 69. Mean Systolic blood pressure values (mm Hg) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	Systolic blood pressure values (mm Hg)			
	Day 0	**Day 45**	**Day 90**	**Product mean**
FFS I	154	143	127	141.333
FFS II	151	146.6	137.4	145
Mean -Days.	152.5	144.8	132.2	
F Days -	75.779 **		**CD- Days.**	3.530
F- Product x Days	8.097 **		**CD- Product x Days.**	4.992

From the above ANOVA table, it was evident that, there was significant variation at 1 per cent level in the Systolic blood pressure levels of subjects supplemented with FFS I & II over a period of 90 days, showing differences over the period of days being evidently significant. Conversely, it could also be noted that, there was no differences among the subjects of FFS I & II groups in the level

of variation in systolic blood pressure, declaring no variation in the products' ability to decrease systolic blood pressure levels of subjects.

On supplementation with FFS I & II to subjects with hypertension, the mean initial systolic BP levels which were 154 mm Hg and 151 mm Hg respectively steadily decreased to 127 mm Hg and 137 (137.4) mm Hg respectively by the end of the period of the study. From the mean values of the products given in the table it could be concluded that both FFS I & II are equally effective in producing favorable results in the case of Systolic Blood Pressure, similar to that of FBS and other lipid profiles.

This concludes that both FFS I & II are equally effective in producing favorable results in the case of systolic blood pressure, similar to that of FBS and lipid profiles. Unlike other parameters like blood glucose and blood lipids, in this supplementation study a significant interaction between the product and the period of study was also notable.

Table 70. Mean diastolic blood pressure values (mm Hg) of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	Diastolic blood pressure values (mm Hg)			
	Day 0	**Day 45**	**Day 90**	**Product mean**
FFS I	97	91	85	91
FFS II	97	94	85	92
Mean -Days.	97	92.5	85	
F - Days.	39.550 **	**CD- Days.**	2.890	

From the table 70, it was evident that, there was significant variation at 1 per cent level in the diastolic blood pressure levels of subjects supplemented with

FFS I & II over a period of 90 days, showing differences over the period of days being evidently significant. On the other hand, it could also be noted that, there was no differences among the subjects of FFS I & II groups in the level of variation in diastolic blood pressure, declaring no variation in the products' ability to decrease diastolic blood pressure levels of subjects.

From the above table it was notable that, the mean initial and final diastolic blood pressure values of subjects supplemented with FFS I & II are 97 mm Hg and 85 mm Hg each respectively.

This concludes that both FFS I & II are equally effective in producing favorable results in the case of diastolic blood pressure.

Similar to that of subjects with hyperglycemia and hypercholesterolemia, to clarify the doubts of initial blood values of the subjects affecting the over the period changes on supplementation, ANOVA was carried out to note the rate of change (%) per day over the previous time and the results are as follows:

Table 71. Mean rate of change (%) systolic blood pressure values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	Day 0 - 45	**Day 45 - 90**	**Product mean**
FFS I	0.9	1.048	0.974
FFS II	0.868	0.952	0.91
Mean -Days.	0.884	1	
F – Days	34.72705 **	**F – products** - 6.568981 *	
CD – days	0.04539	**CD- products.** – 0.0575	

Table 72. Mean rate of change (%) diastolic blood pressure values of 5 subjects supplemented with FFS I & II on day 0, 45 and 90

	Day 0 - 45	Day 45 - 90	Product mean
FFS I	1.38	1.436	1.408
FFS II	1.352	1.438	1.395
Mean -Days.	1.366	1.437	
F – Days 24.2422 **		**CD – days** 0.033249	
F – products - NS			

From the above details, it can be concluded that even the rate of change (%) from initial to intermittent day (45^{th} day) and to the final day (90^{th} day) of the blood pressure, which was inclusive of systolic and diastolic values are concomitant to that of the individual and mean values of the subjects over a period of 90 days. This helps to overcome the fact that, the changes may be due to the variation in the initial values of the subjects or due to individual variations. Also, the rate of change (%) of systolic blood pressure signifies variation among FFS I & II at 5 per cent level, which was not glaringly notable.

During the conduct of the study, frequent monitoring of the subjects showed that, the frequency of infections decreased and the subjects also had a healthy life without any other complications.

4.3.6. Glycemic Index (G.I) of FFS I & II

The goal of the clinical management of type 1 and type 2 diabetes is to control metabolic abnormalities in order to prevent both acute (hyperglycemia, hypoglycemia) and long-term (retinopathy, nephropathy, neuropathy,

cardiovascular disease [CVD]) complications without negatively affecting quality of life (Jenkins *et al.*, 1984). Achieving and maintaining blood glucose (BG) levels as close to normal as possible is crucial for the prevention of long term complications in type 1 and type 2 diabetes and requires an intensive approach to management. Nutrition is of the utmost importance in intensive diabetes management and has been described as the cornerstone of care.

A major focus of the nutritional management of diabetes is the improvement of glycemic control by balancing food intake with endogenous and/or exogenous insulin levels. One way to classify the glycemic response to various carbohydrate containing foods is the glycemic index (GI). However, scientific evidence has linked low-GI diets with improved outcomes, i.e. decreased risk of development of type 2 diabetes, and improvement in metabolic control and quality of life in individuals with diabetes.

The term "glycemic index" describes the acute glycemic response to different types or sources of carbohydrate compared to a reference carbohydrate (glucose or white bread). The GI is, therefore, an index or ranking of the postprandial glycemic response to different sources of carbohydrate in comparison with a reference carbohydrate. With low-GI carbohydrates, blood glucose may remain slightly above fasting levels for a longer period of time compared with high-GI carbohydrates, but cause less of a "spike" in both the BG and insulin response (Wolever, 1990).

Glucose Tolerance Test (GTT) of the subjects was conducted to analyze the GI of FFS I & II.

4.3.6.1. Glucose Tolerance Test (GTT) of the respondents

Table 73. Mean Glucose Tolerance Test (GTT) values of the respondents

Time	Glucose	FFS I	FFS II
Initial (0 min)	115 ± 5	115 ± 5	115 ± 5
30min	278 ± 2.9	175 ± 5	180 ± 10
60min	383 ± 7.6	223 ± 5.8	230 ± 5
90min	308 ± 2.9	185 ± 5	190 ± 10
120min	255 ± 5	146 ± 5.8	153 ± 7.6
Observed peak time (min)	60	60	60
Actual peak time (min)	70.6	65.4	66.2

The GTT values of the subjects revealed that, on fitting a quadratic regression equation (y = 118.3809 +6.8746 x 1 -0.0487 x 2) for glucose with R^2= 0.9447463; (y = 113.8571 +2.9429 x 1 -0.0225 x 2) with R^2 = 0.9418844 for FFS I and (y = 114.4286 +3.0825 x 1 -0.0233 x 2) with R^2 = 0.9402337 for FFS II, it was calculated that, the maximum peak for glucose was at 70.6 min, for FFS I it was 65.4 min and for FFS II, it was 66.2 min. The R^2 values depict the regression lines are much fitting. It can be noted that, when compared to glucose, subjects attained peak values much in advance while consuming FFS I & II. This suggests both FFS I & II are better supplements for subjects with diabetes. Also, the final values for both FFS I & II were much lower than glucose and were under the normal limits.

From the above mean GTT values of the subjects, GI of FFS I & II was calculated.

The GI values of FFS I & II are 48 and 52 respectively. From the standard values cited by Raghuramalu *et al.* (2000), it can be concluded that both FFS I & II falls under the category of intermediate glycemic foods. It can also be noted that both the supplements have much lesser values than the normal cereals, breakfast snacks and roots and tubers, making them a better substitutes in the diets of subjects with diabetes.

Using the glycemic index, glycemic load (G.L.) was also calculated. Glycemic load (G.L.) for one portion size (i.e. 20 gm) of FFS I & II supplemented to the subjects were 5.8 and 6.0 respectively. Glycemic load (G.L.) for three portion size (i.e. 60 gm) of FFS I & II which would be sufficient for a breakfast for the subjects were found to be 17.3 and 18.1 respectively. It is evident that, in both the cases, the glycemic load of the supplements falls under the category of low (GL less than 10) and medium (11 - 19) glycemic load foods respectively. This gives a repetitive confirmation that both FFS I & II prove a better supplement in the dietary management of subjects with lifestyle diseases.

The **salient findings** from the supplementation study are as follows. It was observed that, there was significant variation at 1 per cent level in all the parameters of blood sugar, blood lipids and blood pressure levels of subjects supplemented with FFS I & II over a period of 90 days. Except for PPBS, the findings also proved that, both FFS I & II were equally efficient in varying the blood profiles. In the case of PPBS, FFS I was better than FFS II in lowering the PPBS levels of the subjects supplemented with the FFSs. The mean rate of variation (%) also substantiated the above findings.

Though the variations in the case of LDL, VLDL and HDL did not seem evident, the statistical analysis proved both FFS I & II to be equally effective and significant in altering the blood profiles by the end of the study.

Even though there was notable reduction in the systolic and diastolic blood pressure of subjects supplemented with FFS I & II, in both the cases only a few subjects attained normal value by the end of the study.

This helps to conclude that, both FFS I & II have high therapeutic values and equal potencies in altering the blood sugar, blood lipids and blood pressure levels of subjects when supplemented for a period of 90 days.

In the case of Glucose tolerance test, the final values for both FFS I & II were much lower than glucose and were under the normal limits. This suggests both FFS I & II be better supplements for subjects with diabetes.

The GI values of FFS I & II are 48 and 52 respectively which make them fall under the category of intermediate glycemic foods with much lesser values than the normal cereals, breakfast snacks and roots & tubers, making them better substitutes in the diets of subjects with diabetes.

Glycemic load (G.L.) for one portion size (i.e. 20 gm) of FFS I & II supplemented to the subjects were 5.8 and 6.0 respectively. Glycemic load (G.L.) for three portion size (i.e. 60 gm) of FFS I & II which would be sufficient for a breakfast for the subjects were found to be 17.3 and 18.1 respectively. The glycemic load of the supplements falls under the category of low (GL less than 10) and medium (11 - 19) glycemic load foods respectively. This gives a repetitive confirmation that both FFS I & II prove a better supplement in the dietary management of subjects with lifestyle diseases.

Discussion

5. DISCUSSION

The results of the present investigation entitled "**Development, quality assessment and clinical efficacy of 'Functional Food Supplement' (FFS) for life style disease management**" are discussed in this chapter under the following headings:

5.1. Development of 'Functional Food Supplement' (FFS)

5.2. Quality evaluation of the identified FFSs

5.3. Evaluation of clinical efficacy of the identified FFSs

5.1. DEVELOPMENT OF 'FUNCTIONAL FOOD SUPPLEMENT' (FFS)

Industrialization has led to a dramatic change in the lifestyle of the population, causing an increased incidence of diabetes, obesity, cancers of different organs, vascular diseases, physiological problems, as well as other degenerative diseases. Increasing quantum of work, living speed, longer work schedules and various psychological pressures have pushed people into fast-food cultures with more instant and tasty meals, which have altered the quality of diet. This is very much evident even in our country, as we Indians are more prone to lifestyle diseases much earlier than our Western counterparts and Kerala is crowned the 'capital' of lifestyle diseases.

The current emphasis of food is on the concept of functional foods to promote better health and well-being. An ever-increasing number of consumers are concerned with maintaining the quality of life by using the best effective alternative natural products thus making functional foods the popular choice. Functional foods are also less expensive, more beneficial and do not produce any adverse effects.

Even though there is a wide range of food stuffs that exerts promotive action for counteracting the adverse effects of available measures of disease management, at present they are not effectively used in our daily diet due to

ignorance or oversight. In this context, a systemic approach was carried out to develop functional food supplements for the management of lifestyle diseases.

After an extensive appraisal of literature pertaining to foods with functional properties, Barley, Ragi, Banana, Defatted Soy Flour, Drumstick leaves and Mushroom were taken as the constituents for the development of FFS. Dehydration and fermentation were the two processing techniques applied to standardize FFS. Proportions were optimized based on their nutritional and health promoting properties.

Among the various combinations worked out (Table 01), different levels of screening was done based on their nutritional qualities like low calorie, low fat, adequate carbohydrates, sufficient protein, high fiber and adequate micronutrients suitable for the management of lifestyle diseases and also on their sensory qualities.

The four combinations (combination X, XI, XII and XIV) selected for the dehydration technique of FFS were identified for further investigations on functional properties and sensory evaluation in order to select the best suitable combination. The combinations X, XI, XII and XIV were represented as FFS DT_1, DT_2, DT_3, and DT_4 respectively.

Standardization plays a key role in product development which facilitates growth of food industries. According to Poduval (2002), one of the fore most purpose of standardization is to facilitate the movement of materials and products through all stages of production in any industrial activity starting from the raw material to the finished products, than to the dealer and finally to the retailers and consumers.

Liaqat *et al* (2009) found that the recipe standardization is important to achieve optimal accuracy in determining the nutrient estimation. In the present investigation, the FFS using dehydration technique was standardized by varying

the constituent ratio while in the fermentation technique; the fermenting media, time and consistency of the substrate were altered in the four combinations.

The functional quality assessment of the four combinations of FFS I (dehydration technique) showed the bulk density ranged between 0.75 to 0.82 g/ cm^3 and the combination DT_3 and DT_4 are comparatively less than the other two but is on par with each other. Combination DT_1 (9.33 per cent) had high dispersibility followed by combination DT_3 (8.67 per cent), DT_2 (8.0 per cent) and DT_4 (7.33 per cent) respectively. The rehydration ratio showed that combination DT_1 (0.26) had better rehydration and combination DT_4 (0.23) was less rehydratable. Midhila (2013) reported that, the bulk density of banana blossom flours developed by her ranged between 0.91 to 0.97 g/ cm^3 while their rehydration ratio ranged between 0.25 to 0.50. Saranya (2012) reported that the enriched soup mix (ESM) developed from moringa pulp has a bulk density of 0.31-0.35mg/100g while the rehydration ratio of soup mix developed from moringa ranged from 0.17 to 0.19. Wagner (2000) observed that the appearance of the orange and grape fruit powders prepared by foam mat drying can be improved by increasing bulk density. Suma (2008) reported that the bulk density of dehydrated fruit drink mix from banana varieties Nendran and Palayankodan was 0.663mg/100g and 0.453mg/100g.

The gelatinization time varied from 0.48 to 1.26 min. The combination DT_4 took the least time to gelatinize (0.48 min) while combination DT_2 had the highest gelatinization time of 1.26 min. Also combination DT_4 had the lowest gelatinization temperature (85.3° C) while combination DT_2 had the highest gelatinization temperature (96° C). Resmi (2011) reported that *Njavara* rice showed higher gelatinization time and temperature. Processing loss of combination DT_3 (0.48) and DT_4 (0.52) were the lowest while greater processing loss was noted in combination DT_1 (0.56). The yield ratio was highest for combination DT_3 (0.52) and DT_4 (0.52) while combination DT_1 had the lowest yield ratio of 0.44. Midhila (2013) observed a processing loss of 40 - 45 per cent on drying and flouring in three varieties of banana blossom flour.

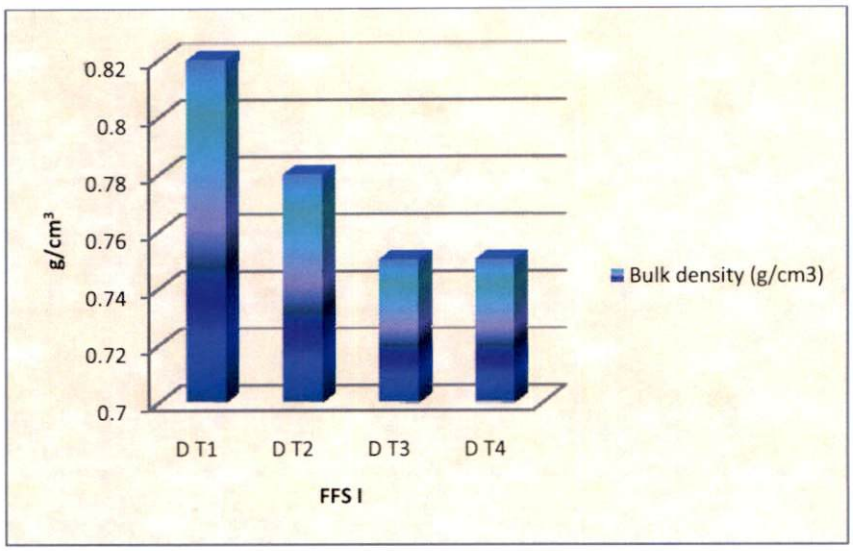


Fig: 03 Bulk density (g/cm^3) of FFS I

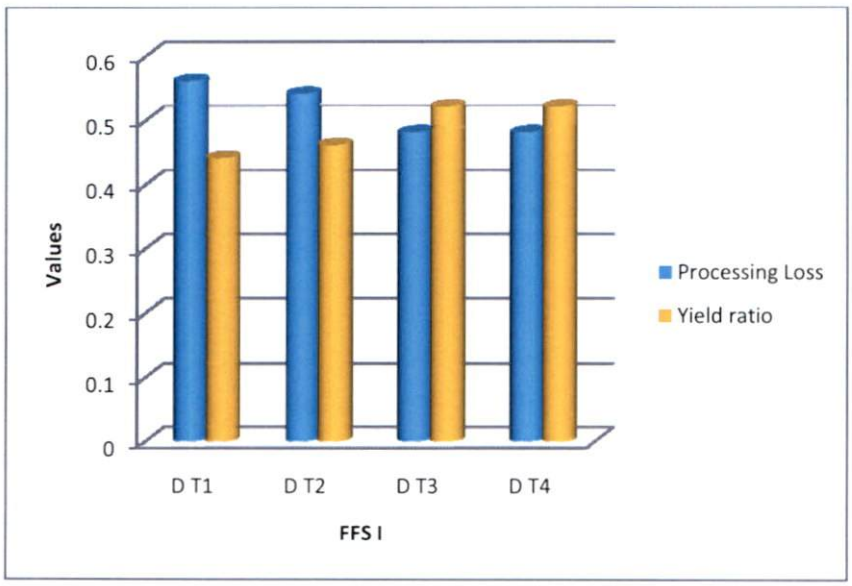


Fig: 04 Processing loss & Yield ratio of FFS I

The sensory evaluation scores of the four combinations of FFS I powder showed that, the colour and appearance of four combinations of FFS powder developed did not exhibit any difference though they were in a score ranging between 3.5 (DT_3) to 4.3 (DT_4). The texture of FFS in powder form did not show any significant differences in scores. The scores were in between 3.2 for DT_1 and 3.7 for DT_4. Maximum score for taste was noticed in FFS DT_4 (4.0), followed by DT_2 (3.3), DT_1 (3.2) and DT_3 (3.0) and also the scores varied significantly at 5 per cent. The flavour of FFS did not differ in scores. DT_1 obtained the least scores of 2.8. DT_2 and DT_3 received a score of 3.2 each. Maximum score was for DT4 (3.7). The overall acceptability score found that combination DT_4 (4.0) was the most preferred, followed by combination DT_2 (3.8) and DT_1 (3.4) respectively. Combination DT_3 (3.2) was the least preferred.

There were no significant differences in colour, appearance, texture and flavour of the porridge. However taste and overall acceptability showed variation at 1 per cent. DT_4 scored the maximum (4.0) while DT_1 scored the least (2.8) in the case of taste.

The overall acceptability score of FFS I porridge of different combinations showed that combination DT_4 (4.2) was the most preferred followed by combination DT_2 (3.6) and DT_1 (3.2) respectively. Combination DT_3 (3.1) was the least preferred. Combination DT_4 scored the highest hedonic scale rating (7.4) followed by combination DT_2 (6.7) and DT_3 (6.3). Combination DT_1 (6.1) was the least liked.

In the case of FFS II (fermentation technique), the functional attributes of the different treatments showed that, bulk density and dispersibility did not have any significant difference among the four treatments. Rehydration ratio was highest for the treatment FT_2 (0.28) followed by FT_3 (0.27) and FT_4 (0.27). Treatment FT_2 had the least rehydration ratio (0.26) and there were significant differences among the values at 1per cent level.

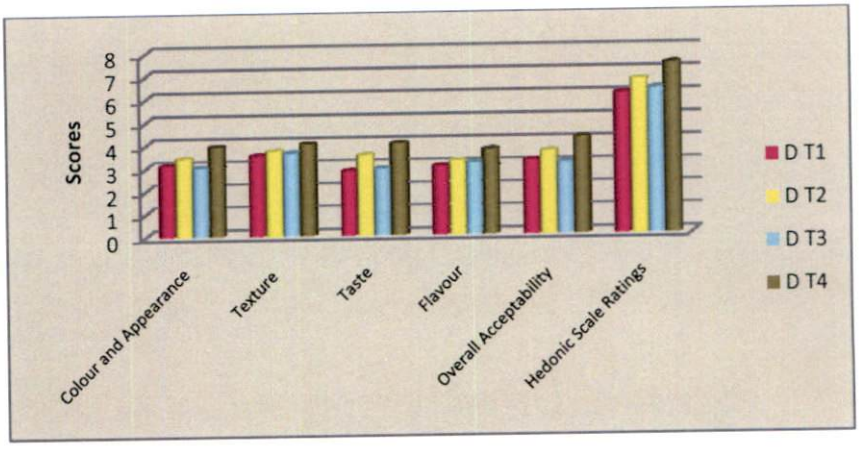


Fig: 05 Sensory evaluation and Hedonic scale ratings of cooked FFS I

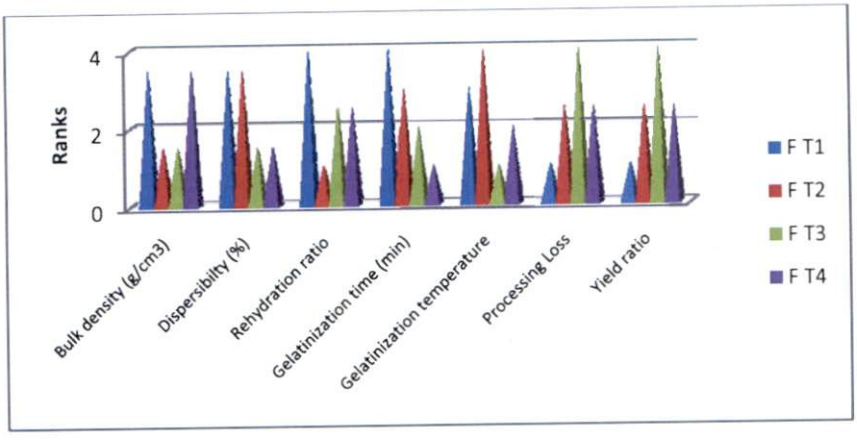


Fig: 06 Rank Charts of Functional Properties of FFS II

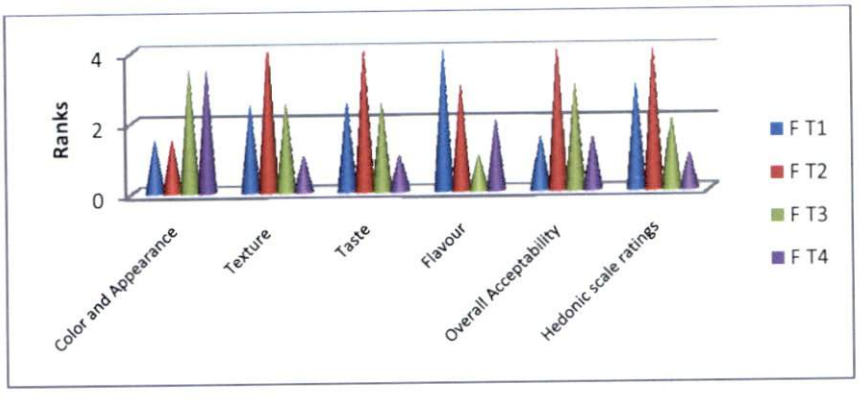


Fig: 07 Rank Charts of Sensory Evaluation of Cooked FFS II

The gelatinization time was highest (2.53 min) for FT_1 and lowest (1.16 min) for FT_4. On considering the gelatinization temperature, treatment FT_2 had the highest (94.0° C) while FT_3 (87.33° C) had the least and all the four treatments were significantly different from each other at 1 per cent level. Treatment FT_3 (0.49) had the highest processing loss followed by FT_2 (0.48) & FT_4 (0.48) and minimal processing loss was noted in treatment FT_2 which was also significantly different. On the other hand, treatment FT_1 (0.54) had the highest yield ratio and FT_2 (0.50) had the least value.

