DEVELOPMENT OF LOW FAT BISCUIT USING FAT REPLACERS

BY

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B. E. (Food Processing Technology)

POST GRADUATE INSTITUTE OF FOOD PROCESSING TECHNOLOGY AND BIO-ENERGY
ANAND AGRICULTURAL UNIVERSITY
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A
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ABSTRACT

DEVELOPMENT OF LOW FAT BISCUIT USING FAT REPLACERS

Name of student **Major Advisor**

Padhiar Hitendrasinh Natvarsinh **Dr. K. B. Kamaliya**

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Biscuits were assumed as sick-man's diet in earlier days. Now, it has become one of the most loved fast food products for every age group. Biscuits are easy to carry, tasty to eat and reasonable at cost. Soft dough biscuits normally contain high amounts of fat ranging from 15 to 25%. Fats are the third largest components after flour and sugar in the bakery products. Fat is still the number one nutritional concern for the most people because the continuous consumption of fat may lead to certain major health disorders such as cardiovascular diseases, obesity, hypertension, some type of cancer, high blood cholesterol etc. To minimize such diseases mainly caused due to excess fat, it is necessary to develop low fat bakery products which would also be useful to maintain the well being of the masses. To reduce the fat content, different types of fat replacers can be used for the production of low fat bakery products. Thus, the present investigation was undertaken to develop low fat biscuits.

In the study fat (Shortening) was replaced with different levels of maltodextrin and whey protein concentrate (WPC) i.e. 40 to 70% (in multiple of 10%) of fat content. Five fat replacement ratios (i.e., 100:0, 75:25, 50:50, 25:75 and 0:100::MD:WPC) and control were taken within each fat replacement level. Three replications of biscuits were carried out for the analysis. The sensory attributes, physical and rheological properties of control and the developed biscuits were studied. The biscuit found sensorily the most acceptable was further analyzed for nutritional composition and

storage study. Sensory and rheological analysis of the selected experimental biscuit as compared to control were carried out during storage study. The cost of both types of biscuits was calculated for industrial application.

Sensory evaluation of biscuits indicate significant difference between crust colour and surface character, crumb colour and taste and aroma at all fat replacement levels. Among all the experimental biscuits, 50:50 fat replacement ratio at 70% fat replacement level scored the highest for the most of all sensory attributes. Therefore, this biscuit was considered for further study.

The results obtained for physical characteristics indicate that, increase in WPC produced biscuits with higher thickness as compared to control, while increase in MD content resulted in increased width, spread ratio and spread factor. These results indicate that effect of both replacers opposite to each other.

Rheological study of the biscuits indicate that all experimental biscuits were significantly differ to control for both characteristics at all replacement ratios while no significant difference was observed among themselves at any fat replacement level. High level of fat replacement increased both hardness and fracturability significantly inspite of addition of fat replacers. Experimental biscuit with 50:50 ratio scored lower among all which was second important result.

Nutritional analysis indicates that selected biscuit is low fat biscuit since it contains 4.19±0.10 % fat which meets the standard for low fat food. The fat content was reduced by 68% while protein content was increased by 26.76%. Calculated energy for the developed low fat biscuit was 401.43 Kcal/100 g which was more than required for the low calorie food as per the definition.

Shelf life study of the biscuits reflected that, there was no significant difference between selected experimental and control biscuit at all the weeks. That means both samples are acceptable after one month storage period. Thus, the developed low fat biscuit could be beneficial to replace the normal biscuit for the persons suffering from various cardio vascular diseases mainly caused due to excess fat and to maintain normal health for healthy individual.

The cost of selected biscuit was found 83.03 Rs./Kg higher but, could be sold at premium price because of health benefits. Such biscuit produced by large scale industry could be marketed as a functional food. Such other health beneficial bakery products could be developed for the well being of the community.

Key words: Low fat biscuit, Fat replacer, Maltodextrin, Whey protein concentrate, Shelf life

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CERTIFICATE – I

Dated: / / 2010

This is to certify that **PADHIAR HITENDRASINH NATVARSINH** (Reg.: 04-0682-2007) has successfully completed the comprehensive examination of Major and Minor subject held on **25 / 02 / 2009** as required under the regulation for **M. Tech** in **Food Processing Technology.**

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CERTIFICATE – II

Dated: / / 2010

This is to certify that the thesis entitled **"Development of low fat biscuit using fat replacers"** submitted for the degree of **Master of Technology** in the subject of **Food Processing Technology** embodies bonafide research work carried out by **Padhiar Hitendrasinh Natvarsinh** (Reg. No.: 04-0682-2007) under my guidance and supervision and that no part of this thesis or research work has been submitted for any other degree. The assistance, guidance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by advisory Committee on /05/2010.

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CERTIFICATE – III

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This is to certify that the thesis entitled "**Development of low fat biscuit using fat replacers**" submitted by **Padhiar Hitendrasinh Natvarsinh** (Reg.: 04-0682-2007) to the Anand Agricultural University, Anand in partial fulfillment of the requirement for the degree of **Master of Technology** in **Food Processing Technology** in the subject of **Food Processing Technology** after suggestion and recommendation by the external examiner was discussed and defended by the candidate before the following members of the Advisory Committee. The performance of the candidate in the oral examination on this thesis has been found satisfactory; we therefore, recommend that the thesis may be approved.

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CERTIFICATE – IV

Dated: /05/2010

This is to certify that **PADHIAR HITENDRASINH NARVARSINH** (Reg.: 04-0682-2007), **P. G. Institute of Food Processing Technology & Bio-Energy, Anand** has made all correction / modifications in the thesis entitled, **"Development of low fat biscuit using fat replacers"** which were suggested by the External Examiner and the Advisory Committee in the oral examination held on 03/05/2010. The final copies of the thesis duly bound and corrected were submitted on /05/2010 are enclosed herewith for approval.

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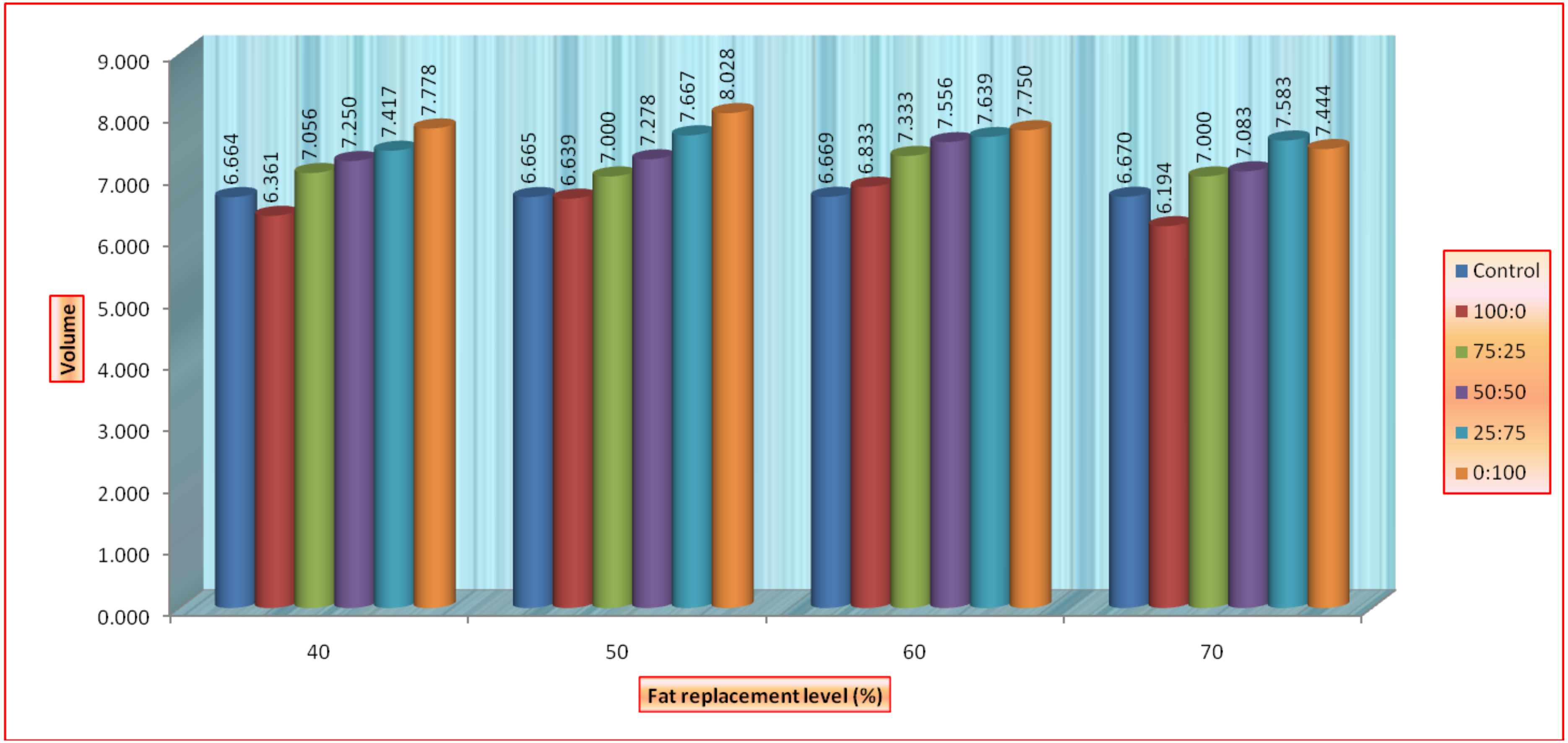

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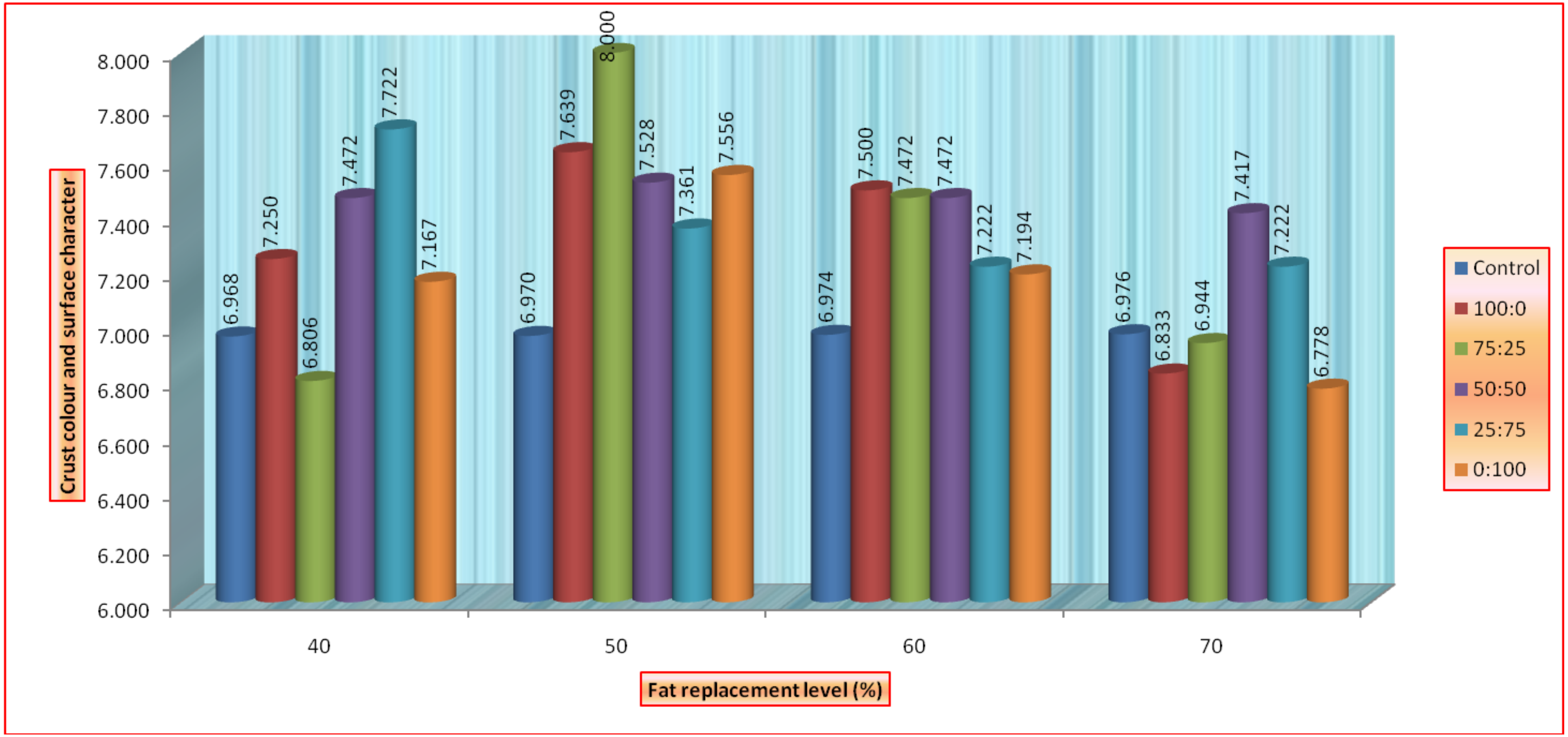

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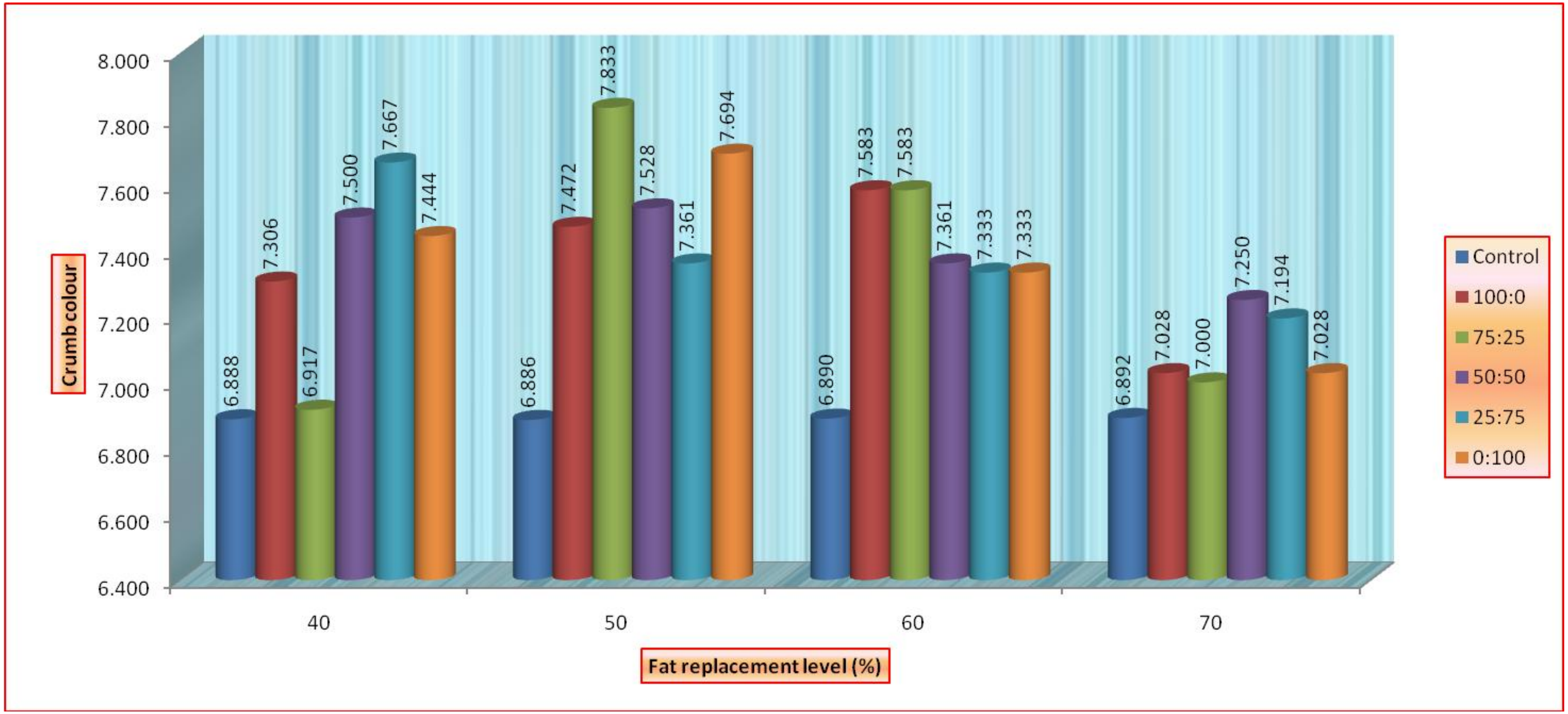

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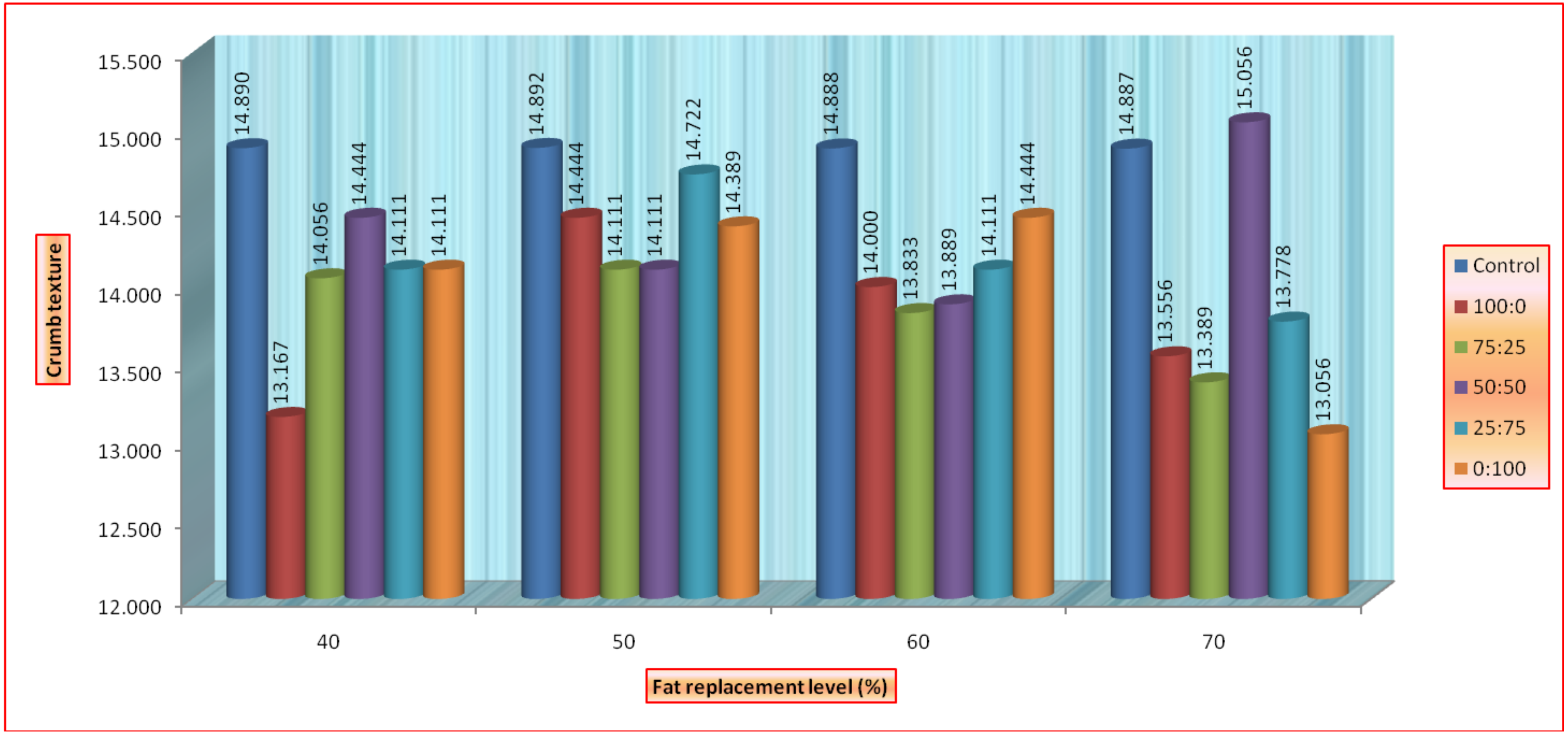

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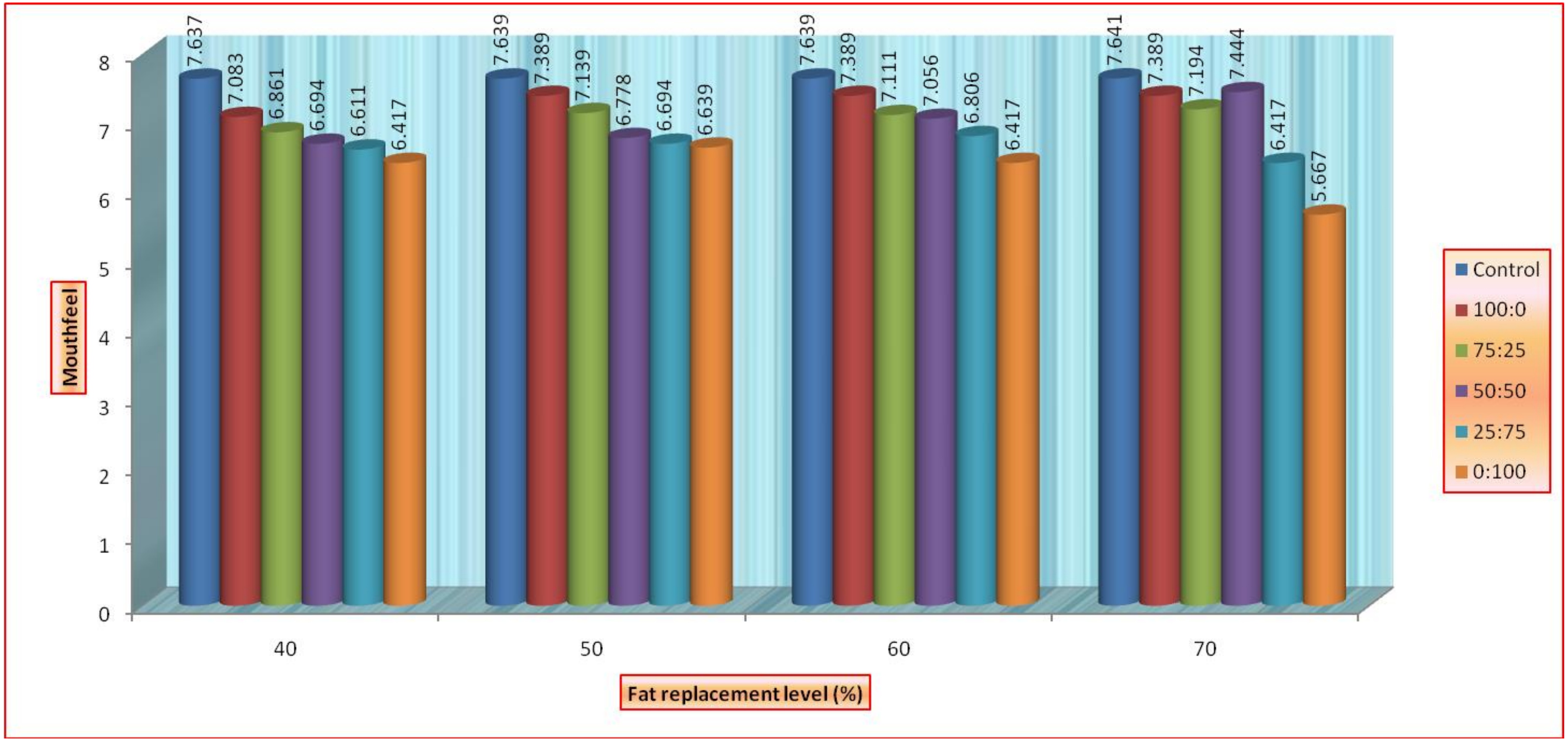