The sensory evaluation scores and hedonic rating of the FFS II powder and porridge showed there was no significant difference in the colour, appearance, texture, taste, flavour and overall acceptability among the four treatments of fermentation. The rank table which was used to select the best combination among the four treatments showed that, F T_4 with its functional property score of (14.5), sensory evaluation score of porridge of (10) and powder (9.0) was most acceptable.

Thus based on the sensory evaluation and functional qualities in both the techniques (dehydration and fermentation), combination IV (D T_4 and F T_4) was selected as the best combinations.

The best identified combinations from each technique (I & II) were investigated in depth for the nutrient content, phytochemical properties, functional qualities, storage stability and clinical efficacy.

5.2. QUALITY ASSESSMENT OF THE IDENTIFIED FFSs

Food quality is a complex concept that is frequently measured using objective indices related to the nutritional, microbiological or physicochemical characteristics of food or in terms of the opinion of designated experts (Cardello, 1995). The quality of the food is a combination of the attributes that determine the degree of acceptability of the product. These include nutritional value, microbiological safety, cost, convenience and organoleptic qualities.

Nambiar and Parnami (2008) reported that development of nutritious and organoleptically acceptable recipes with locally available food is a challenge for the food scientist and the benefits such food based strategies to prevent diseases are manifold.

The quality of the FFS (I and II), was evaluated on nutrient content, functional properties, phytochemical content, storage stability and feasibility of incorporation into various food preparations/products.

5.2.1. Nutrient Analysis of FFS I & II

Kalia and Sood (1996) defined nutritional quality as the combination of chemicals that has significance in determining the degree of acceptability of the product to a user based on its quality and sensory attributes. Nutrients are invisible chemicals in the foods which are necessary for keeping the body healthy.

Marchand and Vandenplas (2000) opined that one way of creating a functional food is by inclusion of ingredients such as probiotics and prebiotics to levels that enable the consumer to derive optimal health benefits.

Stanton *et al.* (2005) reported that fermented foods with their microbial activity play an essential role in conferring the required stability, safety and sensory properties to the product. On the nutritional side, fermentation helps in degradation of anti-nutritional factors and increases mineral bio-availability, protein digestibility of tannin-rich cereals, and degradation of flatulence-causing oligosaccharides.

The effect of fermentation on the various aspects of the FFS and the changes in FFS I & II were studied.

5.2.1.1. Proximate Composition of FFSs

Processing techniques such as germination and fermentation have been found to improve the quality of cereals due to chemical changes that enhance

organoleptic response (Nout, 2000), contents of free sugars, protein and vitamins, as well as bioavailability of minerals (Ochanda *et al.*, 2010), and results in the breakdown of some of the anti-nutritional endogenous compounds (Ahmed *et al.*, 2006). In many instances, usage of only one method may not impart the desired removal of anti-nutritional compounds and a combination of two or more methods is required (Hassan *et al.*, 2007).

Germination has profound effect on nutritional quality of the cereal (Chavan and Kadam, 2000). Germination is a natural biological process of plants by which the seeds come out of latency stage (Sangronis and Machado, 2007). An increase in bioavailability of minerals and weight has been observed during seed germination. Germinated seeds are good source of ascorbic acid, riboflavin, choline, thiamine, tocopheroles and pantothenic acid (Sangronis and Machado, 2007).

Paredes-López and Harry (2002) viewed that fermentation can have multiple effects on the nutritional value of food viz. a significant increase in the soluble fraction of food; quantity and quality of proteins as expressed by biological value, and often the content of water soluble vitamins, while the antinutritional factors show a decline during fermentation.

Institute of Medicine (2002) opined that although numerous studies have attempted to identify the optimal mix of macronutrients for the lifestyle disease management diet, it is unlikely that one such combination of macronutrients exists. The best mix of carbohydrate, protein, and fat appears to vary depending on individual circumstances. The DRI report recommends that, to meet the body's daily nutritional needs while minimizing risk for chronic diseases, healthy adults should consume 45–65 per cent of total energy from carbohydrate, 20–35 per cent from fat, and 10–35 per cent from protein.

The **energy** density of the diet is the energy content per unit of weight or volume, and is correlated with total energy intake (Rolls and Barnett, 2000).

There are substantial data to suggest that total energy intake over the short term varies directly with the energy density of the diet. This suggests that modifying the energy density of the diet could be a way to reduce total energy intake, and therefore reduce lifestyle diseases (Grunwald *et al.,* 2001).

In the present investigation the reduction in the energy content of FFS II (378 kcal) from that of FFS I (384) on fermentation is significant (t value 6.73**). The energy levels of both FFS I & II are equal to the energy contents of most of the cereals, making them a better substitute in the daily diet because of its therapeutic importance.

Adebowale and Maliki (2011) reported that the energy values of the non fermented pigeon pea seed flour had the highest energy value (325.46 kcal/100 g) compared to 315.33 kcal/100 g with fermented flour.

According to Babalola and Giwa (2012) the content of total soluble sugar of raw soybean seed decreased ranging from (2.7 per cent) from the first day of fermentation till (2.28 per cent). The sugar level also decreased which could be as result of its utilization by the metabolizing microbes involved in the fermentation processes. The result these physicochemical changes during fermentation are in line with the work of Giwa *et al.* (2011), who also observed decreases in the sugar content by fermentation period.

Khetarpaul and Chauhan (2000) reported that fermentation of pearl millet flour with yeast and lactobacilli significantly increased the total amount of soluble sugars, reducing and non-reducing sugar content, with a simultaneous decrease in its starch content.

Though the changes were notable in the energy content of FFS I & II, on the health point of view, both the supplements are equally advisable as they have the required amount of the nutrient needed for a healthy life and thereby will aid in the management of degenerative diseases. As discussed earlier, energy intake

management is very much essential in the control of many diseases especially, diabetes and obesity which are the causative factors for other lifestyle diseases.

Proteins are essential component of tissues and cells of the body (Gopalan *et al.*, 2009). Protein can increase insulin response without increasing plasma glucose concentrations in subjects with lifestyle diseases (American Diabetes Association, 2004).

In the present investigation, the protein contents were 21.4 g and 16.5 g for FFS I & FFS II respectively which were significantly different between each other (t 7.90**). The protein content of the supplements is comparable to that of the pulse proteins. This high level of protein in FFSs is recommended for the management of lifestyle diseases especially diabetes. The changes in the protein contents of FFS I & II might be brought about by the processing techniques applied.

The protein efficiency ratio (PER) of wheat was found to increase on fermentation, partly due to the increase in availability of lysine (Hesseltine and Wang, 2001). Urooj and Puttaraj (2000) reported that an improvement in protein digestibility of fermented products is mainly associated with an enhanced proteolytic activity of the fermenting microflora. This is very supportive in the therapeutic effects of FFS II, even though it had lesser protein contents than FFS I, its improved protein digestibility helps in the management of diseases.

A combination of ingredients in the development of FFS I & II with varying techniques has enriched the supplements with good amounts of protein. This in turn would improve the functional and therapeutic importance of both the supplements.

High- protein diets have recently been a proposed as a new strategy for successful weight loss and management of lifestyle diseases (Joer *et al.,* 2001). Abete *et al.* (2010) viewed that, selection of a diet high in fiber, low in energy

density and glycemic load and moderate protein is important for disease management.

Padmaja *et al.* (1994) reported that the protein content of cassava decreased from 2.36 g/100g to 1.61 g/100g during fermentation. A decrease in protein content was observed during the first 2 days of fermentation and thereafter the decrease was not significant (Gupta *et al.*, 1998).

Yousif and El –Tinay (2000) proclaimed that natural fermentation of maize increased non-protein nitrogen and slightly increased protein content. Scientific studies have documented that the protein content of sprouted or germinated plant increased due to degradation of nutrient like carbohydrate and fat in the synthesising of protein (Enujiugha *et al.,* 2003; Fasasi, 2009). Adebowale and Maliki (2011) studied that as fermentation day increased, crude protein and ash increased progressively from 21.8 to 23.9 per cent in pigeon pea.

Energy density is also affected by the macronutrient composition of the diet. Since **fat** is more energy-dense (38 kJ/g) than either protein or carbohydrate (17 kJ/g), reducing the proportion of fat in the diet can have a major impact on reducing the energy density of the diet (Monika and Kiran, 2012).

US Department of Health and Human Services and US Department of Agriculture (2005) suggested that the minimum value of fat protects against energy and nutrient deficiencies, elevated triglyceride levels, and lowers HDL-C levels while the upper limit helps curb saturated fat intake and excess energy consumption.

The results of the present study showed that the developed functional food supplements FFS I & FFS II had lower fat content of 1.88 g and 1.56 g respectively. Since there is solid data which shows that reducing energy density from fat reduces energy intake, functional foods aimed at modifying energy density may be useful in managing obesity and other related lifestyle diseases

(Monika and Kiran, 2012). This enhances the role of both FFS I & II in the management of diseases.

Adebowale and Maliki (2011) concluded that the fat contents were found to be significantly lower in the fermented seed flour than in non-fermented seed flour. Alemu (2009) also reported that fermentation gradually reduced the fat content of Sorghum cultivars of her study. Babalola and Giwa (2012) reported that soybean on fermentation, the fat contents decreased most likely due to their utilization by the growing microorganisms. The decreases in fat content could also be attributed to the metabolizing organism as energy source.

Chinma *et al.* (2009) studied that the decrease in fat contents might be attributed to the increased activities of the lipolytic enzymes during fermentation which hydrolyses fat components into fatty acid and glycerol.

The amount of **carbohydrates** and available insulin may be the most important factor influencing glycemic response after eating and should be considered when developing the eating plan. Monitoring carbohydrate intake, whether by carbohydrate counting or by experience based estimation, remains a key strategy in achieving glycemic control (Alison *et al.*, 2014).

Liu *et al.* (2007) reported that the relationship of dietary carbohydrates to CVD appears to be mediated through indirect mechanisms: contribution to total energy and its effect on overweight and obesity; influence on central obesity; effects on plasma lipids, especially triglycerides and effects on glycemic control. High-carbohydrate diets appear to reduce HDL cholesterol levels and increase the fraction of small dense LDL and triglycerides, both of which may impact adversely on vascular disease.

In the present investigations, the carbohydrate content of FFS I was 60.5 g against 58.0 g of FFS II which was not significantly different. The carbohydrate contents of FFS I & II were much less than that of the cereals and were on par with the pulses. The above findings supports that both FFS I & II with increased

glycemic response and reduced lipid activity that are beneficial for the management of lifestyle diseases.

Wang *et al.* (2003) studied that, microbial fermentation leads to a decrease in the level of carbohydrates as well as some non-digestible poly- and oligosaccharides as observed in the germinating wheat samples. The latter reduces side effects such as abdominal distension and flatulence. Though fermentation processes involved in FFS II had lowered the amounts of carbohydrates when compared to FFS I, the variation was not significant.

Dietary fibre components exert beneficial effects mostly by way of their swelling properties, and by increasing transit time in the small intestine. Consequently, they reduce the rate of release of glucose and its absorption, thus helping in the management of diabetes. Dietary fibre components also bind bile salts, thereby promoting cholesterol excretion from the body and thus reducing blood cholesterol levels, and food toxins in the gut to reduce their toxicity (Gopalan, 2009).

Pereira and Ludwig (2001) reported that high-fibre diets are associated with lower food intake by triggering maximal sensory stimulation in the mouth due to the increased need for chewing. High fibre diets also lead to slower gastric emptying and a slower rate of nutrient absorption and they also reduces the energy density of the overall diet.

The present study showed that the variation in the fibre contents of FFS I (4.0 g) and II (3.33 g) were significant only at 5 per cent level. The fibre contents of both FFS I & II were equal to most of the cereals and pulses. Good amounts of fibre in the FFSs are favourable in improving satiety, maintaining blood glucose and lipid concentrations which are most required for the management of metabolic syndromes or lifestyle diseases.

The decrease in fibre content during fermentation of FFS II could be attributed to the partial solubilisation of cellulose and hemicellulosic type of

material by microbial enzymes. This was supported by (Ejigui *et al*., 2005) who reported that a significant decrease of fat, ash, and fibre contents after four days of maize fermentation.

Total minerals or **ash** content is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of specific inorganic components present within a food, such as Ca, Na, K, Cl etc.

Increase in ash content of FFS II (3.2 g) in comparison to FFS I (3.0 g) were notable in the present study. This proves that, both FFS I & II are equally good stores of most of the minerals which has important roles in the prevention and management of many degenerative diseases.

Similar reports as given by Adebowale and Maliki (2011) who studied that the ash content of the pigeon pea ranged from 4.61 to 5.52 per cent, with non-fermented seed flour had a value of 4.61 per cent while highest value was recorded (5.52 per cent) after 5 days of fermentation. On the other hand Babalola and Giwa (2012) reported that the ash content did not show much change during the processing of soybean on fermentation.

Low **moisture** content in food samples increased the storage periods of the food products (Alozie *et al*., 2009); while high moisture content in foods encourage microbial growth; hence, food spoilage (Temple *et al*., 1996).

The moisture content of both the FFSs denotes that, they can be categorized under low moisture foods, since they contain only 10.95 per cent and 10.8 per cent of moisture respectively.

Babalola and Giwa (2012) reported that the percent moisture content of soybean decreased steadily per day which could be the result of hydrolytic action of the fermenting microbes. These results were on par with the present study, as the moisture content of FFS II was found to be lower than FFS I. However, Adebowale and Maliki (2011) reported that the moisture contents of non-

fermented flour sample was lower than that of fermented flours, which might be due to its low dry matter content. In the present investigation this could be due to the double drying process involved in the preparation of FFS II.

Ijarotimi (2012) reported that the moisture content of germinated wheat flour was the highest (13.23 ± 1.51 g/100g), while that of fermented wheat flour was the lowest (12.67 ± 0.29 g/100g). However, investigations have shown that low moisture content of food samples is a desirable phenomenon, since the microbial activity is reduced (Oyenuga, 2002).

Zekovic *et al.* (2005) observed that **β -glucans** exhibits hypocholesterolemic, anticoagulant properties, anti-cytotoxic, antimutagenic and anti-tumorogenic properties.

Wasser and Weis (1999) had studied that among cereals, the highest content (g per 100 g dry weight) of *β*-glucan has been reported for barley: 2–20 g (65 per cent is water-soluble fraction) and for oats: 3–8 g (82 per cent is water soluble fraction). Other cereals also contain *β*-glucan but in much lower amounts: sorghum 1.1–6.2 g, rye 1.3–2.7 g, maize 0.8–1.7 g, triticale 0.3–1.2 g, wheat 0.5–1.0 g, durum wheat 0.5-0.6 g, and rice 0.13 g (Bacic *et al.*, 2009). Other sources of *β*-glucan include some types of seaweed (Teas, 2001) and various species of mushrooms such as Reishi, Shiitake, and Maitak.

In the present study FFS II was found to contain higher amounts of β – glucans (1.68 g) compared to FFS I (1.60 g). The judicious selection of ingredients like barley and oyster mushroom in the development of the FFSs contributed to their β –glucan contents. Also these indigenous ingredients are better substitutes in the daily diet than oats.

With the above mentioned nutritional benefits, the developed functional food supplements are highly recommended in the market for their therapeutic value in disease management.

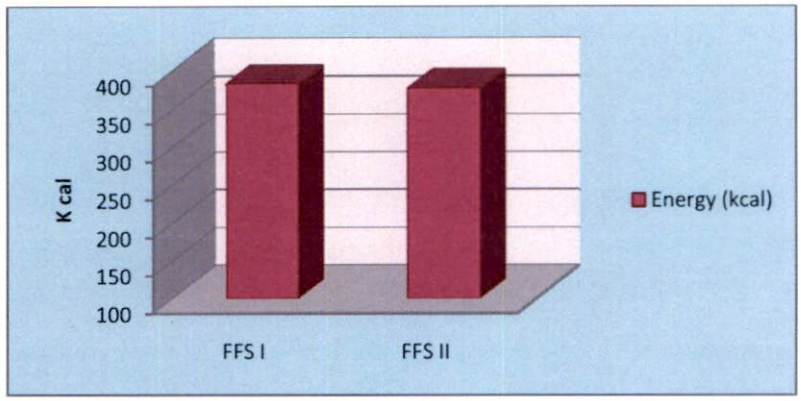


Fig: 08 Energy (kcal) content of FFS I & II

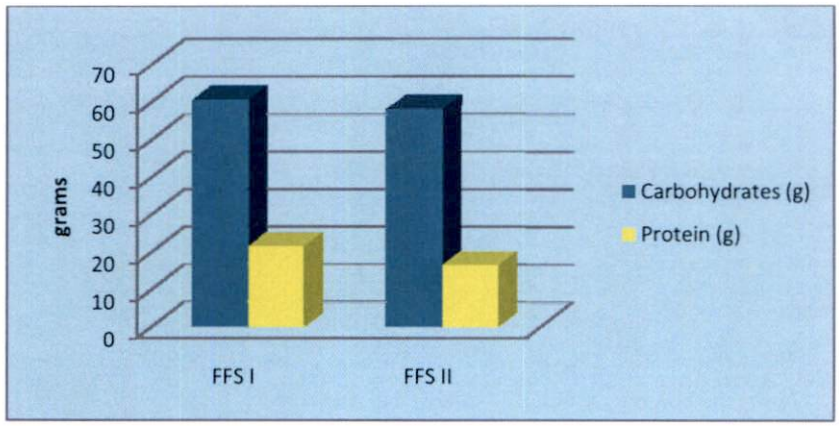


Fig: 09 Carbohydrates & protein content of FFS I & II

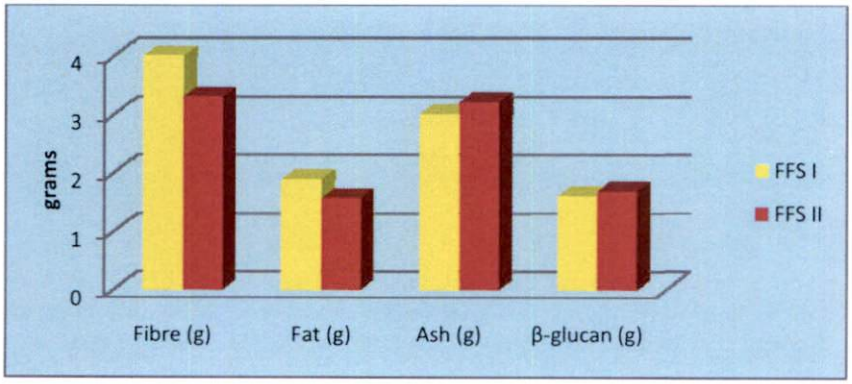


Fig: 10 Fiber, fat, ash & β-glucan content of FFS I & II

5.2.1.2. Vitamin Composition of Functional Food Supplements I & II

The vitamin content analysis carried out in the present investigation showed that, FFS II on fermentation had produced a remarkable increment in the β-carotene (2910 µg), thiamine (1.63 mg), riboflavin (1.3 mg), niacin (2.68 mg) and folic acid (40.0 mg) levels. Whereas, the vitamin content of FFS I was β-carotene (1948 µg), thiamine (0.8 mg), riboflavin (0.77 mg), niacin (1.88 mg) and folic acid (29.56 mg). Changes in vitamin E (3.35 µg) of FFS I to that of FFS II (3.0 µg) were noted. On the other hand, FFS II had a comparatively lower Vitamin C (8.73 mg) content than FFS I (13.1 mg).

The present study showed that, the developed FFSs is a hoard of vitamin sources which would substantiate their role in the management of lifestyle diseases.

Lichtenstein *et al.* (2006) were of the opinion that folate intake and to a lesser extent intake of vitamins B6 and B12 are inversely associated with blood homocysteine levels. In observational studies, increased blood levels of homocysteine are associated with an increased risk of CVD.

Chan *et al.* (2001) suggested that in addition to the role of various secondary metabolites, vitamins too play a pivotal role in conferring antioxidant capacity. Vitamin E is considered to be an efficient chain-breaking antioxidant that produces a relatively nonreactive chromanoxyl radical.

Doba *et al.* (2005) reported that vitamin C is a hydrophilic antioxidant, and is considered to be a poor antioxidant within the lipophilic plasma membrane. Vitamin C plays a valuable role in the regeneration of vitamin E and thereby acts to reduce the rate of oxidative consumption of vitamin E (Wrona *et al.*, 2003). β-Carotene is another hydrocarbon carotenoid and quencher of singlet oxygen at a low partial pressure of oxygen (Tsuchihashi *et al.*, 2001).