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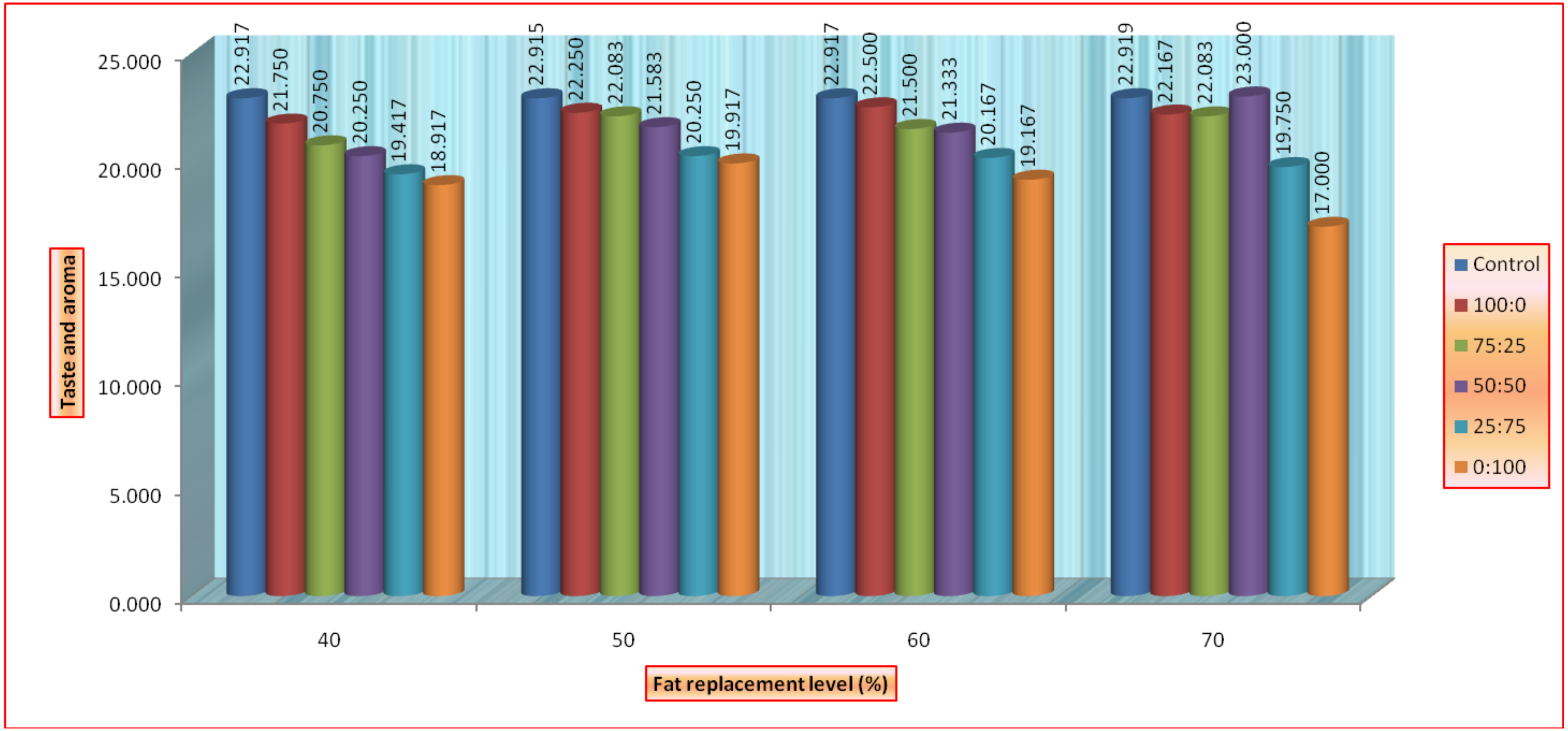

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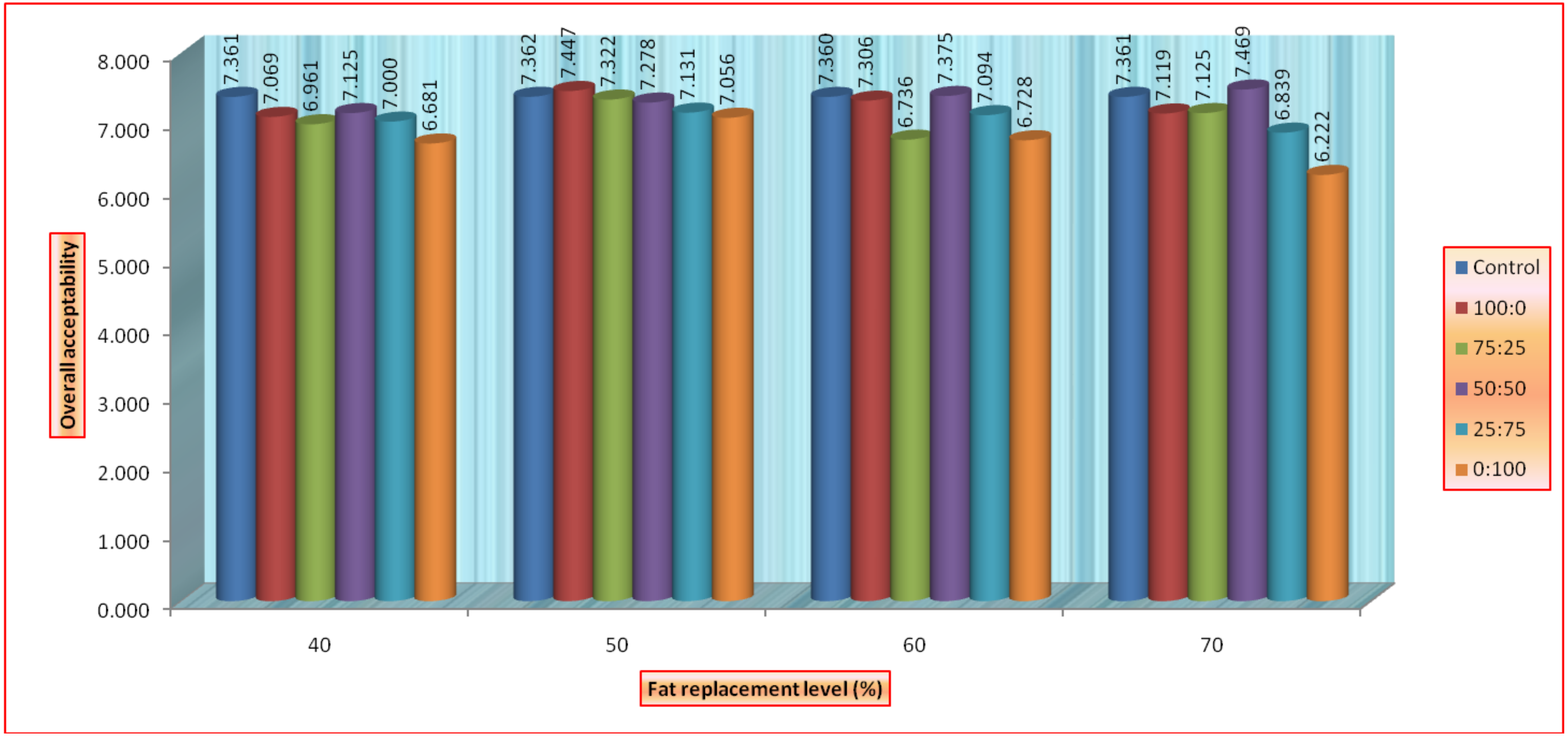

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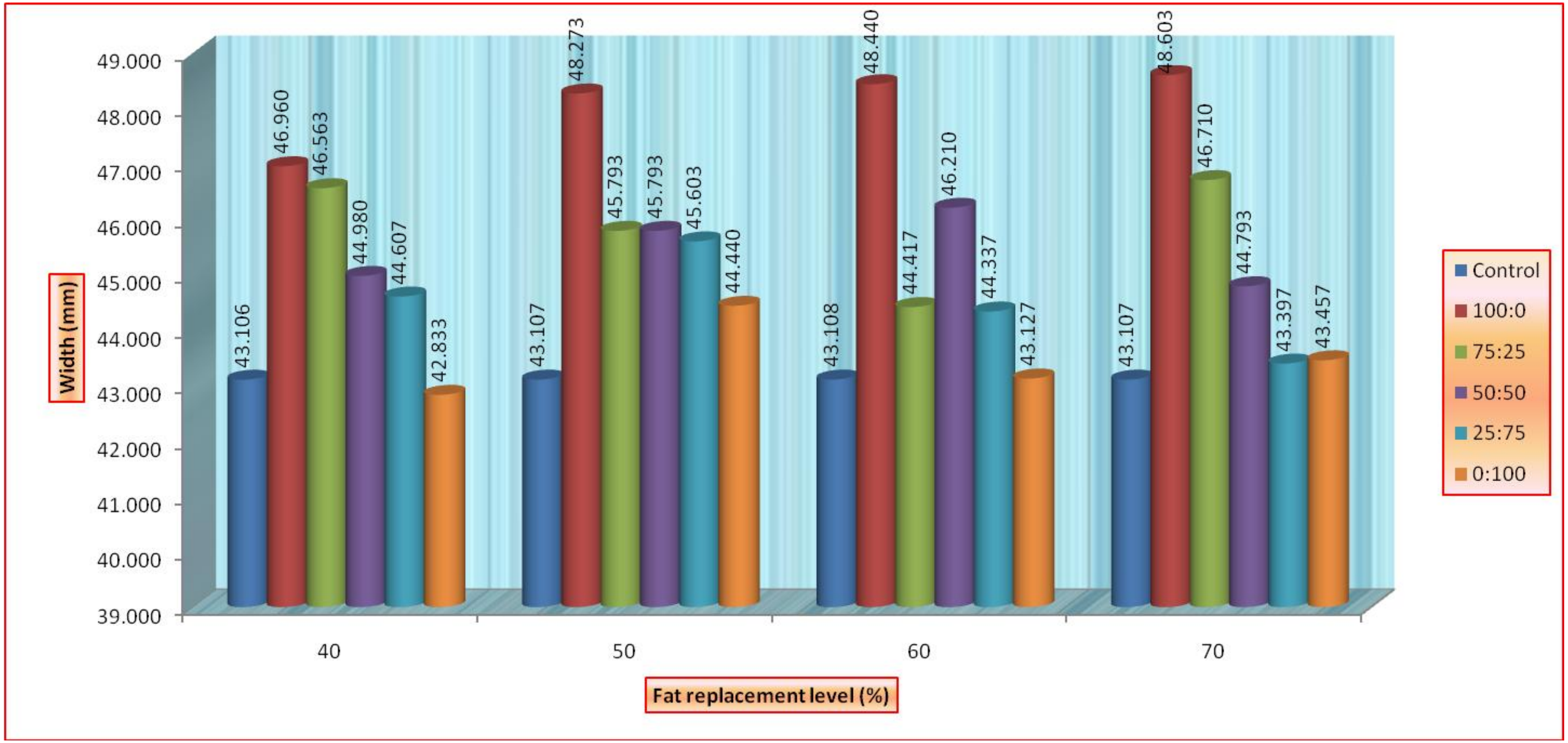

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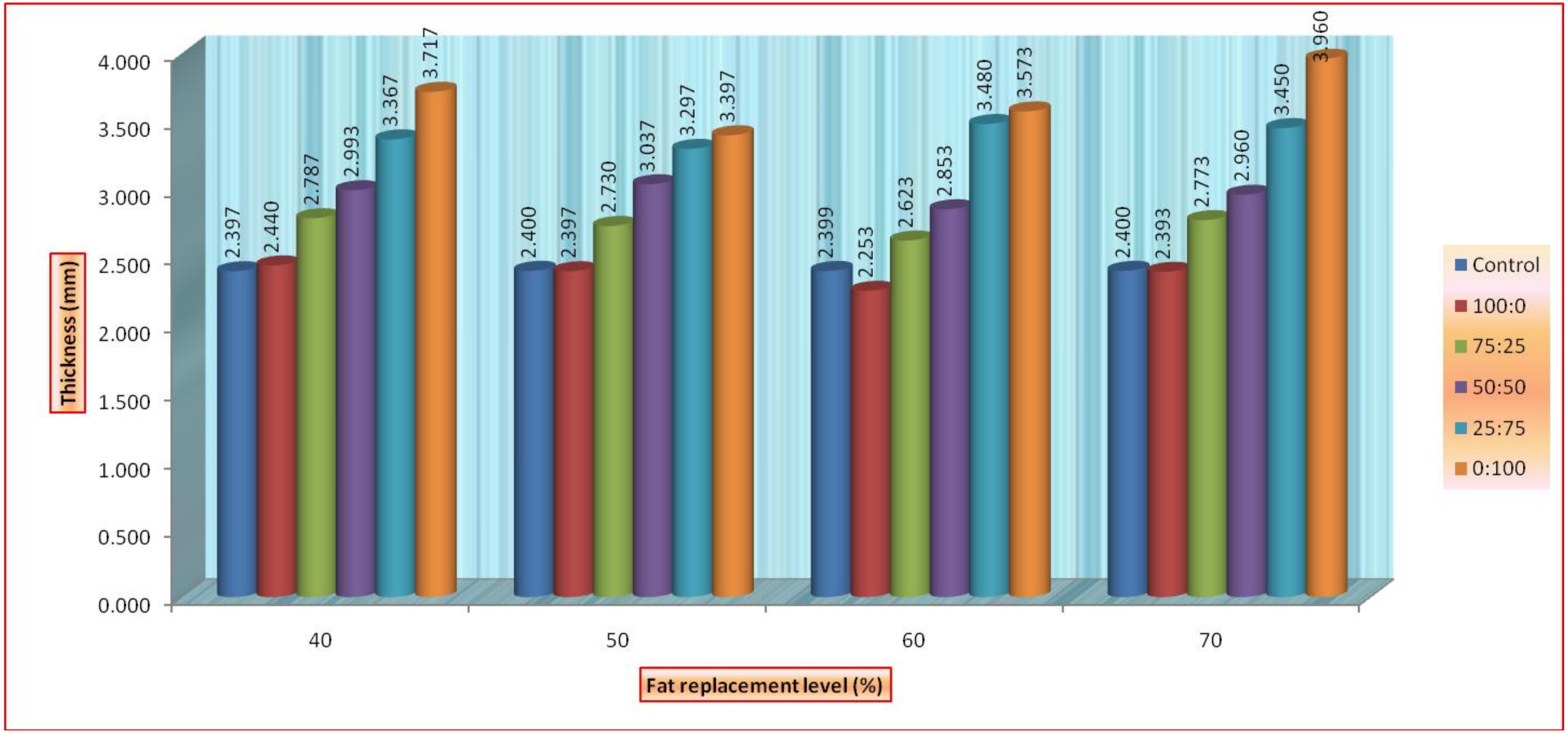

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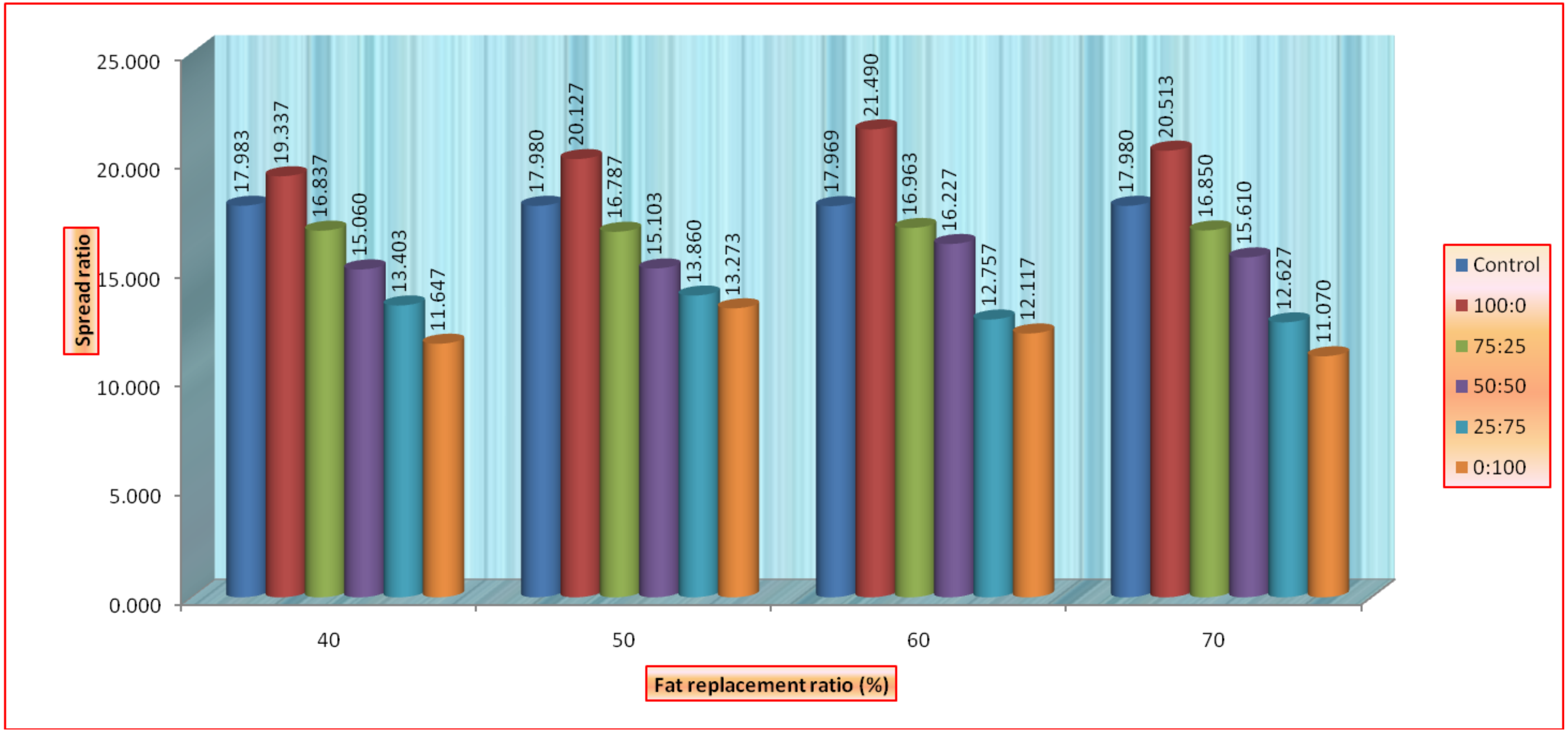

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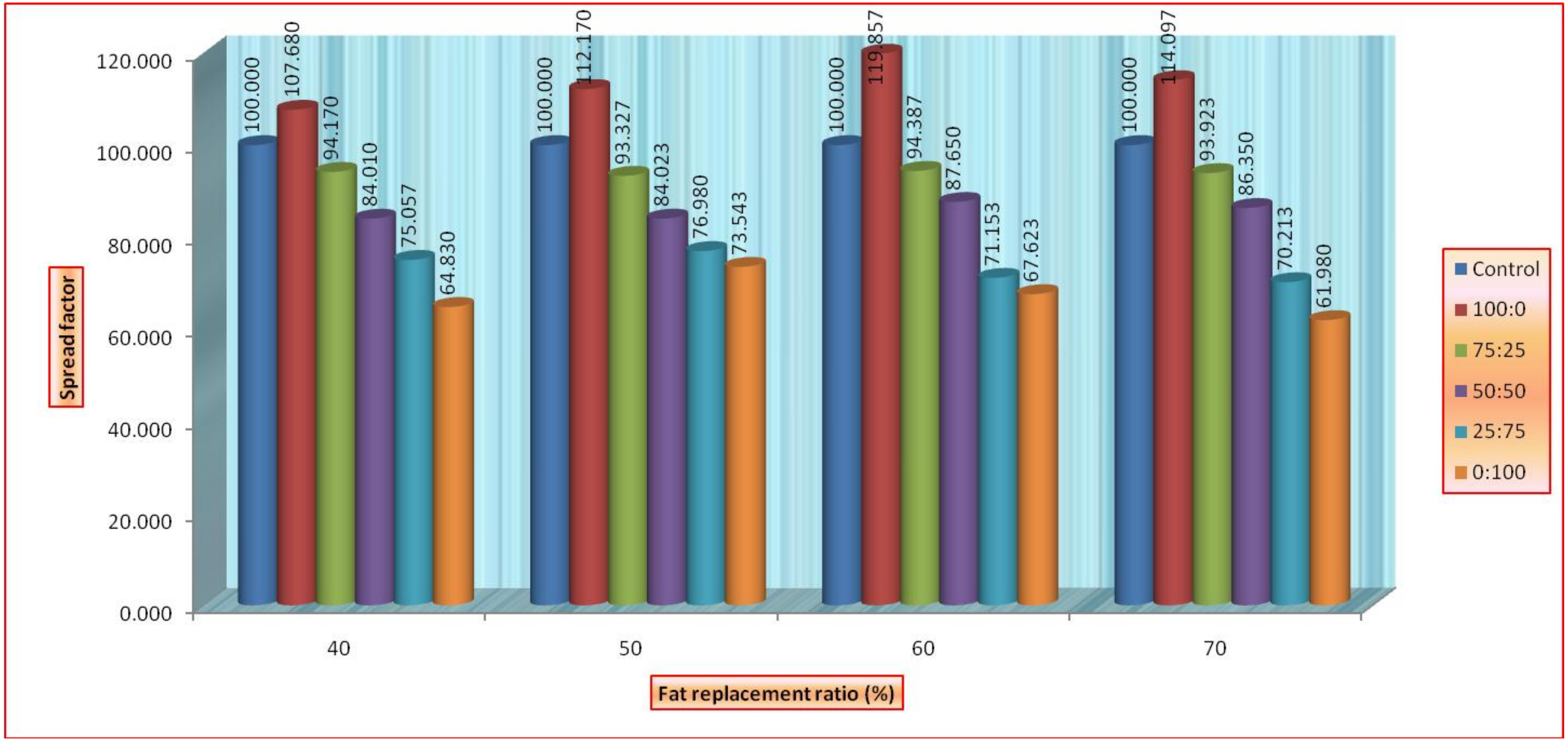

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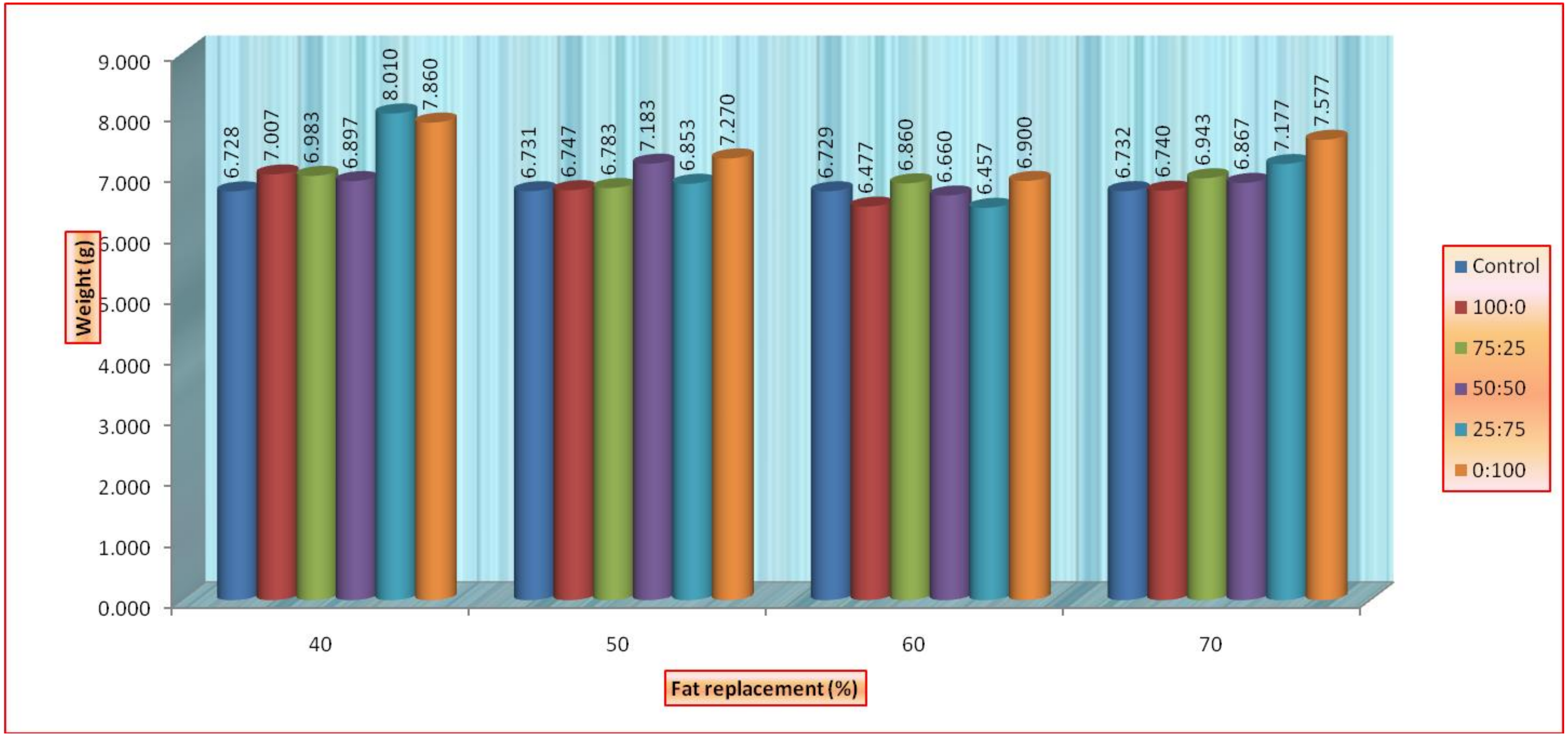