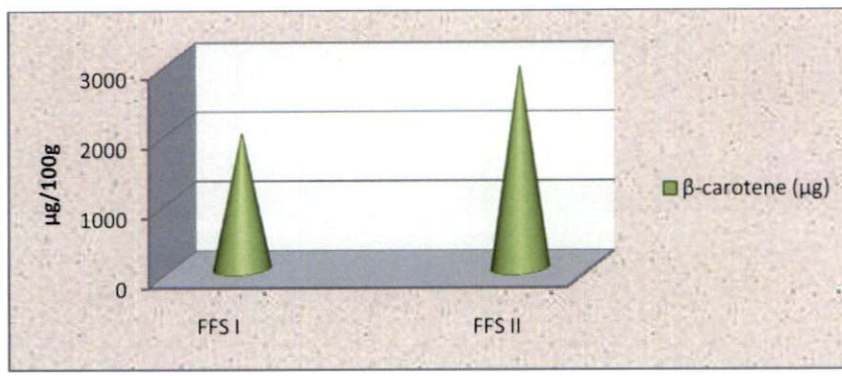


Fig: 11 β-carotene content of FFS I & II

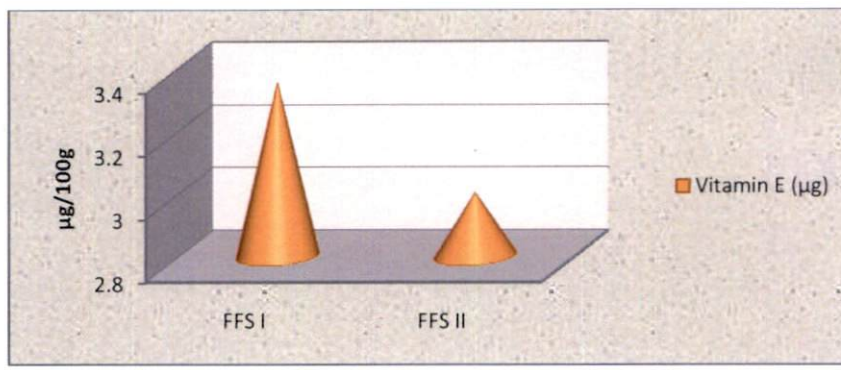


Fig: 12 Vitamin E content of FFS I & II

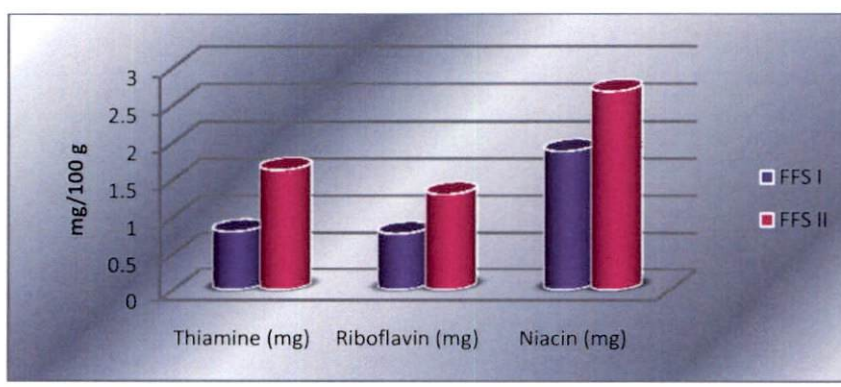


Fig: 13 B complex vitamins of FFS I & II

The high levels of various vitamins in both the FFS I & II substantiate its role as antioxidant and its impact on CVD management.

Afify *et al.* (2012) observed that significant changes were brought about in the Vitamin E and β-carotene content of white sorghum varieties on soaking and fermentation. Germinated seeds are good source of ascorbic acid, riboflavin, choline, thiamine, tocopheroles and pantothenic acid (Sangronis and Machado, 2007).

Murdock and Fields (2002) investigated that during fermentation certain micro-organisms produce vitamins at a higher rate. The content of thiamine and riboflavin in dhokla and ambali was about 50 per cent higher after fermentation. The levels of vitamin B12, riboflavin and folacin were increased by lactic acid fermentation of maize flour. Fermented whole onion plant retained 97 per cent of vitamin A activity (Speek *et al.*, 2002).

Steinkraus (2003) reported that several B vitamins including niacin (B3), panthothenic acid (B5), folic acid (B9), and also vitamins B1, B2, B6 and B12 are released by Lactic Acid Bacteria in fermented foods. Cereal-based products such as *ogi*; *mageu*; and *kenkey* have been reported to have an improved B-vitamin content (Iwuoha and Eke, 2006).

5.2.1.3. Mineral Composition of Functional Food Supplements I & II

The mineral contents like copper, zinc, potassium, sodium, calcium, magnesium, phosphorus and manganese of FFS I reduced from 7.6 mg/100g to 3.6 mg/100g, 8.64 mg/100g to 2.88 mg/100g, 497 mg/100g to 425.4 mg/100g, 498 mg/100g to 475 mg/100g, 472 mg/100g to 458 mg/100g, 467 mg/100g to 378 mg/100g, 141 mg/100g to 109 mg/100g and 2.38 mg/100g to 2.12 mg/100g respectively in FFS II. However, Selenium was found to be significantly higher in FFS II (0.73 µg) than FFS I (0.67 µg) at 1 per cent significance level. However, Selenium which is an antioxidant was found to be significantly higher in FFS II

(0.73 µg) than FFS I (0.67 µg). The decrease in mineral contents may be due to leaching out during processing involved in the development of FFSs. Similar results were reported by Mubarak (2005), in his study on nutritional composition and antinutritional factors of mung bean seeds as affected by processing steps practised at household levels.

Chromium, potassium, magnesium, and possibly zinc deficiency may aggravate carbohydrate intolerance (Mooradian *et al.*, 2009). In the late 1990s, two randomized placebo- controlled studies in China found that chromium supplementation had beneficial effects on glycemia. Data from recent small studies indicate that mineral supplementation may have a role in the management of glucose intolerance, gestational diabetes mellitus (GDM), and corticosteroid-induced diabetes (Cefalu and Hu, 2008; Althuis *et al.*, 2009).

Walti *et al.* (2003) reported that magnesium is found to have a link with carbohydrate metabolism, being a cofactor for several enzymes as well as in glucose transport across the cell membrane and aid insulin for its secretion at multiple levels, binding with receptor and activation tyrosine kinase. Reduced magnesium concentrations have been observed in diabetic adults and children despite good nutritional status (Sales and Pedrosa, 2006).

Adequate calcium consumption throughout the lifecycle will help in attainment and maintenance of peak bone mass and in the prevention of chronic diseases like osteoporosis, hypertension and certain types of cancer later in life (NIH Consensus statement, 2000).

The above studies stress the role of minerals in the case of disease prevention, management and various other metabolic activities. The developed food supplements (FFS I & II) with fairly good amounts of most of the minerals will be helpful in disease prognosis and management.

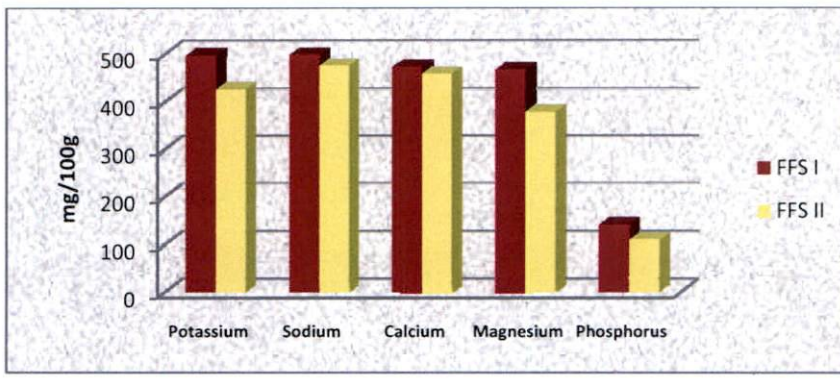


Fig: 14 Macro minerals of FFS I & II

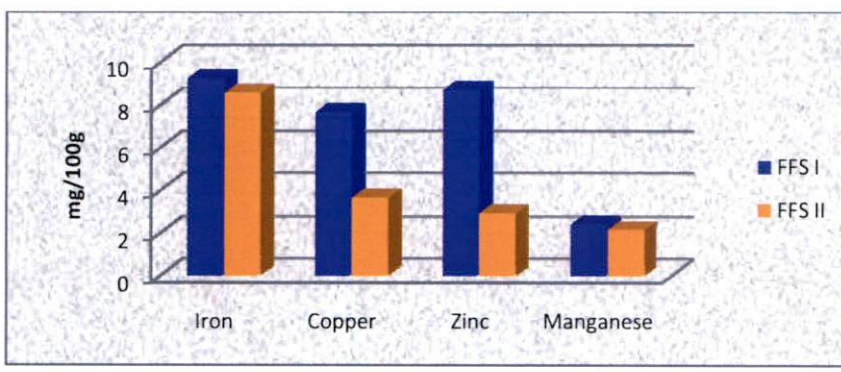


Fig: 15 Micro minerals of FFS I & II

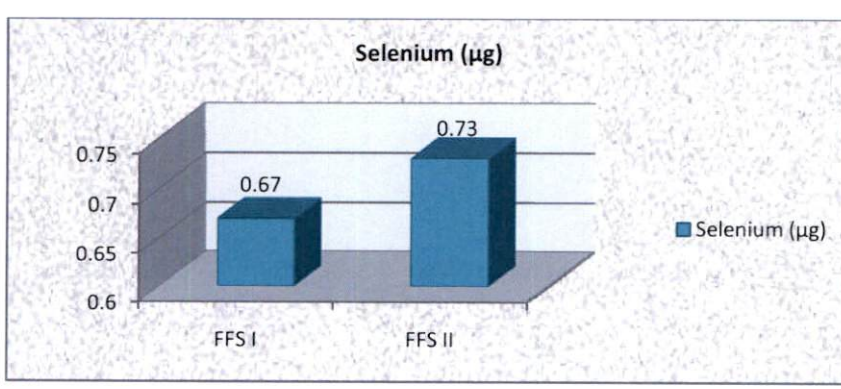


Fig: 16 Trace minerals of FFS I & II

Though the following studies discussed the causes for variation in the mineral contents of the FFSs, it is notable that both the functional supplements are equally important in the disease management. The lower levels of minerals in FFS II will be overcome by its increased bio availability brought about by fermentation.

Vaishali *et al.* (2004) who studied effect of natural fermentation on *in vitro* zinc bioavailability in cereal-legume mixtures found that fermentation increased the zinc solubility and the zinc uptake by intestinal segment to a significant level.

In their study, Gabriel and Akharaiyi (2007) reported that in comparison, the mineral composition of germinated wheat flour sample was higher in calcium, potassium, sodium, magnesium and phosphorous than fermented and raw wheat flour sample respectively.

5.2.1.4. Phytochemical Composition of Functional Food Supplements I & II

Plant phytochemicals exhibit diverse pharmacological and biochemical actions when ingested by animals and man (Amadi *et al.*, 2006; Soetan, 2008). Phytochemicals have been linked to many other positive health effects in humans and animal studies including coronary heart disease, diabetes, high blood pressure, inflammatory processes, infection, psychotic diseases, ulcers and macular degeneration. Many phytochemicals may have multiple actions on human health. For example, flavonoids present in many fruits not only have anticancer properties but have also been shown to have anti-allergic, cardiovascular protection properties, anti-inflammatory properties and antiviral properties (Benavente-Garcia, 2001).

The results of phytochemical analysis of the present investigation proved that, the flavonoids content of FFS I was 4.6 per cent as against 1.23 per cent for FFS II which was highly significant at 1 per cent level. The alkaloid content of FFS I was (0.8 per cent) against 0.2 per cent for FFS II. The polyphenol content of FFS I was 73.25 mg whereas fermentation reduced its content to 48.25 mg in

FFS II. The tannin contents of FFS I and II were 10.09 mg and 5.77 mg respectively. The oxalate contents of FFS I and II were 5.32mg and 1.92 mg respectively.

Flavonoids are most commonly known for their antioxidant activity. They are modifiers which modify the body's reactions to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobical and anticancer activity (Balch and Balchi, 2000; Ekam and Ebong, 2007), and may be useful in therapeutic roles (Jisika *et al*, 2000). Alkaloids are organic compounds that contain nitrogen, and are physiologically active with sedative and analgesic properties. They are used in relieving pains, anxiety and depression (Jisika *et al*, 2000).

Yang *et al.* (2001) reviewed the inhibition of carcinogenesis by dietary polyphenolic compounds and questioned the link between the antioxidative and anticarcinogenic properties, asserting that polyphenols may inhibit carcinogenesis. Tannins (tannic acids) and saponins are responsible for the antibacterial activity of the plant seed extracts (Gloor, 1997). Saponins are used in veterinary vaccines as adjuvant (e.g. foot-and-mouth disease vaccines) helping to enhance immune response. People suffering from coronary heart disease are encouraged to consume moderately oxalate rich foods as it helps to reduce blood cholesterol (Savage, 2000).

Most of these compounds in plants could be removed by several processing methods such as soaking, germination, boiling, autoclaving, fermentation, genetic manipulation and other processing methods (Soetan, 2008). The toxic effects of oxalate, phytate and tannins could be avoided, provided the plant food is cooked before consumption (Enechi and Odonwodu, 2003).

Earlier, most of the phytochemicals were considered as anti nutrients and various processing techniques were applied to reduce their content. Later studies were carried out to justify the pharmacological effects of the phytochemicals in various diseases. Though there was considerable variation brought about in the

phytochemical contents by the different processing techniques involved in the development of the FFSs, their high bio availability and increased therapeutic action was much favourable.

Sindhu and Khetarpaul (2001) opined that fermentation is an important process which significantly lowers the content of antinutrients (phytates, tanins and polyphenol) of cereal grains. Reduction in phytate may increase the amount of soluble iron, zinc, calcium several folds (Blandino *et al.,* 2003).

Nout and Ngoddy (2001) observed that during fermentation, the reduction of phytic acid, tannin and polyphenol is due to the enzymes like phytase, polyphenol oxidase etc. present in the food grain or micro flora content.

Reduced phytate content was reported by Harland and Harland, (2000) in yeast fermented bread; locust bean seeds (Eka, 2001); natural lactic fermented maize meal (Chompreeda and Fields, 2001); dough fermentation for whole grain flour (Roos *et al.*, 2000); increase in fermentation temperature of pearl millet (Kheterpaul and Chauhan, 2001); (Giami, 2004) for fluted pumpkin seeds; Adeniran *et al.* (2013) for lima bean and locust bean seeds. Reduction in phytic acid during fermentation could be due to the enzymatic action of the fermenting microorganisms which hydrolyze phytate into inositol and orthophosphate.

Decrease in phytate with increase in fermentation time was also reported by (Mulimani *et al.*, 2003) for fermented black gram and fermented soybean respectively. It has also been reported that phytase hydrolyses phytate to inositol and phosphoric acid and this process releases some elements such as phosphorus thus increasing the mineral availability.

Dykes and Rooney (2006) reported that fermentation reduced phenolic compounds and tannins in finger millet by 20 and 52 per cent respectively. Fermentation coupled with methods such as decortication, soaking and germination reduced the tannins in sorghum, other cereals and in beverages made from these cereals (Dlamini *et al.,* 2007). Fermentation of porridges from whole

and decorticated tannin sorghum led to significant reduction of total phenols (Chethan *et al.*, 2008).

The research results of Afify *et al.* (2012) showed that reduction of total phenols, total flavonoids and tannins after soaking of sorghum varieties were notable, which may be attributing to leaching of phenols into the soaking medium. The lower level of total phenols and total flavonoids after soaking may be due to the release of phenolic compounds into soaking water (Akillioglu and Karakaya, 2010).

Adeniran *et al.* (2013) concluded that, tannin content decreased with fermentation in lima beans and locust beans. The decrease could be attributed to the hydrolysis of polyphenolic compounds of tannin complexes during fermentation. In her study, Ijarotimi (2012) reported that the tannin, oxalate and trypsin composition of germinated wheat flour sample were higher and significant different from that of raw and fermented wheat flour samples respectively.

Scientific studies have established that processing methods such as cooking, dehulling, soaking, fermetation and germination, improve the nutritional quality of food products by reducing or eliminating the anti-nutrient composition of the food products (Syed *et al.*, 2011).

The above studies were in support to the present investigation indicating considerable reduction in tannin content of FFS II due to the various processing steps involved in the preparation when compared to FFS I.

Considering all these details discussed above, it could be noted that the reduction in the phytochemical levels of FFS II was due to the processing steps involved in it. However, it must also be taken into consideration that, though there was decrease in the phytochemicals in FFS II, fermentation has brought about favourable changes in the other nutrients and also it has increased the activities of the available phytochemicals. Thus FFS I with its higher

phytochemical contents and FFS II with its higher potency and activity are on the same scales in exhibiting the disease preventing properties as discussed earlier.

5.2.1.5. Amino Acid Profile of Functional Food Supplements I & II

Amino acid profiling of FFS I & II depicted that, Glutamic acid (132.9 nmoles/ml) is the highest amino acid content in FFS I followed by arginine (97.1 nmoles/ml), Glycine (88.5 nmoles/ml), Leucine (52.9 nmoles/ml), Serine (51.6 nmoles/ml) etc. On the other hand FFS II elicited high amounts of Glycine (107.6 nmoles/ml), followed by Arginine (96.3 nmoles/ml), Glutamic acid (78.3 nmoles/ml), Serine (69.1 nmoles/ml), Leucine (60.3 nmoles/ml) etc. Histidine is the most limiting amino acid in both FFS I (16.0 nmoles/ml) and II (3.9 nmoles/ml).

The Total Essential Amino acid (TEAA) content of FFS I & II were, 291 nmoles/ml and 248.5 nmoles/ml. Whereas, the Total Non Essential Amino acid (TNEAA) content of FFS I & II were, 467.6 nmoles/ml and 414.4 nmoles/ml respectively. In FFS I Isoleucine (109) is the most limiting amino acid, whereas in FFS II it is Histidine (40). Phenylalanine is the most abundant amino acid in both FFS I (206) & II (102). FFS I follows an amino acid sequence of Iso leucine, Valine, Methionine while in FFS II it is Histidine, Iso leucine, Valine. FFS I have a higher EAA index and Nutritional index % of 42.57 and 9.12 respectively when compared to FFS II (31.26) and 5.16 respectively.

Kendler (2006) observed that several amino acids are considered to be essential nutrients for maintaining normal cardiovascular function because inadequate levels of some amino acids under different pathophysiological conditions are associated with heart dysfunction.

Appleton (2002) studied that amino acid arginine, is a precursor for the synthesis of nitric oxide in virtually all cell types, and is also believed to exert cardiovascular benefits. The plasma levels of arginine are reduced in diabetic

patients, and have been linked to disturbances in both fasting and post challenge glucose levels (Menge *et al.*, 2010).

Nout and Ngoddy (2001) identified that certain amino acids may be synthesised and the availability of B group vitamins may be improved on fermentation. Fermentation of cereals by lactic acid bacteria has been reported to increase free amino acids and their derivatives by proteolysis and/or by metabolic synthesis (Mugula *et al.*, 2003).

Adams (2000) found that fermentation has been shown to improve the nutritional value of grains such as wheat and rice, basically by increasing the content of the essential amino acids lysine, methionine and tryptophan. Fermentation of rice by lactic acid bacteria enhances lysine content (Lee *et al.*, 1999).

Ijarotimi (2012) reported that the total amino acid composition of germinated wheat sample was higher than fermented wheat flour sample, but the value was lower than that of raw wheat sample; and this is due to the breaking down of other nutrients like carbohydrate to synthesis amino acids that the germinating seeds needed for its biochemical activities and growth.

Studies of Nanson and Field (2004) depicted that during the fermentation of corn meal the concentrations of available lysine, methionine, and tryptophan increased. Mubarak (2005) in his study reported that soaked and cooked processes of mung bean seeds were slightly decreased in lysine and also found that cooking reduced the sulfur-containing amino acids and tryptophan. All processing methods increased the concentration of leucine. These studies favourably supported the data of the current investigation.

The FFS I & II with its high amino acid profile and indices have a greater role to be played in the metabolic pathways in varying nutrients and thereby have a greater role in the management of metabolic syndrome. The amino acid

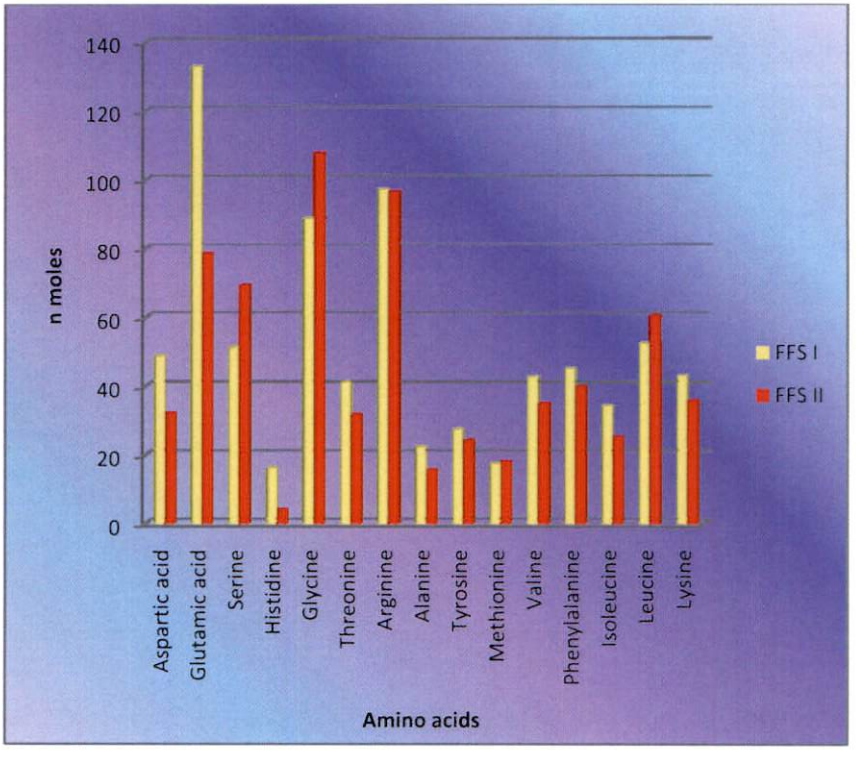


Fig: 17 Amino acid profile of FFS I & II

profiling of the food supplements also substantiates the improved protein qualities of the products developed.

It must also be noted that, both FFS I & II contains very good amounts of arginine, which is proved to possess beneficial effects on heart health and blood sugar management.

5.2.1.6. Total Antioxidant Capacity (TAC) of FFSs

Knight (2005) opined that in healthy individuals, free radical production is continuously balanced by natural antioxidative defence systems. Disruption of the balance between reactive oxygen species (ROS) production and elimination, due, among other things, to aging, leads to the process called oxidative stress leading to many diseases including cardiovascular diseases, atherosclerosis, cataracts, chronic inflammation, and neurodegenerative diseases, such as Alzheimer's or Parkinson's disease.

Rechner *et al.* (2002) reported that ROS and free radicals are also considered as inducers of lipid peroxidation and cause the deterioration of foods. Although organisms have endogenous antioxidant defences produced during normal cell aerobic respiration against ROS, other antioxidants are taken from the diet, both from natural and synthetic origin.

Halliwell *et al.* (2002) suggested that antioxidants, which can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very important in the prevention of many diseases.

Plant-derived products contain a wide range of phytochemicals, including antioxidants, which are thought to have a protective role against risk of oxidative stress-related diseases such as cancer and cardiovascular diseases (Koksal and Gulcin, 2008). Therefore, a diet rich in bioactive compounds, including natural

antioxidants, has been associated with reduced risk of heart disease, cancer and diabetes (Kaur and Kapoor, 2002).

In addition to reports documenting antioxidant activity, the effect of food processing and storage on antioxidant activity has also been reported (Roy *et al.*, 2009).

The present study reported that there was significant variation in the total antioxidant activity of both FFS I and II on using different solvent media. Also there are significant differences among the total antioxidant activity of FFS I and II. Results indicated that the total antioxidant activity of FFS I was 0.811 µg / g and that of FFS II was 0.607 µg / g. FFS I (1.09 µg / g) had the highest total antioxidant activity when aqueous ethanol was used. For FFS II (0.83 µg / g) the maximum antioxidant activity was derived while using absolute ethanol.

Extracting antioxidants from plant material most often involves the method of solvent extraction. The choice of solvent has been shown to have a significant influence on the concentration of antioxidants extracted (Ahmad *et al.*, 2011).

Yim *et al.* (2009) studied that, among the various solvent extracts used like, methanol, ethanol, acetone, water and hexane, extraction time and temperature with water as extraction solvent were found to have a critical role in extracting total phenolics in edible wild and cultivated mushrooms, which in turn are believed to possess antioxidant activity with possible food application.