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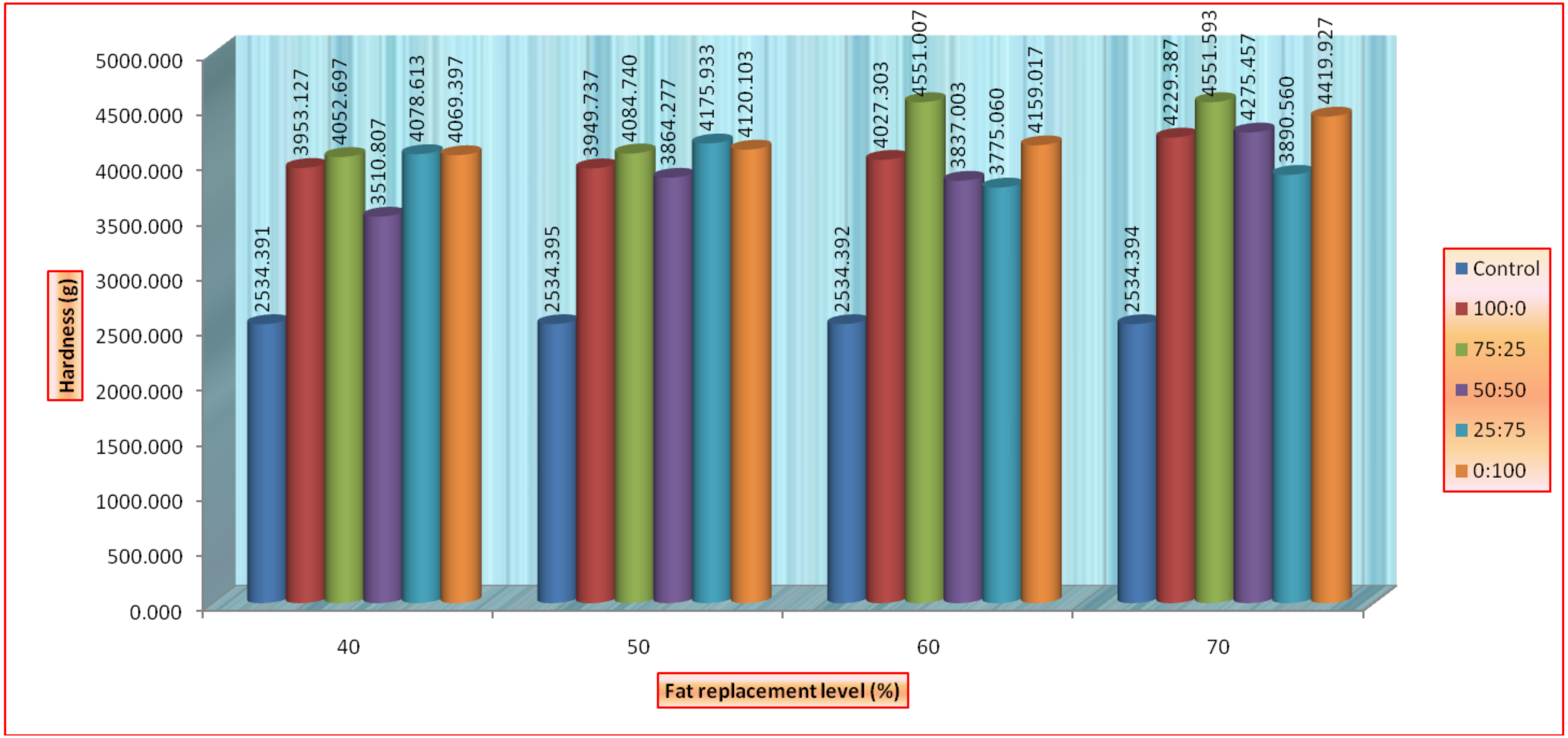

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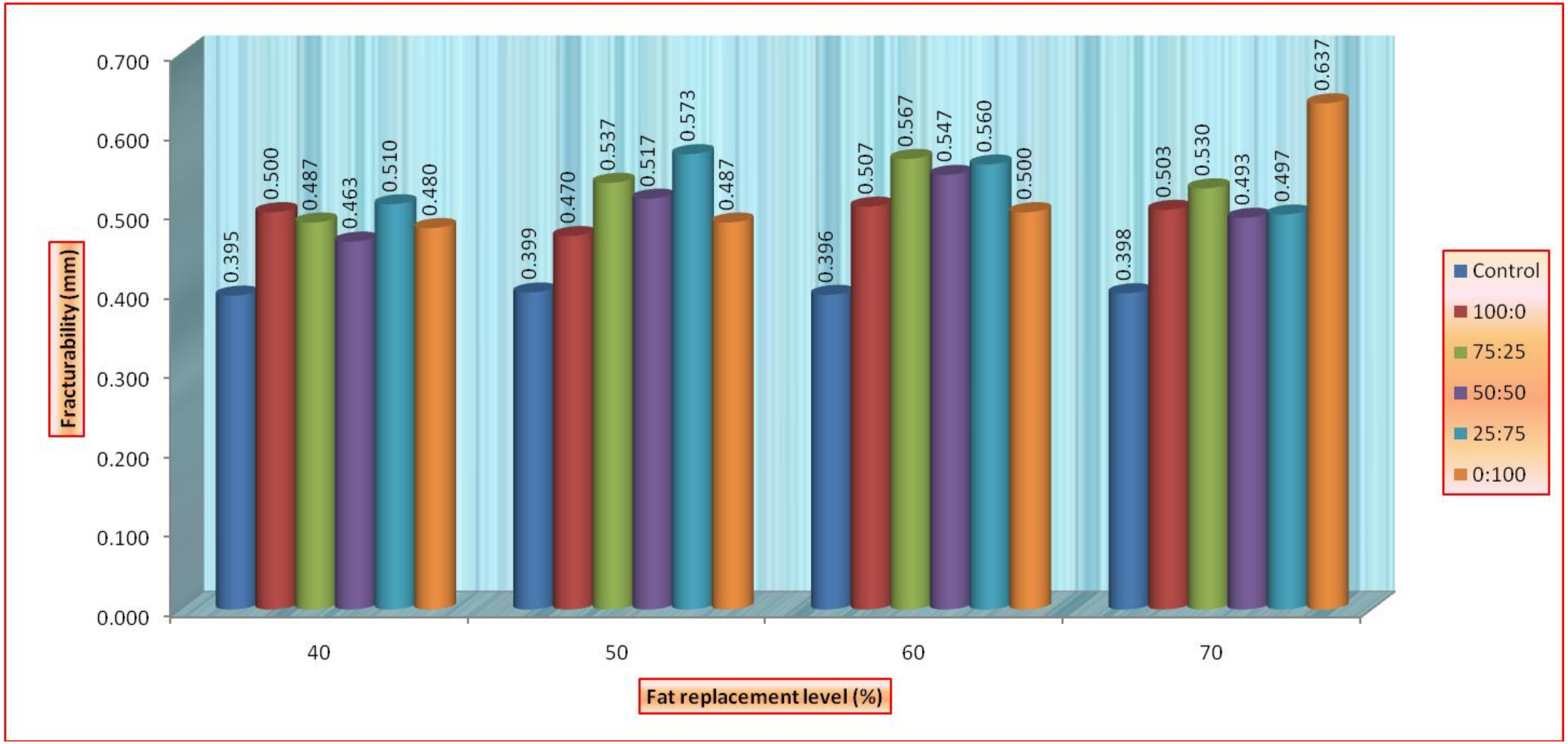

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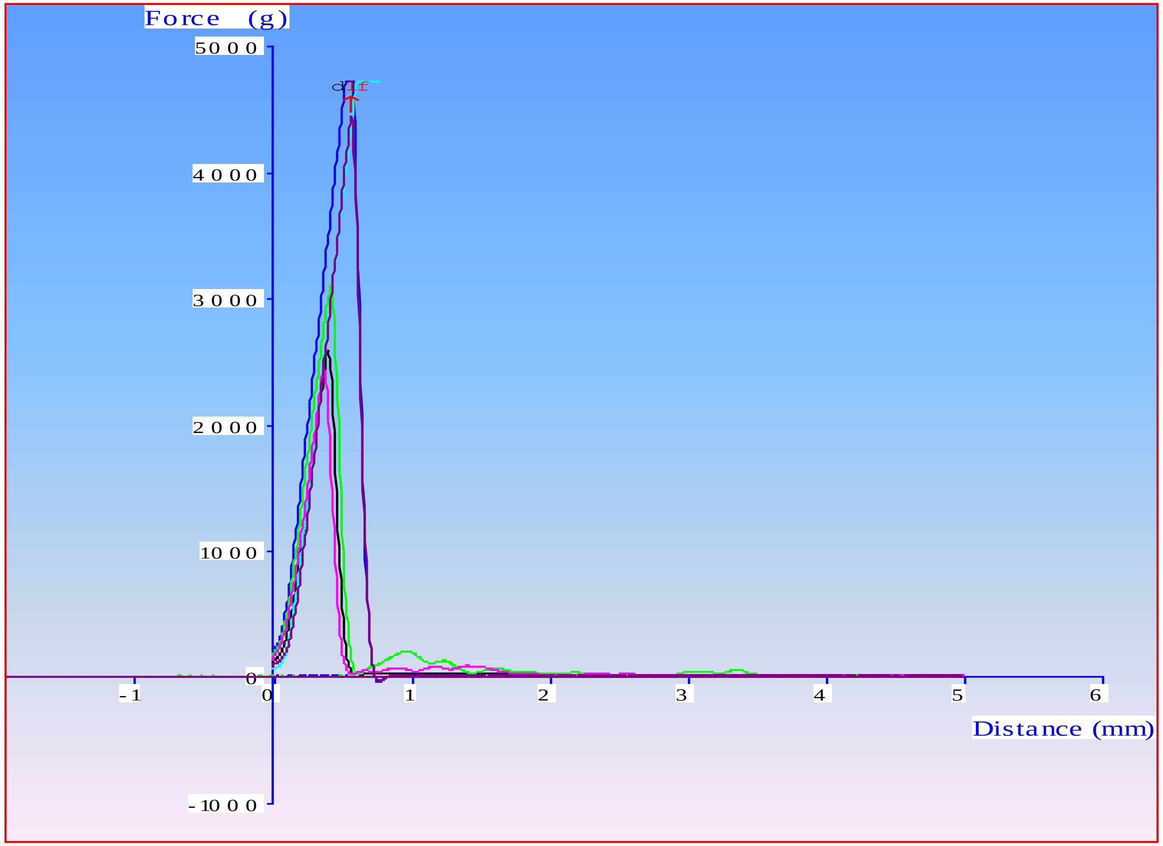

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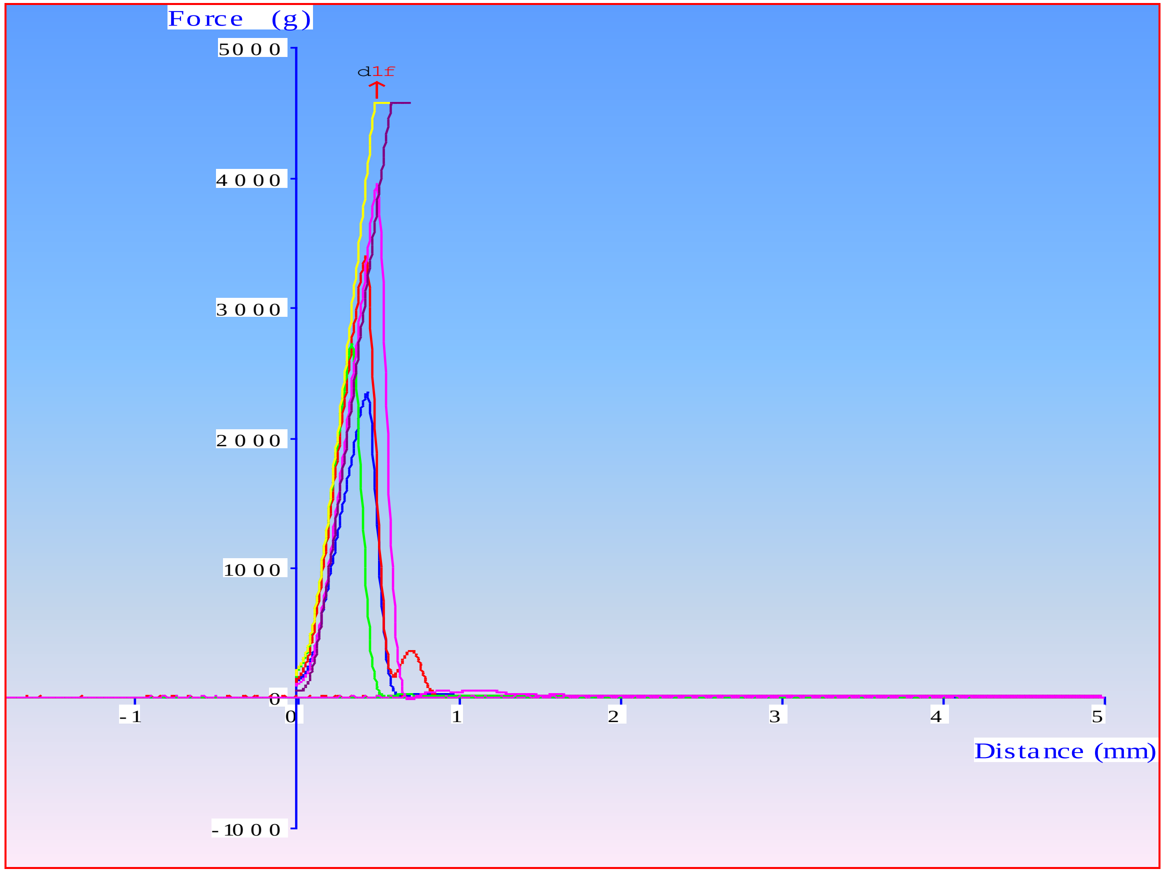

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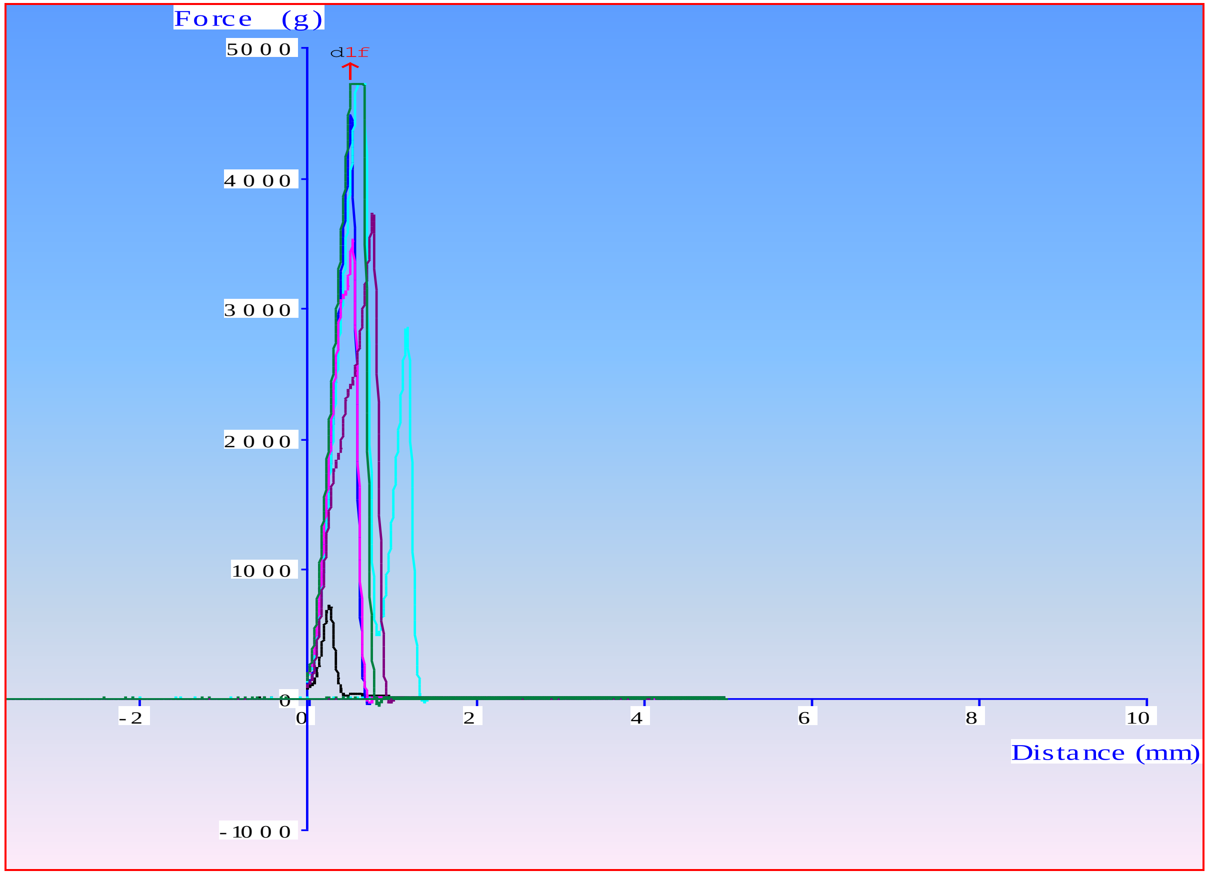

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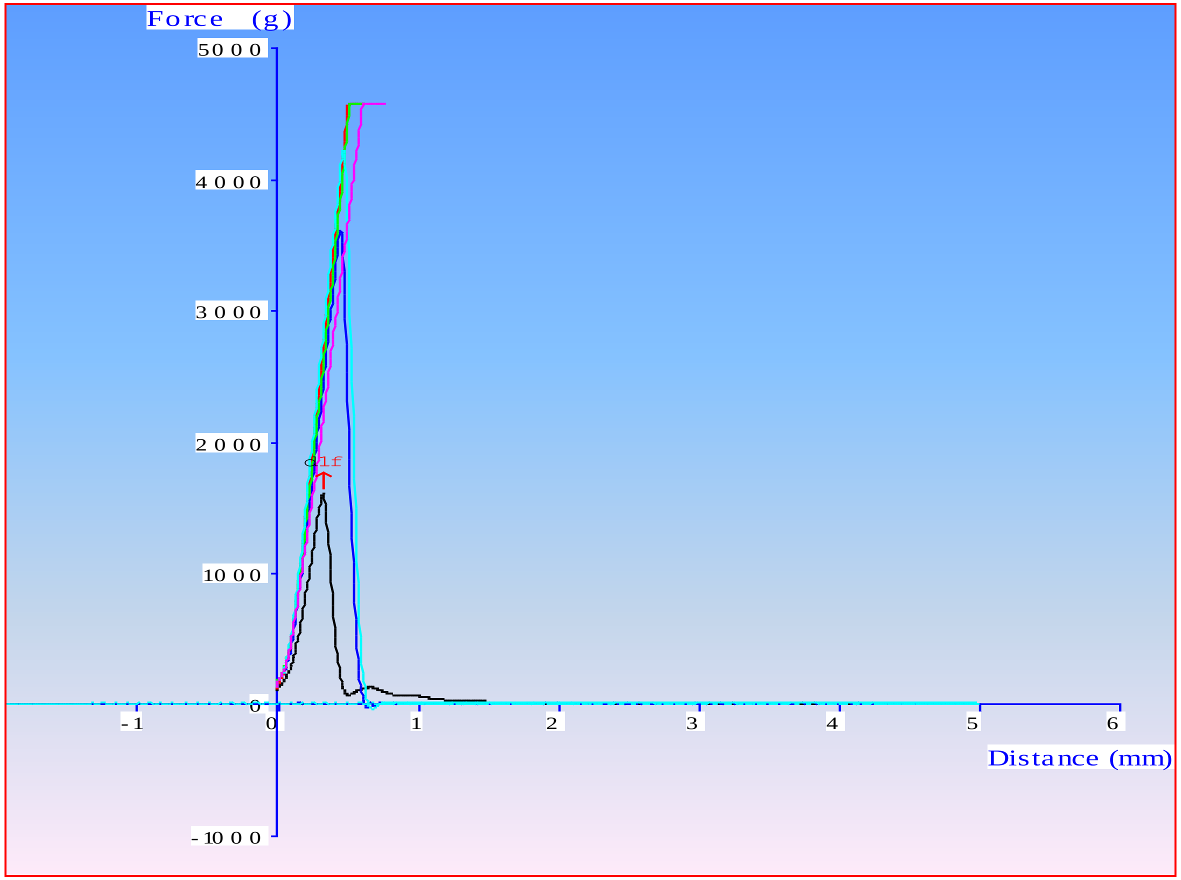

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Hitendrasinh Padhiyar

Place: Anand
Date: /05 / 2010

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percent
^{0}C	Degree centigrade
AACC	American Association of Cereal Chemists
ANOVA	Analysis of variance
AO	
CF	
cm	Centimeter
CMC	Carboxy methyl cellulose
CRD	Completely randomized design
DATEM	Diacetyl tartaric acid esters of mono and diglycerides
DE	Dextrose Equivalent
e.g.	for example
etc.	et cetera
FDA	Food and Drug Administration
FFC	Full-fat control
FICCI	Federation of India Chambers of Commerce and Industry
FSIS	Food Safety and Inspection Service
FWPC	fermented whey protein concentrate
g	gram
g%	gram percentage
GDP	Groth Dromestic Produce
GG	guar gum
GMS	Glycerol monostearate
GRAS	Generally Recognized as Safe
HDP	Heavy duty platform
HIV	Human immunodeficiency virus
hr	hour
kcal	Kilo calorie
Kg	Kilogram
Kgf	Kilo gram fource
Max.	maximum
MD	Maltodextrin
MDG	mono- and diglycerides
mg	miligram
mg%	milligram percentage
Min.	minimum
ml	milliliter
mm	milimeter
MP	Microparticulate protein
N	Newton (for force)
N	Normality (for chemical solutions)
NaCas	Sodium caseinates
pps	parts per second
RF	reduced-in-fat
RH	relative humidity
rpm	revolution per minute
Rs.	Rupees
RWF	refined wheat flour
S	second
SMS	Stable Micro Systems
SSL	Sodium stearoyl lactylate

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CHAPTER I
INTRODUCTION

Food is a critical contributor to physical well-being and a major source of pleasure, worry and stress. This is time now to revisit the concept of foods, beyond basic nutritional needs. Today, food has indeed become central for the cultural pleasure of feeding a family and greeting friends in a social setting. But on the other hand, a healthy diet consisting of foods with functional properties can help to promote well-being and even reduce the risk of developing certain diseases. WHO stresses the importance of a healthy diet in preventing non-communicable diseases (Howlett 2008).

The development of the food industry in India stems from the consistently increasing agricultural output. With the second largest global arable land area, India is one of the key food producing countries in the world, second only to China (Swiss Business Hub India 2008). India is the largest producer of milk, pulses, sugarcane and tea in the world and the second largest producer of wheat, rice, fruits and vegetables (Indian Brand Equity Foundation 2009). The total food production in India is expected to double in the next decade. There are many opportunities for large investments in food and food processing technologies, skills and equipments. Health food and health food supplements are the rapidly rising segments of this industry (Indian msme ecosystems 2008).

1.1 Food processing industry

India's food processing industry is one of the largest industries in the country - it is ranked fifth in terms of production, consumption, export and expected growth. The Indian food industry is estimated over US$ 200 billion and is expected to grow to US$ 310 billion by 2015 (Indian Brand Equity Foundation 2009). According to the Ministry of Food Processing, this industry contributed 9% to India's GDP and had a share of 6% in the total industrial production (Swiss

Business Hub India 2008). According to 2007 estimates, the food processing industry employs – directly or indirectly – more than 12 million people across the country and has the potential to absorb many more.

The food processing sector recorded a growth rate of 13.14 percent during 2006-07. The industry grew at 18 percent during 2007-08, according to Mr. Subodh Kant Sahai, Minister of food processing industries, scoring a 10 percent jump over 2004. The Minister further added that the sector is expected to grow at the rate of 20 percent by the year 2015 (Indian msme ecosystems 2008).

India presents several potential growth areas in the food processing sector. Based on potential growth opportunities and the enabling environment in terms of policy support, three key segments have been identified that indicate high attractiveness (Swiss Business Hub India 2008):

(A) Mass market basic foods:

1 Fruits and vegetables segment

2 Meat, poultry and fisheries segment

(B) Mass market value-added products: Dairy, bakery

(C) Niche market foods: Snack Foods, ready-to-make foods, packaged foods

India is one of the world's major food producers but accounts for only 1.7% (valued at US$ 7.5 billion) of world trade in this sector – this share is slated to increase to 3% (US$ 20 billion) by 2015 (Indian msme ecosystems 2008). Bakery products are highly potential for value addition and thus it could be a leading contributing sector for the development of food industry.

1.2 Bakery industry

In recent years, in India, bakery products have become popular among different cross sections of population due to increased demand of convenience foods. Bakery products constitute the largest segment of grain based processed foods (Murughan and Ramesh 2007 b) still it remains the cheapest of the processed ready to eat product in India (Baisya 2007).

Bakery industry in India is one of the largest food industries with an annual turn over of about Rs. 5000 Crore (Lawrence 2008). In India, the growth rate of the bakery industry is around 17% in April – June of 2009 (Indian Biscuits Manufactures Associations 2009). It is estimated that as on today there are around 65,000-70,000 bakeries working in large, medium and small scale sector (Malhan 2005). Still the per capita consumption of bakery products in India is just around 2.3 kg of bread and 1.8 kg of biscuit as compared to 50 to 150 kg of bread and 10 to 15 kg of biscuit in developed countries of the world, indicating thereby a great potential for the development of the bakery industry (Kamaliya and Rema 2003).

Present market size of various segments of bakery industry is mentioned in the Table - 1.

According to a recent "Food and Beverages" survey conducted by the Federation of India Chambers of Commerce and Industry (FICCI), the segments which are expected to record high growth i.e. between 10-20 percent include (New Zealand Trade and Enterprise 2009):

- Branded flour (atta) (16 percent),
- Bakery items like bread, cakes (11 percent),
- Biscuits (16 percent),
- Fruit juices, pulp and concentrate (18 percent)
- Sauces/ketchups (17 percent).

1.3 Indian biscuits industry

India Biscuits Industry came into limelight and started gaining a sound status in the bakery industry in the later part of 20th century when the urbanized society called for ready made food products at a tenable cost. Biscuits were assumed as sick-man's diet in earlier days. Now, it has become one of the most loved fast food products for every age group. Biscuits are easy to carry, tasty to eat and reasonable at

Table 1.1 Production of bakery products

Product	Market size ('000 tonnes)	Growth (%)
Bread	1,500	8%
Biscuit	1,200	8%
Cake	350	6%

(Source: Murughan and Ramesh 2007 b)

cost. States that have the larger intake of biscuits are Maharashtra, West Bengal, Andhra Pradesh, Karnataka and Uttar Pradesh. Maharashtra and West Bengal, the most industrially developed states, hold the maximum amount of consumption of biscuits. Even, the rural sector consumes around 55 percent of the biscuits in the bakery products (Maps of India.com's India business directory 2009).

India is the second largest producer of biscuits after USA. Bread and biscuits account for 80% of total bakery products produced in the country (Murughan and Ramesh 2007 b). The organized and unorganized sectors of the biscuit industry are in the proportion of 55:45 ratio (Indian Biscuits Manufactures Associations 2009). Soft dough biscuits account for over 70% of total production of biscuits in India. These types of biscuits normally contain high amounts of fat ranging from 15 to 25%. Due to its high cost, fat alone contributes to about 50% of total raw material cost (Rao and Srivastava 1993).

1.4 Fat and bakery products

Most of the bakery products are rich in fat and sugar and are made up of refined wheat flour thus they are calorie dense (Drewnowski et al 1998). Fats are the third largest components after flour and sugar in the bakery products (Agrawal and Khare 2005). The presence of fat in food is associated with a wide range of textural characteristics from smooth and creamy to chewy, crunchy and brittle. Fat provide texture and bulk to food, hold water, provide a mechanism for heat transfer at high temperature and act as a carrier for fat-soluble flavour molecules (Drewnowski et al 1998). Fat, oil, margarine or any other such material used in bakery products is collectively termed as "shortening". The functions of shortening in biscuits and cookies are (Singhal 1997):

- Improves dough machining and sheeting.
- Lubricates and controls the flow of dough.
- Provides shortness and rich bite.
- Enhances the flavour, appearance and nutritive value.

- Improves aeration and leavening.
- Improves the keeping quality.

The effect of fat on dough quality and on the finished product depends not only on their composition but also on quality of flour and on native lipids (Alla et al 1998).

Fat is still the number one nutritional concern for the most people because the continuous consumption of fat may lead to certain major health disorders such as cardiovascular diseases, obesity, hypertension, some type of cancer, high blood cholesterol etc. (Akoh 1998). People are also becoming health conscious. According to Food and Drug Administration (FDA) the consumption of fat must be reduced to 30% of total calories of diet (Agrawal and Khare 2005). Thus there is an urgent need to reduce the fat content from the diet. Bakery products led themselves easy for modification. This behaviour is quite suitable for the development of value added modified products to be termed as health food.

The baking industry has responded to the demands of consumers by developing low or reduced fat products defined as those foods that have at least one-third fewer calories than an equivalent serving of a normal counterpart. The reduced calorie product also must not be nutritionally inferior to the standard similar product (Klopfenstein et al 1995). To minimize consumer confusion, FDA and Food Safety and Inspection Service (FSIS) have defined descriptive term such as “free”, “low” and “reduced” that may be used on food labels from which some are listed in the table 1.2 (Mehta & Mehta 2005).

1.5 Fat replacer

A *fat replacer* is an ingredient that can be used to provide some or all of the functions of fat, yielding fewer calories than fat (Akoh 1998). Fat replacers need to be able to replicate all or some of the functional properties of fat in a fat-modified food. Fat replacers chemically resemble fats, proteins or carbohydrates and are generally

Table 1.2 Nutrient content descriptor that may be used on food labels

Description	**Definition**
Low	A serving (and 50 g of food, if the serving size is small) contains not more than 40 calories; 140 mg of sodium; 3 g of fat; 1 g of saturated fat and 15% of calories from saturated fat or 20 mg of cholesterol.
Reduced	A nutritionally altered product contains 25% less of a nutrient or 25% fewer calories than a reference food cannot be used if the reference food already meets the requirement for a "low claim".
Less	A food contains 25% less of a nutrient or 25% lower calories than a reference food.
% fat free	A product must be low fat or fat free and the percentage must accurately reflect the amount of fat in 100 g of food. Thus, 2.5 g of fat in 50 g of food results in a "85% fat free" claim.

(Sourse: Mehta & Mehta 2005)

categorized into two groups—fat substitutes and fat mimetics.

Fat substitutes are macromolecules that physically and chemically resemble triglycerides (conventional fats and oils) and which can theoretically replace the fat in foods on a one-to-one or gram-for-gram basis. Fat mimetics are substances that imitate organoleptic or physical properties of triglycerides but which cannot replace fat on a one-to-one or gram-for-gram basis. Fat mimetics, often called protein or carbohydrate based fat replacers, are common food constitutes, e.g. starch and cellulose, but may be chemically or physically modified to mimic the function of fat (Akoh 1998). Different type of fat replacers and their general functions are given in the table 1.3.

To minimize the diseases like cardiovascular diseases, obesity, hypertension, some type of cancer, high blood cholesterol etc. mainly caused due to excess fat, it is necessary to develop low fat bakery products for the well being of the masses. To reduce the fat content, different type of fat replacer can be used for production of low fat bakery products include Sucrose polyesters, Emulsifier, 'Caprenin', polydextrose, 'Oatrim', maltodextrin, 'Simplesse' etc. (Akoh 1998). Out of these carbohydrate based fat replacers (like maltodextrin) and protein based fat replacers (like 'Simplesse') were used singly to replace fat in food by many scientists but the combination of both is rarely experimented.

1.5.1 Maltodextrin

Maltodextrins are Generally Recognized as Safe (GRAS), non sweet, nutritive (4kcal/g on a dry basis) mixtures of saccharide polymers of varying chain lengths. They are produced by partial hydrolysis of starch obtained from corn or potato starch. Maltodextrins are used to build solids and viscosity, bind/control water and contribute smooth mouth feel in fat replacing systems for table spreads, margarine, imitation sour cream, salad dressings, baked goods, frostings, fillings, sauces, processed meat and frozen desserts (Akoh 1998).

Table 1.3 Functions of fat replacer in bakery products

Fat Replacer	**General Functions**
Lipid based (e.g. Sucrose poly fatty acid)	Emulsify, provide cohesiveness, tenderize, carry flavour, prevent staling, prevent starch retrogradation, condition dough
Carbohydrate based (e.g. Maltodextrin, Polydextrose)	Retain moisture, retard staling
Protein based (e.g. 'Simplesse', 'Dairy Lo')	Texturize

(Source: Akoh 1998)

1.5.2 Whey protein concentrate

Whey protein concentrate (WPC) is defined as a substance obtained by removal of sufficient non protein constituents such as water, minerals and lactose etc. from whey so that the finished product contained not less than 25% proteins (table 1.4). Whey proteins are being preferred for developing low calorie food products because of continuing rise in non-fat dry milk products, fluctuating cost and quality of commodities like eggs, increased intolerance to some of the food and food ingredients and religious reservations.

Sweet whey is excellent in piecrust dough, giving improved tenderness and flakiness. Shortening and water levels may be kept at a minimum because of tenderizing and low water requirements of whey cookies made with whey have excellent flavour and colour (Bakshi et al 2002).

A thermally denatured whey protein concentrate ('Dairy Lo') interacts with water and prevents iciness, provides opacity, controls viscosity, stabilizes air cells along with a fat sparing effect in dairy product when used at a level of 2 to 5%. 'Simplesse', made from milk whey protein or egg white is used as a fat replacer in various products (Bhatt et al 2008 a).

1.5.3 Lecithin

Lecithin is a by-product of the processing of crude soybean oil; it is the "gum" that is removed during the degumming step of oil refining. The crude gum is treated and purified to give the various commercial lecithin products that are available to the food processor today (Bailey 1996 b). The use of lecithin in doughs is said to produce better dough machinability, yield a more uniform crust colour, give a more tender crust to the baked product (Pyler 1988 b).

A combination of protein, starches and hydrocolloids has been suggested to have synergistic effects for lowering fat and retaining

Table 1.4 Composition of whey protein concentrate

Constituent	**Percent**
Protein	25 (min)
Fat	0.2 – 10
Ash	2 – 15
Lactose	60 (max)
Moisture	16

(Source: Bakshi et al 2002) (U.S. Standards) textural characteristics of the products. A combination of fat replacers can have tremendous potential in the development of fat modified foods with greater acceptability while lowering the total energy and fat intake (ADA report 2005).

Objectives:-

Keeping above facts in mind and with a view to develop a value added product using two fat replacers (Maltodextrin and Whey Protein Concentrate), this research study is proposed to be undertaken with the following objectives:

1) To evaluate the suitability of fat replacers for preparation of low fat biscuit.
2) To study the influence of fat replacers on the organoleptic, physical and textural characteristics of the biscuits.
3) To optimize the quantity of fat replacers for production of low fat biscuit.
4) To study the nutritional quality and storage stability of the selected biscuits.

CHAPTER II
REVIEW OF LITERATURE

Demand of processed food is increasing rapidly, with increasing urbanization. Among the processed foods, bakery products, in particular biscuits command wide popularity in rural as well as urban areas among all the age groups. This is due to longer shelf life, prepared in local bakeries also, easy marketing, low cost, varied taste and texture etc. (Gupta and Singh 2005).

Market drivers for the bakery industry are given below (Murughan and Ramesh 2007 a):

(1) Changing life style
(2) Longer working hours
(3) Large and growing middle class
(4) More entertainment
(5) Growing population of non-agricultural – due to urbanization
(6) Rapid economic development – higher disposable income
(7) Cultural shift – towards less formal eating habits
(8) Increased eating – on- the- move: small packs, resalable packs
(9) Growing number of fast food chains
(10) Growing number of working women
(11) Global brand
(12) The changing age profile (65 million people expected to enter 20-34 year age group by 2010) and
(13) Increasing exposure to western type products and life style.

Baked foods have a great potential as meal replacers, convenience foods, health foods and fulfills all function of any high quality food can contribute (Selvarajan 2004). With changing lifestyle trends like eating out, having meals at odd hours have increased and about 29.66%. Indians eat out frequently of which 48.14% consume high fat diet. The change in eating trend can be seen from the growth in demand for bread and biscuits, which account for over 85% of the bakery products (Bhatt et al 2008 a). From which biscuits are considered as the fat rich food products.

Most of the bakery products are rich in fat and sugar and are made up of refined wheat flour thus they are calorie dense (Drewnowski et al 1998). Fats are the third largest components after flour and sugar in the bakery products (Agrawal and Khare 2005). The bakery and the confectionary industry is one of the major industries for processed foods in India and perhaps the largest consumer of edible oils and fats. (Singhal 1997).

2.1 Functions of fat in the diet and in the body

Fat is a glyceride, decompose into fatty acid and glycerol. Three molecules of fatty acid are combined with one molecule of glycerol forming triglyceride. Fats and oils belong to the second major class of organic compounds, collectively called lipids, that play a vital part in the biological scheme of nature (Pyler 1988 a). Fats performing vital functions in the diet and human body which are given below (Murughan and Ramesh 2007 a):

(1) provides essential fatty acids
(2) stimulates appetite
(3) lends tenderness to foods such as meat and baked goods
(4) provides twice as many calories per gram, compared to either protein or carbohydrate, thus it is concentrated source of energy
(5) provides satiety
(6) protects the internal organs from physical trauma, as a part of the body's adipose tissue
(7) transports fat-soluble vitamins in the body, it is also essential for the conversation of pro-vitamin A or carotene to its active form - retinol
(8) provides raw material for biochemical compounds or products (e.g. vitamin D) needed by the body
(9) insulates against extreme temperature through fat layers under the skin.

Oils and fats can also play a role in structure development, lubrication, aeration, heat transfer, moisture retention, shelf life preservation and can enhance eating quality, volume, crumb development, texture and flavour of the food product (Weinwright 1999).

2.2 Functions of fat in biscuits

"Shortening" is a baker's term; fat in a bakery item "shorten" (tenderizes) the texture of the finished product. The use of shortening as an ingredient finds its basis in the fat's ability to perform the different functions namely: (a) tenderizing and imparting shortness to the crumb structure, (b) aiding in the aeration of the product, (c) stabilizing batters and creams by emulsification, (d) improving the overall palatability of the product and (e) extending the keeping quality or shelf life of the finished product (Pyler 1988 b). Quantity of fat used in the formula will determine the hardness of dough and hardness of biscuit (Dubey 2000). Flavour, texture, and appearance of baked products are affected by types and amounts of fat used (Pyler 1988 a). Fat contributes to cookie spread and to general product appearance, it enhances aeration for leavening and volume and makes the cookies more easily breakable (Brijs et al 2009). Fat enhances the flavours of other ingredients as well as contributing its own flavour as in the case of butter in butter cookies (Geetha and Narayanan 2004).

High fat consumption is associated with various health disorders such as obesity, cancer, high blood cholesterol and coronary heart disease (Brijs et al 2009). It is estimated that the death caused due to cardiovascular disease may rise to 40% by 2015 (Bhatt et al 2008 a). This presents, challenges for the baking industry, since fat cannot be easily replaced, especially in a complex food system such as cookies (Brijs et al 2009) and consumers want fat taken out of food without the taste and texture of the food being adversely affected. When fat is to be removed and replaced with water and other ingredients, a total formulation approch must be used to build in all the desired properties (Bakhsi et al 2001).

Booth Reseach Services conducted a national survey in US in 2000 and found that 79% of adult US population consumes low or no fat products. Consumers also want variety of low or no fat foods with characteristics of full fat counterparts (Kaur and Sroan 2003). Thus to avoid the problems related to obesity and to have a low calorie diets the demand for low fat or no fat food is also increasing day by day (Bhatt et al 2008 a).

2.3 Effect of fat replacement

The mouth feel properties of oils and fats are important characteristics to consider when reducing or replacing fat in formulations. Oils and fats may add to modify or reduce flavour and sweetness perception. Fats can also have an effect upon processing and handling. The characteristics generally of importance in biscuits (cookies) include crispness, shortness, the correct texture for the biscuit type whether soft or hard and good flavour release. Fats or shortenings are used to reduce the hardness of the biscuit and thus, simply reducing fat in the recipe results in a harder biscuit. This can also affect the cutting and machining of the dough whereby the finished biscuit may be misshapen. Furthermore, for the fat reduction to be of any value- either economically or dietetically- it needs to be significant, for example, 20 to 30% of the total fat (Mitchell 1996).

Reduction of fat in cookies resulted in a chewy texture and a low moisture content (Oreopoulou et al 2002). Fat reduction often results in a cookie with a nontraditional snap characteristics and an intermediate final moisture content (Lyon et al 2003). The bite of the fat free crackers and cookies is slightly hard as would be expected from elimination of shortening but the product breaks down easily during mastication (Dubey 2003).

Flavours in food are either lipid-soluble or water-soluble. Fat acts as carrier of lipid-soluble flavours (by dissolving them and reducing their vapour pressure, thus stabilizing flavour profile) and also as precursor for flavour development (as during frying or lipolysis). Therefore replacement of fat in food results in lack of flavour medium, as fat mimetics are capable of carrying water-soluble compounds only, thus many fat-soluble flavours may stand out.

Problems associated with low fat or no fat food having fat replacers are vast which include: poor aeration and thus poor cell structure, lack of fat like lubricity, loss of viscosity and body due to low solids, moisture loss in absence of fat and poor flavour release. Consequently, reformulation is required to address complexity of these problems in designer food (Kaur and Sroan 2003).

2.4 Fat replacers

Today, reducing intake of dietary fat is a major dietary goal for many consumers, government agencies and health groups. Consumers are increasingly looking for foods and beverages naturally low fat or with reduced fat or free from fat. To cater this ever-increasing demand for low or no fat foods, researchers have developed replacement for fat. But the role played by fat to contribute to organoleptic properties and appearance of food is very complex thus, it is a difficult ingredient to replace. No single fat replacer can reproduce all the characteristics of natural fat. Therefore, achieving fat reduction in foods often involves a “systems approach” in which combinations of ingredients and processing techniques are used to compensate for specific functions of the fat being replaced (Hann 1997).

In the search to offer consumers, the foods which will be lower in fat content, food technologists have developed several ingredients which can be used as fat replacers. Now a day most of the low fat foods are formulated using carbohydrate-based fat replacers which are generally derived from natural products and so there are less problems with their acceptability by consumers. In addition, the incorporation of emulsifiers has been proposed to reduce interfacial tension and increase the effectiveness of the fat, allowing less to be used (Klopfenstein et al 1995).

Fat replacers are ingredients used to replace fat in the food system. On the basis of origin and chemical nature fat replacers are categorized as:

1) Carbohydrate-based fat replacers
2) Protein-based fat replacers
3) Fat- or lipid-based fat replacers/synthetic fat substitutes.

Carbohydrate-based fat replacers are derived from cereals, grains and plants and include both digestible and indigestible complex carbohydrates. They can be divided into several subsections viz; maltodextrins, microcrystalline cellulose, methylcellulose gums, hydrocolloid gums, polydextrose, pectin and inulin (Bhatt et al 2008 b).

Protein-based fat replacers are derived from a variety of protein sources, including egg, milk, whey, soy, gelatin and wheat gluten. Some of these protein-based fat mimetics are microparticulated (sheared under heat) to form microscopic coagulated round deformable particles that mimic the mouth feel and texture of fat (Akoh 1998). These fat mimetics can be used in low baked goods; dairy products, such as fat free ice creams, frozen desserts, and milkshakes; reduced fat versions of butter; sour cream; low fat cheese; yogurt; salad dressing; margarine; mayonnaise; coffee creamers; soups and sauces (ADA reports 1998).

Fat-based fat replacers or synthetic fat substitutes are heat stable formulations in which configuration of fat molecule is altered by substituting either glycerol or fatty acids. When glycerol is substituted with non-traditional backbones, it becomes resistant to enzymes in gut and is not digested e.g., a sucrose polyester (Kaur and Sroan 2003). Emulsifiers are also fat-based fat replacers. Emulsifiers are surface active molecules capable of replacing up to 50% fat in a formulation e.g., mono- and di-acylglcerol, lecithin, sodium stearoyle-2-lactylate, sucrose fatty acid esters and polyglycerol esters etc.

Apart from these basic ones with particular chemical base, many combination products of fat replacers are also available in the market. These have been produced to overcome inefficiencies of one single chemical/origin based fat-replacers (Kaur and Sroan 2003). Such combination products are listed in table 2.1.

Examples of fat-replacers approved as GRAS include various carbohydrate polymers, gums, gels and starches; microparticulated proteins; whey proteins and fat emulsifiers (ADA reports 1998).

2.4.1 Maltodextrin (MD)

Maltodextrins (MDs) have been available to the food technologist as filling and bulking agents for many decades (Harkema 1996). In 1983, the U.S. Food and Drug Administration (FDA) issued regulations defining maltodextrins as nonsweet, nutritive saccharide polymers consisting of D-glucose units linked primarily by α-1,4 bonds with a dextrose equivalent (DE) of less than 20. Currently, the term matodextrin is used widely in the general literature to describe enzymatically-prepared, partially-hydrolyzed starches with a DE below 20 from any botanical source (Roller 1996).

MD in powder form derived from carbohydrate sources, such as corn, potato, wheat and tapioca, yields 4cal/g (Savitha et al 2008). These products are easily digestible, easy to blend with other dry ingredients, easy to dissolve and have low viscosity in solution (Harkema 1996). MDs could be used in producing emulsions as texture modifiers, bulking agents and particularly in food emulsions to a certain extent for substitution of fat (Savitha et al 2008). The key physical characteristic associated with maltodextrins useful in fat replacement is their ability to form sort, spreadable, thermoreversible gels with melt-in-the-mouth properties that give a fat-like mouth feel to food products (Roller 1996).

One part of a low-DE maltodextrin and three parts of water can often replace four parts of fat or oil. This can reduce the calories original provided by fat to as little as 11%. Low-DE maltodextrins

Table 2.1 Combination products of fat replacers

Fat replacer	Commercial Names	Food Categories
Polyester and Protein	Prolestra®, Nutrifat®, Finess®	Ice creams, salad dressings, mayonnaise, sauces, snacks and baked products
Blend of Hydrolysed Starches	Nutrifat® C & PC, Instant PC, PC Supreme	Ice creams, salad dressings, processed meats and sauces
Blend of Food Gums	Keystone 3100™	Dairy based drinks, ice creams and yogurts
Blend of Milk Proteins, Emulsifiers & Modified Food Starches	N-Flate®	Cake mixes
Blend of Modified Food Starches	Ultrafreese 400 ™	Low fat frozen desserts

(Source: Kaur and Sroan 2003)

enhance creaminess, provide body and give a fatty mouth coating to the food product in which they are used. Low-DE maltodextrins can be used directly as powders or in the form of pre-prepared gels, depending on the processing conditions and the desired characteristics of the final product (Harkema 1996).

The gross chemical composition of maltodextrins is related to the botanical source from which they are derived. Lipids can be responsible for the presence of off-flavours, high turbidity, higher pasting temperatures and lower viscosity of starches while proteins may lead to mealy flavours and a tendency to foam (Roller 1996).

Hydrocolloids in combination with maltodextrin, emulsifiers, whey solids etc. can help reformulate products having low fat content (Selvarajan 2004). The complete or partial replacement of an emulsified shortening with a maltodextrin gel (for instance in cake batters) may require the addition of an emulsifier and perhaps, some other minor changes in the formulations, in order to obtain optimum product quality (Dubey 2003).

2.4.2 Whey protein concentrate (WPC)

Replacement of unwanted fat in the diet with protein is an excellent nutritional exchange. The exchange of protein or carbohydrate for fat is an excellent way to reduce both calorie intake and the percentage of calories derived from fat. In those products where microparticulate protein (MP) replaces animal fat, a significant reduction in saturated fat and cholesterol content is also achieved (Singer 1996).

Addition of whey protein to bakery products raises protein content, balances essential amino acids and increases nutritional value. Wheat-flour protein quality can be improved by addition of whey protein supplements, which are high in lysine. Most recently, whey protein and its main fractions have been investigated for many unique health-enhancing or disease-combating properties, including immunopotentiation through increased intracellular glutathione synthesis, effects in reducing cancer cell proliferation, counteracting the wasting syndrome in HIV positive individuals, antimicrobial properties of some of the minor whey proteins and other effects (Jooyandeh et al 2009).

Whey proteins also have many functional benefits in the bakery products. They contribute moisture holding, foaming, gelling, structure forming and other benefits to bakery products. In some cases they can replace egg albumin. On addition of dairy proteins to bakery products, browning reactions occur affecting flavour and stability as well as colour. Browning is mainly a sugar–protein interaction or a Maillard-type reaction, forming brown polymers or melanoidins (Arendt et al 2005). Thus use of milk solids and whey solids can lead to many benefits (Selvarajan 2004).