Rahim *et al.* (2013) in their study found that, among the nine solvents used for the evaluation of yield of extracts, detection of phytochmicals and antioxidant activity of the *T. stocksianum* flower, the highest weight of extract was obtained by n-hexane (21 g), followed by butanol (20 g), methanol (19.3 g), water (17 g), acetone (14.5 g), chloroform (13.5 g), petroleum (12.3 g) and ethanol (11.4 g).

Jamuna *et al.* (2011) studied that on the basis of results of total antioxidant capacity of the various fruit extracts the majority of the antioxidant capacity of a fruit may be derived from the active compounds such as polyphenols - flavonoids and tannins.

Rice-Evans *et al.* (2005) opined that the antioxidant activity of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators.

Stewart *et al.* (2000) reported that the flavonoids, a large family of low molecular weight polyphenolic compounds, include the flavones, flavonols, flavonones, isoflavones, flavan-3-ols and anthocyanins. Many flavonoids may help to provide protection against the oxidation at the cellular level as antioxidants by interfering in enzyme activity, chelating of redox-active metals and effective scavengers of hydroxyl and peroxyl radicals as well as quenching superoxide radicals and singlet oxygen (Afanas'ev *et al.*, 2009).

Gyamfi and Aniy (2002) viewed that tannins are known to inhibit lipid peroxidation and lipoxygenases *in vitro*, and has ability to scavenge radicals such as hydroxyl, superoxide and peroxyl, which are known to be important in cellular pro-oxidant states.

In addition to the role of various secondary metabolites vitamins too play a pivotal role in conferring antioxidant capacity. Vitamin E is chain-breaking antioxidant (Chan *et al.*, 2001); Vitamin C is a hydrophilic antioxidant (Doba *et al.*, 2005) and plays a valuable role in the regeneration of vitamin E and thereby acts to reduce the rate of oxidative consumption of vitamin E (Wrona *et al.*, 2003); β- Carotene is another hydrocarbon carotenoid and quencher of singlet oxygen at a low partial pressure of oxygen (Tsuchihashi *et al.*, 2001).

It could be concluded that, though various solvent extracts exhibited varying levels of TAC in FFS I & II, the antioxidant potency of both the supplements were highly significant. FFS I & II exhibit higher activities in

preventing the reactive oxygen species (ROS) and free radicals from oxidizing. This in turn prevents lipid peroxidation and various chronic illnesses. This proves both FFS I & II to be equally good at scavenging properties. This might be brought about by the high levels of vitamins like beta carotene, Vitamin C & E and also selenium. Some of the phytochemicals like flavanoids, polyphenols etc are also found to exhibit greater antioxidant properties.

5.2.1.7. DPPH Scavenging Activity of FFS I & II

During oxidative stress and exposure to radiation, excessive free radicals are produced that are known to cause damage to the biomolecules. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical is widely used as a reliable tool to measure free-radical scavenging and thus antioxidant activity of plant materials (Sanchez-Moreno *et al.*, 1999).

The present study showed that the DPPH scavenging activity of FFS I & II proved that they had higher levels inhibitory effects. Though there was significant variation between the IC 50 values of FFS I (585.35) & II (639) and also in comparison with standard ascorbic acid (477.86), their potency were found to be higher.

Studies showed that, different solvent extracts showed varying levels of DPPH in samples. Methanol is found to be the most apt solvent for extraction of most of the compounds that exhibits scavenging activities.

Anwar *et al.* (2013) reported that Cauliflower extracts obtained from air-dried, sun-dried, and oven-dried samples using different extraction solvents exhibited appreciable scavenging activity in the range 62.6-70.0 per cent. For this study, the DPPH radical scavenging activity varied considerably in relation to both the extracting solvents and drying processes. The oven-dried cauliflower extracted by aqueous methanol has the highest scavenging activity at 70.0 per cent.

In their investigation, Khan *et al.* (2012) reported that, methanol extract of *Sonchus asper* showed the highest scavenging activity (lowest IC50; 2.5 ± 0.05) followed by chloroform extract, ethyl acetate and hexane extracts. The observed differential scavenging activities of the extracts against various systems may be referred to the different mechanisms of the radical antioxidant reactions in the different assays.

According to Tomsone *et al.* (2012) eight solvents with different polarity were used to study the DPPH activity of Horse radish, and results of multivariate dispersion analyzes showed that solvent significantly influence DPPH scavenging activity, but extraction methods does not have significant influence.

Literature data showed that DPPH scavenging activity differs depending on used solvent and food matrix. Researchers studied selected tropical fruits from Malaysia and stated that DPPH scavenging activity of pineapple ranged from 12.7 per cent to 93.7 per cent, banana ranged from 32.8 per cent to 79.1 per cent, but guava ranged from 67.5 per cent to 94.6 per cent (Alothman *et al.*, 2009).

López *et al.* (2011) reported that the highest activity DPPH was observed in the aqueous algae extract. The selected tropical fruits from Malaysia showed the highest DPPH scavenging activity for pineapple and guava 90 per cent acetone extract. Whereas Alothman *et al.* (2009) reported that the highest DPPH antiradical activity for bananas showed 70 per cent ethanol extract.

Presence of favourable amounts of antioxidants like vitamins, minerals and other phytochemicals are responsible for the high scavenging activities of both FFS I & II.

The developed functional food supplements with favourable sources of proximate compositions, vitamins, minerals, phytochemicals and antioxidant properties proves its role as a functional product in prevention and management of various chronic and lifestyle diseases like diabetes, CVD, hypertension, cancers

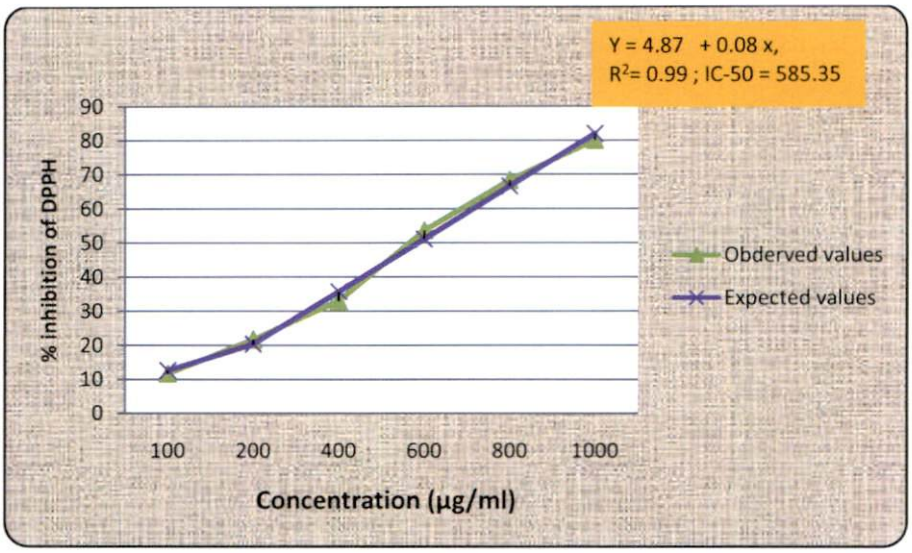


Fig: 18 Dose inhibition curve and IC_{50} values FFS I by fitting regression equation

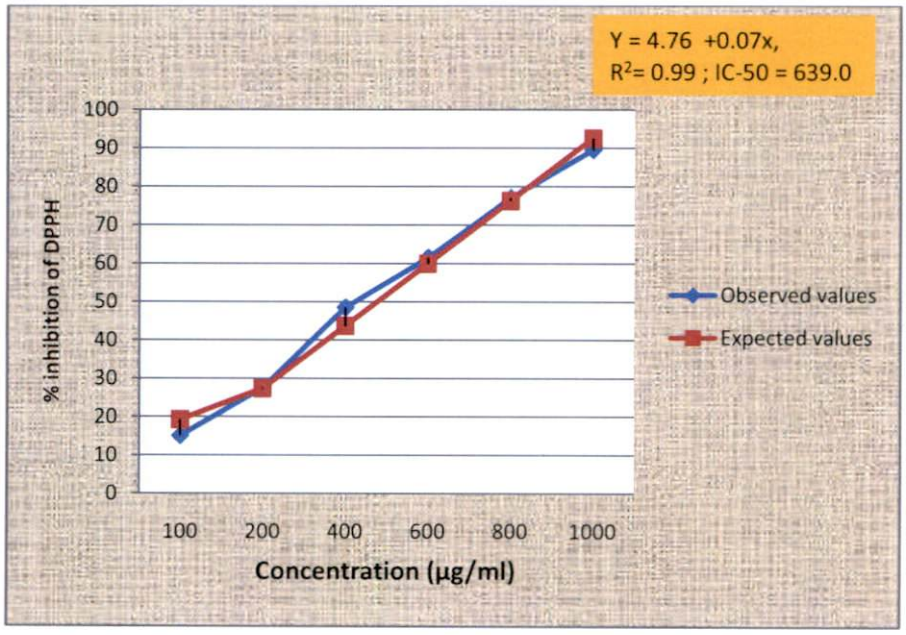


Fig: 19 Dose inhibition curve and IC_{50} values FFS II by fitting regression equation

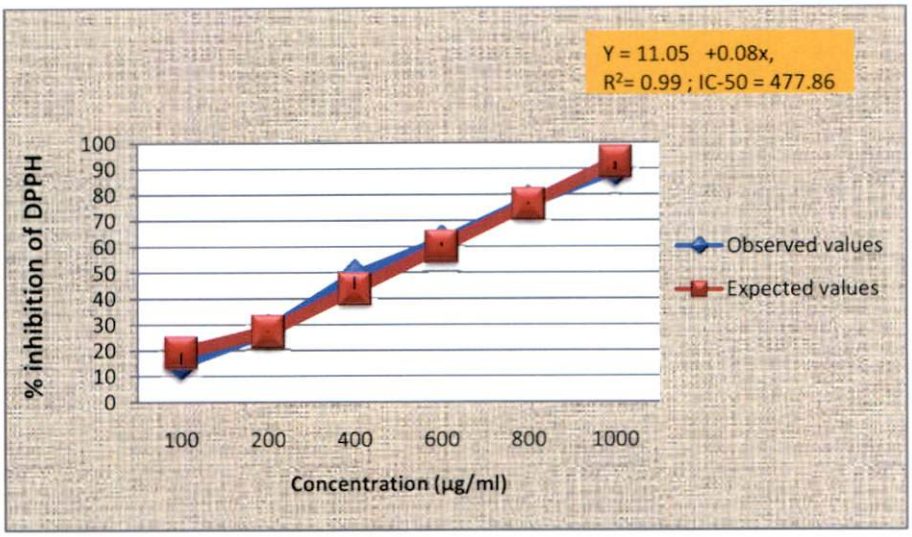


Fig: 20 Dose inhibition curve and IC_{50} values standard (Ascorbic acid) by fitting regression equation

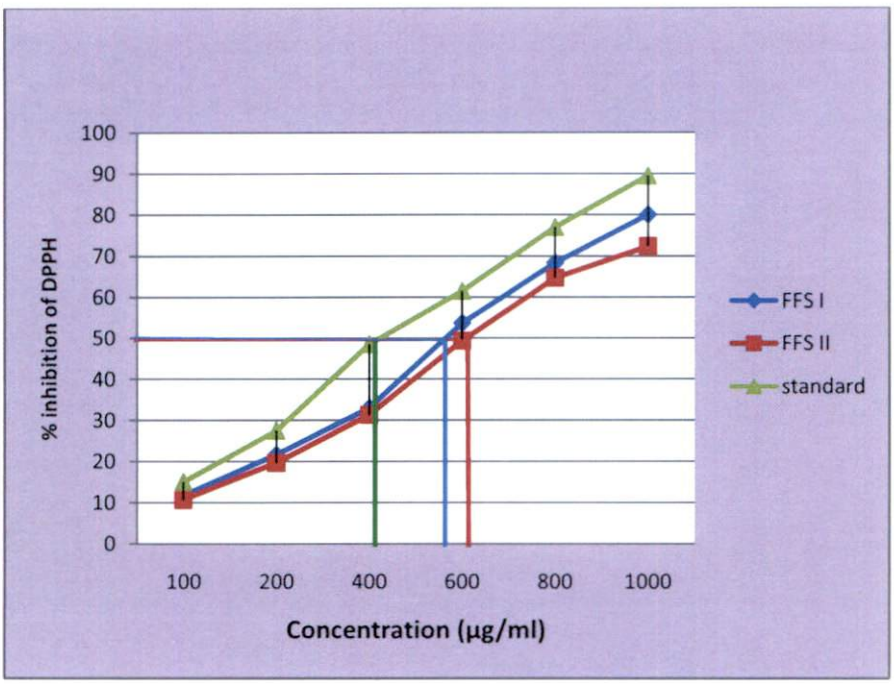


Fig: 21 Comparison of dose inhibition curve and IC_{50} values FFS I & II with standard by fitting regression equation

etc. Their higher scavenging and inhibitory effects are also notable from their ability in immune modulation and antioxidation.

5.2. 2. Functional Properties of FFSs

Functional qualities help in the qualitative assessment and acceptability of any new product. Functional properties determine the overall behaviour of food during production, processing, storage and consumption (Agunbbiade, 2006). Functional properties of food materials are very important for the appropriateness of diet, particularly, for the growing children (Omueti *et al.*, 2009).

To observe the functional qualities bulk density, dispersibility, rehydration ratio, gelatinization time and temperature, processing loss, yield ratio, swelling index, water absorption capacity, water absorption index and water solubility index were ascertained.

In the present investigation, the functional properties depicted that, the functional characters like bulk density (0.90 g/cm^3), rehydration ratio (0.27), gelatinization time (1.16 min) and temperature (88.25^0 C), Water Absorption Capacity (WAC) (1.59) and Water Solubility Index (0.14) significantly increased in the fermentation technique of FFS II on comparison with FFS I, which had the following values like bulk density (0.75 g/cm^3), rehydration ratio (0.23), gelatinization time (0.48 min) and temperature (85.25^0 C), Water Absorption Capacity (WAC) (1.44) and Water Solubility Index (0.13). On the other hand, dispersibility (2.75 per cent), swelling capacity (6.66) and Water Absorption Index, WAI (2.65) decreased significantly in FFS II when compared to FFS I values like dispersibility (7.25 per cent), swelling capacity (7.04) and Water Absorption Index, WAI (2.68). However, there were no differences noted in the processing loss and yield ratio of the two FFS.

Bulk density is an indication of the porosity of a product which influences packaging design and has been found to be a function of flour wettability (Solsuki, 1962). According to Padmasshree *et al.* (1987), higher bulk density is

desirable for greater ease of dispersibility of flours. Though FFS II had a higher 0.90 g/cm^3 when compared to FFS I (0.75 g/cm^3), FFS II had a very low dispersibility of 2.75 per cent against FFS I (7.25 per cent).

According to Osundahunsi and Aworh (2002) the lower loose bulk density implies that less quantity of the food samples would be packaged in constant volume thereby ensuring an economical packaging. However, the packaged bulk densities would ensure more quantities of the food samples being packaged, but less economical. Nutritionally, loose bulk density promotes easy digestibility of food products, particularly among children with immature digestive system. This proves FFS I to be more economical compared to FFS II, however no such differences were noted in the cost calculations of the supplements.

Ijarotimi (2012) in her study found that, raw and fermented wheat flour sample recorded a Bulk density (BD) ranged between 0.80 ± 0.04 and 0.86 ± 0.02. These findings are in line with the present study, as the bulk density of FFS II (0.90 g/cm^3) increased from that of FFS I (0.75 g/cm^3).

On the contrary, Adebowale and Maliki (2011) reported that, bulk density values decreased gradually with fermentation periods in pigeon pea. Similarly Alka *et al.* (2012) revealed reduction in bulk density of sorghum, pearl millet and maize on fermentation.

Swelling index is a measure of the ability of starch to imbibe water and swell. Adebayo-Oyetoro *et al.* (2012) reported that for unfixed fermentation where lower than the values for fixed fermentation.

Sanni *et al.* (2001) reported that the swelling index of granules reflect the extent of associative forces within the granules therefore the higher the swelling index, the lower the associative forces. Swelling power is an indication of the water absorption index of the granules during heating (Loos *et al.*, 2001).

Swelling capacity decreased with increasing fermentation period. This is comparable to the results of the present study.

Jacguier *et al.* (2006) reported that the starch granules start to swell rapidly only after the temperature reached the onset of the gelatinisation temperature. The swelling power of flour samples is often related to their protein and starch contents (Woolfe, 2002). Higher protein content in flour may cause the starch granules to be embedded within a stiff protein matrix, which subsequently limits the access of the starch to water and restricts the swelling power. The obtained results do not fit these previous observations with flours lower in protein and higher in total starch content having a higher swelling ability.

Singh *et al.* (2003) investigated that in addition to protein content, a higher concentration of phosphorous may increase hydration and swelling power by weakening the extent of bonding within the crystalline domain. Furthermore, the amylopectin is primarily responsible for granule swelling, thus higher amylose content would reduce the swelling factor of starch (Tester and Morisson, 2000).

According to the study of Ijarotimi (2012), swelling capacity (SC) ranged between 0.0 ± 0.03 for raw sample and 1.23 ± 0.21 for germinated sample. On the contrary, FFS II had least swelling power (6.66) compared to FFS I (7.04) and was significantly variable (t 30.60**).

Water absorption capacity is an index of the maximum amount of water that a food product would absorb and retain (Marero *et al.*, 2001). Studies have shown that the microbial activities of food products with low water absorption capacity would be reduced (Giami and Bekeham, 2001). Hence the shelf-life of such product would be extended.

Niba *et al.* (2001) had described that the water retention capacity of a starch granule gives the degree of exposure of the internal structure of the starch granules to water. It is an important processing parameter and has implication for viscosity, bulking and consistency of product.

Moreover, Diwakar *et al.* (1999) mentioned that during cooking, gelatinization of the carbohydrates and swelling of the crude fibre might occur which could also lead to increased water absorption. The present study also depicted a picture of increase in WAC in FFS II (1.59) on fermentation when compared to the unfermented FFS I (1.44).

Adebowale and Maliki (2011) supported the present findings and revealed that water absorption capacity of pigeon pea increased with the fermentation periods.

The above study also supports the present findings that, FFS II had a higher water retention capacity and higher bulk density when compared to FFS I.

Investigations of Ijarotimi (2012) proved that, Water absorption capacity (WAC) of fermented wheat flour (415 per cent) was higher than germinated wheat flour (315 per cent) and raw wheat flour (405 per cent) samples.

On the other hand, (WAC) was increased after talbina treatment of barley varieties were noted, and that increase might be due to ß-glucans which form viscous gels on hydration. Likewise, Munck (1999) found non starch polysaccharide ß-glucan may influence (WAC). This also substantiates the present finding that, FFS II had higher ß-glucan content than FFS I and in turn a higher water absorption capacity.

Kinsella (2006) reported that, the function of proteins could hold water by hydrogen bonding or by physical entrapment. On the contrary, Pelemba *et al.* (2002) found a decrease in (WAC) with increasing temperature and they explained that by decomposition or degradation of starch. Ding *et al.* (2006) also stated that the WAC decreased with increasing temperature if starch melting prevails over the gelatinization phenomenon.

However in this study, FFS II had a higher gelatinization temperature of 88.25^0 C as against FFS I (85.25^0 C) but higher WAC.

According to Kamal *et al.* (2012), Water Absorption Capacity (WAC) was decreased after germination treatment in both varieties of barley, and described that decrease might be due to degradation of starch in sprouting seeds.

Anderson (1982) reported that cooking temperature increased **Water Absorption Index (WAI)** for several grains. Results of this study do not agree with those findings. However, it must be noted that there was no significant difference between FFS I (2.68) and FFS II (2.65) in this regard. It may be suggested that WAI is mainly influenced by the affinity of the flour particles for water because finer particles form a greater gel matrix with water trapped into it (Anderson *et al.*, 1969).

Similar to that of the present study, where WSI of FFS II (0.14) was significantly varied from FFS I (0.13) (t 3.27**). Kamal *et al.* (2012) revealed that, **Water solubility index (WSI)** increased in barley, due to hydrolysis for polysaccharides during malting. Likewise, the hydro-thermal treatment for barley flour (talbina) had an increase in (WSI), which happened as a result of increase the ratio of ß-glucan solubility.

The present study puts forward a mixed view in the case of functional qualities of the FFSs. Among the various functional parameters assessed, their combined effects in both the FFSs make them better products that could be easily marketed. Functional characters are one of the most important criteria in fixing the market acceptability of the product. Also, the functional properties like bulk density, dispersibility, gelatinization, swelling capacity and other have significant roles to be played to release a product in the market as one with therapeutic properties.

5.2.3. Pasting Properties of FFSs

Viscoelastic studies have been used to determine the gelatinisation of suspensions from a variety of starches as well as their pasting characteristics during heating and subsequent cooling (Afoakwa and Sefa-Dedeh, 2002). It has been shown (Tsai *et al.*, 2003) that upon heating, the viscosity increased suddenly after a certain temperature.

With regard to pasting properties, FFS I depicted, the pasting temperature as 84.45^0 C and pasting point 59.25 cP. However, these properties could not be detected for FFS II. The peak viscosity which is the ability of starch to swell freely before their physical breakdown ranged between 1190.75 for FFS I and 223.75 for FFS II. FFS II had the lowest (4.0 RVU) breakdown viscosity when compared to FFS I (206.75 RVU). The final viscosity, which is the change in the viscosity after holding cooked starch, ranged between 1958.75 for FFS I and 453.75 RVU for FFS II. The setback value of the FFS I & II were 975 and 234 RVU, respectively.

Mohan *et al.* (2010) reported that the pasting behaviours are also influenced by the interaction between the chemical components and the crystallinity, size, structure, distribution, and water binding capacity of the starch granules.

Oyewole and Afolami (2001) viewed that pasting temperature gives an indication of the gelatinization time during processing. It is the temperature at which the first detectable viscosity is measured and an index characterized by initial change due to the swelling of starch.

Starches with lower pasting temperatures are generally considered to be easier to cook and are also associated with low paste stability, which is usually considered to be an undesirable property (Afoakwa and Sefa-Dedeh, 2002).

Pasting temperature has been reported to relate to water binding capacity, a higher pasting temperature implies higher water binding capacity, higher gelatinization and lower swelling property of starch due to high degree of association between starch granules (Oyewole, 2001). These findings are also in correlation with the present investigation.

Afoakwa *et al.* (2010) studied that pasting temperature was observed to decrease for the fermented maize–millet malt blends when the sprouting time for the millet malt was increased. In their study, Adebayo-Oyetoro *et al.* (2012) reported that the pasting temperature of lafun produced from fixed and unfixed fermentation methods also increased as the length of fermentation increases irrespective of the method used.

In regard to the pasting temperatures, the sweet potato flour had the highest (80.98°C) with the yam flour having the lowest (72.75°C), which could be related to the starch concentrations of the samples.

In the present study, the pasting temperature of FFS II was undetectable, which might be due to its very low values. However, FFS I had a pasting temperature of 84.45^0 C, proving higher paste stability as well.

Aprianita *et al.* (2009) studied that, taro and yam flours had the highest peak time, which may indicate a greater structural rigidity in comparison to sweet potato flour (Leon *et al.*, 2006).