WPC is capable of forming a tough, resilient structure surrounding the foam cells and also acts as an excellent surfactant and film forming agent and hence has to be considered as a potential ingredient for formulating products like bread, rolls, biscuits, cakes and other bakery products. Foaming properties of WPC are also advantageous in the production of hard biscuits and crackers because it helps in producing nicely shaped product and secures a good oven rise. WPC finds application in frozen baked cakes and cookies as an emulsifier to improve texture. WPC can also be used as fat replacer in most of the meat products. Gelation properties of WPC can be used to modify the structural properties of food products, such as hardness, cohesiveness and elasticity (Brueckner and Jayaprakasha 1999). In some biscuits WPC can replace 10-20% of the wheat flour (Puranik 2003). One of these mimetics, *Simplesse®,* is manufactured from whey protein concentrate by a patented microparticulation process. Simplesse provides fat-like creaminess in high-moisture applications, but like other proteins it tends to mask flavour (Akoh 1998).

2.4.3 Lecithin

The first surfactants (emulsifiers) to be used extensively in the baking industry were the lecithin (Pyler 1988 b). In general terms, emulsifiers are used to facilitate processing or to improve the texture, consistency and shelf-life of finished foodstuffs. Emulsifiers form films or spread on surfaces, build or increase viscosity through hydration and soften or weaken structures created by polysaccharides or proteins. Emulsifiers can also play a significant role in enabling reformulation using lower levels of fat in processed water-in-oil (w/o) emulsions and oil-in-water (o/w) systems. Typical examples of products in which proportion of fat can be reduced significantly, i.e., by 30% or higher, include yellow fat spreads, biscuits, cakes, baking emulsion, ice cream and salad dressings (Mitchell 1996).

Commercial lecithin is obtained chiefly from corn and soybean oils. Commercial lecithin normally contains 35 to 40% corn or soybean oil that serves as a carrier for the phosphatides and acts as a protective medium against oxidation (Pyler 1988 b).

The natural emulsifier lecithin is a complex mixture of phospholipids that provide the majority of its surface-active properties. In some cases, lecithin acts synergistically to improve the functionality of SSL and monoglycerides. In addition, lecithin provides drier dough that machine better and release well from rotary die faces (Klopfenstein et al 1995).

Defatted, processed and modified lecithin in powder form has extensive uses in many bakery products. They contribute to better emulsification, silky texture, non-stick properties and many other benefits unique to lecithin (Selvarajan 2004). It is readily soluble in fats and oils but insoluble in water – hence the practice of first incorporating the material in the baking fats when using it in dough or batter.

In common with most other surfactants, the use level of lecithin is quite low, being on the order of 0.15 to 0.2% based on flour in bakery products with a shortening content of less than 8% while in high fat products it is from 1 to 2% based on the shortening (Pyler 1988 b).

A combination of protein, starches and hydrocolloids has been suggested to have synergistic effects for lowering fat and retaining textural characteristics of the products. A combination of fat replacers can have tremendous potential in the development of fat modified foods with greater acceptability while lowering the total energy and fat intake (ADA report 2005). Combination of various fat mimetics (to provide smoothness); maltodextrin, corn syrup and gums (to develop cling, firmness and body); and emulsifiers (to provide tenderness, lubrication and to reduce set back), must be used to develop optimum system. Flavours can be added to overcome loss in flavour profile (Kaur and Sroan 2003).

Based on these theoretical aspects, many workers had tried out to produce reduced fat bakery products using various fat mimetics either single or in combination with other additives like emulsifiers.

2.5 Fat replacement

2.5.1 Fat replacement in biscuits

Rao and Srivastava (1993) observed decrease in overall quality score (from 48.5 to 30.0) and in spread (from 5.5 to 5.25 cm) while increase in hardness (from 0.9 to 1.3 kgf) when they decreased fat from 20% to 7.5% in soft dough biscuit formula. The spread, crispness and breaking strength were improved by addition of additives (GMS, SSL and lecithin). However, lecithin at 0.5% in combination with 7.5% hydrogenated fat was found to be the most effective for improving the overall quality of low fat biscuits.

Alla et al (1998) studied that addition of fat softened the dough, increased length and reduced thickness and weight of the biscuits. They further observed that, up to 20% the increase in length seemed considerable and it tended to stabilize beyond 20%. Thickness decreasing seemed to be more significant for fat contents between 10 and 20%.

Baltsavias et al (1999) determined the mechanical properties of short-dough biscuits prepared using a standard fat (37.0, 28.4, 20.9

and 16.4%), a firmer one (28.5 and 20.9%) and sunflower oil (20.5 and 16.3%) in three point bending tests. The biscuits with 28.4% standard fat considered as control. They found that reduction of fat content increased the modulus and the fracture stress of biscuits.

Manohar and Rao (1999) concluded that, emulsifiers (glycerol monostearate, lecithin and sodium stearoyl lactylate at the level of 5g/kg of flour) in general had a greater beneficial effect, particularly in the case of biscuits made from medium hard wheat flour. They also found the beneficial effect of using emulsifiers in producing a softer dough, with improved machinability and better textural characteristics of biscuits.

Arendt et al (2003 b) developed a biscuit containing reduced fat and sugar levels by using 'Novelose 330' (resistant starch), 'Raftilose' (sugar replacer / fructo oligosaccharide), sodium caseinate (dairy protein) and 'Simplesse' (protein-based fat replacer) at 10-14%, 10-15%, 25-30% and 25-30%, respectively. The sodium caseinate and the 'Simplesse' significantly increased the dough hardness values. The trials, which included the sodium caseinate, 'Raftilose' and 'Simplesse' at the medium to low levels and 'Novelose' at higher levels produced significantly thinner biscuits that were closest to the standard biscuit. A positive correlation was revealed between biscuit thickness and peak force required to break the biscuits ($p<0.01$), indicating that as biscuit thickness increased, it became more difficult (required more force) to snap the products as measured by the three-point break.

Conforti and Lupano (2004) studied the effects of two different WPCs and found that the presence of WPC with a high protein content decreased the fracture stress of biscuits. Arendt et al (2005) observed that addition of protein powders (WPC and sodium caseinate (NaCas) at the rate of 5, 10 and 15% of flour weight) in a short dough biscuit increased surface brownness, shrinkage during baking and hardness. The hardness of the biscuits increased as the level of protein powder was increased.

The effects of fat replacement with either maltodextrin or polydextrose on rheology of biscuit dough and on the quality of biscuits studied by Leelavathi et al (2007). Fat in the biscuit formulation was reduced from 20% (control) to 10%, 8% and 6% levels. For the preparation of low fat biscuits, fat content in the biscuit formulation was reduced by 50%, 60% and 70%, respectively. The texture analyzer showed an increase in dough hardness from 20.78 N to 44.08 N. Replacing fat with equal quantities of maltodextrin and polydextrose reduced the dough consistency and dough hardness, to some extent. Effect of fat reduction had a negative effect on biscuits texture which was improved significantly, when fat was replaced with maltodextrin.

Savitha et al (2008) showed that replacement of sugar with increasing amount of MD from 10 to 40% along with 0.05% sucralose influenced dough rheology.

Burseg et al (2009) prepared anethole-flavoured biscuits with different compositions (flour, five levels of fat, and two levels of sugar). They observed that both fat content and sugar form had a significant effect ($p<0.05$) on mechanical properties of the biscuits. The study suggested that aroma–sweetness interactions were overlaid by other sensory inputs such as perceived texture (hardness, particle size, moistness) and aroma transfer related to the chewing process. Perceived sweetness intensity was higher for high-fat biscuits suggesting enhanced fat–sweetness interactions. The opposite effect was observed for aroma perception.

Whey protein concentrate (at levels of 10g/formula) fortified biscuits and muffins from cassava based composite flours were prepared by Jisha and Padmaja (2009). The crude protein content of the experimental biscuits ranged from 9.63% to 11.00%. Spread ratio and spread factor were the least (9.27 and 60.99, respectively) for the biscuits made with unmalted cassava/finger millet mixes, while use of Termamyl pseudo-malted cassava/finger millet raised the spread ratio to 11.11 and spread factor to 73.09.

2.5.2 Fat replacement in cookies

Armbrister and Sester (1994) studied the sensory and physical properties of chocolate chip cookies made with vegetable shortening or fat replacers (polydextrose, acid-treated cornstarch, citrus peel pectin, mono- and diglycerides, microparticulated milk protein and potato maltodextrin) at 50 and 75 levels. The control cookie was significantly more fracturable than all cookies with fat replacers, except for that with 50% polydextrose. Sensory firmness was highest for control cookies. Vanill-like, sweet aromatics and caramelized flavours were higher ($p<0.05$) in the control cookies than in any cookies made with fat replacers. Cookies made with the MDG, potato maltodextrin and pectin at 50 and 75% replacement levels respectively did not differ significantly in contour from that of the control cookies, but all other variations differed significantly ($p<0.05$).

Klopfenstein et al (1995) found the principal effects of carbohydrate based fat substitutes on shortbread cookie i.e., higher moisture content, greater toughness and lower specific volume. Fat substitution of 35% had the least negative effects on the physical attributes. The combinations of fat replacer with emulsifier ('N-Flate'/SSL and 'Litess'/DATEM) showed minimal differences in cookie breaking strength in comparison with the traditional shortbread cookie at the three levels of fat substitution (35, 45 and 55% of shortening weight) and 0.5% emulsifier.

Patel and Rao (1996) found that there was significant improvement in spread ratio of cookies with increasing amount of fat (25, 28.8, and 32%). The hardness values of cookies reduced significantly with increasing addition of fat. The data indicated that the quality of cookies improved with the use of 0.5% lecithin.

Baldree et al (2002) prepared fat-free chocolate bar cookies by using okra gum or apple-sause as a fat ingredient substitutes to replace margarine and egg yolk in high fat cookies and found that both fat-free products contained greater than 3 times moisture as compared to full fat cookies. Colour and smell of fresh fat-free cookies containing okra gum did not significantly differ from full-fat cookies. Fat replacers in cookies at levels greater than 50%, similar reductions in flavour acceptability were noted.

Oreopoulou et al (2002) used carbohydrate- or protein-based fat mimetics (an improved polydextrose, 'Litesse', a maltodextrin with low dextrose equivalent (DE ¼ 3), 'C_deLight MD 01970' (Cerestar); an oat derived product rich in b-glucans, 'Dairytrim' (Meyhall Chemical AG, Netherlands); an oligofructose (inulin), 'Raftiline' (ORAFTI Active Food Ingredients, France); and a blend of microparticulated whey proteins and emulsifiers, 'Simplesse Dry 100' (The Nutrasweet Kelco Company, UK)) to replace up to 50% of fat in cookies. The brittleness of the cookies was increased with the addition of all fat mimetics, but a moderate increase was obtained with 'C_deLight', 'Simplesse' or 'Raftiline'.

The butter oil in the cookie formula could be partially replaced by 'N-Oil' (a tapioca dextrin) or 'N-Flate' (a modified corn starch and a balanced blend of emulsifiers) plus 'Firm-Tex' (modified food starch and guar gum in a nonfat dried milk at 1:1 ratio up to 50% level to produce high quality, low fat Betifore-type cookie. The developed low fat cookie had a positive effect in reducing the total serum cholesterol, LDL-cholesterol and triglycerides serum levels in rats (EL-Soukkary et al 2003).

Lyon et al (2003) prepared oatmeal and chocolate chip cookies modified with sugar and fat replacers to replace 50% of the sugar or fat respectively. They found that both reduced-in-fat (RF) cookies were harder than the Full-fat control (FFC). FFC cookies were thin with a wide diameter. Fat replacement resulted in thicker cookies with reduced diameters. They found that sugar and fat replacement had a greater effect on texture than on flavour.

Overall sensory characteristics as well as texture and surface characteristics of amaranth flour incorporated (25%) cookies were improved upon addition of glycerol mono stearate (GMS) or soy lecithin at 0.5% level on flour basis. The spread ratio (6.52 to 8.49) and tenderness of the cookies increased while breaking strength decreased (4.021 to 3.807 kg) with the inclusion of GMS and soy lecithin. The increase in the overall quality score of the cookies indicate that the cookie made using emulsifiers, either individually or in combination were improved in all respects (Rahim et al 2005).

George and Suyong (2006) observed decrease in the diameter and increase in the height of cookies by replacing shortening with 'Nutrim oat bran' (jet-cooked oat bran). There was no significant difference in cookie hardness among samples with up to 20% shortening replacement but the cookies became lighter in colour as the 'Nutrim OB' content was increased.

Perry and Swanson (2007) prepared oatmeal and chocolate chip cookies by using a non-sucrose sweetener blend (dextrose/acesulfame-K) and/or prune puree to replace 50% of the sugar and/or fat, respectively. Panelists indicated that all formulations of each cookie type were acceptable with ratings above mid-point on the scale for flavour and texture acceptability. In chocolate chip cookies, sensory results ($p<0.05$) suggest an increase in off-flavours and bitterness and a decrease in sweet and buttery attributes with modification. Total fat reduction was 32% and 45% in the oatmeal and chocolate chip cookies, respectively. Caloric reductions were 1415% in the modified oatmeal cookies, and 1213% in the modified chocolate chip cookies.

Brijs et al (2009) studied structural and textural properties of sugar-snap cookies made with a decrease in fat level from (15.8% to 8.7% on dough base) and increase in sugar level (from 31.2% to 34.4%). The cookie diameter increased and its height decreased with increasing sugar or fat levels. X-ray microfocus computed tomography porosities and cell sizes increased with fat level, but cell size distribution, cell wall thickness and distribution were not affected by fat level, indicating that fat primarily incorporates air. The different fat levels used showed the fat to have an effect on the (amount of) air incorporation during cookie dough making. Furthermore, higher air incorporation was probably related to lower dough viscosity, and, as a result, to larger cookie diameters. In addition, higher fat levels reduced cookie break strength, which was not only due to a higher porosity, indicating that fat influences the strength of the cell walls (but not their thickness).

2.5.3 Fat replacement in other baked products

Barbeau et al (1991) found that cupcakes prepared with a commercial fat substitute, 'N-Flate' were less tender and had less cell uniformity than those prepared with shortening. Variations prepared with 63% polydextrose (AFP) were less moist, had a more bitter crust and crumb, and showed greater cell uniformity than those prepared with sugar.

Khalil (1998) studied physical and sensory characteristics of cakes prepared with either the carbohydrate-based fat replacers 'N-Flate' (a balanced blend of nonfat dry milk, polyglycerol esters, modified food starch and guar gum), 'Paselli MD 10' (a potato maltodextrin) and 'Litesse' (an improved polydextrose FCC) at the rate of 0, 25, 50 and 75% of fat weight or fat replacers plus emulsifier (mono- and diglycerides; 0 and 3% of flour weight). The combination of the emulsifier with either 'Paselli MD 10' or 'Litesse' also enhanced the specific gravity. Cakes prepared with 'Paselli MD 10' had the highest volumes, specific volume, standing heights and compressibilities. Incorporation of emulsifier with fat replacers improved cake volumes, standing heights and compressibilities. Incorporation of emulsifier with fat replacers did not affect the crust colour, crumb colour and flavour, but significantly ($p<0.05$) improved softness and eating quality. Cake prepared with 'Paselli MD 10' had the best physical characteristics among fat replacers.

Kaur et al (2000) studied effect of emulsifiers and hydrocolloids as fat replacers in baked products. The emulsifiers viz., glycerol mono strerate (GMS) and sodium stearoyl-2-lactylate (SSL) and hydrocolloids viz., guar gum (GG) and carboxy methyl cellulose (CMC) at the levels of 0.25 and 0.50% of flour weight basis were included in the formulas to reduce the fat contents by 10 and 20% in muffins and cookies, respectively and omitting fat in bread. The qualities of products prepared after reduction of fat and addition of emulsifiers and hydrocolloids were better than their respective controls. Breads prepared by additives without fat at 0.50% level were superior to their respective controls but had less volume as compared to bread made with additive (0.25%) and fat combination. Muffins prepared with 20.0% reduced fat and additives at 0.50% level got more score. While low calorie cookies were prepared by incorporation of GG, CMC, SSL and GMS in the formula at 0.25 and 0.50% levels and reducing 10 and 20% of shortening respectively, as compared to control.

Archilla et al (2001) indicated that maltodextrin, as fat replacer in combination with 'high-fructose corn sweetener 90' was effective in maintaining moisture and water activity levels similar ($p<0.05$) to the full-fat muffin but were firmer ($p<0.05$) in texture profiles than the full-fat counterpart. Maltodextrin alone significantly ($p<0.05$) impeded the staling rate during storage.

Bell and Campbell (2001) prepared low fat, sugar-free cakes by replacing shortening (11.2%) and sucrose (30.0%) with 'Lactitol', a sugar alcohol containing 2 kcal/g, at the rate of 29.8% with emulsifier and hydrocolloids. Overall acceptability of the low fat, sugar free cake significantly lower than the regular cake, both in the blind test and the informed test. The acceptability was increased significantly ($p<0.05$) when judges knew the identity of the cake, in case of the blind test whereas no changes were found for the regular cake.

Arendt et al (2003 a) studied the performance of different fat replacers (Inulin powder, Inulin gel and 'Simplesse') at various levels (2.5 and 5.0%) in wheat bread and dough compared to a control containing fat and found that breads containing the inulin gel (2.5%) were similar in quality characteristics to the control breads containing fat. The panelists indicated that 25% fat replacement with pawpaw fruit puree is sensorily acceptable as compared to control containing

100% vegetable shortening in a plain shortened cake (Duffrin and Wiese 2003).

Inglettt et al (2005) found increase in specific gravity of the cakes and decrease in viscosity as more shortening (at the level of 20, 40, and 60%) was replaced with 'Oatrim' (oat β-glucan amylodextrins). The cakes prepared up to 20% by weight of Oatrim did not evidenced significant changes in softness ($p<0.01$) and generally exhibited similar physical properties to the control cake. Jaipal et al (2007) indicated that the maltodextrin gel (from sorgum starch) up to a 30% replacement with shortening resulted in satisfactory cakes.

Jooyandeh et al (2009) found that sensory characteristics of bread samples supplemented with whey permeate at 25, 50, 75 and 100% level to wheat flour were better but samples supplemented with fermented whey protein concentrate (FWPC) more than 50% caused significant decrease in sensory scores. Fat, protein, ash and moisture contents of bread increased with increasing levels of substitution and this effect was more in FWPC fortified breads.

2.6 Storage study

The shelf life on any type of biscuit depends on its chemical composition, packaging material selected and the environment factors such as temperature, relative humidity (RH) and light. In general, biscuits have low moisture contents and significant amounts of fat. Hence, they are prone to loss of crispness by absorption of moisture and rancidity development due to oxidation.

Chauhan et al (2000) packed biscuits in two different types of packaging materials namely, polypropylene (160 gauge) and laminate of cellophane (150 gauge) and butter paper and stored for 60 days under ambient conditions of average minimum and maximum temperature of 24.8^0 and 34.2^0C and humidity 63 and 83.8%, respectively. The samples were analyzed on 15, 30, 45 and 60 days of storage for changes in physical parameters and overall acceptability. During storage, hardness, crispness and overall acceptability scores of biscuits decreased gradually. Biscuits packed in polypropylene could be stored for 45 days under ambient conditions whereas in laminated packaging, the shelf-life of biscuits was 30 days.

These studies indicate that, maltodextrin (carbohydrate-based fat replacer) and modified WPC (protein-based fat replacer) are the most popular fat replacers to develop the low fat bakery products. The researchers suggested that a combination of protein, starches and hydrocolloids have synergistic effects for lowering fat and retaining textural characteristics of the products. A combination of fat replacers can have tremendous potential in the development of fat modified foods with greater acceptability with lowering the total energy and fat intake. WPC fortified products would be beneficial in improving the health and well being of human beings and thereby introduce it as a "functional food". Recently, craze towards western culture changed the food habits to use more and more bakery products. Thus this is high time to develop low fat or no fat bakery products in particular biscuits since they are rich in fat but having added advantages like longer shelf-life, prepared in local bakeries also, easy marketing, low cost, varied taste and texture etc.

CHAPTER III

MATERIALS AND METHODS

The main objective of the study was to develop low fat biscuits and thereby introduce it as a “functional food”. Normally biscuits are prepared with refined wheat flour (*maida),* sugar and shortening as the principle ingredients, thus they are calorie dense. Continuous consumption of such biscuits may lead to some major chronic diseases such as cardiovascular diseases, obesity, hypertension, some type of cancer, high blood cholesterol etc. Therefore, commercial formula was modified to develop the low fat biscuits. The physico-chemical and nutritional characteristics of the raw material was evaluated. While the physical characteristics of finished product, nutritional characteristics of selected finished product and textural characteristics of the finished biscuits and selected biscuits were evaluated. Sensory evaluation was conducted to check the acceptability of the experimental biscuits and selected biscuits during storage period. This chapter encompasses detail of materials and methodologies employed during the investigation. Methodologies related to the technological aspects as well as the physical, chemical and statistical analysis are enunciated hereunder.

3.1 Materials

The various raw ingredients required to develop the biscuits were surveyed for their availability and quality. Only good quality raw ingredients were procured in sufficient quantity. Immediately after purchase, the materials were stored properly and used as and when required. However, perishable ingredients were purchased as required during experiment.

3.1.1 Refined wheat flour

After proper analysis, the good quality refined wheat flour was procured from the local market. The quantity of the flour purchased varied depending upon the use as and when needed. The flour was sieved twice before use to clean it properly.

3.1.2 Fat (shortening)

Fat was procured from the market and used during the entire experimental period.

3.1.3 Sugar

Fine powdered food grade sugar purchased from market was used for the entire experimental period.

3.1.4 Dextrose syrup

Dextrose syrup was procured from the local market for the entire study.

3.1.5 Non fat dry milk powder

Non fat dry milk powder manufactured by Amul dairy was purchased from the local market.

3.1.6 Soy lecithin

Soy lecithin used for making of the biscuits was purchased from the local market.

3.1.7 Leavening Agents

Both the leavening agents (Ammonium bicarbonate and Sodium bicabonate) were procured from the reliable source.

3.1.8 Salt

Sodium chloride, a common salt, was procured from market and used throughout the experimental period.

3.1.9 Essence

Vanilla essence used for the preparation of biscuits was procured from the reliable source.

3.1.10 Water

Double distilled water was used for the purpose of making the biscuits.

3.1.11 Fat replacers

a) Maltodextrin: Food grade maltodextrin (DE<20), manufactured by M/s HiMedia Laboratories Pvt. Ltd. was procured from the reliable chemical store for the entire study.
b) Whey protein concentrate: WPC 70% (PROCON 3700) was supplied by M/s Mahaan Proteins Ltd., New Delhi at free of cost.

3.1.12 Packaging material

Metallised food bags and transparent food grade polyethylene bags were procured from local market. Polyethylene bags were used as common packaging material for the biscuits while metallised food bags were used for storage study of the biscuits.

3.2 Preparation of biscuits

Control biscuit was prepared basically using rubbing method according to Kamaliya and Kamaliya (2001) while the formulation used was according to Klopfenstein et al (1995). The same is depicted in the table 3.1.

Good quality raw ingredients were procured form the market and used for product preparation. The refined wheat flour and powdered sugar sieved twice separately to remove the foreign particles. The fat was creamed till light and fluffy. To that refined wheat flour was gradually added, rubbed thoroughly till major portion of flour particles get coated with fat. Powdered sugar was added to that and mixed gently till thoroughly dispersed in rubbed flour. Dextrose syrup and milk powder were suspended in water while sodium chloride was dissolved in the water. The leavening agents and vanilla essence were also dissolved in the same solution and immediately transferred into the fat-flour-sugar mixture. Then, it was mixed gently and kneaded into a smooth dough. The dough was sheeted on platform to a thickness of 3 mm using wooden rolling pin. The dough-sheet was cut into hexagonal shape using a metallic cutter of 28 mm length. Each raw biscuit was transferred and systematically arranged on a baking sheet about 10 mm apart from each other. The tray was transferred, as quickly as possible, to a preheated baking oven at 208^{0}C and baked for 8 minute. The biscuits were immediately transferred on a cooling rack. The cooled biscuits were packed in polyethylene pouches, sealed, stored at ambient room temperature and evaluated after 24 hour. All precautions were taken for the development of ideal characteristics in the control product as indicated in the step of preparation in the references originally cited.

After the successful preparation of the control biscuit, the experimental biscuits were prepared by replacing shortening with maltodextrin and WPC at 40, 50, 60 and 70% levels in the formula at the ratio of 0:100, 75:25, 50:50, 25:75 and 100:0.

To prepare the experimental biscuits lecithin was first creamed with fat and then mixed with maltodextrin and WPC. Rest of the procedure followed similar to the control biscuits. The flow chart and selected steps of the biscuit processing is depicted in the figure 3.1 and plate 3.1 and 3.2, respectively, alongside.

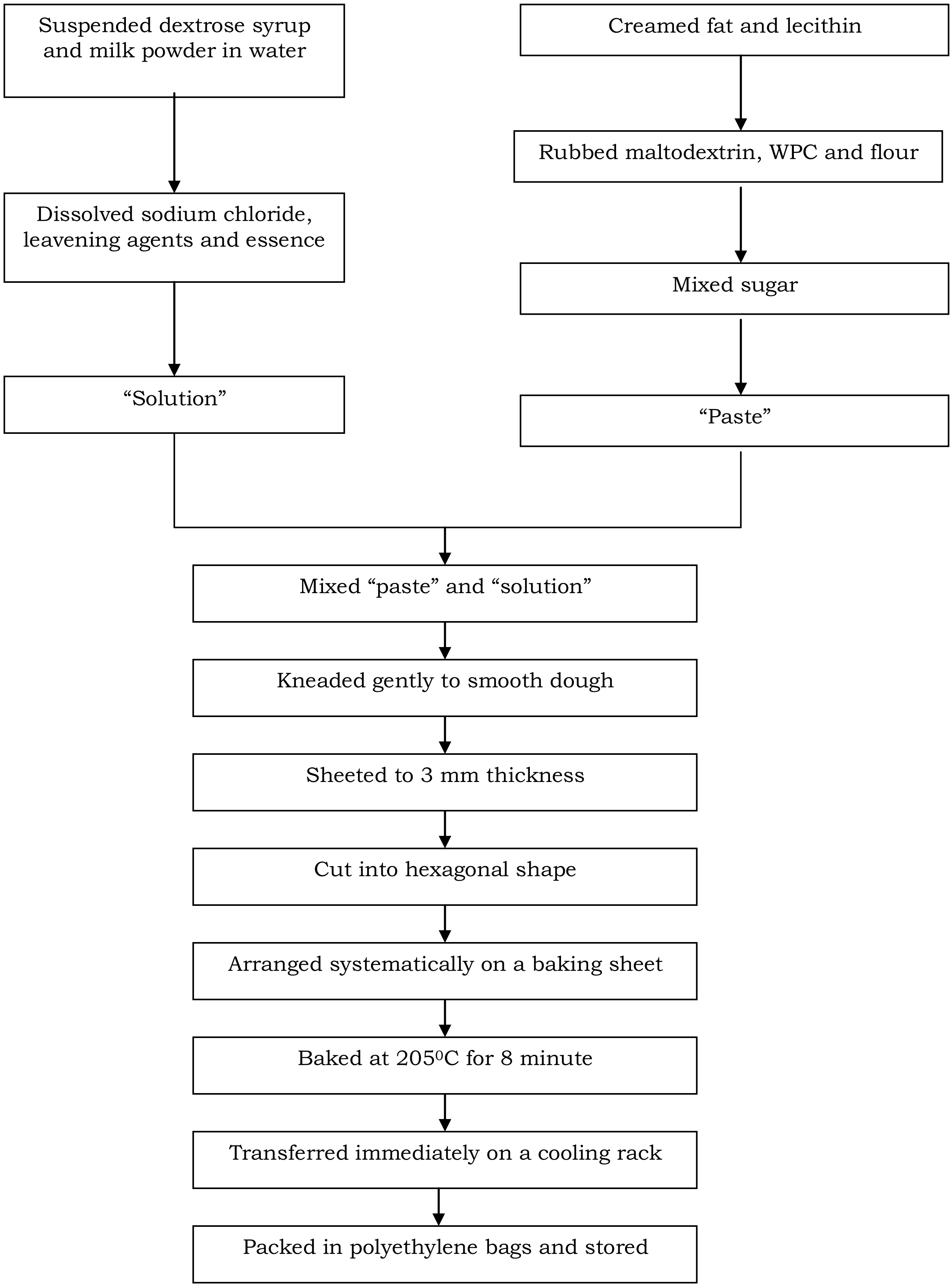

Fig.3.1: Flow chart for biscuit preparation

3.3 Experimental design

3.3.1 Variables

1] Product: Low fat biscuits

2] Fat replacers: 1. Maltodexrin

2. Whey protein concentrate

3] Ratio (Shortening : Maltodextrin : Whey protein concentrate): As per the table no. 3.2 to 3.5.

4] Baking temperature: 205^0C

5] Baking time: 8 minute

6] Replications: 3

7] Location: PGI - FPT&BE, Anand Agricultural University, Anand.

Table 3.2. Shortening : Maltodextrin : Whey protein concentrate (40% fat replacement)

Shortening	Maltodextrin	Whey protein concentrate
100	0	0
60	40	0
60	30	10
60	20	20
60	10	30
60	0	40

Table 3.3. Shortening : Maltodextrin : Whey protein concentrate (50% fat replacement)

Shortening	Maltodextrin	Whey protein concentrate
100	0	0
50	50	0
50	37.50	12.50
50	25	25
50	12.50	37.50
50	0	50

Table 3.4. Shortening : Maltodextrin : Whey protein concentrate (60% fat replacement)

Shortening	Maltodextrin	Whey protein concentrate
100	0	0
40	60	0
40	45	15
40	30	30
40	15	45
40	0	60

Table 3.5. Shortening : Maltodextrin : Whey protein concentrate (70% fat replacement)

Shortening	Maltodextrin	Whey protein concentrate
100	0	0
30	70	0
30	52.50	17.50
30	35	35
30	17.50	52.50
30	0	70

3.3.2 Observations

A. Analysis of fat content of WPC, Shortening and Refined Wheat Flour

B. Physico-chemical properties of flour

- Water absorption power
- Wet gluten
- Dry gluten
- Sedimentation value
- Alkaline water retention capacity

C. Sensory evaluation of finished products

- Volume
- Crust colour and surface character
- Crumb colour
- Crumb texture
- Mouthfeel
- Taste and aroma
- Overall acceptability

D. Physical characteristics of finished products

- Width
- Thickness
- Spread ratio
- Percent spread factor
- Weight

E. Textural properties of finished product

- Hardness
- Fracturability

F. Nutritional properties of the selected products

(1) Proximate analysis

- Moisture
- Protein
- Fat
- Carbohydrate
- Ash

(2) Energy value

G. Storage stability of the selected low fat biscuits

(1) Organoleptic changes

(2) Rheological changes

H. Cost analysis of the biscuits

3.4 Experimental procedure

3.4.1 Variation in fat level

The standardized recipe for the biscuit preparation as given by Klopfenstein et al (1995) was modified by replacing shortening with maltodextrin and WPC at 40, 50, 60 & 70% levels. The ratio of maltodextrin and WPC was 0:100, 75:25, 50:50, 25:75 and 100:0. There were no changes in the quantity of other raw ingredients (except water quantity) as well as processing conditions during the preparation.

3.4.2 Variation in water quantity

Looking to the more water absorption power of the fat replacers, the level of water addition was increased as the level of fat replacer increased till the dough having similar consistency to the control dough was obtained. The quantity of water used is given table 3.1.

3.5 Analytical techniques

Refined wheat flour, shortening and WPC were analysed for fat content. Refined wheat flour was also analysed for water absorption power, wet gluten, dry gluten, sedimentation value and alkaline water retention capacity. The physical properties i.e. width, thickness and weight were measured while spread ratio and spread factor were calculated. Rheological properties of the finished products and the selected products for the storage study were analysed by using TA.XT2 texture analyzer of Stable Micro Systems (SMS). Selected biscuits were analysed for moisture, protein, fat, carbohydrate and ash content as their nutritional composition. Those were also analysed for energy value. The methods of analysis are described hereafter in this chapter.

3.5.1 Physico-chemical Characteristics

Raw ingredients and their composite flour mixtures were analysed for physico-chemical characteristics. Wet and dry gluten, water absorption power, sedimentation value and alkaline water retention capacity were the physico-chemical properties analysed.

3.5.1.1 Water absorption power (WAP)

Water absorption power was determined following method of Kamaliya and Kamaliya (2001). In this method known amount of sample (25 g) i.e. refined wheat flour was taken in a porcelain cup. Distilled water was filled in a burette (25 ml capacity) and allowed to run (about 13-14 ml) in to the cup gradually. The mixture was continuously stirred using a glass-road. Then water was added drop by drop, with continued stirring until firm dough ball of appropriate consistency (similar to used for biscuit preparation) was obtained. Care was taken that no material adheres to the utensils. The quantity of water (ml) required was measured and used to calculate water absorption power on 14% flour moisture basis and expressed in percentage.

Calculation:

$$\text{Water absorption power (\%) (14\% moisture basis)} = \frac{\text{Quantity of water absorbed by flour (ml)}}{\text{Sample weight (g)}} \times \frac{\text{Std. solid matter in flour (i.e. 86)}}{\text{(100-Percent sample moisture)}} \times 100$$

3.5.1.2 Gluten

Wet and dry gluten was determined following hand washing method of AACC (2000).

3.5.1.2.1 Wet gluten

The dough-ball prepared while determining water absorption power was allowed to stand in water at room temperature (approximately 26-27^{0}C) for 60 minute. The dough was then kneaded gently in a stream of tap water over bolting cloth placed on a fine sieve until all the starch and soluble matters were removed. That has required about 15-20 minute. Care was taken that no scattered parts of gluten were washed away along with water. To determine the absence of starch in washed gluten, 1 to 2 drops of squeezed water from gluten was allowed to drop into a beaker containing perfectly clear water. In the presence of starch, cloudiness appears. The gluten that was obtained by washing was kept in water for 60 minute. It was pressed between two palms until it just began to stick, was rolled into a ball, placed on a weighed flat-bottom aluminium dish and the weight obtained was considered as wet gluten weight and expressed as (on 14% flour moisture basis) percentage after appropriate calculations.

Calculation:

$$\text{Wet gluten (g\%) (14\% moisture basis)} = \frac{\text{Wet gluten weight (g)}}{\text{Sample weight (g)}} \times \frac{\text{Std. solid matter in flour (i.e. 86)}}{\text{(100-Percent sample moisture)}} \times 100$$

3.5.1.2.2 Dry gluten

Wet gluten was then transferred to an oven for drying at 100^{0}C for 24 hr, cooled and weighed. The drying process was repeated till difference between two successive weights was less than 1 mg. The final weight was recorded as dry gluten and expressed in percent (on 14% flour moisture basis) after appropriate calculations.

Calculation:

$$\text{Dry gluten (g\%) (14\% moisture basis)} = \frac{\text{Dry gluten weight (g)}}{\text{Sample weight (g)}} \times \frac{\text{Std. solid matter in flour (i.e. 86)}}{\text{(100-Percent sample moisture)}} \times 100$$

3.5.1.3 Sedimentation value

Sedimentation value was determined as per the method of AACC (1995). Known amount of sample i.e. flour (3.2 g) was placed in 100 ml glass-stoppered graduated cylinder. Simultaneously a timer was started and 50 ml of 0.4 mg% bromophenol blue solution was added. Then it was mixed thoroughly by moving a stoppered cylinder horizontally lengthwise, alternately right and left, through a space of a 18 cm, 12 times in each direction within 5 second. Care was taken that flour swept completely into the suspension during the mixing. At the end of the first 2 minute (as time schedule shown at the end) the contents were mixed completely by inverting the cylinder and reverting it, as if it were pivoted at the centre. This action was performed smoothly, exactly 18 times in 30 second. Then it was allowed to stand for 1.5 minute. To this 25 ml of isopropyl alcohol : lactic acid : distilled water mixture::20:8:62 (V/V) solution was added and mixed immediately by inverting the cylinder four times, similar to the method mentioned above. Again it was allowed to stand for 1.75 minute followed by mixing for 30 second and allowing to stand for 1.5 minute. This was again mixed for 15 second, then the cylinder was placed immediately in an upright position in front of light in such a way that the marker could be clearly seen. Exactly after 5 minute the volume was read out in millimetre of sediment from the cylinder. This gives the uncorrected sedimentation value, which was corrected by expressing it on 14% moisture basis of the sample.

Calculation:

$$\text{Corrected sedimentation value} = \text{Sedimentation value [uncorrected]} \times \frac{\text{Std. solid matter in flour (i.e. 86)}}{\text{(100-Percent sample moisture)}}$$

3.5.1.4 Alkaline water retention capacity

Alkaline water retention capacity of sample i.e. flour was determined following the AACC (1995) method. Weighed a 15 ml centrifuge tube with stopper. Exactly 1 g flour of known moisture content was transferred into the centrifuge tube. To that 5 ml 0.1N sodium bicarbonate solution was added and stoppered. It was shaken vigorously to suspend the flour, placed on the table and the timer was started.

The tube was shaken at an interval of 5, 10, 15 and 20 minutes and then it was centrifuged at 5000 rpm for 15 minute. Centrifugation should not be stopped suddenly. The tube was taken out from the

Table 3.6. Time schedule for testing sedimentation value:

Time shown in stop-watch (minute)

Sample	**Added water (started stopwatch)**	**Mixed for 30 second**	**Added Acid and mixed 4 times**	**Mixed for 30 second**	**Mixed for 15 second**	**Set up right**	**Reading* taken**
1	0:00	2:00	4:00	6:00	8:00	8:15	13:15
2	0;30	2:30	4:30	6:30	8:30	8:45	13:45
3	1:00	3:00	5:00	7:00	9:00	9:15	14:15
4	1:30	3:30	5:30	7:30	9:30	9:45	14:45

Each operation was started at the indicated time.

* Read the uncorrected sedimentation values exactly after 5 minute.

centrifuge without disturbing the contents. The supernatant was decanted by draining the tube at 45^0 angle for 5 minute, blotted the tip of the tube with tissue paper. The tube was placed upside down (90^0) on a tissue paper in the test tube stand for 5 minute to drain off the water completely. The neck of the tube was blotted again. At the end the tube was weighed with the stopper.

Calculation:

AWRC(%)

$$= \frac{(\text{Weight of tube with gel (g)}) - (\text{Weight of empty tube (g)})}{\text{Sample weight (g)}} \times \frac{\text{Std. solid matter in flour (i.e. 86)}}{100 - \%\text{ sample moisture}} \times 100$$

3.5.2 Physical Properties

3.5.2.1 Biscuit Width

The width of biscuits was determined as per AACC (1995) method no. 10-15 D.

After baking, the biscuits were allowed to cool for 30 minute, on a mesh like structure. After that, 8 biscuits were placed edge to edge on a smooth platform such as the top of a sunmica table. The length of all 8 biscuits together was measured as “width” and recorded in mm. Each biscuit was rotated at 90^0 angle and again width was measured. Average of 8 biscuits was calculated on both occasions and the final average was considered as the width of the individual biscuit.

3.5.2.2 Biscuit thickness

The thickness of biscuit was determined as per AACC (1995) method no. 10-15 D.

After baking, the biscuits were allowed to cool for 30 minute, on a mesh like structure. After that, 8 biscuits were stacked one on top of another and height of all 8 biscuits together was measured as “thickness” using a vernier calliper and recorded in mm. The biscuits were re-stacked in a different order and again measured for thickness.

Average of 8 biscuits was calculated on the both the occasions and the final average was considered as width of individual biscuit.

3.5.2.3 Spread Ratio

The spread ratio of the biscuit was calculated as per the formula of AACC (1995) method no. 10-15 D.

$$\text{Spread ratio} = \frac{\text{Biscuit width (mm)}}{\text{Biscuit thickness (mm)}}$$

3.5.2.4 Spread Factor

The spread factor of biscuit was calculated using the following formula suggested by Awasthi et al. (1999).

$$\text{Percent spread factor} = \frac{\text{Spread ratio of biscuit prepared from blend}}{\text{Spread ratio of biscuit prepared from control}} \times 100$$

3.5.2.5 Weight

Average weight of randomly selected 8 biscuits was taken.

3.5.3 Textural properties

The three-beam technique was used to assess textural quality (hardness and fracturability) of the biscuits (Lyon et al 2003). Both properties of the biscuits were measured with the help of 3-Point Bending Rig (HDP/3PB) using 5kg load cell Heavy Duty Platform (HDP/90) using TA.XT2 texture analyzer of Stable Micro Systems (SMS).

<u>Settings</u>: Mode: Measure Force in Compression
Option: Return to Start
Pre-Test Speed: 1.0 mm/s
Test Speed: 3.0 mm/s
Post-Test Speed: 10.0 mm/s
Distance: 5 mm
Trigger Force: Auto - 50g
Data Acquisition Rate: 500pps

Test Set-Up:

Two adjustable supports of the rig base plate were placed at suitable distance (35mm) apart so as to support the sample. The distance was noted and kept constant through out the study. The base plate was then secured onto the Heavy Duty Platform. The Heavy Duty Platform was maneuvered and locked in a position that enables the upper blade to be equidistant from the two lower supports. The sample was placed centrally over the supports just prior to testing. The test was run by using key-board or software program after setting all the parameters as given above. After breakdown of the biscuit due to the pressure developed by the probe, the results for the two properties (hardness and fracturability) were saved/noted. Then the pieces of biscuit were removed and test was run for the second sample.

3.5.4 Nutritional Composition

3.5.4.1 Moisture

Moisture content was determined by evaporation (loss on drying) method according to AOAC (1980).

A known weight of food sample (5g) was dried to a constant weight in an oven at 101-105^{0}C overnight using coded clean crucible and the loss of weight was equated to the moisture content of the food after cooling the crucible in a desiccator. Where overnight drying has not been undertaken the drying process was repeated for further 30 minute period until successive weighing differed by less than 0.1% of the original mass of food sample was obtained.

Calculation:

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where,

W_1 = Initial weight of empty crucible

W_2 = Weight of crucible + food sample before drying

W_3 = Final weight of crucible + food sample after drying

% Total solids (Dry matter) = 100 - % Moisture

3.5.4.2 Protein

Nitrogen estimation was carried out by micro-Kjeldahl method (Oser 1976). Then nitrogen was converted into protein.

A known amount of sample, i.e. about 0.5 g of foodstuff, was digested with 10 ml of concentrated sulphuric acid along with a pinch of digestion mixture. After digestion volume was made up to 100 ml with glass-distilled water. A known amount of digested sample was then taken in Kjeldahl flask together with 10 ml of 50 % NaOH and 10 ml of distilled water and it was distilled for 10 minute to liberate the ammonia from the sample. The liberated ammonia was collected in 5 ml of 2 % boric acid containing mixed indicator. The ammonium borate solution thus formed was then titrated with 0.02 N sulphuric acid to a purplish-grey end-point. A blank was run in a similar manner without the sample using distilled water only.

Calculation:

$$\text{Total nitrogen content \% (w/w)} = \frac{V}{W} \times 0.0014 \times 100$$

Where,

V = Volume of 0.1 N H_2SO_4 (ml)

W = Wt. of sample (g)

Protein content = % Total nitrogen x 6.25

3.5.4.3 Fat

Fat estimation was carried out by gravimetric solvent extraction procedure using to Soxhlet method. This involved extracting the fat from the food with minimum exposure to heat and light using an appropriate solvent, removing the solvent and weighing the residue, i.e. the fat.

About 5 g of well ground dried sample was taken in an extraction thimble. The thimble was placed in the extractor of the Soxhlet apparatus and was connected to a weighed flask containing 1½ cycle petroleum ether. This was in turn connected to a reflux condenser. The sample was extracted under reflux for 4-5 hour (around 15-16 cycles) and the petroleum ether extract was evaporated to dryness. The flask containing the fat was dried in a hot air oven at 60-70^{0}C for few hour, cooled in a desiccator and was weighed.

Calculation:

$$\text{Fat \%} = \frac{[\text{Wt. of flask + fat (g)}] - [\text{Wt. of empty flask (g)}]}{\text{Wt. of sample in (g)}} \times 100$$

3.5.4.4 Carbohydrate

Total carbohydrate was estimated by the difference method (Narender et al 2007).

3.5.4.5 Ash

Ash content was estimated by the method of AOAC (1984). The total mineral content of a food was estimated as the ash content, which is the inorganic residue remaining after the organic matter has been burnt away.

About 4 to 6 g of the food was weighed accurately into previously ignited, cooled and weighed crucible. That was heated gently over a burner until the food was charred. The crucible was transferred to a muffle furnace at about 550^{0}C and left until a white or light gray ash resulted. If the residue was black in colour, it was moistened with a small amount of water, to dissolve salts, dried in an oven and the ashing process was repeated.

Calculation:

$$\text{Ash \%} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where,

W_1 = Initial weight of empty crucible

W_2 = Weight of crucible + food sample before ashing

W_3 = Final weight of crucible + ash

3.5.4.6 Energy value

Energy value of the biscuit was calculated as per Atwater general factor system (FAO report 2003).

3.6 Sensory evaluation

A sensory judging panel with six panellists from among the faculty members of the institute was constituted. The experimental samples were served to the judges. The panellists were instructed how to rate each sample on the composite scoring test as suggested by Ranganna (1986). The proforma for scoring used was prepared on the basis of the proforma prescribed by CFTRI, Mysore. The ratings were converted into scores. Biscuits were analysed for sensory attribute on the next day of preparation. The panellists evaluated volume, crust colour and surface character, crumb colour, crumb texture, mouth feel, taste and aroma and overall acceptability.

3.7 Statistical analysis

The standard SPSS programme was run to analyze the data. All the data were tested for significance using the ANOVA and two way completely randomized design (CRD) among means of various parameters (Steel and Torrie 1980). Data for nutritional and storage study were tested for significance using t – Test.

3.8 Cost analysis

The method adopted for economic analysis was as per Kamaliya (2005). Basic cost of raw ingredients used for product preparation was calculated on the current cost at the time of preparation of the raw ingredients in the wholesale commercial market. For calculations of cost price, 50% of basic cost was added as overhead charges to this and the total cost was considered as the production cost. The selling price was calculated by adding 25% profit to production cost. The experimental product could be sold easily at a premium rate. Therefore, a premium-selling price was calculated with additional marginal premium charges of just 10% more than normal selling price and expressed as percent of extra income gained as compared to the control product.

CHAPTER IV

RESULTS AND DISCUSSION

This section delineates the results obtained from various experiments carried out during investigation. The data generated were statistically analyzed and the results are discussed and interpreted for certain conclusions.

The present study was planned to develop low fat biscuit using two fat replacers. In the study fat content of biscuit was standardized. Fat (Shortening) was replaced with different levels of maltodextrin and whey protein concentrate (WPC) i.e. 40 to 70% (in multiple of 10%) of fat content. Five fat replacement ratios (i.e., 100:0, 75:25, 50:50, 25:75 and 0:100::MD:WPC) and control were taken within each fat replacement level. Three replications of biscuit preparations were carried out for the analysis.

Fat content of WPC, shortening and refined wheat flour (RWF) were analyzed. RWF was also analyzed for the physico-chemical properties. The sensory attributes, physical and rheological properties of control and the developed biscuits were studied. The biscuit found sensorily acceptable was further analyzed for nutritional composition and also used for storage study. Sensory and rheological analysis of the selected experimental biscuit as compared to control were carried out during storage study.

4.1 Fat content of WPC, shortening and refined wheat flour

The present study was planned to develop biscuits with low fat content. Therefore, the major two ingredients (i.e. shortening and refined wheat flour) which could affect the fat content of the biscuits were analyzed for fat content using gravimetric solvent extraction procedure (Soxhlet method). The values of three replications obtained are presented in table 4.1.

Fat content of shortening and refined wheat flour was found 93.88±0.28 and 1.05±0.115 percent, respectively. Value of fat content of WPC was considered 6.0 percent as per the certificate provided by the manufacturing multinational company.

4.2 Physico-chemical properties of refined wheat flour

Physico-chemical properties of RWF affect the physical properties of biscuits prepared from it. Therefore, RWF was analyzed for water absorption power (WAP), wet gluten, dry gluten, sedimentation value and alkaline water retention capacity (AWRC). The analysis was carried out in three replications and values obtained are presented in Table 4.2.

4.2.1 Water absorption power of RWF

Water plays an important role in bakery production, because during processing, water is the main factor in controlling the rheological properties of the dough. Moreover, its presence in the baked products influences on their palatability, freshness and keeping quality. Hence it is necessary to determinate the WAP (Kamalaiya and Kamaliya 2001). WAP of RWF was 61.63±1.11 percent.

4.2.2 Wet and dry gluten content of RWF

Gluten is responsible for the rheological properties of dough because it forms the skeleton of the dough and ultimately formats the structure of the baked products. Cookies do not require strong gluten. Refined wheat flour contained 33.26±0.50 and 11.53±0.31 percent wet and dry gluten, respectively which were more or less similar as reported by Pawshe (2008).

4.2.3 Sedimentation value of RWF

Sedimentation value used to estimate the combined effect of gluten quantity and quality (Kamaliya 2005). The sedimentation value

Table 4.1 Fat content of the ingredients

Ingredients	Fat (%)
Whey Protein Concentrate	6.00*
Shortening	93.88±0.28
Refined Wheat Flour	1.05±0.115

* As per the certificate provided by the manufacturing company

Values are mean score of 3 replications

Table 4.2 Physico-chemical properties of the refined wheat flour

Property	Value
WAP (%)	61.63±1.11
Wet gluten (g %)	33.26±0.50
Dry gluten (g %)	11.53±0.31
Sedimentation value (ml)	22.30±0.38
Alkaline water retention capacity (%)	69.47±0.37

Values are mean score of 3 replications

for RWF was found to be 22.30±0.38 ml as similar to results obtained by Tripathi (2003).