Peak viscosity is linked to the ease of cooking of sample analyses. The presence of malt in the fermented blends decreased the viscosities as compared to the sample without the malts. The results of Afoakwa *et al.* (2010) showed that the addition of 5 per cent maize malt caused decreases from 140 to 90 BU whereas that of 10 per cent maize malt decreased slightly from 120 to 110 BU after 4 days of malting.

In the present investigation, the peak viscosity of FFS I was found to be 1190.75, which was found to be considerably decreased in FFS II (223.75) on fermentation and other processing techniques. The observed reduction in peak viscosity of the supplement (FFS II) is suspected to be resulted from the action of amylases from the malted ragi (Helland *et al.*, 2002; Traore′ *et al.*, 2004).

Moongngarm (2011) in her study reported that, as the steeping and germination time increased, the values of peak viscosity, breakdown, set back, and final viscosity of germinated rice decreased compared to ungerminated rice. This was probably due to the degradation of starch by enzyme activity during the germination process.

Aprianita *et al.* (2009) in their comparative study reported that, sweet potato flour had lowest peak viscosity due to lower rigidity of starch granules which in turn caused instability and consequently disruption upon the heating and stirring treatment (Leon *et al.*, 2006) as opposed to the taro flour which could be due to the small granule size, which also led to higher swelling power and subsequently higher viscosity.

The viscosity attained by a sample after holding the temperature constant at 95^0 C for 30 min (95^0 C HOLD) gives an indication of the ease of breakdown of the hot cooked paste. According to Afoakwa *et al.* (2010), generally, the addition of cereal malt to the fermented maize decreased the viscosity of the resulting gruel at 95^0 C HOLD.

Traore′ *et al.* (2004) reported that alpha amylase activity increased remarkably during malting of sorghum, maize and millet. However, the drying process tremendously reduced the level of alpha amylase activity in sorghum while that of maize increased by 26 per cent during drying.

Similarly, in the present study, the combined effect of fermentation and drying could have brought about the changes in the hold viscosity of FFS II which was comparatively lower than FFS I.

Shimelis *et al.* (2006) reported that final viscosity is used to indicate the ability of starch to form various paste or gel after cooling and that less stability of starch paste is commonly accompanied with high value of breakdown.

The investigations of Adebayo-Oyetoro *et al.* (2012) depicted that, the final viscosity ranged from 356.05RVU to 348.24RVU for unfixed fermented lafun while fermented cassava has the least value of 327.60RVU.

Results of the present study showed that, the final viscosity, which is the change in the viscosity after holding cooked starch, ranged between 1958.75 for FFS I and 453.75 RVU for FFS II. This implied that starch paste from FFS I which has the highest for final viscosity was less stable after cooling compared to the FFS II, hence retrogradation occured faster. The variation in the final viscosity might be due to the simple kinetic effect of cooling on viscosity and the re-association of starch molecules in the samples (Ikegwu *et al.*, 2009).

According to Reka and Andras (2008), the breakdown viscosity is related to the stiffness of swollen granules whilst the amylopectin is responsible for susceptibility of swollen granules to disintegration when the gelatinized starch slurry is heated and stirred. The heating of the slurry not only causes starch gelatinization but also it activates the enzymes to hydrolyze the starch. As a result of this, the viscosity of the slurry decreases significantly.

Result of the set back viscosity of the starch samples of Adebayo-Oyetoro *et al.* (2012) ranged between (166.45RVU-149.96RUV) for fixed fermentation with sample fermented for 48h having the highest value of 166.45 RVU while unfixed fermented lafun for 96h had the lowest value of 149.90RVU. Sanni *et al.* (2001) reported that lower set back viscosity during the cooling of FFS II indicate higher resistance to retrogradation.

Similar to that of the functional qualities, the pasting properties of the food supplements developed also implies a varying effect. The changes brought about by fermentation in FFS II was notable, as FFS II produced a comparatively

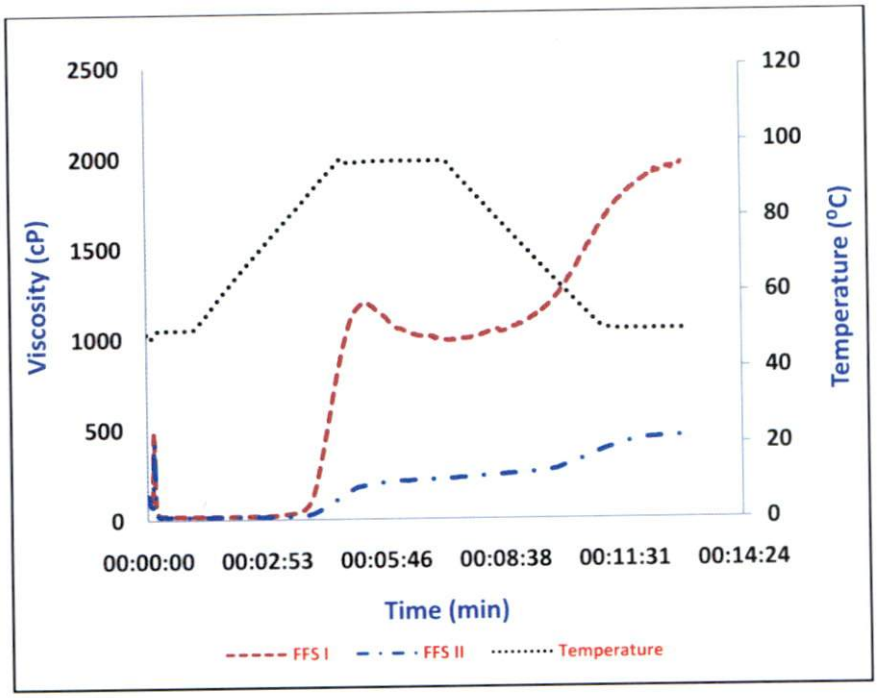


Fig: 22 Change in viscosity of FFS I & II depending on time and temperature

thinner gruel or porridge than FFS I. This can be brought about by the interaction between the chemical components and the crystallinity, size, structure, distribution, and water binding capacity of the starch granules. Changes in amylase content, gelatinization, retrogradation etc might be the other contributing factors. However sensory evaluation of the food supplements (FFS I & II) did not show any significant variation among the products and recipes. This proves that, both FFS I & II are well in balance as products with better marketability. Also the varying properties of FFS I & II could be helpful in producing different recipes, as each recipe demands varying cooking techniques. This would further help the consumers in avoiding the monotony of recipes with an increased therapeutic effect.

5.2.4. Textural Properties of FFSs

The reports of the present study when investigated for the textural characteristics of the food supplements developed revealed that, FFS I scored 9.06 on the hardness scale. FFS I showed a cohesiveness value of 0.499. Adhesiveness value of FFS I was (- 37.515). The springiness and gumminess values of FFS I is 5.4 and 4.79 respectively. The chewiness range of FFS I showed 25.86. Texture Profile Analysis showed very low values for FFS II which could not be detected in hardness, cohesiveness, elasticity, gumminess etc., of the fermented flour paste.

The altered textural properties were attributed to greater starch granule stability due to short amylose-like fragments formed by enzymatic hydrolysis of amylopectin (Schwartz, 2006).

Xia *et al.* (2014) studied that the hardness, springiness and adhesiveness of fermented whole-soya bean cotyledon sufu were 249.703 ± 0.500 g, 0.606 ± 0.008 and 8.393 ± 0.032 J, respectively. The corresponding values for traditional Sufu were 264.863 ± 0.572 g, 0.615 ± 0.007 and 7.516 ± 0.031 J,

respectively. This shows significant variation on textural properties on fermentation.

Numfor *et al.* (2005) studied the physicochemical changes in cassava starch and flour associated with fermentation and related to textural properties of its flour pastes. Texture profile analysis showed a decreased in hardness, cohesiveness, elasticity, and gumminess of the fermented flour paste. The altered textural properties were attributed to greater starch granule stability due to short amylose-like fragments formed by enzymatic hydrolysis of amylopectin.

Sensory evaluation of recipes developed from FFS I & II however did not portray any of the above mentioned changes in the supplements. Though FFS I marked some of the textural properties applied, it must be noted that the values are much lesser, thus making not much variation from that of FFS II. To conclude that, FFS I and II are almost similar in characters and thereby no differences can be attributed to the market acceptability of the two products.

5.2.5. Colour Attributes of FFSs

Colour is one of the important visual attribute that has been used to judge the overall quality of foods for a very long time. If the colour is unattractive, a potential consumer may not be impressed by any other attributes. The first impression of food is usually visual and a major part of willingness to accept a food depends on its appearance.

Colour changes can give information about the extent of browning reactions such as maillard reaction, caramelization, degree of cooking and pigment degradation during processing (Ilo *et al.,* 1999).

With regard to colour preference of samples, lightness factors are expressed in terms of 'L' values which indicate lightness or darkness on a scale of 100 to 0. The chromatin break coordinates, which represent hue and chroma, are expressed by 'a' and 'b', respectively.

In the present study, the mean L* value ranged between 73.72 for FFS I being the highest and 64.77 for FFS II which had the lowest L* value. This indicates that a substantial level of colour change had occurred during drying that yielded dark brown powder, particularly in the FFS II. The presence of glucose, fructose and protein, an extension of Maillard reaction had occurred within FFS II. In addition, certain enzymes such as polyphenol oxidase may be present that could contribute a certain stage of enzymatic browning that took place during drying. There is some evidence that a* values of FFS I (1.71) were lower than those of FFS II (3.50). However, as a result of the browning reactions during drying, FFS II exhibited higher b* values than FFS I.

A low value for chroma and a high value for lightness are desired for the product to meet the consumer preference. In terms of product colour, it was observed that FFS I had a high value of whiteness (L = 73.72) and a low value of chroma (a = 1.71). Thus, in this study colour of FFS I can meet consumer preference due to the highest whiteness and low chroma values.

Abbas *et al.* (2010) studied that the mean L* value for all Banana Peel flour ranged between 37.6 to 49.0 and DR and CR had the lowest L* value (~ 37.6). This indicates that a substantial level of colour change had occurred during drying that yielded dark brown powder, particularly in the ripe samples. It was found that banana paste dehydrated with vacuum dehydration had darker colour (lower L value) and more intense yellow colour (higher b value) as a result of condensation due to moisture loss, enzymatic and nonenzymatic browning (Thipayarat, 2007).

Salehifar and Shahedi (2007) reported that, the addition of oat flour in wheat bread resulted in a loss of flour brightness (L*) and a decrease in flour yellowness (b*), probably due to oat bran colour in the flour. The redness of the flour was not changed with the addition of oat flour (a*).

It could be noted that, with regards to the colour attributes of the food supplements, FFS I produced a comparatively lighter colour than FFS II. However the acceptability of the product lies in its final output. Since, sensory evaluation of the recipes developed from FFS I & II did not produce significant variation, it could be concluded that, both the supplements are on par with each other.

5.2.6. Particle Size of FFSs

Singh *et al.* (2003) suggested that the particle size of starch is one of the most important characteristics, which may influence other physicochemical properties such as swelling power; paste clarity, and water-binding capacity.

Comparing the particle size, for FFS I, the largest particle was in the size of 534.7 in comparison to FFS II which had a particle size of 560.6 r. nm. The particles of FFS I ranged between 400 to 650 r. nm while for FFS II it ranged between 400 to 800 r. nm. The maximum width of the particles of FFS I was 32.51 r. nm and 29.07 r. nm for FFS II.

With these factors in mind the use of FFS I & II may be equally applicable for several different applications within the food industry, particularly products that require starch that offers a smaller particle size allowing for smooth textured starch gel (Tattiyakul *et al.*, 2005).

Manary (2006) reported that, the size of the particles in the therapeutic mixture has to be less than 200 μm for the mixture to maintain its consistency. However, no such criteria were substantiated for food supplements.

O'Dea *et al.* (2002) viewed that, food particle size has become increasingly more important to the food industry. Many food ingredients exist in some particulate form whether it is powders, emulsions, suspensions, and/or pellets. Moreover, the shape and size of these particles as well as their distribution affect flavor, texture, and appearance of foods that we eat. Size and shape of ingredients also affect the stability of a given product as well as process

ability and functionality of the desired end product (http://ajcn.nutrition.org/content/47/4/675.short).

Particle size controls a number of chemical and physical properties which include; reaction and dissolution rate, packing density, sedimentation, appearance, and texture. Non-biologically, particle size is another way to increase reaction rates (http://www.leatherheadfood.com/droplet-and-particle-size).

Particle size also effects solubility and is directly related to bioavailability. This is particularly important for the food and pharmaceutical industries. The smaller the particle whether drug or food, the greater the chance of it being absorbed by the gut (http://www.fda.gov/Food/Guidance ComplianceRegulatory Information/GuidanceDocuments/FoodIngredientsandPackaging/ucm300661.htm.

Usually, larger particles don't pack as well in that they have more space between them because they have a larger surface area. Smaller particles tend to pack more densely because they have a smaller surface area (http://ajcn.nutrition.org/content/47/4/675.short).

Particle size and distribution is an important characteristic contributing to product appearance affecting the overall bulk properties of the food item such as visual texture and density as well as color. Larger particle size indicates a chewy food, whereas smaller particle size indicates crunchy and less moist. Large particle size tends to have less distinct flavor, whereas fine particles have more flavor (http://www.leatherheadfood.com/droplet-and-particle-size).

Heaton *et al.* (2001) reported that the increased insulin response to finely ground flour may be relevant to the cause of diseases linked to hyperinsulinemia (excess blood insulin levels) and to the management of diabetes.

For a product to be acceptable in the market, it must be able to satisfy a large round of varying characteristics. It must not only focus on the intended need, but also other characteristic features like nutrients, product quality, cooking

properties, colour, functional properties etc. A product which aims purely to be a therapeutic one may not be successful in the market, if the other characteristics like product acceptability, stability etc were not taken into consideration (http://www.leatherheadfood.com/food-innovation-research).

So the food supplements I & II developed in this study, not only proves its qualities in terms of nutrients and therapeutic value, but the acceptability of the products in the market are also analyzed based on their functional, physico – chemical, sensory and storage qualities.

5.3. SHELF LIFE ASSESSMENT OF FFSs

A guide to calculating the shelf life of foods (2005) stated that, shelf life is a guide for the consumer of the period of time that food can be kept before it starts to deteriorate, provided any stated storage conditions have been followed. The shelf life of a product begins from the time the food is prepared or manufactured. Its length is dependent on many factors including the types of ingredients, manufacturing process, type of packaging and how the food is stored.

Kumar (2001) suggested that there are many ways in which quality and nutrients can be lost. They may not necessarily result in the product being harmful but can mean that it is no longer of an acceptable standard. Moisture gain/loss, chemical change, light induced change, temperature changes, physical damage, spoilage by rodents and insects, flavours and odours from storing food near other strongly smelling products, product tampering etc.

Shelf life study is an objective, methodical means to determine how long a food product can reasonably stay safe without any appreciable change in quality. Hence in present investigation moisture, peroxide value and microbial growth were examined periodically up to a period of six months.

Robertson (2000) reported that, the free space volume has an important influence on the rate of oxidation of foods that a large package area and a low bulk density result in greater oxygen transmission.

Moisture is one of the important parameter which determines the shelf life quality of food product. Low moisture is highly important for longer storage period (Shankar, 2003).

The moisture content of FFS I during the initial storage period was (10.8 per cent) which gradually increased to 11.3 per cent in the final (6^{th}) month of storage. Similarly, FFS II had an initial moisture content of 10.68 per cent against the final moisture percent of 10.97. Even though the variation seems negligible over the period of storage, the changes were significant in both FFSs.

Midhila (2013) studied the effect of storage on the dried banana blossom flour. She found that with advancement in the storage period, the moisture level enhanced. But the increase in moisture content did not influence the quality of the developed products because the increase in moisture content was negligible.

Saranya (2012) reported that the moisture content of stored Enriched Soup Mix (ESM) was found to enhance gradually during the storage period. But the increase in moisture content does not influence the quality of the RTC product.

Sharma (2006) reported that the primary products of lipid oxidation are hydro peroxides which are generally present as peroxides. Thus it seemed reasonable to determine the concentration of peroxide as a measure of extend of oxidation and thus rancidity. The nature of auto oxidation degradation depends on the extent of un-saturation of lipids. Thus in the present study, very minimal levels of peroxide appeared in FFS I by the end of 5^{th} month (0.22 meq/kg) and 6^{th} month (0.23 meq/kg). On the other hand, FFS II elucidated peroxide contents (0.21 meq/kg) only in the final (6^{th}) month of the storage studies. However, it could be noted that the peroxide contents in the FFS I & II were much minimal than the permitted limits.

Midhila (2013) reported in the developed banana RTC product there was an increase in peroxide value with increase in storage time owing to the oxidative deterioration of lipids in the coconut.

Krokida (2001) reported that the peroxide value increased during storage. Neelofer (2004) reported that the peroxide value of therapeutic and malted health drink mix were 0.32 meq/100g and 0.54meq/100g. Saranya (2012) reported that the negligible amount of peroxide content was observed for the ESM developed from moringa. Similar results were reported by Resmi (2012) in the peroxide content of processed *Njavara* grits over a period of three months of storage studies.

Investigations have shown that low moisture content of food samples is a desirable phenomenon, since the microbial activity is reduced (Oyenuga, 2002). Low moisture content in food samples increased the storage periods of the food products (Alozie *et al.*, 2009); while high moisture content in foods encourage microbial growth; hence, food spoilage (Temple *et al.*, 2001).

The findings of the present study implies that, even by the end of six months of storage studies, the increase in moisture content of both FFS I & II though significant in the 6^{th} month from that of the initial, they are still much lesser and can be categorized as non perishable foods. It must also be noted that, the peroxide contents in the FFS I & II were notable only by the end of the study period and much minimal than the permitted limits. This implies better shelf life and storage stabilities of both the supplements developed. Even the observed changes in the moisture and peroxide can be attributed to the loosening of the sealed packages kept for study as time passes. Vacuum packaging or aluminium foil packages could definitely enhance the storage period.

5.3.1. Total Microbial Population of FFSs

Microbial quality is one of the most critic quality parameters in a dynamic system such as food. There are different threats in food quality originating from

microbial sources. Spoilage causing organisms causes off odour and off taste and lead to economic losses (Rao, 2003). The concept of spoilage by microorganisms are the primary cause of the end of shelf life and that hence reducing initial microbial populations is a strategy to extend shelf life (Zagory, 2003).

Serial dilution followed by spread plating was employed to detect the presence of microorganisms. In the present investigation it is evident that during the six months of storage period no actinomycetes were found to be appeared in the developed products i.e. FFS I & II. But bacterial colonies were observed during the 5^{th} month (1×10^{-1} cfu/g) and 6^{th} month (2×10^{-2} cfu/g) of storage. Simultaneously, fungi were detected in the 6^{th} month (1×10^{-1} cfu/g) of the storage studies. Even though bacteria and fungi were detected, it was present only in permissible limits. No pathogenic organisms could be detected in the FFS I.

However, FFS II was found to be sterile and free of microbial contamination even after the 6^{th} month of the storage studies. This might be brought about by the double drying technique involved in the processing of FFS II. The above findings prove that both FFS I & II are safe and stable up to a period of six months and more.

Nasheeda (2006) reported that the bacterial population of banana powder packed in poly propylene covers ranged between 5.68 – 6.88× 103 cfu/g. Suma (2008) reported that the bacterial count in fruit drink mix developed from banana ranged between 5.42 – 8 $\times 10^6$ cfu/g, where as fungal growth was recorded to be in the range 2.59 – 4.26 $\times 10^4$ cfu/g. Saranya (2012) and Resmi (2012) also noted no or much less amounts of microbes in the products developed by them.

5.4. COST OF PRODUCTION OF FFS (I & II)

Poonia and Dabur (2009) studied that the economic returns from the functional foods can offer improved opportunities for all the members in the food supply chains: from raw material producers and processors to retailers. Functional foods can be an opportunity for economic growth for many developing countries

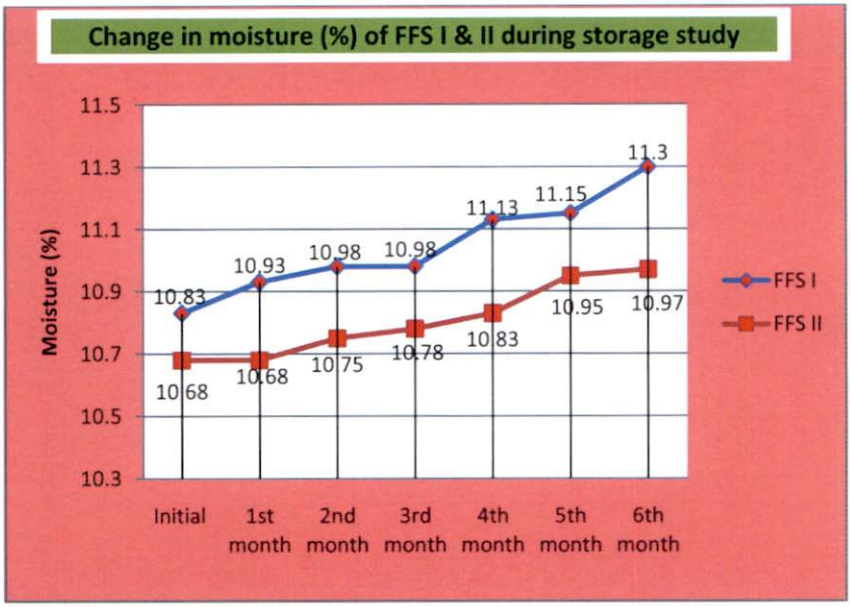


Fig: 23 Change in moisture (%) of FFS I & II during storage study

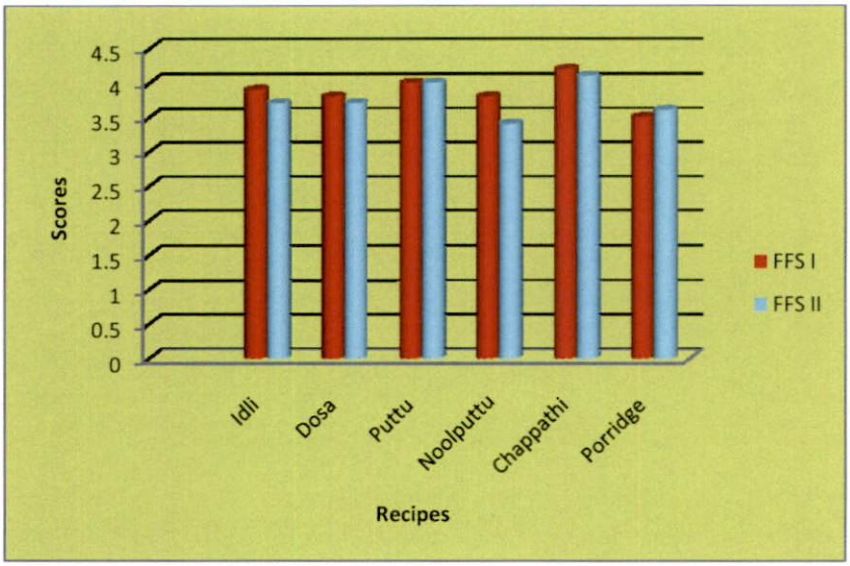


Fig: 24 Overall acceptability scores of recipes standardized from FFS I & II

endowed with rich biodiversity and traditional knowledge of the health effects of certain indigenous plant species.