4.2.4 Alkaline water retention capacity of RWF

AWRC applicables to predicting flour quality for sugar-snap cookies, which are made slightly alkaline by leavening agents (AACC 1995). It varies with type of flour (Kamaliya 2005). The alkaline water retention capacity of refined wheat flour was observed to be 69.47±0.37 percent which was similar to the results reported by Tripathi (2003).

4.3 Sensory evaluation of the biscuits

The success of any modified food depends on the sensory acceptability and their similarity to the original product. Most consumers are not ready to change their taste. Therefore, reduced fat products need to be at least as acceptable as the full-fat ones even though they may have different taste. Thus, sensory evaluation of the biscuits was conducted using composite scoring test by a panel of 6 judges to study the effect of fat replacers on the sensory characteristics of the biscuits and select one experimental biscuit for further study.

4.3.1 Effect of the fat replacers on the volume of the biscuits:

The first characteristic to catch the eyes of customer is volume (Kamaliya and Kamaliya 2001). Therefore, mean values of the volume of the biscuits prepared using different fat replacement levels (i.e., 40, 50, 60 and 70 %) in varied ratios (i.e., 100:0, 75:25, 50:50, 25:75 and 0:100) of two fat replacers (MD and WPC) and of the control biscuit were scored by panel of experts and presented in the table 4.3. Results showing the effect of fat replacers on biscuit volume are presented in figure 4.1 and represented photographically in plate 4.1 and 4.2.

Statistical analysis of the volume of the biscuits shows that none of the biscuits were significantly differ ($p \leq 0.05$) from each other at all fat replacement levels while there was no significant difference ($p \leq 0.05$) between control and 100% MD containing biscuit at all levels of fat replacement. Figure 4.1 shows that volume of all the experimental biscuits gradually increased with increase in amount of WPC for each fat replacement level which is similar to the physical attributes of the biscuits, i.e., thickness. The reason is foaming properties of WPC which helps in producing nicely shaped product and secures a good oven rise (Brueckner and Jayaprakasha 1999). It was observed that biscuit prepared with 100% MD scored lower than the biscuit prepared with 100% WPC at each fat replacement level.

Table 4.3 Sensory score of volume of biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	6.664	6.665	6.669	6.670	6.667[a]
100:0	6.361	6.639	6.833	6.194	6.507[a]
75:25	7.056	7.000	7.333	7.000	7.097[b]
50:50	7.250	7.278	7.556	7.083	7.292[bc]
25:75	7.417	7.667	7.639	7.583	7.576[cd]
0:100	7.778	8.028	7.750	7.444	7.75[d]
Overall mean of FR level @	7.088	7.213	7.296	6.995	GM= 7.148
SEM (mean)	0.268				
SEM (Overall mean of FR ratio)	0.134				
SEM (Overall mean of FR level)	0.109				
SEM (GM)	0.055				
F value (Overall mean of FR ratio)	13.53*				
F value (Overall mean of FR level)	1.483[NS]				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean, NS: Statistically not significant

@ Means bearing the same superscript within the column/raw do not differ significantly ($p \leq 0.05$)

Values are mean score of a composite scoring test by a panel of 6 judges x 3 replications

* ($p \leq 0.05$)

Maximum score: 10

Sensory score for each ratio was gradually increased up to peak value and fallen thereafter gradually.

4.3.2 Effect of the fat replacers on the crust colour and surface character of the biscuits:

Crust colour and surface character is another attribute of the biscuit which catches eyes of the customer. Thus the crust colour and surface character of all the experimental and control biscuits were evaluated by panel of judges and mean values of score given by them are presented in the table 4.4. Results showing the effects of fat replacers on same attribute presented graphically in the figure 4.2 and photographs for the same are represented in plate 4.3 and 4.4.

All experimental biscuits scored higher compared to control at 40, 50 and 60% fat replacement level at all the ratios except 75:25::MD:WPC at 40% fat replacement level. The improved scores for colour and appearance could be due to the Maillard reaction (Narendra et al 2007).

The surface characteristic of the biscuits observed the best at 50:50::MD:WPC though the score was lower. Values of crust colour and surface character of the experimental biscuits at 50% and 60% fat replacement levels were significantly differ ($p \leq 0.05$) to the biscuits at 40 and 70% fat replacement levels. Control biscuit was significantly differ at all fat replacement ratios except 0:100::MD:WPC. However, all the biscuits with different fat replacement ratios do not significantly differ ($p \leq 0.05$) to each other in crust colour and surface character attributes. Similar observations were made by Armbrister and Sester (1994) when they evaluated cookies for texture and appearance attributes.

Crust colour and surface character of the biscuit prepared with 100% MD scored higher compared to 100% WPC containing samples at all fat replacement levels. Panel of the judges noted that surface character of the biscuit prepared with the least ratio of WPC was better and further improved with decreased amount of WPC. Biscuit

Table 4.4 Sensory score of crust colour and surface character of biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	6.968	6.970	6.974	6.976	6.972[a]
100:0	7.250	7.639	7.500	6.833	7.306[b]
75:25	6.806	8.000	7.472	6.944	7.306[b]
50:50	7.472	7.528	7.472	7.417	7.472[b]
25:75	7.722	7.361	7.222	7.222	7.382[b]
0:100	7.167	7.556	7.194	6.778	7.174[ab]
Overall mean of FR level @	7.231[ab]	7.509[c]	7.306[bc]	7.028[a]	GM= 7.269
SEM (mean)	0.225				
SEM (Overall mean of FR ratio)	0.113				
SEM (Overall mean of FR level)	0.092				
SEM (GM)	0.046				
F value (Overall mean of FR ratio)	2.431*				
F value (Overall mean of FR level)	4.686*				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean

@ Means bearing the same superscript within the column/raw do not differ significantly (p ≤ 0.05)

Values are mean score of a composite scoring test by a panel of 6 judges x 3 replications

* (p ≤ 0.05)

Maximum score: 10

made with 75:25::MD:WPC at 50% fat replacement level scored the highest while biscuit made with 0:100::MD:WPC at 70 % fat replacement level scored the lowest among all the biscuits prepared.

4.3.3 Effect of the fat replacers on the crumb colour of the biscuits:

Harmony between crust and crumb colour attracts the customer. Lively, lustrous, clean and uniform colour is desirable for biscuit. Thus the mean values of sensory score given by a panel of experts to the crumb colour of the experimental and control biscuits are presented in the table 4.5 and depicted in figure 4.3.

Sensory evaluation showed that the control biscuit had creamy white crumb colour which is significantly differ ($p \le 0.05$) to all the experimental biscuits at all the ratios but no significant difference ($p \le 0.05$) was observed among the experimental biscuits themselves. Sensory score for each ratio was gradually increased up to peak value and fallen thereafter gradually. Among all experimental biscuits, biscuit with 75:25::MD:WPC ratio scored the highest 7.833±0.239 and the lowest 7.000±0.23 at 50 and 70% fat replacement level, respectively.

4.3.4 Effect of the fat replacers on the crumb texture of the biscuits:

The mean values of the crumb texture and surface character of all the experimental biscuits and the control biscuit are given in the table 4.6. Results showing the effects of fat replacers on same attribute presented graphically in the figure 4.4.

All the fat replacement levels do not differ significantly ($p \le 0.05$) to each other while control biscuit was significantly differ ($p \le 0.05$) to the experimental biscuits at all the ratios except 50:50::MD:WPC. Similar observations were made by Armbrister and Sester (1994) while preparing cookies. Among all the biscuits, 50:50::MD:WPC at 70 % level of fat replacement scored the highest (15.056±0.478) while

Table 4.5 Sensory score of crumb colour of biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	6.888	6.886	6.890	6.892	6.889^{b}
100:0	7.306	7.472	7.583	7.028	7.347^{a}
75:25	6.917	7.833	7.583	7.000	7.333^{a}
50:50	7.500	7.528	7.361	7.250	7.410^{a}
25:75	7.667	7.361	7.333	7.194	7.389^{a}
0:100	7.444	7.694	7.333	7.028	7.375^{a}
Overall mean of FR level @	7.287^{ab}	7.463^{b}	7.347^{b}	7.065^{a}	GM= 7.291
SEM (mean)	0.239				
SEM (Overall mean of FR ratio)	0.119				
SEM (Overall mean of FR level)	0.097				
SEM (GM)	0.049				
F value (Overall mean of FR ratio)	2.771*				
F value (Overall mean of FR level)	2.945*				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean

@ Means bearing the same superscript within the column/raw do not differ significantly ($p \leq 0.05$)

Values are mean score of a composite scoring test by a panel of 6 judges x 3 replications

* ($p \leq 0.05$)

Maximum score: 10

Table 4.6 Sensory score of crumb texture of biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	14.890	14.892	14.888	14.887	14.889[b]
100:0	13.167	14.444	14.000	13.556	13.792[a]
75:25	14.056	14.111	13.833	13.389	13.847[a]
50:50	14.444	14.111	13.889	15.056	14.375[ab]
25:75	14.111	14.722	14.111	13.778	14.181[a]
0:100	14.111	14.389	14.444	13.056	14.000[a]
Overall mean of FR level @	14.130	14.444	14.194	13.954	GM= 14.181
SEM (mean)	0.478				
SEM (Overall mean of FR ratio)	0.239				
SEM (Overall mean of FR level)	0.195				
SEM (GM)	0.098				
F value (Overall mean of FR ratio)	2.921*				
F value (Overall mean of FR level)	1.084[NS]				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean, NS: Statistically not different

@ Means bearing the same superscript within the column/raw do not differ significantly ($p \leq 0.05$)

Values are mean score of a composite scoring test by a panel of 6 judges x 3 replications

* ($p \leq 0.05$)

Maximum score: 20

control biscuit scored the higher among all the fat replacement ratios at each fat replacement level except 70% fat replacement level. A trend similar to the crumb colour attribute was observed that sensory score for each ratio was gradually increased up to peak value and fallen thereafter gradually.

Panel of judges stressed some points which were similar to results obtained by other researchers. Reduction of fat had most significant effect over crispness of biscuit, as it become hard (Rao and Srivastava 1993), give a product with a chewy texture and a low moisture content (Oreopoulou et al 2002) and nontraditional snap characteristics (Lyon et al (2003). The bite of the experimental sample was slightly hard as would be expected from elimination of shortening but the product broken down easily during mastication (Dubey 2003).

4.3.5 Effect of the fat replacers on the mouthfeel of the biscuits:

Problems associated with low fat or no fat food having fat replacers are vast which include: lack of fat like lubricity; loss of viscosity and body due to low solids; moisture loss in absence of fat; poor flavour release etc. as explained by Kaur and Sroan (2003). Thus, the mouthfeel of the biscuits prepared using MD and WPC fat repalcers at different levels at varied ratios and of the control biscuit were evaluated and their mean values are given in the table 4.7.

The values of mouthfeel of the biscuits gradually decreased with decrease in amount of MD at all fat replacement levels except the 50:50 ratio at 70% fat replacement level which was the highest among all the experimental biscuits. The results indicate that only MD containing experimental biscuits scored more than only WPC containing samples. The score gradually higher with increased amount of MD in the ratios at each fat replacement level. Most of the ratios at the 50% fat replacement scored better than the other fat replacement levels. Increase in amount of WPC in the ratio at each fat replacement level decreased mouthfeel score. These biscuits appeared to be very hard and unpleasant to eat.

Table 4.7 Sensory score of mouthfeel of biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	7.637	7.639	7.639	7.641	7.639^{a}
100:0	7.083	7.389	7.389	7.389	7.313^{ab}
75:25	6.861	7.139	7.111	7.194	7.076^{b}
50:50	6.694	6.778	7.056	7.444	6.993^{b}
25:75	6.611	6.694	6.806	6.417	6.632^{c}
0:100	6.417	6.639	6.417	5.667	6.285^{d}
Overall mean of FR level @	6.884	7.046	7.069	6.958	GM= 6.990
SEM (mean)	0.242				
SEM (Overall mean of FR ratio)	0.121				
SEM (Overall mean of FR level)	0.099				
SEM (GM)	0.049				
F value (Overall mean of FR ratio)	15.79*				
F value (Overall mean of FR level)	0.738^{NS}				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean, NS: statically not significant

@ Means bearing the same superscript within the column/raw do not differ significantly ($p \leq 0.05$)

Values are mean score of a composite scoring test by a panel of 6 judges x 3 replications

* ($p \leq 0.05$)

Maximum score: 10

4.3.6 Effect of the fat replacers on the taste and aroma of the biscuits:

Taste is the most desirable sensory attribute in bakery and confectionary group of food products. Any manipulations that reduce the fat level of cookies will result in diminished acceptance. Therefore, taste and aroma of all the experimental biscuits and the control biscuit were evaluated by judges and score given by them are presented in the table 4.8. Results showing the effects of fat replacers on biscuit taste and aroma are shown in figure 4.6.

The control cookie had significantly more vanilla-like flavour than the other variations and significantly more sweet aromatics than any of the cookies using fat replcers (Armbrister and Sester 1994). Results of present investigation indicate that the biscuits prepared with 50:50 and 100:0 ratio of MD:WPC at 70% fat replacement level scored the highest and the lowest, respectively, among all the biscuits. The values of this property of the biscuits gradually decreased with increase in amount of WPC at each level of fat replacement except the 50:50 ratio at 70% fat replacement level which was the highest among all the biscuits including control. When other reserachers used fat replacers in cookies at levels greater than 50%, similar reductions in flavour acceptability were observed (Baldree et al (2002); Lyon et al (2003)). The low scores for the flavour of biscuits incorporated with WPC could be due to its flavour binding capacity (Narendra et al 2007).

Flavours in food are either lipid-soluble or water-soluble. Fat acts as carrier of lipid-soluble flavours (by dissolving them and reducing their vapour pressure, thus stabilizing flavour profile) and also as precursor for flavour development (as during frying or lipolysis). Therefore, replacement of fat in food results in lack of flavour medium, as fat mimetics are capable of carrying water-soluble compounds only, thus many fat-soluble flavours may stand out (Kaur and Sroan 2003). Secondly, all variations of the shortening reduced

Table 4.8 Sensory score of taste and aroma of biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	22.917	22.915	22.917	22.919	22.917[b]
100:0	21.750	22.250	22.500	22.167	22.167[ab]
75:25	20.750	22.083	21.500	22.083	21.604[a]
50:50	20.250	21.583	21.333	23.000	21.542[a]
25:75	19.417	20.250	20.167	19.750	19.896[c]
0:100	18.917	19.917	19.167	17.000	18.750[d]
Overall mean of FR level @	20.667[a]	21.500[b]	21.264[ab]	21.153[ab]	GM= 21.146
SEM (mean)	0.697				
SEM (Overall mean of FR ratio)	0.348				
SEM (Overall mean of FR level)	0.284				
SEM (GM)	0.142				
F value (Overall mean of FR ratio)	19.54*				
F value (Overall mean of FR level)	1.521*				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean

@ Means bearing the same superscript within the column/raw do not differ significantly ($p \le 0.05$)

Values are mean score of a composite scoring test by a panel of 6 judges x 3 replications

* ($p \le 0.05$)

Maximum score: 30

cookies also had significantly less caramelized flavour than did the control (Armbrister and Sester 1994).

4.3.7 Effect of the fat replacers on the overall acceptability of the biscuits:

The mean values of the overall acceptability of the biscuits prepared using different fat replacement levels (i.e., 40, 50, 60 and 70 %) in varied ratios (i.e., 100:0, 75:25, 50:50, 25:75 and 0:100) of two fat replacers (MD and WPC) and of the control biscuits are given in the table 4.9.

There is no significant effect of fat replacement level on the overall acceptability of the experimental biscuits. Figure 4.7 shows that 100% MD containing samples more acceptable than the 100% WPC at all the fat replacement levels. Another finding from the graph was that score among the samples containing fat replacers was decreased for first ratio followed by the control, decreased for the second ratio (except the fat replacement level 70%), increased for the next ratio and finally decreased one by one for the last two ratios among all the four fat replacement levels except the fat replacement level at 50% in which score continuously decreased from the first ratio to the last.

Among all the levels, 50:50 ratio scored the highest except 50% fat replacement level in which it scored the third highest. This indicates that score of overall acceptability decreased with increase in amount of WPC for all the ratios other than 50:50 ratio at all the fat replacement levels. Score for the 50:50 ratio gradually increased with increased fat replacement level and scored highest (7.469±0.241) at 70% fat replacement level.

The results obtained for sensory characteristics reflected that, the maximum level of fat replacement that could be used to obtain an acceptable quality of low fat biscuit was 70% and 50:50::MD:WPC among ratios. On this basis this sample was selected for nutritional analysis and storage study.

Table 4.9 Sensory score of overall acceptability of biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	7.361	7.362	7.360	7.361	7.361[b]
100:0	7.069	7.447	7.306	7.119	7.235[ab]
75:25	6.961	7.322	6.736	7.125	7.036[ab]
50:50	7.125	7.278	7.375	7.469	7.312[ab]
25:75	7.000	7.131	7.094	6.839	7.016[a]
0:100	6.681	7.056	6.728	6.222	6.672[c]
Overall mean of FR level @	7.033	7.266	7.100	7.023	GM= 7.105
SEM (mean)	0.241				
SEM (Overall mean of FR ratio)	0.121				
SEM (Overall mean of FR level)	0.098				
SEM (GM)	0.049				
F value (Overall mean of FR ratio)	4.482*				
F value (Overall mean of FR level)	1.301[NS]				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean, NS: Statistically not differenet

@ Means bearing the same superscript within the column/raw do not differ significantly ($p \leq 0.05$)

Values are mean score of a composite scoring test by a panel of 6 judges x 3 replications

* ($p \leq 0.05$)

Maximum score: 10

4.4 Physical characteristics of biscuits

Physical properties are important for selecting packaging material and package designing. In addition to the sensory qualities, physical properties of biscuit also help to judge consumer acceptability. Therefore, in this study width, thickness and weight were measured while spread factor and spread ratio were calculated as per formula to study the effect of fat replacers and discussed in this section.

4.4.1 Effect of the fat replacers on the width of the biscuits:

The diameter of the baked cookie is important in commercial bakeries because the finished product must fit a previously designed container (Bailey 1996 a). Therefore, the width of control and all the experimental biscuits were measured and their mean values are expressed in the table 4.10 and plotted in figure 4.8.

Biscuit containing 100 % MD scored the highest width among all the experimental biscuits at each level of fat replacement. The width was increased as the level of incorporation of MD was increased in experimental biscuits as similar to the results obtained by Savitha et al (2008). A reverse trend was observed when WPC was used for fat replacement as similar to Arendt et al (2005) except 70 % fat replacement level. Thus experimental biscuit prepared with 100% WPC at 40% fat replacement level scored the lowest width (42.83±1.027) among all the experimental biscuits. Biscuit containing 100% WPC was found much more similar in width to the control than the other experimental biscuits at all the levels of fat replacement.

There might be two probable reasons for lower spread. First, the lower level of fat incorporating lesser air, results in higher dough viscosity, logically outcome in larger cookie diameter (Brijs 2009). Second important factor is the lower glass transition temperature which occurred just before dough expansion and resulted in cookies with greater spread, as explained by Armbrister and Sester (1994).

Table 4.10 Width of the biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	43.106	43.107	43.108	43.107	43.107[a]
100:0	46.960	48.273	48.440	48.603	48.069[c]
75:25	46.563	45.793	44.417	46.710	45.871[b]
50:50	44.980	45.793	46.210	44.793	45.444[b]
25:75	44.607	45.603	44.337	43.397	44.486[ab]
0:100	42.833	44.440	43.127	43.457	43.464[a]
Overall mean of FR level @	44.842	45.502	44.939	45.011	GM= 45.073
SEM (mean)	1.027				
SEM (Overall mean of FR ratio)	0.514				
SEM (Overall mean of FR level)	0.419				
SEM (GM)	0.210				
F value (Overall mean of FR ratio)	12.55*				
F value (Overall mean of FR level)	0.491[NS]				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean, NS: Statistically not different

@ Means bearing the same superscript within the column/raw do not differ significantly ($p \leq 0.05$)

Values are mean score of 3 replications

* ($p \leq 0.05$)

4.4.2 Effect of the fat replacers on the thickness of the biscuits:

The height of the biscuit is another crucial factor effecting on proper packaging, depending upon the type of packaging equipment used. The amount of shortening (relative to flour) in the dough affect diameter and height (Bailey 1996 a). Therefore, mean values of the thickness of all the experimental biscuits and the control biscuit are given in the table 4.11. Results showing the effects of fat replacers on biscuit's thickness presented graphically in the figure 4.9.

Biscuit prepared with 100% WPC at maximum level of replacement scored the highest (3.96±0.144) thickness while biscuit containing 100% MD at 60% replacement level scored the lowest (2.25±0.144) among all the experimental biscuits. Thickness of the biscuits gradually increased with increasing level of WPC through ratio while reversed trend was observed for the MD. Results of study carried out by Brijs et al (2009) supports results obtained in the present study. They conclude that, increasing fat contents correlated linearly with increasing cookie diameter and as a consequence with decreasing cookie height.

Arendt et al (1999) concluded that, even though the production process, namely sheeting height and disc diameter were the same for all biscuits, the thickness of the final product varied significantly during fat replacement. Armbrister and Sester (1994) produced a thin batter and a flat thin cookie when fat replaced with polydextrose. They also concluded that, even though each of the replacers is considered hygroscopic, how the water was held and its ability undoubtedly influenced the spread and other textural properties.

4.4.3 Effect of the fat replacers on the spread ratio of the biscuits:

As per the definition, spread ratio directly depends upon the width and thickness of the biscuit. Both properties were varied according to amount and type of fat replacers. Therefore, spread ratio of control and experimental biscuits were calculated and their mean

Table 4.11 Thickness of the biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	2.397	2.400	2.399	2.400	2.400^{a}
100:0	2.440	2.397	2.253	2.393	2.371^{a}
75:25	2.787	2.730	2.623	2.773	2.728^{b}
50:50	2.993	3.037	2.853	2.960	2.961^{c}
25:75	3.367	3.297	3.480	3.450	3.398^{d}
0:100	3.717	3.397	3.573	3.960	3.662^{e}
Overall mean of FR level @	2.951	2.876	2.864	2.989	GM= 2.92
SEM (mean)	0.144				
SEM (Overall mean of FR ratio)	0.072				
SEM (Overall mean of FR level)	0.059				
SEM (GM)	0.029				
F value (Overall mean of FR ratio)	53.38*				
F value (Overall mean of FR level)	1.04^{NS}				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean, NS: Statistically not different

@ Means bearing the same superscript within the column/raw do not differ significantly ($p \leq 0.05$)

Values are mean score of 3 replications

* ($p \leq 0.05$)

values are depicted in the table 4.12. Results showing the effect of fat replacers on biscuit spread ratio are presented in figure 4.10.

The spread ratio for biscuit containing 75:25::MD:WPC ratio was much more similar to the control biscuit as compared to other ratios at each level of fat replacement. Biscuit prepared with 100% MD scored the highest among all the ratios at each level of fat replacement while biscuit containing 100% WPC scored the lowest for spread ratio. Biscuit prepared with 100% MD at 60% fat replacement level scored the highest while the biscuit prepared with 100% WPC at 70% fat replacement level scored the lowest. Spread ratio recorded a gradual fall with reduction in fat level as similar to results reported by Rao and Srivastava (1993).

A trend was observed that spread ratio of all the experimental biscuits decreased with increase in amount of WPC and reverse trend was observed for MD among all the ratios at each level of fat replacement. This was result of increase in width of experimental biscuits. Leelavathi et al (2007) found that values for spread ratio were decreased with increase in amount of MD which is opposite to results obtained in the present study. This might be due to difference in method of biscuit preparation, i.e. rubbing method was used in the present study while Leelavathi et al (2007) used the creaming method. This may be related to competition between sugar and flour for the water available in the formula. In the creamed procedure, sugar particles are coated with fat and thus are impeded from solution. The sugar particles are not surrounded by fat in the non-creamed procedure or when using oil and they tend to dissolve more rapidly, leaving less chance for the flour to compete for the available water (Abboud et al 1985). Patel and Rao (1996) found that there was significant improvement in spread ratio of cookies with increasing amount of fat.