Hasler *et al.* (2002) reported that retail prices of functional foods are generally higher ranging between 30 to 500 percent above the comparable conventional foods. Moreover, demand for functional foods within the developing countries is growing, presenting a lucrative opportunity to develop domestic markets.

Functional foods sell at higher prices and contain larger profit margins than conventional foods. This makes the food sector attractive for the players of food supply chains including marketing, storage and transportation (Kotilainen *et al.*, 2006).

The cost of one Kg of FFS I & II was found to be Rs. 200/-. While the cost of one portion size (20 g) of FFS I & II each incurred a cost of Rs. 4.0/- only. Consumers of any category would not hesitate to spend an amount of Rs. 4.0/- per day considering their health rather than drugs which not only produces higher side effects but are costly also.

On the health point of view, the rates of both FFS I & II are much less when compared to the proprietary products available in the market. So both FFS I & II are better products and economically viable when scaled against health.

5.5. SUITABILITY OF DEVELOPING VARIOUS FOOD PREPARATIONS/ PRODUCTS USING FFS (I & II)

Suitability of incorporating the FFS (I and II) into various preparations/products was tried out and subject to organoleptic evaluation for the sensory characteristics like colour and appearance, flavour, texture, taste and overall acceptability using a panel of trained members.

Commonly consumed breakfast items like Idli, Dosa, Chappathi, Noolputtu, Puttu and porridge were standardized in the laboratory by the incorporation of FFS I & II. The acceptability of the standardized recipes by the incorporation of FFS I & II were assessed by a panel of trained members using a score card on a five point scale.

There were no significance differences in color & appearance, flavor and overall acceptability of the recipes developed from FFS I. But in the aspects of texture and taste, there were significant variations among the recipes. Among the recipes, puttu (4.3) got the maximum score for taste, followed by chappathi (4.2), idli (4.0) and dosa (4.0) which were on par with each other, and then noolputtu (3.8) and porridge (3.5) scored the least. Similarly, among the recipes developed from FFS II, puttu (4.1) got the maximum score for taste, followed by chappathi (4.0), dosa (3.8), idli (3.7) and noolputtu (3.7) which were on par with each other, and then finally porridge (3.2) scored the least.

From the above findings it could be concluded that, both FFS I & II were equally acceptable in whichever form of recipes could be supplemented in the common breakfast items of the consumers.

Any food supplement will be acceptable by the population, only if it could be easily incorporated in the daily diet of the consumers, without altering their cultural practices.

In this context, the developed food supplements FFS I & II, could be incorporated in the dietary habits of the population affected by lifestyle diseases even without altering their cultural practices and thus could be promoted in the market level.

Sawant *et al.* (2013) reported that, the mean scores of sensory evaluation of the RTC extruded snacks from finger millet based composite blends were within the acceptable range on a 9 point scale of rating.

Malar and Narayanan (2013) reported that, of all the millet incorporated products, rice dosa and appam were much acceptable. Rest of the items namely, wheat dosa, chappathi and porridge were also acceptable though there was a slight alteration in sensory qualities.

Pratheepa and Beatrice (2013) reported that, when compared to rice idli whey water incorporated idli had a better taste, appearance, color and flavor. The overall acceptability was higher for whey water incorporated idli (4.7) when compared to rice idli (4.0).

Portion sizing was fixed based on a pilot study done in the laboratory mainly focusing on the sensory acceptance on the different level of incorporation of FFS I & II separately in different recipes.

20 gm of the FFSs (i.e. one part) were supplemented with two parts of the basic ingredients for recipe formulation. 20 gm of FFS I was found to contain, 76.8 Kcal of energy, 12 g of carbohydrates, 4.2 g of protein and 0.4 g of fat. While, 20 gm of FFS II was found to contain, 75.6 Kcal of energy, 11.6 g of carbohydrates, 3.4 g of protein and 0.32 g of fat.

Feasibility of substitution of the FFS (I & II) in the Food Exchange List, especially in the breakfast items was computed based on their nutrient contents. Keeping the above points in mind, FFS I & II could be used in the cereal, pulse and other exchange lists. This would help to avoid monotony of diets for the subjects with lifestyle diseases.

5.6. EVALUATION OF CLINICAL EFFICACY OF FFS (I & II)

The clinical efficacy of the FFS (I & II) was ascertained through case studies. Five subjects each for three disease conditions namely, hyperglycemia, hypercholesterolemia and hypertension; and two different FFS developed were selected for the case studies. 20 g of FFS I & II developed was supplemented in the breakfast of subjects for a period of three months.

Details on preliminary information regarding their socio-economic profile, health status, dietary and life style pattern and nutritional status were assessed prior to supplementation.

Based on each of the disease condition of the subjects, i.e. hyperglycemia, hypercholesterolemia and hypertension, the subjects were selected according to the socio-economic profile from the main list. Socio economic profiles like, age, sex, religion etc were considered carefully to group the respondents for the study.

The details on the dietary habits of the subjects revealed that, all the respondents were non-vegetarians. Most of the subjects were having three main meals and one snack per day and had a diet plan of their own. Majority of the subjects had their food especially breakfast and dinner in front of the television. Consumption of fruits was optional and also seasonal.

Almost all the subjects consumed fish once a day, especially in the lunch. It was evident that, the subjects belonging to the Muslim community consumed red meat mostly. Even though the subjects with diabetes (pre-diabetes) consumed tea or coffee without sugar, their meals or snacks involved fried foods and foods of dense calories. Also intake of oil through snacks and other fried foods was high in most of the subjects.

Food frequency of the subjects showed that, their eating pattern is varied and inconsistent. It also depicts a picture of unhealthy eating pattern, which might be the baseline reason for their morbidity conditions.

Only eight out of the thirty subjects had normal BMI, i.e. less than 25. All the other subjects falls under grade I obesity, since they had a BMI of 25 – 29.9. Also the waist – hip ratio denotes upper body obesity in most of the subjects. Even for the subjects with normal BMI, the waist – hip ratio was on the higher side, denoting the predominant distribution of fat in the upper part of the body.

Dhar and Sarwate (2013) reported that, there was a linear association existing between adiposity and blood pressure of hypertensive middle aged women. This shows, BMI as an important indicator for hypertension. Similar observations were made by Deshmukh *et al.* (2006). Shahbazpur (2003) and Gus *et al.* (2004) have also reported earlier significant positive association of BMI with systolic and diastolic pressure.

Mehta (2013) reported that the BMI and Waist Hip Ratio were related to the clinical health parameters and dietary habits of the members of the Marvadi Jain community.

Nande *et al.* (2010) reported that, waist circumference and hip circumference was found to be directly proportional to weight gain and BMI. Waist circumference gives a better prediction of visceral and total fat and of diseases risk.

Pratheepa and Beatrice (2013) reported that, the mean Waist Hip Ratio of 0.92 of the subjects suggested that the values were above normal and also show a substantial increase in the risk for metabolic complications.

These studies support that, the health and dietary habits of the subjects are one of the foremost causative factors in the development of lifestyle diseases like diabetes, hyperlipidemia, hypertension etc.

5.6.1. Impact Evaluation of FFS I & II on Hyperglycemic Subjects

Blood parameters like Fasting Blood Sugar (FBS) and Post Prandial Blood Sugar of the subjects was monitored before, in between and at the end of the supplementation study to understand the impact of supplementation of FFS I & II. A total of ten subjects with hyperglycemia were supplemented with FFS I & II accordingly.

The biochemical investigations proved that there was there was significant variation at 1 per cent level in the FBS levels of subjects supplemented with FFS I & II over a period of 90 days. The mean initial (Day 0) FBS values for the subjects supplemented with FFS I & II were 129 mg/dl and 131 mg/dl respectively. By the end of supplementation on day 90, the values significantly reduced to 104 mg/dl and 106 mg/dl for FFS I & II respectively.

But on the other hand, it could also be noted that, there is no differences among FFS I & II in the level of variation in FBS of the subjects. This concludes that both FFS I & II are equally effective in producing favorable results. It can also be noted that, in both the cases of FFS I & II, the final values matched the FBS values of normal subjects or those who are under control.

The investigations also found there is significance variation at 1 per cent level in the PPBS levels of subjects supplemented with FFS I & II over a period of 90 days. The mean initial (Day 0) PPBS values for the subjects supplemented with FFS I & II were 204 mg/dl and 212 mg/dl respectively. By the end of supplementation on day 90, the values significantly reduced to 161 mg/dl and 174 mg/dl for FFS I & II respectively.

It is also evident that, there was significant variation among the FFS I & II in reducing the PPBS of the subjects. On a whole, FFS I was found to be more effective in reducing the PPBS of the subjects when compared to FFS II.

Even the rate of change (%) of both FBS & PPBS is concomitant to that of the values of the subjects over a period of 90 days.

Kumari and Sinha (2004) studied the impact of supplementation of fenugreek incorporated therapeutic food containing bengal gram, green gram, horse gram, dry peas and fenugreek seeds on blood sugar levels of NIDDM patients and found both fasting and postprandial blood sugar levels were reduced.

Thilakavathi and Muthuselvi (2010) studied that, subjects fed with bajra incorporated chappathi registered low blood glucose response followed by white oat chappathi when compared to standard wheat chappathi. Also when fenugreek was added to these, the change was still more evident.

Premakumari and Haripriya (2010) found that supplementation of wheat germ, bran and grass had beneficial effect in alleviating specific health issues like diabetes, obesity and could be used as an immune booster in low immunity.

Jaziya (2011) reported that incorporation of five gram dried oyster mushroom (*Pleurotus florida*) powder in the daily diet, reduced blood glucose and blood lipid levels. This might be attributed to the beta glucan content and other phytochemicals of oyster mushroom. Similarly Anju (2013) reported that, incorporation of five gram dried milky mushroom (*Calocybe indica*) powder in the daily diet, reduced blood glucose and blood lipid levels of the subjects supplemented with it.

Resmi (2012) inferred that, supplementation of Njavara in the diets of the subjects with diabetes showed significant variation over a period of three months.

Premakumari *et al.* (2013) reported that, after 90 days of supplementation with value added rice bran incorporated RTE mixes, there was considerable variation in the FBS and PPBS levels of the subjects.

Pratheepa and Beatrice (2013) studied that, whey incorporated idli produced significant change on the plasma glucose levels of the subjects when compared to rice idli.

The above results strongly suggest that, management of blood sugar could be made possible with selected natural food supplements with great therapeutic value. In this context, it can be recommended that the developed food supplements FFS I & II could be added to the list of food supplements.

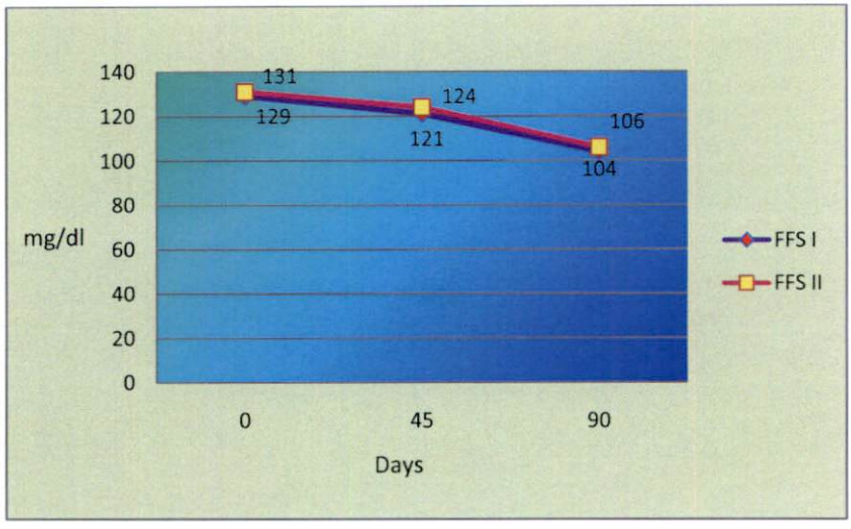


Fig: 25 Effect of supplementation of FFS I & II on mean FBS of subjects

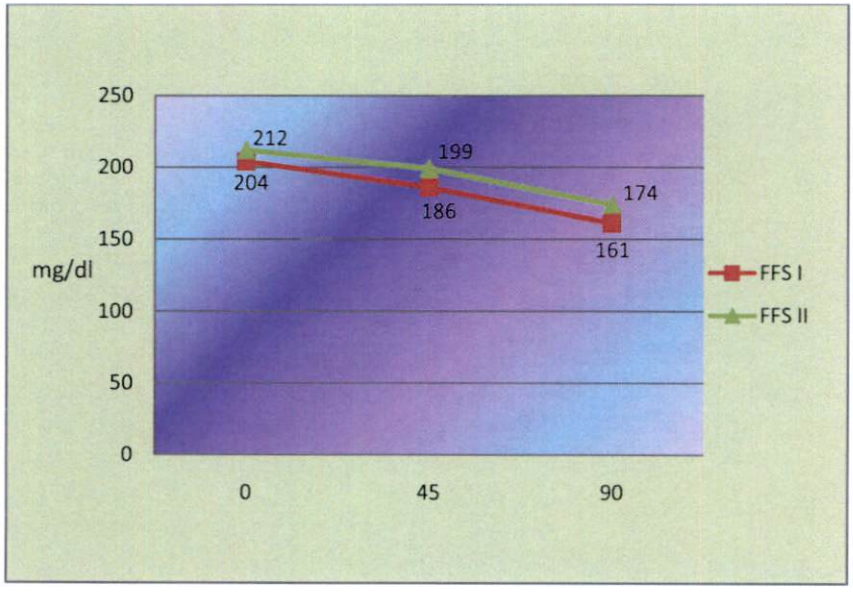


Fig: 26 Effect of supplementation of FFS I & II on mean PPBS of subjects

5.6.2. Impact Evaluation of FFS I & II on Hypercholesterolemic Subjects

Blood parameters like Total Cholesterol (TC), Low density Lipoprotein (LDL), Very Low density Lipoprotein (VLDL), High density Lipoprotein (HDL) and Triglycerides (TG) of the subjects were monitored before, in between and after the conduct of the study to understand the impact of supplementation of FFS I & II. A total of ten subjects with hypercholesterolemia were supplemented with FFS I & II for a period of three months.

From the present supplementation study, it could be inferred that, significant variation at 1 per cent was observed in the total cholesterol, LDL, VLDL, TG and HDL levels of subjects supplemented with FFS I & II over a period of 90 days, showing differences over the period of days being evidently significant. Conversely, it could also be noted that, there is no differences between the subjects of FFS I & II groups in the level of variation in the above mentioned lipid parameters.

The mean initial Total Cholesterol (TC) values of the subjects supplemented with FFS I & II were 240 mg/dl for each, which had reduced to 190 mg/dl and 193 mg/dl respectively.

The mean initial LDL values of the subjects supplemented with FFS & II were 155 mg/dl and 157 mg/dl respectively. By the end of 90 days of the study, the values had decreased to 146 mg/dl and 148 mg/dl for FFS I & II respectively.

It could be observed that, the mean initial VLDL values of the subjects supplemented with FFS & II were 40 (39.6) mg/dl and 42 mg/dl respectively. By the end of 90 days of the study, the values had decreased to 36 mg/dl and 38 (37.6) mg/dl for FFS I & II respectively.

When subjects were supplemented with FFS I & II, the mean initial TG levels which were 182 (182.4) mg/dl and 182 mg/dl respectively, gradually declined to 168 (168.4) mg/dl and 170 (169.6) mg/dl respectively by the 90^{th} day.

On supplementation with FFS I & II to subjects with hypercholesterolemia, the mean initial HDL levels which were 40 (40.4) mg/dl and 40 (39.8) mg/dl respectively steadily increased to 45 mg/dl and 46 (45.6) mg/dl by the end of the study.

These findings suggest that, both FFS I & II are equally good at producing favorable changes in the lipid profile of the subjects.

Aruoma *et al.* (2010) investigated that a Fermented papaya preparation (FPP) supplement was beneficial over oxidative stress-induced cell damage and inflammation implicated by cancers, diabetes, arthritis, cardiovascular dysfunctions, neuro degenerative disorders.

A supplementation study conducted by Thilakamani and Mageshwari (2011) indicated that a formula with Italian millet flour, whole wheat flour, corriander leaves and groundnut oil given to young women with risk of CVD decreased total cholesterol and low density lipoprotein cholesterol.

Reduction in total cholesterol and other blood lipid levels were reported by Jaziya (2011) by incorporation of 5 gm dried oyster mushroom (*Pleurotus florida*) powder and Anju (2013) by incorporation of 5 gm dried milky mushroom (*Calocybe indica*) powder in the daily diet in the subjects supplemented with it.

Resmi (2012) in her study inferred that, supplementation of Njavara in the diets of the subjects with hypercholesterol showed significant variation in the total cholesterol levels over a period of three months of the study. Nobuhiko *et al.* (2013) suggested that Mung bean Protein Isolate (MPI) may improve the plasma lipid profile by normalizing insulin sensitivity.

Premakumari *et al.* (2013) studied that, after 90 days of supplementation of rice bran containing recipes, the subjects showed a significant reduction in the total cholesterol, LDL and TG. However, variations in the HDL were insignificant.

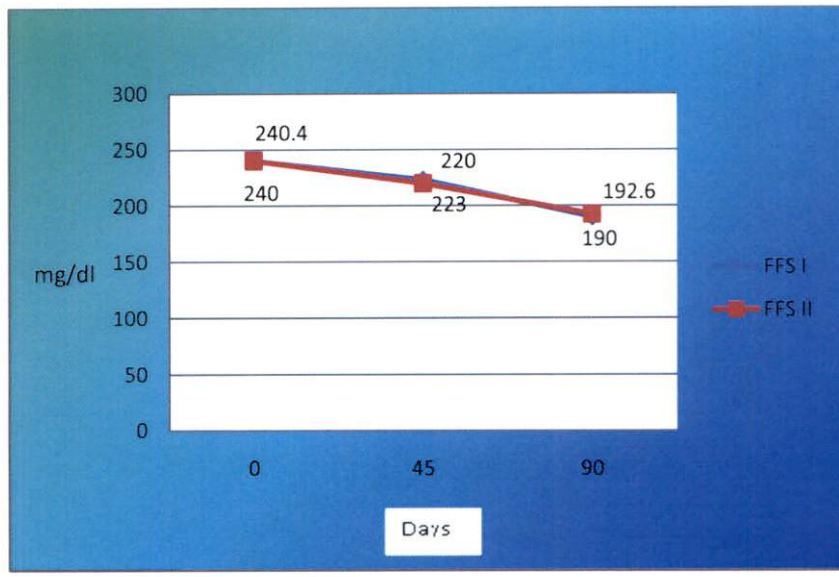


Fig: 27 Effect of supplementation of FFS I & II on mean Total Cholesterol of subjects

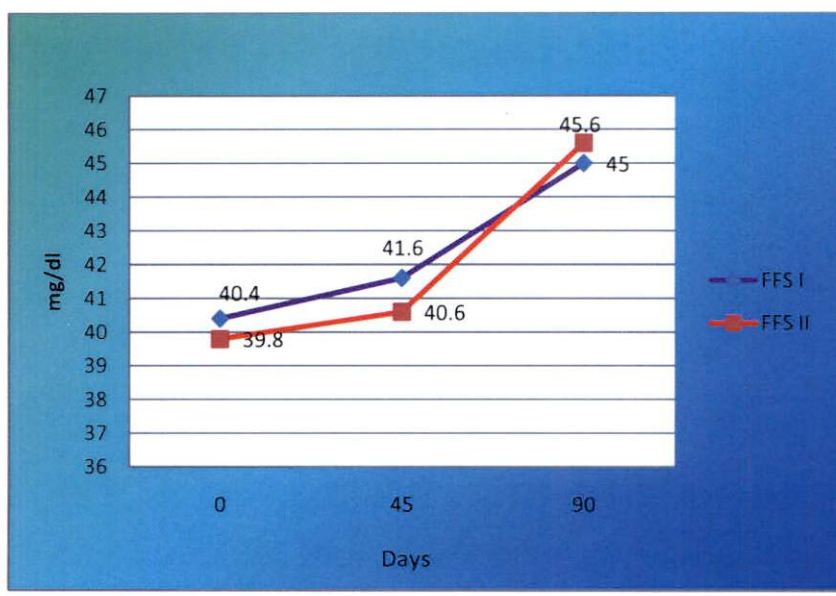


Fig: 28 Effect of supplementation of FFS I & II on mean HDL of subjects

5.6.3. Impact Evaluation of FFS I & II on Hypertensive Subjects

Blood parameters like systolic and diastolic blood pressure of the subjects were monitored before, in between and after the conduct of the study to understand the impact of supplementation of FFS I & II. A total of ten subjects with hypertension were supplemented with FFS I & II respectively.

Similar to that of blood glucose and blood lipids, there is significant variation at 1 per cent in the systolic and diastolic blood pressure levels of subjects supplemented with FFS I & II over a period of 90 days, showing differences over the period of days being evidently significant. On the other hand, it could also be noted that, there is no differences among the subjects of FFS I & II groups in the level of variation. When compared to the glucose and lipid parameters, though the changes in blood pressure were less, the reduction in systolic and diastolic pressure by the end of the study was significant.

On supplementation with FFS I & II to subjects with hypertension, the mean initial systolic BP levels which were 154 mm Hg and 151 mm Hg respectively steadily decreased to 127 mm Hg and 137 (137.4) mm Hg respectively by the end of the period of the study. While the mean initial and final diastolic blood pressure values of subjects supplemented with FFS I & II are 97 mm Hg and 85 mm Hg each respectively.

This concludes that both FFS I & II are equally effective in producing favorable results in the case of blood pressure levels of the subjects, similar to that of the study on blood glucose and lipid profiles of the subjects.

Contradicting to the studies conducted by Jaziya (2011), Resmi (2012) and Anju (2013), who had reported that their supplementation studies did not produce significant variation in the blood pressure levels of the subjects even after three months, FFS I & II could bring down the blood pressure levels to a remarkable level. It may be presumed that this might be due to the combined effects of the various ingredients in the supplements. However, the blood pressure levels of the

subjects supplemented with FFS I & II did not reduce to that of the normal levels were notable.

5.6.4. Glycemic Response of FFS I & II

The Glycemic Index of the food is affected by various factors. Less gelatinized the starch, slower the rate of digestion; the fibrous coat around beans acts as a physical barrier, slowing down the access of digestive enzymes; more amylose contained in food the less water the starch will absorb, the slower the rate of its digestion; the larger the particle size, the harder it is for water and enzymes to penetrate. Viscous, soluble fibers increase the viscosity of the intestinal contents, and slow the interaction between the starch and the enzymes (Alison *et al.*, 2014).

Urooj and Puttaraj (2000) studied the *in vivo* glycaemic responses to six cereal-based foods traditionally consumed in South India were evaluated in patients with non-insulin-dependent diabetes mellitus (NIDDM) and healthy volunteers. The postprandial responses to the foods at 30, 60 and 120 min were significantly lower than those to the reference glucose, in both groups. The peak glucose responses for three foods, i.e. chapatti, idli and poori, occurred 60 min postprandially in both groups. The glycaemic index (GI) values ranged from 67 to 90 in NIDDM and from 44 to 69 in healthy subjects with no significant differences within the groups. Significant relationships were observed between peak responses and area under the curve for foods in patients with NIDDM and *in vitro* rate of starch hydrolysis.

Thilakavathi and Muthuselvi (2010) studied that, all the millet (bajra, thenai, varagu and white oats) incorporated chappathis registered low glycemic index compared to standard. Also millet incorporated chappathis with fenugreek had low GI than those without it. The GI of the chappathis incorporated with thenai, bajra, varagu and white oats were 93.3, 90.5, 92.5 and 91.8 respectively.

In the present study, from the mean GTT values of the subjects, GI of FFS I & II was calculated. The GI values of FFS I & II are 48 and 52 respectively and they fall under the category of intermediate glycemic foods.

Macdonald (1999) studied that, of all the grains, barley has the lowest glycemic indexes. Pearled barley (GI = 36) and cracked barley (GI = 72) have lower glycemic indexes than sweet corn (GI = 78), rolled barley (GI = 94), and instant white rice (GI = 128). Barley is a low glycemic source of carbohydrates and a great source of fibre (1.5 per cent), both of which are advantageous in maintaining good glucose levels and weight control. Presence of barley in FFS I & II might be a contributing factor for their medium GI levels.

Indika *et al.* (2012) reported that, grape seed extract enhances postprandial plasma antioxidant status and reduces the glycemic response to a meal, high in fat and carbohydrate in subjects with the Metabolic Syndrome.

The Glucose Tolerance Test (GTT) of the subjects revealed that the maximum peak for glucose was at 70.6 min, for FFS I it was 65.4 min and for FFS II, it was 66.2 min. It can be noted that, when compared to glucose, subjects attained peak values much in advance while consuming FFS I & II. The postprandial responses to the foods at 30, 60 and 120 min were significantly lower than those to the reference glucose, in both groups. This suggests both FFS I & II be better supplements for subjects with diabetes. Also, the final values for both FFS I & II were much lower than glucose and were under the normal limits.

Glycemic load (G.L.) for one portion size (i.e. 20 gm) of FFS I & II supplemented to the subjects were 5.8 and 6.0 respectively. Glycemic load (G.L.) for three portion size (i.e. 60 gm) of FFS I & II which would be sufficient for a breakfast for the subjects were found to be 17.3 and 18.1 respectively. The glycemic load of the supplements falls under the category of low (GL less than 10) and medium (11 - 19) glycemic load foods respectively. This gives a

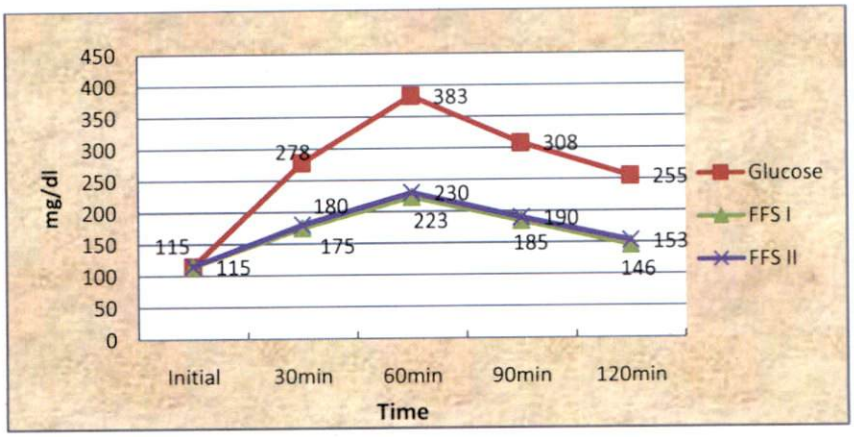


Fig: 29 Comparison between mean Glucose Tolerance Test (GTT) of FFS I, II & Glucose (Standard)

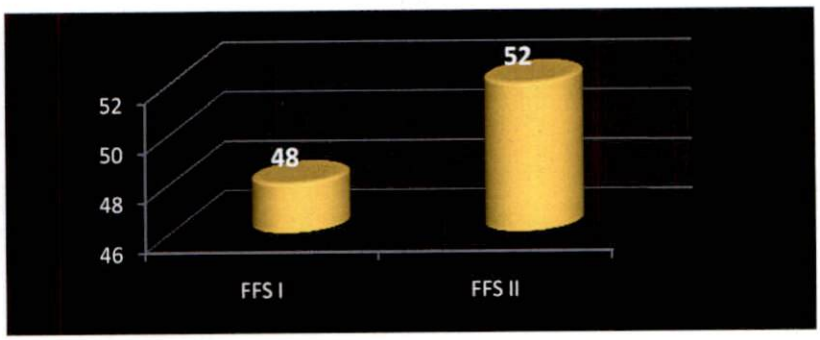


Fig: 30 Glycemic Index (GI) of FFS I & II

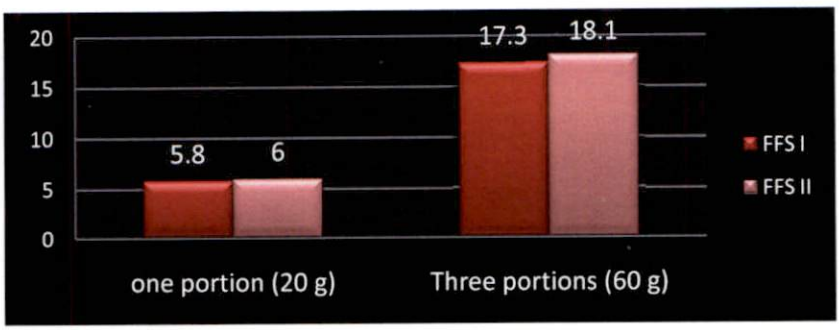


Fig: 31 Glycemic load of FFS I & II

repetitive confirmation that both FFS I & II prove a better supplement in the dietary management of subjects with lifestyle diseases.

To conclude, the several measures used to correct the imbalances of life style degenerative diseases, do not become healthy alternatives as besides being expensive, produce wide spectrum of adverse effects. There is a wide range of food stuffs that exerts promotive action for counteracting these adverse effects but at present is not used in our daily diet due to ignorance or oversight. Incorporating a food supplement along with medicines creates a more favorable option for the patients in the prevention and management of lifestyle diseases.

Developing a functional food supplement using available food items that are rich in bioactive compounds has the twin ability of nourishment and therapeutic action. A combination of natural food items serves a better purpose than a single ingredient. Putting together the essential ingredients in a nut shell package also covered the defect of planning a cumbersome menu which becomes practically inapplicable. This brings in the importance of FFS I & II, which has to find its way into the market for the benefits of the consumers. Other natural and indigenous food sources are to be examined for their therapeutic effects. Based on the availability in the premises, even single ingredients mentioned in the study can be consumed daily for improved health and disease prevention.

Summary

6. SUMMARY

Lifestyle-related diseases are engulfing the hearth of our population in an enormous pace. Development of the methods to prevent the lifestyle-related diseases is very important. Lowering the incidence of these diseases through the use of various foods containing natural bioactive resources are valuable in this context. Functional foods have been explored as a tool for management of lifestyle diseases. Multi-functional food factors that are valuable in controlling various lifestyle-related diseases were judiciously selected for the study.

The present investigation entitled "**Development, quality assessment and clinical efficacy of 'Functional Food Supplement' (FFS) for life style disease management**" was carried out for developing a functional food mix using available food items that are rich in bioactive compounds which will have the twin ability of nourishment and therapeutic action. A combination of food ingredients will serve a better purpose than a single ingredient. Putting together the essential ingredients in a nut shell package will also cover the defect of planning a cumbersome menu which becomes practically inapplicable.

The objective of the study was to develop a functional food mix from natural resources as an attempt to accomplish desirable therapeutic outcomes with reduced side effects and to validate the health benefits of the developed functional food supplement among human subjects in order to promote and to commercialize the product. The experiment was carried out in the Department of Home Science, College of Agriculture, Vellayani, Thiruvananthapuram during the period of 2011-2013. Major findings of the study are summarized below.

Focusing on the main objective of the study, the constituents for the FFS contained barley, ragi and banana, defatted soy flour, drumstick leaves powder and mushroom powder in different proportions. Dehydration and fermentation were the two processing techniques applied to standardize FFS. Proportions were optimized based on their nutritional and health promoting properties.

Among the various combinations worked out, different levels of screening was done based on their nutritional qualities like low calories, low fat, adequate carbohydrates, sufficient protein, high fiber and adequate micronutrients suitable for the management of lifestyle diseases and also on their sensory qualities.

The four combinations (combination X, XI, XII and XIV) represented as FFS DT_1, DT_2, DT_3, and DT_4 respectively were selected for the dehydration technique of FFS were identified for further investigations on functional properties and sensory evaluation in order to select the best suitable combination.

In the case of fermentation technique, combination XIV was identified for further investigation. This combination was further subjected to four different fermentation techniques (natural fermented dough, natural fermented batter, yeast fermented dough and yeast fermented batter) and named as FFS FT_1, FT_2, FT_3, and FT_4 respectively.

The functional quality assessment of the four combinations of FFS I (dehydration technique) showed the bulk density ranged between 0.75 to 0.82 g/ cm^3 and the combination DT_3 and DT_4 are comparatively less than the other two but is on par with each other. Combination DT_1 (9.33 per cent) had high dispersibility followed by combination DT_3 (8.67 per cent), DT_2 (8.0 per cent) and DT_4 (7.33 per cent) respectively. The rehydration ratio showed that combination DT_1 (0.26) had better rehydration and combination DT_4 (0.23) was less rehydratable.

The gelatinization time varied from 0.48 to 1.26 min. The combination DT_4 took the least time to gelatinize (0.48 min) while combination DT_2 had the highest gelatinization time of 1.26 min. Also combination DT_4 had the lowest gelatinization temperature (85.3° C) while combination DT_2 had the highest gelatinization temperature (96° C). Processing loss of combination DT_3 (0.48) and DT_4 (0.52) were the lowest while greater processing loss was noted in

combination DT_1 (0.56). The yield ratio was highest for combination DT_3 (0.52) and DT_4 (0.52) while combination DT_1 had the lowest yield ratio of 0.44.

The sensory evaluation scores of the four combinations of FFS I powder showed that, the colour and appearance of four combinations of FFS powder developed did not exhibit any difference though they were in a score ranging between 3.5 (DT_3) to 4.3 (DT_4). The texture of FFS in powder form did not show any significant differences in scores. The scores were in between 3.2 for DT_1 and 3.7 for DT_4. Maximum score for taste was noticed in FFS DT_4 (4.0), followed by DT_2 (3.3), DT_1 (3.2) and DT_3 (3.0) and also the scores varied significantly at 5 per cent. The flavour of FFS did not differ in scores. DT_1 obtained the least scores of 2.8. DT_2 and DT_3 received a score of 3.2 each. Maximum score was for DT4 (3.7). The overall acceptability score found that combination DT_4 (4.0) was the most preferred, followed by combination DT_2 (3.8) and DT_1 (3.4) respectively. Combination DT_3 (3.2) was the least preferred.

There were no significant differences in colour and appearances, texture and flavour of the porridge. However, taste and overall acceptability showed variation at 1 per cent. DT_4 scored the maximum (4.0) while DT_1 scored the least (2.8) in the case of taste.

The overall acceptability score of FFS I porridge of different combinations showed that combination DT_4 (4.2) was the most preferred followed by combination DT_2 (3.6) and DT_1 (3.2) respectively. Combination DT_3 (3.1) was the least preferred. Combination DT_4 scored the highest hedonic scale rating (7.4) followed by combination DT_2 (6.7) and DT_3 (6.3). Combination DT_1 (6.1) was the least liked.

In the case of FFS II (fermentation technique), the functional attributes of the different treatments showed that, bulk density and dispersibility did not have any significant difference among the four treatments. Rehydration ratio was highest for the treatment FT_2 (0.28) followed by FT_3 (0.27) and FT_4 (0.27).

Treatment FT_2 had the least rehydration ratio (0.26) and there were significant differences among the values at 1per cent level.

The gelatinization time was highest (2.53 min) for FT_1 and lowest (1.16 min) for FT_4. On considering the gelatinization temperature, treatment FT_2 had the highest (94.0° C) while FT_3 (87.33° C) had the least and all the four treatments were significantly different from each other at 1 per cent level. Treatment FT_3 (0.49) had the highest processing loss followed by FT_2 (0.48) & FT_4 (0.48) and minimal processing loss was noted in treatment FT_2 which was also significantly different. On the other hand, treatment FT_1 (0.54) had the highest yield ratio and FT_2 (0.50) had the least value.

The sensory evaluation scores and hedonic rating of the FFS II powder and porridge showed there was no significant difference in the colour and appearance, texture, taste, flavour and overall acceptability among the four treatments of fermentation. The rank table which was used to select the best combination among the four treatments showed that, F T_4 with its functional property score of (14.5), sensory evaluation score of porridge of (10) and powder (9.0) was most acceptable.

The best identified combinations from each technique (I & II) were investigated indepth for the nutrient content, phytochemical properties, functional qualities, storage stability and clinical efficacy.

The nutrient analysis of the developed functional food supplements showed that, there reduction in the energy content of FFS II (378 kcal) from that of FFS I (384 kcal). The protein and fat contents were 21.4 g and 1.88 g for FFS I as against 16.5 g and 1.56 g respectively for FFS II. The variation in the fibre contents of FFS I (4.0 g) and II (3.33 g) were significant at 5 per cent level only. Though FFS II is found to contain higher amounts of β –glucans (1.68 g) compared to FFS I (1.60 g), statistically the difference was insignificant. The moisture content of both the FFS denoted that, they can be categorized under low moisture foods, since they contain only 10.95 per cent and 10.8 per cent of

moisture respectively. The carbohydrate content of FFS I was 60.5 g against 58 g of FFS II which was not significantly different.

The vitamin content analysis carried out in the present investigation showed that, FFS II on fermentation had produced a remarkable increase in the β-carotene (2910 µg), thiamine (1.63 mg), riboflavin (1.3 mg), niacin (2.68 mg) and folic acid (40.0 mg) levels. Whereas, the vitamin content of FFS I was β-carotene (1948 µg), thiamine (0.8 mg), riboflavin (0.77 mg), niacin (1.88 mg) and folic acid (29.56 mg). Changes in vitamin E (3.35 µg) of FFS I to that of FFS II (3.0 µg) were noted. On the other hand, FFS II had a comparatively lower Vitamin C (8.73 mg) content than FFS I (13.1 mg).

In the case of mineral composition, the mineral contents like iron, copper, zinc, potassium, sodium, calcium, magnesium, phosphorus and manganese of FFS I reduced from 9.2 mg/100g to 8.5 mg/100g, 7.6 mg/100g to 3.6 mg/100g, 8.64 mg/100g to 2.88 mg/100g, 497 mg/100g to 425.4 mg/100g, 498 mg/100g to 475 mg/100g, 472 mg/100g to 458 mg/100g, 467 mg/100g to 378 mg/100g, 141 mg/100g to 109 mg/100g and 2.38 mg/100g to 2.12 mg/100g respectively in FFS II. However, Selenium was found to be significantly higher in FFS II (0.73 µg) than FFS I (0.67 µg).

The results of phytochemical analysis proved that, there was reduction in flavonoids from 4.6 per cent (FFS I) to 1.23 per cent (FFS II) was highly significant. T he alkaloid content of FFS I was (0.8 per cent) against 0.2 per cent for FFS II. The polyphenol content of FFS I was 73.25 mg whereas fermentation reduced its content to 48.25 mg in FFS II. The tannin contents of FFS I and II were 10.09 mg and 5.77 mg respectively. The oxalate contents of FFS I and II were 5.32mg and 1.92 mg respectively.

Amino acid profiling of FFS I & II depicted that, Glutamic acid (132.9 nmoles/ml) is the highest amino acid content in FFS I followed by arginine 97.1 nmoles/ml, Glycine (88.5 nmoles/ml), Leucine (52.9 nmoles/ml), Serine (51.6

nmoles/ml) etc. On the other hand FFS II elicited high amounts of Glycine (107.6 nmoles/ml), followed by Arginine (96.3 nmoles/ml), Glutamic acid (78.3 nmoles/ml), Serine (69.1 nmoles/ml), Leucine (60.3 nmoles/ml) etc. Histidine is the most limiting amino acid in both FFS I (16.0 nmoles/ml) and II (3.9 nmoles/ml).

The Total Essential Amino acid (TEAA) content of FFS I & II were, 291 nmoles/ml and 248.5 nmoles/ml. Whereas, the Total Non Essential Amino acid (TNEAA) content of FFS I & II were, 467.6 nmoles/ml and 414.4 nmoles/ml respectively. In FFS I Isoleucine (109) is the most limiting amino acid, whereas in FFS II it is Histidine (40). Phenylalanine is the most abundant amino acid in both FFS I (206) & II (102). FFS I follows an amino acid sequence of Iso leucine, Valine, Methionine while in FFS II it is Histidine, Iso leucine, Valine. FFS I have a higher EAA index and Nutritional index % of 42.57 and 9.12 respectively when compared to FFS II (31.26) and 5.16 respectively.

The salient findings of the quality assessment of FFS I & II based on the total antioxidant activity showed that, FFS I (1.09 µg / g) had the highest total antioxidant activity when aqueous ethanol was used. For FFS II (0.83 µg / g) the maximum antioxidant activity was derived while using absolute ethanol. Results indicated that the total antioxidant activity of FFS I was 0.811 µg / g and that of FFS II was 0.607 µg / g. However it could be noted that there is not much variation in the activity levels of aqueous ethanol (0.82 µg /g) and absolute ethanol in the case of FFS II. In both FFS I (0.57 µg / g) & II (0.41 µg /g), hot water extraction produced the least antioxidant activity.

Similarly DPPH scavenging activity of FFS I & II proved that they had higher levels inhibitory effects. Though there was significant variation between the IC 50 values of FFS I (585.35) & II (639) and also in comparison with standard ascorbic acid (477.86), their potency were found to be higher. Also, regression analysis showed that, the expected values were in line with the observed values in all the three cases.

The selection indices showed that among the developed functional food supplements, FFS I was found to be the best in terms of the nutritional properties. Though the selection index of vitamins scored higher for FFS II, in terms of all the other characters like macronutrients, fibre + β –glucans, phytochemicals, minerals, EAA Index, Total Antioxidant Capacity and DPPH free radical scavenging activity, FFS I was found to obtain the highest scores.

On assessing the functional properties and physico – chemical properties it depicted that, the functional characters like bulk density (0.90 g/cm^3), rehydration ratio (0.27), gelatinization time (1.16 min) and temperature (88.25^0 C), Water Absorption Capacity (WAC) (1.59) and Water Solubility Index (0.14) significantly increased in the fermentation technique of FFS II on comparison with FFS I, which had the following values like bulk density (0.75 g/cm^3), rehydration ratio (0.23), gelatinization time (0.48 min) and temperature (85.25^0 C), Water Absorption Capacity (WAC) (1.44) and Water Solubility Index (0.13). On the other hand, dispersibility (2.75 per cent), swelling capacity (6.66) and Water Absorption Index, WAI (2.65) decreased significantly in FFS II when compared to FFS I values like dispersibility (7.25 per cent), swelling capacity (7.04) and Water Absorption Index, WAI (2.68). However, there were no differences noted in the processing loss and yield ratio of the two FFS.

With regard to pasting properties, for FFS I, the pasting temperature (84.45^0 C) and pasting point (59.25 cP) were notable. However, these properties could not be detected for FFS II. The peak viscosity which is the ability of starch to swell freely before their physical breakdown ranged between 1190.75 for FFS I and 223.75 for FFS II. FFS II had the lowest (4.0 RVU) breakdown viscosity when compared to FFS I (206.75 RVU). The final viscosity, which is the change in the viscosity after holding cooked starch, ranged between 1958.75 for FFS I and 453.75 RVU for FFS II. The setback value of the FFS I & II were 975 and 234 RVU, respectively.

Texture Profile Analysis showed very low values for FFS II which could not be detected in hardness, cohesiveness, elasticity, gumminess etc., of the fermented flour paste. FFS I scored 9.06 on the hardness scale. FFS I showed a cohesiveness value of 0.499. Adhesiveness value of FFS I was (- 37.515). The springiness and gumminess values of FFS I is 5.4 and 4.79 respectively. The chewiness range of FFS I showed 25.86.

The colour attributes when analyzed portrayed that, the mean L* value ranged between 73.72 for FFS I and 64.77 for FFS II which had the lowest L* value. a* values of FFS I (1.71) were lower than those of FFS II (3.50). However, as a result of the browning reactions during drying, FFS II (22.0) exhibited higher b* values than FFS I (15.33). A low value for chroma and a high value for lightness are desired for the product to meet the consumer preference.

Comparing the particle size, for FFS I, the largest particle was in the size of 534.7 in comparison to FFS II which had a particle size of 560.6 r. nm. The particles of FFS I ranged between 400 to 650 r. nm while for FFS II it ranged between 400 to 800 r. nm. The maximum width of the particles of FFS I was 32.51 r. nm and 29.07 r. nm for FFS II.

From the storage studies of the developed supplements it was found that, for FFS I the moisture percentage increased from 10.83 per cent from the initial to 11.3 per cent and in FFS II the initial moisture percentages recorded were 10.68 per cent which gradually increased to 10.97 per cent in the end of the period of the study.

The peroxide content was not observed for both FFS I & II for the first four months of the study. FFS I reported peroxide contents in the 5^{th} month (0.22 meq/kg) and 6^{th} month (0.23 meq/kg). On the other hand, FFS II elucidated peroxide contents (0.21 meq/kg) only in the final (6^{th}) month of the storage studies. However these values were much minimal.

During the six months of storage period, no actinomycetes were found to be appeared in the developed products i.e. FFS I & II. But bacterial colonies were observed from the 5th month (1×10^{-1} cfu/g) and 6th month (2×10^{-2} cfu/g). Simultaneously, fungi were detected in the 6th month (1×10^{-1} cfu/g) of the storage studies. Even though bacteria and fungi were detected, it was present only in permissible limits. No pathogenic organisms could be detected in the FFS I. However, FFS II was found to be sterile and free of microbial contamination even after the 6th month of the storage studies. Even after six months of storage study, both FFS I & II had scored high scores for acceptability. This further emphasises the increased the scope of marketability of the FFSs in terms of sensory attributes also.

Though functional foods are found to sell at higher prices in the market, on assessing the cost of production of the developed functional supplements, the cost of one Kg of FFS I & II was found to be Rs. 200/-. While the cost of one portion size (20 g) of FFS I & II incurred a cost of Rs. 4.0/- only. On the health point of view, the rates of both FFS I & II are much less when compared to the proprietary products available in the. So both FFS I & II are better products and economically viable when scaled against health.

Suitability of incorporating the FFS (I and II) into various preparations/products was tried out and subject to organoleptic evaluation for the sensory characteristics like color and appearance, flavor, texture, taste and overall acceptability using a panel of trained members.

Commonly consumed breakfast items like Idli, Dosa, Chappathi, Noolputtu, Puttu and porridge were standardized in the laboratory by the incorporation of FFS I & II. The acceptability of the standardized recipes by the incorporation of FFS I & II were assessed by a panel of trained members using a score card on a five point scale.

There were no significance differences in color and appearance, flavor and overall acceptability of the recipes developed from FFS I. But in the aspects of texture and taste, there were significant variations among the recipes. Among the recipes, puttu (4.3) got the maximum score for taste, followed by chappathi (4.2), idli (4.0) and dosa (4.0) which were on par with each other, and then noolputtu (3.8) and porridge (3.5) scored the least. Similarly, among the recipes developed from FFS II, puttu (4.1) got the maximum score for taste, followed by chappathi (4.0), dosa (3.8), idli (3.7) and noolputtu (3.7) which were on par with each other, and then finally porridge (3.2) scored the least. From the above findings it could be concluded that, both FFS I & II were equally acceptable in whichever form of recipes might be supplemented in the breakfast of the consumers.

Portion sizing was fixed based on a pilot study done in the laboratory mainly focusing on the sensory acceptance on the level of incorporation of FFS I & II separately in different recipes. 20 gm of the FFSs (i.e. one part) were supplemented with two parts of the basic ingredients for recipe formulation. 20 gm of FFS I was found to contain, 76.8 Kcal of energy, 12 g of carbohydrates, 4.2 g of protein and 0.4 g of fat. While, 20 gm of FFS II was found to contain, 75.6 Kcal of energy, 11.6 g of carbohydrates, 3.4 g of protein and 0.32 g of fat.

Feasibility of substitution of the FFS (I & II) in the Food Exchange List, especially in the breakfast items was computed based on their nutrient contents. Keeping the above points in mind, FFS I & II could be used in the cereal, pulse and other exchange lists. T his would help to avoid monotony of dicts for the subjects with lifestyle diseases.

The clinical efficacy of the FFS (I & II) was ascertained through case studies. The study was conducted in the Elamkulam Panchayath, Malappuram district of Kerala. Clinical efficacy of FFS (I & II) was determined in the three disease conditions viz. Hyperglycemia, Hypertension and Hyperlipidemia which is highly prevalent in our state.

Five subjects each for three disease conditions and two different FFS developed were selected for the case studies. FFS I & II developed was supplemented in the breakfast of subjects for a period of three months. After the selection process, preliminary information regarding their socio-economic profile, health status, dietary and life style pattern and nutritional status were collected through a suitably structured questionnaire.

The details on the dietary habits of the subjects revealed that, all the respondents were non-vegetarians. Most of the subjects were having three main meals and one snack per day and had a diet plan of their own. Consumption of fruits was optional and also seasonal. Almost all the subjects consumed fish once a day, especially in the lunch. Even though the subjects with diabetes (pre-diabetes) consumed tea or coffee without sugar, their meals or snacks involved fried foods and foods of dense calories. Also intake of oil through snacks and other fried foods was high in most of the subjects. Food frequency of the subjects showed that, their eating pattern is varied and inconsistent. It also depicts a picture of unhealthy eating pattern, which might be the baseline reason for their morbidity conditions.

Only eight out of the thirty subjects had normal BMI, i.e. less than 25. All the other subjects falls under grade I obesity, since they had a BMI of 25 – 29.9. Also the waist – hip ratio denotes upper body obesity in most of the subjects. Even for the subjects with normal BMI, the waist – hip ratio was on the higher side, denoting the predominant distribution of fat in the upper part of the body.

Impact of the supplementation of FFS (I & II) on the subjects was monitored initially (before), intermittently (after 45 days) and finally (after) the conduct of the study. Clinical parameters like Fasting Blood Sugar, Post Prandial Blood Sugar, Glycemic Index, blood pressure and lipid profile, general health and morbidity of the subjects was monitored before, in between and after the conduct of the study.

The biochemical investigations proved that there was there was significant variation at 1 per cent level in the FBS levels of subjects supplemented with FFS I & II over a period of 90 days. The mean initial (Day 0) FBS values for the subjects supplemented with FFS I & II were 129 mg/dl and 131 mg/dl respectively. By the end of supplementation on day 90, the values significantly reduced to 104 mg/dl and 106 mg/dl for FFS I & II respectively.

But on the other hand, it could also be noted that, there is no differences among FFS I & II in the level of variation in FBS of the subjects. This concludes that both FFS I & II are equally effective in producing favorable results. It can also be noted that, in both the cases of FFS I & II, the final values matched the FBS values of normal subjects or those who are under control.

The investigations also found that there is significance variation at 1 per cent level in the PPBS levels of subjects supplemented with FFS I & II over a period of 90 days. The mean initial (Day 0) PPBS values for the subjects supplemented with FFS I & II were 204 mg/dl and 212 mg/dl respectively. By the end of supplementation on day 90, the values significantly reduced to 161 mg/dl and 174 mg/dl for FFS I & II respectively.

There was significant variation among the FFS I & II in reducing the PPBS of the subjects. On a whole, FFS I was found to be more effective in reducing the PPBS of the subjects when compared to FFS II. Even the rate of change (%) of both FBS & PPBS is concomitant to that of the values of the subjects over a period of 90 days.

From the present supplementation study, it was inferred that, significant variation at 1 per cent was observed in the total cholesterol, LDL, VLDL, TG and HDL levels of subjects supplemented with FFS I & II over a period of 90 days, showing differences over the period of days being evidently significant. Conversely, it could also be noted that, there is no differences between the

subjects of FFS I & II groups in the level of variation in the above mentioned lipid parameters.

The mean initial Total Cholesterol (TC) values of the subjects supplemented with FFS I & II were 240 mg/dl for each, which had reduced to 190 mg/dl and 193 mg/dl respectively. The mean initial LDL values of the subjects supplemented with FFS & II were 155 mg/dl and 157 mg/dl respectively. By the end of 90 days of the study, the values had decreased to 146 mg/dl and 148 mg/dl for FFS I & II respectively. It could be observed that, the mean initial VLDL values of the subjects supplemented with FFS & II were 40 (39.6) mg/dl and 42 mg/dl respectively. By the end of 90 days of the study, the values had decreased to 36 mg/dl and 38 (37.6) mg/dl for FFS I & II respectively. When subjects were supplemented with FFS I & II, the mean initial TG levels which were 182 (182.4) mg/dl and 182 mg/dl respectively, gradually declined to 168 (168.4) mg/dl and 170 (169.6) mg/dl respectively by the 90^{th} day.

On supplementation with FFS I & II to subjects with hypercholesterolemia, the mean initial HDL levels which were 40 (40.4) mg/dl and 40 (39.8) mg/dl respectively steadily increased to 45 mg/dl and 46 (45.6) mg/dl by the end of the study. These findings suggest that, both FFS I & II are equally good at producing favorable changes in the lipid profile of the subjects.

Similar to that of blood glucose and blood lipids, there is significant variation at 1 per cent in the systolic and diastolic blood pressure levels of subjects supplemented with FFS I & II over a period of 90 days, showing differences over the period of days being evidently significant. On the other hand, it could also be noted that, there is no differences among the subjects of FFS I & II groups in the level of variation. When compared to the glucose and lipid parameters, though the changes in blood pressure were less, the reduction in systolic and diastolic pressure by the end of the study was significant.

On supplementation with FFS I & II to subjects with hypertension, the mean initial systolic BP levels which were 154 mm Hg and 151 mm Hg respectively steadily decreased to 127 mm Hg and 137 (137.4) mm Hg respectively by the end of the period of the study. While the mean initial and final diastolic blood pressure values of subjects supplemented with FFS I & II are 97 mm Hg and 85 mm Hg each respectively. This concludes that both FFS I & II are equally effective in producing favorable results in the case of blood pressure levels of the subjects, similar to that of the study on blood glucose and lipid profiles of the subjects.

The GI values of FFS I & II are 48 and 52 respectively and they fall under the category of intermediate glycemic foods. The GTT of the subjects revealed that the maximum peak for glucose was at 70.6 min, for FFS I it was 65.4 min and for FFS II, it was 66.2 min. It can be noted that, when compared to glucose, subjects attained peak values much in advance while consuming FFS I & II. The postprandial responses to the foods at 30, 60 and 120 min were significantly lower than those to the reference glucose, in both groups. This suggests both FFS I & II be better supplements for subjects with diabetes. Also, the final values for both FFS I & II were much lower than glucose and were under the normal limits.

Glycemic load (G.L.) for one portion size (i.e. 20 gm) of FFS I & II supplemented to the subjects were 5.8 and 6.0 respectively. Glycemic load (G.L.) for three portion size (i.e. 60 gm) of FFS I & II which would be sufficient for a breakfast for the subjects were found to be 17.3 and 18.1 respectively. The glycemic load of the supplements falls under the category of low (GL less than 10) and medium (11 - 19) glycemic load foods respectively. This gives a repetitive confirmation that both FFS I & II prove a better supplement in the dietary management of subjects with lifestyle diseases.

All the above result confirms the importance of FFS I & II, which has to find its way into the market for the benefits of the consumers.

Findings of the present investigation strongly recommend that both FFS I & II developed proved to be efficient in the dietary management of the subjects with lifestyle diseases as the developed FFSs are proved to contain therapeutic and health promoting properties. The study recommends that natural food ingredients can be effectively utilized for the development of functional food supplements for the management of lifestyle diseases. With the virtue of the studied parameters, the FFSs could be promoted for commercialization.

Future Line of Work:

In vivo studies to prove the therapeutic role of the developed functional food supplements in managing the lifestyle diseases can be taken as a future prospective. Large scale studies to strengthen and validate the clinical role FFS has to be undertaken. Commercialization and market acceptability of the developed FFS has to be promoted for the benefit of subjects who are obsessed with food but are instead stuck to medicines for treatment and management of diseases.

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DEVELOPMENT, QUALITY ASSESSMENT AND CLINICAL EFFICACY OF 'FUNCTIONAL FOOD SUPPLEMENT' (FFS) FOR LIFE STYLE DISEASE MANAGEMENT

by

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(2011-24-101)

Abstract of the
thesis submitted in partial fulfillment of the
requirement for the degree of

DOCTORATE OF PHILOSOPHY IN HOME SCIENCE

(Food Science and Nutrition)

Faculty of Agriculture

Kerala Agriculture University, Thrissur

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2014

Abstract

ABSTRACT

With a global increase in the prevalence of lifestyle diseases, both nutrition and functional foods play key roles in its prevention and management. Functional foods from natural sources are cost effective, sustainable and reduce the risk factors. In this context, the present investigation entitled "Development, quality assessment and clinical efficacy of 'Functional Food Supplement' (FFS) for life style disease management" was conducted to develop a Functional Food Supplement (FFS) using locally available food ingredients that are not included in our daily diet due to ignorance or over sight but are rich in bioactive compounds with the twin ability of nourishment and therapeutic action.

The constituents selected for the FFS contain barley, ragi, banana, defatted soy flour, drumstick leaves and mushroom. Different proportions of the ingredients were worked out based on their nutritional qualities, amino acid scores, fibre content and other health promoting properties and sensory qualities. Dehydration and fermentation were the two processing techniques applied to standardize the FFSs.

From the twenty combinations worked out, after different levels of screening, four combinations were selected under the dehydration technique. In the fermentation technique, one combination (combination XIV) selected from the dehydration techniques subjected to four different treatments was identified for further investigation. Best suitable combination from each processing technique was identified based on functional properties and sensory qualities. Thus DT_4 (B: R: Bp: DSF: DLp: Mp = 3.5:2.0:1.5:2.5:0.25:0.25) from FFS I was selected for in-depth investigation. While in the case of FFS II yeast fermented batter (FT_4) was identified as the best combination. The identified FFS I & II were subjected to indepth investigations such as quality analysis based on nutrient content, functional properties, phytochemical content, storage stability and clinical efficacy on the lifestyle diseases.

Significant differences were found in the nutrient contents of FFS I & II. FFS I had higher energy content of 384 kcal, protein 21.4 g and fat 1.88g. Nutrient status of FFS II showed (378 kcal) of energy, 16.5 g of protein and 1.56 g of fat which were considerably lower than that of FFS I. Fibre and β – glucan content of FFS I were (4.0 g) and (1.60 g) respectively. Though FFS II is found to contain higher amounts of β–glucans (1.68 g) compared to FFS I, it had significantly lower fibre content of 3.33 g. The variation noted in the carbohydrate content of FFS I was 60.5 g against 58 g of FFS II which were statistically insignificant.

FFS II on fermentation had produced a remarkable increase in the β-carotene (2910 µg), thiamine (1.63 mg), riboflavin (1.3 mg), niacin (2.68 mg) and folic acid (40.0 mg) levels. However, vitamin E (3.35 µg) and Vitamin C (8.73 mg) content of FFS I was significantly higher than FFS II. FFS I had a significantly higher composition of all the macro (potassium, sodium, calcium, magnesium, and phosphorus), micro (iron, copper, zinc, manganese) and trace elements (selenium) compared to FFS II. The total antioxidant activity and DPPH free radical scavenging activity of both FFS I & II developed proved to be in favour of the disease management. Phytochemical contents of FFS I was significantly higher than FFS II. Amino acid profile depicted that, Glutamic acid (132.9 nmoles/ml) is the highest amino acid in FFS I while FFS II elicited high amounts of Glycine (107.6 nmoles/ml). The Total Essential Amino acid (TEAA) content, Essential Amino Acid index and Nutritional index per cent were higher in FFS I when compared to FFS II.

Assessment of functional qualities of the developed functional food supplements based on the parameters viz. pasting properties, textural properties, colour attributes and particle size showed that, both FFS I & II were equally acceptable and had higher market potentials. Moisture, peroxides and microbial contents were bare minimum during storage, promoting them for better marketability. The cost of one Kg of both FFS I & II was Rs. 200/- and the cost

of one portion size of the products was only Rs. 4.0/- indicating better economic viability when scaled up against health.

Efficacy of the developed FFS was tested in the selected subjects from Elamkulam panchayat of Malappuram district, with lifestyle diseases viz. hyperglycemia, hypercholesterolemia and hypertension. Impact of the supplementation (20 gm per day) of FFS (I & II) on selected subjects was closely monitored through clinical parameters like fasting and post prandial blood sugar, Glycemic Index, blood pressure and lipid profile and general health and morbidity. The findings proved that both FFS I & II were equally good in lowering the FBS, PPBS, Total Cholesterol, LDL, VLDL, Triglycerides and blood pressure levels of the subjects. Both FFS I & II also had a favourable role in enhancing HDL levels. Except, blood pressure all the other parameters of the subjects had come to normal values at the end of supplementation period. Glycemic Index values of FFS I & II and Glycemic load were low which further supports the therapeutic function of the products.

Findings of the present investigation strongly recommend that both FFS I & II developed proved to be efficient in the dietary management of the subjects with lifestyle diseases as the developed FFSs are proved to contain therapeutic and health promoting properties. The study recommends that natural food ingredients can be effectively utilized for the development of functional food supplements for the management of lifestyle diseases. With the virtue of the studied parameters, the FFSs could be promoted for commercialization.

In vivo studies to prove the therapeutic role of the developed functional food supplements in managing the lifestyle diseases can be taken as a future prospective. Large scale studies to strengthen and validate the clinical role FFS has to be undertaken. Commercialization and market acceptability of the developed FFS has to be promoted for the benefit of subjects who are obsessed with food but are instead stuck to medicines for treatment and management of diseases.

Appendices

APPENDIX – I

Score card for organoleptic qualities of recipes from FFS I & II

Criteria	Score						
		I	II	I	II	I	II
Colour & Appearance	**5**						
Excellent	**4**						
Very Good	**3**						
Good	**2**						
Fair	**1**						
Poor							
Texture							
Excellent	**5**						
Very Good	**4**						
Good	**3**						
Fair	**2**						
Poor	**1**						
Taste							
Excellent	**5**						
Very Good	**4**						
Good	**3**						
Fair	**2**						
Poor	**1**						
Flavour							
Excellent	**5**						
Very Good	**4**						
Good	**3**						
Fair	**2**						
Poor	**1**						
Overall acceptability	**5**						
Excellent	**4**						
Very Good	**3**						
Good	**2**						
Fair	**1**						
Poor							
Total							

Date: **Name:**

Signature:

APPENDIX II

QUESTIONNAIRE TO ELICIT INFORMATION ON DIETARY PATTERN, LIFESTYLE, HEALTH AND DISEASE OF THE SUBJECTS SELECTED FOR THE STUDY

I GENERAL INFORMATION:

Name: Age:
Sex: Male □ Female□
Religion: Occupation:
Family income (monthly): Incomes spend on food:
Number of members in the family:

II DIETARY HABITS:

Vegetarian □ Non-vegetarian □

Number of meals/day: Main meals:

Snacks: None □ 1 □ 2 □ 3 □

Do you have a diet plan of your own? Yes □ No □

MEAL PATTERN:

	ITEM	QUANTITY	INGREDIENTS	QUANTITY
Early Morning				
Break fast				
Mid morning				
Lunch				
Mid afternoon				
Evening				
Dinner				
Bed time				

FOOD FREQUENCY TABLE:

ITEM	Quantity (Gram)	Daily	Weekly twice/thrice	Weekly once	Fortnightly	Monthly	Occasionally
Whole Cereals							
Pulses							
Fruits							
Vegetables							
Green leafy vegetable							
Milk & milk products							
Chicken							
Mutton							
Beef							
Pork							
Fish							
Egg							
Fried foods							
Baked foods							
Processed foods							
Ghee/dalda							

Type of oil used for cooking: Coconut oil □ Vegetable oil □ Rice bran oil □ Palmolein □ Other (specify) □

Do you have food while watching TV? Yes □ No □ If yes, mostly □ sometimes □ rarely □

III HEALTH INFORMATIONS:

Disease	Tick (√/×)	Indicators	Current level	Duration (years/ months)	Medication (years/ months)	Family History Of Disease		
						Father	Mother	Others (specify)
Hypertension		Bp						
Diabetes		FBS						
		PPBS						
Cholesterol		Total						

Morbidity status of the subject: Frequency of occurrence of infections/any other ailments

Home remedies if any to control diabetes/cholesterol/blood pressure:

Smoking: Yes □ No □ Quantity/day:

Alcohol: Yes □ No □ Quantity/day:

Exercise: Daily □ Weekly twice/thrice □ Weekly once □ None □

Type of exercise:

Special foods taken to control disease conditions:

Assistance for house hold work:

IV ANTHROPOMETRIC MEASUREMENTS:

Height: **Weight:**

BMI: **Waist-Hip Ratio:**

V OTHER DETAILS:

1. Do you feel stressed at minute difficulties?

Yes □ No □ If yes, Mostly □ Sometimes □ Rarely □

2. Health checkups: None □ yearly once □ Occasionally □ On demand □

3. Blood profile monitoring: Regularly □ Occasionally □ On demand □ None □

4. Record your earnest opinion about dietary modifications:

APPENDIX III

Glucose Tolerance Test (GTT)

GTT is carried out after 12 hrs of overnight fasting. Glucose 75g in adults is orally administered. Before the glucose load and 15 minutes to 120 minutes after the administration of glucose blood samples were collected and glucose levels were estimated.

The diagnostic criteria for diabetes and impaired glucose tolerance, the fasting blood sugar levels vary between 80-110 mg/100ml. The blood sugar levels increase after the glucose load and come down to base level within 2 hrs.

WHO criteria with glucose load 75g for adult or 1.75g/Kg/body wt (To a maximum of 75g).

	Glucose concentration		
	Venous whole blood	**Capillary whole blood (mg/dl)**	**Venous plasma (mg/dl)**
	Normal	**Diabetes Mellitus**	**Confirmed**
Fasting	120	120	140
2 hours after glucose load	180	200	200
	Impaired glucose tolerance		
Fasting	120	120	140
2 hours after glucose load	120-180	140-200	140-200

APPENDIX IV

Dietary habits of the respondents

Sl no	Dietary habits	Number/Percent of respondents		
		Hyperglycaemic	Hypercholesterimic	Hypertensive
1	**Dietary habits**			
	Vegetarian	0 (0)	0 (0)	0 (0)
	Non vegetarian	10 (100)	10 (100)	10 (100)
	Total	10 (100)	10 (100)	10 (100)
2	**Number of meals / day**			
	Less than three	0 (0)	0 (0)	0 (0)
	Three	10 (100)	09 (90)	10 (100)
	More than three	0 (0)	01 (10)	0 (0)
	Total	10 (100)	10 (100)	10 (100)
3	**Number of snacks / day**			
	None	0 (0)	0 (0)	0 (0)
	One	06 (60)	08 (80)	07 (70)
	Two	04 (40)	02 (20)	03 (30)
	Three	0 (0)	0 (0)	0 (0)
	Total	10 (100)	10 (100)	10 (100)
4	**Own diet plan**			
	Yes	07 (70)	06 (60)	07 (70)
	No	03 (30)	04 (40)	03 (30)
	Total	10 (100)	10 (100)	10 (100)

5	**Watching television while having food**			
	Yes	04 (40)	08 (80)	06 (60)
	No	06 (60)	02 (20)	04 (40)
	Total	10 (100)	10 (100)	10 (100)
6	**Cooking oil**			
	Coconut oil	05 (50)	06 (60)	05 (50)
	Vegetable oil	01 (10)	02 (20)	01 (20)
	Rice bran oil	01 (10)	02 (20)	01 (30)
	Palmolein	0 (0)	0 (0)	0 (0)
	Coconut oil + vegetable oil	03 (30)	01 (10)	02 (20)
	Other	0 (0)	0 (0)	01 (10)
	Total	10 (100)	10 (100)	10 (100)

APPENDIX V

Food frequency table of the respondents

Food Item	Frequency					
	Daily	Weekly twice	Weekly once	Fortnightly	Monthly	Occasionally /rarelly
Whole Cereals	20 (66)*	5 (17)	5 (17)	-	-	-
Pulses	30 (100)	-	-	-	-	-
Fruits	10 (33)	10 (33)	05 (17)	05 (17)	-	
Vegetables	30 (100)	-	-	-	-	-
Green leafy vegetable	-	20(66)	05 (17)	05 (17)	-	-
Milk & milk products	30 (100)	-	-	-	-	-
Chicken	-	02 (06)	10 (33)	10 (33)	05 (17)	03 (10)
Mutton	-	-	-	-	05 (17)	25 (83)
Beef	-	-	-	4(13)	4(13)	4(13)
Pork	-	-	-	-	-	4(13)
Fish	14 (47)	12 (40)	4 (12)	-	-	-
Egg	-	12 (40)		18 (60)	-	-
Fried foods	30 (100)	-	-	-	-	-
Baked foods	30 (100)	-	-	-	-	-
Processed foods	30 (100)	-	-	-	-	-
Ghee/dalda	-	-	-	10 (33)	15 (50)	05 (17)

* Numbers in parenthesis indicates percentage of respondents

APPENDIX VI

Health Habits of the respondents

Health Habits	Number/Percent of subjects		
	Hyperglycaemic	Hypercholesterimic	Hypertensive
SMOKING			
Yes	0 (0)*	02 (20)	02 (20)
No	10 (100)	08 (80)	08 (80)
ALCOHOL			
Yes	0 (0)	02 (20)	03 (30)
No	10 (100)	08 (80)	07 (70)

* Numbers in parenthesis indicates percentage of respondents

Physical exercise of the respondents

Exercise	Number/Percent of subjects		
	Hyperglycaemic	Hypercholesterimic	Hypertensive
Daily	04 (40)*	06 (60)	04 (40)
Weekly twice/thrice	02 (20)	02 (20	50 (50)
Weekly once	02 (20)	01 (10)	01 (10)
None	02 (20)	01 (10)	0 (0)

* Numbers in parenthesis indicates percentage of respondents