Rahim et al (2005) explaind the reason that spread ratio depends upon the dough viscosity. Dough viscosity is affected by the starch gelatinization. Delayed starch gelatinization reduces the dough

Table 4.12 Spread ratio of the biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	17.983	17.980	17.969	17.980	17.980[b]
100:0	19.337	20.127	21.490	20.513	20.367[c]
75:25	16.837	16.787	16.963	16.850	16.859 [b]
50:50	15.060	15.103	16.227	15.610	15.500[d]
25:75	13.403	13.860	12.757	12.627	13.162[a]
0:100	11.647	13.273	12.117	11.070	12.027 [a]
Overall mean of FR level @	15.711	16.188	16.256	15.775	GM= 15.982
SEM (mean)	0.880				
SEM (Overall mean of FR ratio)	0.440				
SEM (Overall mean of FR level)	0.359				
SEM (GM)	0.180				
F value (Overall mean of FR ratio)	49.35*				
F value (Overall mean of FR level)	0.604[NS]				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean, NS: Statistically not different

@ Means bearing the same superscript within the column/raw do not differ significantly ($p \leq 0.05$)

Values are mean score of 3 replications

* ($p \leq 0.05$)

viscosity and improves the spread of the cookies. This might be the reason why the biscuit prepared with increasing amount of MD achieved better spread. Secondly, high protein content of WPC increased the protein content of the dough and played a role in water binding and development of gluten network which increase in thickness (Manohar and Rao 1999 a) and may affect the spread of the biscuits.

4.4.4 Effect of the fat replacers on the spread factor of the biscuits:

The mean values of the spread factor of all the experimental and control biscuits are given in the table 4.13 and presented graphically in figure 4.11.

Spread factor is defined as the ratio of spread ratio of blend to the spread ratio of control which decreased with increasing amount of WPC in the formulation for each fat replacement level while the spread factor increased with an increase in MD as similar to the results obtained by Savitha et al (2008). It is observed that, the spread factor increased significantly as compared to control biscuit when MD alone was replaced to fat at different levels. This may be result of decrease in the viscosity of biscuit dough resulted in higher spread factor when MD was added to formulaltions (Savitha et al 2008). Biscuits prepared with 100% MD at 60% fat replacement level scored the highest among all the experimental biscuits while biscuits prepared with 100% WPC at 70% fat replacement level scored the lowest. This is exactly opposite to thickness and in consequence with definition.

4.4.5 Effect of the fat replacers on the weight of the biscuits:

Weight of biscuit is important in commercial bakeries to comply with government act of weight and measures as well as marketing point of view i.e., pre-designed container must contain similar number of biscuits of equal size. Therefore, biscuits were weighed and depicted as means in table 4.14. The same are presented in figure 4.12.

Table 4.13 Spread factor of the biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	100.00	100.00	100.00	100.00	100.000[b]
100:0	107.680	112.170	119.857	114.097	113.451[c]
75:25	94.170	93.327	94.387	93.923	93.952[b]
50:50	84.010	84.023	87.650	86.350	85.508[d]
25:75	75.057	76.980	71.153	70.213	73.351[a]
0:100	64.830	73.543	67.623	61.980	66.994[a]
Overall mean of FR level @	87.624	90.007	90.112	87.761	GM= 88.876
SEM (mean)	5.170				
SEM (Overall mean of FR ratio)	2.585				
SEM (Overall mean of FR level)	2.111				
SEM (GM)	1.055				
F value (Overall mean of FR ratio)	44.43*				
F value (Overall mean of FR level)	0.420[NS]				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean, NS: Statistically not different

* Significantly not different (p ≤ 0.05) within raw/column

@ Means bearing the same superscript within the column/raw do not differ significantly (p ≤ 0.05)

Values are mean score of 3 replications

* (p ≤ 0.05)

Table 4.14 Weight of the biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio@
Control	6.728	6.731	6.729	6.732	6.730^{a}
100:0	7.007	6.747	6.477	6.740	6.743^{a}
75:25	6.983	6.783	6.860	6.943	6.893^{a}
50:50	6.897	7.183	6.660	6.867	6.902^{a}
25:75	8.010	6.853	6.457	7.177	7.124^{ab}
0:100	7.860	7.270	6.900	7.577	7.402^{b}
Overall mean of FR level @	7.248^{b}	6.928^{ab}	6.681^{a}	7.006^{ab}	GM= 6.965
SEM (mean)	0.350				
SEM (Overall mean of FR ratio)	0.175				
SEM (Overall mean of FR level)	0.143				
SEM (GM)	0.071				
F value (Overall mean of FR ratio)	2.156*				
F value (Overall mean of FR level)	2.677*				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean

@ Means bearing the same superscript within the column/raw do not differ significantly ($p \leq 0.05$)

Values are mean score of 3 replications

* ($p \leq 0.05$)

Weight of all the experimental biscuits were more than the control except the biscuits containing 100:0, 50:50 and 25:75 (MD:WPC) ratio at 60% fat replacement level. Brijs et al (2009) reported that cookie weight decreased linearly with increased fat level in the recipe which was similar to results obtained during present study. Figure 4.12 shows that 100% WPC containing biscuits scored higher than the biscuits prepared with 100% MD at each fat replacement level. Similar trend was observed between 25:75::MD:WPC and 75:25::MD:WPC ratio for all the fat replacement levels except 60% fat replacement level.

The results obtained for physical characteristics indicate that, increase in WPC produced biscuits with higher thickness as compared to control, while increase in MD content resulted in increase in width, spread ratio and spread factor. These results indicate that effects of both replacers found helpful to increase the size of biscuits. That would be highly beneficial for marketing point of view.

4.5 Textural properties of biscuits

Texture of food relate to the mechanical work that occurs in food processing operations, as they do when they later interact in the consumer's mouth. For instance, during mastication, it is desirable to weaken the structure so that it will properly disintegrate when forces are applied. So two properties i.e., hardness and fracturability are measured using texture analyzer and discussed below.

4.5.1 Effect of the fat replacers on the hardness of the biscuits:

The maximum force necessary to break a biscuit describes the hardness or brittleness of a biscuit. The mean values of the hardness of the biscuits prepared with different fat replacement levels (i.e., 40, 50, 60 and 70 %) at varied ratios (i.e., 100:0, 75:25, 50:50, 25:75 and 0:100) of two fat replacers (MD and WPC) and of the control biscuit are given in the table 4.15 and presented graphically in figure 4.13. Figure 4.15 to figure 4.18 are the selected graphs automatically plotted in texture analyzer while analyzing textural properties of experimental and control biscuits. Plate 4.5 represents the photograph of texture analyzer and its action.

All the experimental biscuits were significantly ($p \leq 0.05$) different to the control at different ratios of fat replacement. Similar results were obtained by Rahim et al (2005) and Leelavathi et al (2007). The hardness value of the experimental biscuits changed due to decreased shortening action as stated by Patel and Rao (1996). Experimental biscuit prepared with 50:50 ratio of both the fat replacers scored the lowest hardness at the 40 and 50 % fat replacement levels while in case of 60 and 70% fat replacement levels, the same ratio scored the second highest followed to 25:75::MD:WPC ratio. A specific trend was observed that, hardness of the experimental biscuits containing 100% MD was lower than the experimental biscuits containing 100% WPC at all the levels of fat replacement. This is similar to the conclusion drawn by Arendt et al (2005) that, as the level of protein substitution

Table 4.15 Hardness of the biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	2534.391	2534.395	2534.392	2534.394	2534.393[b]
100:0	3953.127	3949.737	4027.303	4229.387	4039.888[a]
75:25	4052.697	4084.74	4551.007	4551.593	4310.009[a]
50:50	3510.807	3864.277	3837.003	4275.457	3871.886[a]
25:75	4078.613	4175.933	3775.06	3890.56	3980.042[a]
0:100	4069.397	4120.103	4159.017	4419.927	4192.111[a]
Overall mean of FR level @	3699.839	3788.197	3813.964	3983.553	GM= 3821.388
SEM (mean)	341.793				
SEM (Overall mean of FR ratio)	170.896				
SEM (Overall mean of FR level)	139.536				
SEM (GM)	69.768				
F value (Overall mean of FR ratio)	14.436*				
F value (Overall mean of FR level)	0.723[NS]				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean, NS: Statistically not different

@ Means bearing the same superscript within the column/raw do not differ significantly ($p \leq 0.05$)

Values are mean score of 3 replications

* ($p \leq 0.05$)

increased, peak force value also increased, i.e. the biscuit became harder.

Area under the curve in figure 4.15 to 4.18 integrates both maximum force required and time taken to penetrate in the biscuit. That represents hardness which increased when fat was replaced in the experimental biscuits. According to Oreopoulou (2002), the reduction of fat in the formulations lead to samples with higher σ_{max}, therefore resulted cookies were harder than control. Lyon et al (2003) also found that both reduced fat cookies were harder than the full fat counterpart cookies. Savitha et al (2008) found that the breaking strength values increased with increase in MD content of the biscuits.

The high protein content of WPC, the carbohydrate content of MD and the overall higher contents of powders in non-fat solids provide a plausible explanation: protein and sugar bind water and thus reduce the loss of water during baking. Shortening, on the other hand, is pure fat that contains no water binding substances. The protein in WPC may contribute to the formation of a stronger structure, and additionally, the sugars in all powders may bind water and thus contribute to dough hardness (Arendt et al 2003 c). Second factor is high gelling ability of milk proteins, holding recipe water, fat and other ingredients in a tight matrix, resulting in firmer biscuits as explained by Narendra et al (2007). These might be the reasons why the experimental biscuits prepared at different levels of fat replacement in different ratios are significantly different ($p \leq 0.05$) to the control.

4.5.2 Effect of the fat replacers on the fracturability of the biscuits:

Generally fracturability can be defined as the force with which the cookie shatters but here the distance at the point of break is the resistance of the sample to bend and so relates to the 'fracturability' of the sample (i.e. the mean distance compressed before breaking value is called fracturability as per the definition given by the equipment

Table 4.16 Fracturability of the biscuits prepared with different levels and ratios of fat replacers

FR level (%) / FR ratio (MD:WPC)	40	50	60	70	Overall mean of FR ratio @
Control	0.395	0.399	0.396	0.398	0.397[b]
100:0	0.500	0.470	0.507	0.503	0.495[a]
75:25	0.487	0.537	0.567	0.530	0.530[a]
50:50	0.463	0.517	0.547	0.493	0.505[a]
25:75	0.510	0.573	0.560	0.497	0.535[a]
0:100	0.480	0.487	0.500	0.637	0.526[a]
Overall mean of FR level @	0.473	0.497	0.513	0.509	GM= 0.498
SEM (mean)	0.038				
SEM (Overall mean of FR ratio)	0.019				
SEM (Overall mean of FR level)	0.015				
SEM (GM)	0.008				
F value (Overall mean of FR ratio)	7.577*				
F value (Overall mean of FR level)	1.377[NS]				

Where,

FR: Fat replacement, GM: Grand mean, SEM: Standard error of mean, NS: Statistically not different

@ Means bearing the same superscript within the column/raw do not differ significantly ($p \le 0.05$)

Values are mean score of 3 replications

* ($p \le 0.05$)

manufacturing company). The mean values for the fracturability of experimental and control biscuits are depicted in the table 4.16.

No biscuits with fat replacers simulated the textural attributes of the control biscuits. None of the variations differ significantly between levels. Biscuit containing 40 % fat replacement with 50:50::MD:WPC scored the lowest fracturability (0.463±0.038) while with 70 % fat replacement at the ratio of 0:100::MD:WPC scored the highest fracturability (0.637±0.038) among all the experimental biscuits. A trend was observed that, fracturability for the samples containing fat replacers was increased for first two ratios followed by the control, decreased for the third ratio, again increased for the next ratio and finally decreased among all the four fat replacement levels except 75:25::MD:WPC ratio for the 40 % fat replacement level and 0:100::MD:WPC ratio for the 70 % fat replacement level. The instrumental compression results were also in agreement with the results found by sensory evaluations.

It is possible that differences in fracturability resulted, in part, from differences in the water held in the system. Armbrister and Sester (1994) explained very well this fact in their study. This was probably related to two factors: 1) modification of sucrose crystallization and 2) altered water mobility and its holding-release behaviour. How and when the water is released in the oral cavity is influenced by the specific fat replacer and its interactions with the other ingredients. This factor determined, at least partially, the texture and appearance attributes of the experimental biscuits. Water associated with fat replacers generally created smoother and softer biscuits. How the water was held by the particular fat replacer could influence how much was free to dissolve the sugars present.

Other two factors had also played a role in fracturability. One was the development of complex gluten network when fat replacement was more and second was the thickness of the biscuits. This may be related to fat reduction, increased moisture content and allowing increased gluten development (Lyon et al 2003). Thicker biscuits take

more force (Manohar and Rao 1999 a), more compressed distance and more time just before the breaking. Physical parameters indicated that the thickness of all the experimental biscuits were higher than the control biscuits.

Rheological study of the biscuits indicates that all experimental biscuits were significantly differ to control for both characteristics at fat replacement ratios while no significant difference was observed among them at fat replacement levels. High level of fat replacement increased both hardness and fracturability significantly. Addition of fat replacers at 50:50 ratio scored the lowest at all the ratios.

4.6 Nutritional properties of the biscuits

4.6.1 Proximate analysis of the biscuits:

An experimental biscuit selected, i.e., 50:50::MD:WPC at 70 % fat replacement level, on the basis of sensory evaluation was analyzed along with control biscuit for proximate nutritional composition. The results obtained are depicted in table 4.17.

4.6.1.1 Fat content of the biscuits:

Fat content of biscuits was analyzed using automatic soxhlet apparatus (Auto-soxhlet). Photographs of the same as well as fat extracted during analysis are presented in plate 4.6. According to Mehta and Mehta (2005) low fat food contains 3 g fat per 50 g food sample if serving size is small. Results of the present study indicate that, fat content of control biscuit was 13.09±0.23% and reduced to 4.19±0.10% in the experimental biscuit. The selected experimental biscuit could be considered as low fat biscuit as per definition since it contain 2.245 g (less than 3 g) fat per 50g samples. Fat content was reduced 68% which means a major objective to reduce fat content was achieved successfully.

4.6.1.2 Moisture content of the biscuits:

Decrease in fat content automatically increases the quantity of the other nutritional constitutes of the biscuits. Moisture content in the control and experimental biscuits was observed 2.73±0.23 and 3.68±0.14%, respectively. That is similar to Klopfenstein et al (1995); Oreopoulou et al (2002); Srivastava and Rao (1993). Secondly, high water binding capacity of fat replacers had played role in increasing moisture content of the biscuits.

4.6.1.3 Protein content of the biscuits:

Protein content of the experimental biscuit (9.71±0.33%) was higher than that of control (7.66±0.13%). Similar increase in protein content was reported by Narender et al (2007). The increase in the

Table 4.17 Chemical composition of control and selected experimental biscuits

Product / Constitute	Control Biscuits (%)	Selected Experimental Biscuits (%)	Percent Increase
Moisture	2.73±0.23	3.68±0.14	34.79
P value	0.07766		-
Carbohydrate	75.56±0.12	81.22±0.36	7.49
P value	0.00301		-
Protein	7.66±0.13	9.71±0.33	26.76
P value	0.01190		-
Fat	13.09±0.23	4.19±0.10	(-67.99)
P value	0.00048		-
Ash	0.97±0.007	1.19±0.02	22.68
P value	0.02276		-

Values are mean score of 3 replications

Data analyzed by t-Test

protein content of WPC supplemented biscuits was due to the appreciably higher protein content (70 %) of WPC.

4.6.1.4 Carbohydrate content of the biscuits:

Carbohydrate content of control and selected experimental biscuit was 75.56±0.12 and 81.22±0.36 %, respectively. Observed increase in carbohydrate content was due to decreased fat content and increased MD content.

4.6.1.5 Ash content of the biscuits:

Ash content of the control and selected experimental biscuit was 0.97±0.007 and 1.19±0.02 %, respectively. The increase in ash content is obviously due to higher content of WPC which is source of mineral content (Narender et al 2007).

4.6.2 Energy value of the selected biscuits:

Calculated energy for the developed low fat biscuit found 401.43 Kcal/g which is more than required for the low calorie food as per the definition (Mehta and Mehta 2005). In the present study, low fat biscuit was not low calorie food and it is not necessary that all low fat food must be the low calorie food as explained by Mehta and Mehta (2005).

Equation for energy calculation is given below as per Atwater general factor system (FAO 2003).

$$\text{Energy} = (4 \times \text{carbohydrate (g)}) + (9 \times \text{fat (g)}) + (4 \times \text{protein (g)})$$

$$\text{Energy}_{(\text{Control})} = (4 \times 75.56) + (9 \times 13.09) + (4 \times 7.66)$$

$$= 450.69 \text{ Kcal/100 g}$$

$$\text{Energy}_{(\text{Experimental})} = (4 \times 81.22) + (9 \times 4.19) + (4 \times 9.71)$$

$$= 401.43 \text{ Kcal/100 g}$$

Nutritional analysis indicates that, selected experimental biscuit is low fat biscuit. Fat content was decreased by 68% which is major achievement of the study and fulfillment of the major objective.

4.7 Storage study of the biscuits

The shelf life of biscuits depend on its chemical composition, packaging material and environmental factors such as temperature, relative humidity and light. In general, biscuits have low moisture and significant amount of fat. Hence, they are prone to loss of crispness by absorption of moisture and development of rancidity due to oxidation. The rancidity of biscuits is accelerated by high temperature which can be controlled by using suitable packaging material (Chauhan et al 2000).

For the shelf life evaluation, control and the experimental biscuit selected on the basis of sensory evaluation were stored for one month at atmosphere condition. Metallised food bag was selected as packaging material because of its low water vapour transmission rate (WVTR). It also prevents aroma transfer. Both biscuits were analyzed for changes in sensory and rheological characteristics at each week of the storage period.

4.7.1 Organoleptic changes in the biscuits during storage:

The sensory evaluation of biscuits were carried out by a panel of six judges. The changes in volume, crust colour, crumb colour, crumb texture, taste and aroma, mouthfeel and overall acceptability of biscuits are given in table 4.18. Scores for all the sensory attributes of the control and experimental biscuits slightly decreased with increase in storage period. However, no undesirable changes were noticed in the biscuits after one month of storage which was similar to the results reported by Narendra et al (2007).

Chauhan et al (2000) reported that a moisture content of more than 7% (on dry weight basis) was critical with respect to the loss of crispness. In the present study, both (control and experimental) biscuits contained less than 7% moisture (table 4.17). That might be the reason for no noticeable changes found in the biscuit during storage.

Volume, crust colour and crumb colour of the experimental biscuits were scored higher than the control for all the weeks even though they are significantly not differ. Control biscuits scored higher than experimental biscuits for crumb texture, taste and aroma, mouthfeel and overall acceptability for all the weeks.

4.7.2 Rheological changes in the biscuits during storage:

The mean values of the rheological characteristics of the stored control and experimental biscuits prepared with 50:50::MD:WPC ratio at 70 % fat replacement level are given in the table 4.19.

Hardness value of both biscuits reduced gradually during storage period which is similar to trends reported by Chauhan et al (2000). From the first week, experimental biscuits scored significantly higher than the control for the hardness and found higher during complete storage period.

Fracturability of the control biscuits scored lower than the experimental biscuits at the beginning of the storage. Fracturability of the control biscuits was significantly differ ($p \leq 0.05$) than the experimental biscuits at each week. With increase in storage time, fracturability value of both the biscuits were decreased gradually.

Shelf life study of the biscuits reflected that, there was no significant difference found between control and experimental biscuits during storage. That means both samples were acceptable up to one month storage period.

4.8 Cost analysis of the selected biscuits

Cost of the control and selected experimental biscuit was calculated and depicted in table 4.20. The selling price of control and experimental biscuit was found 63.10 and 83.03 Rs./Kg, respectively and thus there was 31.58% extra gain by selling experimental biscuit as compared to control.

The biscuits prepared with 50:50::MD:WPC ratio at 70% fat replacement level was selected on the basis of sensory evaluation and were found acceptable up to one month. Cost is not much and could be recovered by selling it at premium price because of health benefits. Thus it could be promoted as functional food by industry.

Table 4.19 Rheological characteristics of control and selected experimental biscuit during storage

Week	Biscuit	Hardness (g)	Fracturability (mm)
Week 0	Control	2804.970±187.96	0.520±0.070
	Experimental biscuit	4555.167±0.103	0.623±0.043
	P value	0.000740270	0.277749727
Week 1	Control	2776.290±175.387	0.476±0.061
	Experimental biscuit	4555.163±0.101	0.613±0.049
	P value	0.000532027	0.156485932
Week 2	Control	2742.143±118.102	0.386±0.012
	Experimental biscuit	4571.613±0.876	0.546±0.014
	P value	0.000101384	0.001057565
Week 3	Control	2700.093±100.856	0.383±0.017
	Experimental biscuit	4569.107±0.139	0.536±0.048
	P value	0.000049900	0.040165432
Week 4	Control	2626.643±63.267	0.380±0.020
	Experimental biscuit	4566.203±1.411	0.513±0.013
	P value	6.7500E-06	0.005716532

Values are mean score of 3 replications
Data analyzed by t-Test

CHAPTER V

SUMMARY AND CONCLUSIONS

India is one of the key food producing countries in the world, second only to China. The total food production in India is expected to double in the next decade. India's food processing industry ranked fifth in terms of production, consumption, export and expected growth. There are many opportunities for large investments in food and food processing technologies, skills and equipments. Health food and health food supplements are the rapidly rising segments of this industry.

In recent years, in India, bakery products have become popular among different cross sections of population due to increased demand of convenience foods. It is one of the cheapest of the processed ready to eat product in India. Bakery products are highly potential for value addition and health promotion thus it could be a leading contributing sector for the development of food industry. Bakery industry in India is one of the largest food industries with an annual turnover of about Rs. 5000 Crore.

Biscuits were assumed as sick-man's diet in earlier days. Now, it has become one of the most loved fast food products for every age group. Biscuits are easy to carry, tasty to eat and reasonable at cost. India is the second largest producer of biscuits after USA. Soft dough biscuits account for over 70% of total production of biscuits in India. These types of biscuits normally contain high amounts of fat ranging from 15 to 25%. Due to its high cost, fat alone contributes to about 50% of total raw material cost.

Fats are the third largest components after flour and sugar in the bakery products. The presence of fat in food is associated with a wide range of textural characteristics from smooth and creamy to chewy, crunchy and brittle. Fat is still the number one nutritional concern for the most people because the continuous consumption of fat may lead to certain major health disorders such as cardiovascular diseases, obesity, hypertension, some type of cancer, high blood cholesterol etc.

To minimize the diseases mainly caused due to excess fat, it is necessary to develop low fat bakery products for the well being of the masses. To reduce the fat content, different type of fat replacer can be used for production of low fat bakery products.

In aforesaid views, the present study was planned to develop a nutritionally modified and low fat biscuit. The product thus produced could serve the following distinct purposes:

1. Provide low fat food to the community to improve health status of the public.
2. Availability of new functional bakery product due to presence of WPC.
3. Reduce the cost of new product for bakery industries and consumers.

Keeping above facts in mind and with a view to develop a value added heath beneficial product using two fat replacers (Maltodextrin and Whey Protein Concentrate), this research study was proposed to be undertaken with the following objectives:

1) To evaluate the suitability of fat replacers for preparation of low fat biscuit.
2) To study the influence of fat replacers on the organoleptic, physical and textural characteristics of the biscuits.
3) To optimize the quantity of fat replacers for production of low fat biscuit.
4) To study the nutritional quality and storage stability of the selected biscuits.

The experiment was conducted at P.G. Institute of Food Processing Technology and Bio-energy, Anand Agricultural University, Anand during the year 2008-2009. Two way completely randomized design was followed with two independent factors viz. (i) different fat replacement levels (40, 50, 60 and 70 %) and (ii) different fat replacement ratios (0:100, 75:25, 50:50, 25:75 and 100:0) with three replications. Two fat replacers were used i.e., Maltodextrin and Whey protein concentrate. Biscuits prepared using rubbing method. Fat replacers were incorporated with fat during creaming. The dependent parameters considered under the study were sensory characteristics, physical characteristics and rheological characteristics. The sensorily selected biscuits were analyzed for the proximate nutritional value where as energy value was calculated. Storage study was carried out to compare changes between control and selected biscuits.

Role of fat is important in the maintenance of good health. The present study was planned to develop low fat biscuit. Therefore, fat content of selected raw materials was analyzed using gravimetric solvent extraction method, which found as below:

1. Fat content of shortening and refined wheat flour was 93.88±0.280 and 1.05±0.115 percent, respectively.

2. Value of fat content of WPC was considered 6.0 percent as per the certificate provided by the manufacturing multinational company.

Physico-chemical properties of refined wheat flour influences on rheological, physical and sensory characteristics of biscuits. Therefore, RWF was analyzed in 3 replications for water absorption power, wet gluten, dry gluten, sedimentation value and alkaline water retention capacity. The following results were obtained for the different physico-chemical properties.

1. Water absorption power of refined wheat flour was 61.63±1.11 percent.

2. Refined wheat flour contained 33.26±0.50 and 11.53±0.31 percent wet and dry gluten, respectively.

3. The sedimentation value for refined wheat flour was found 22.30±0.38 ml.

4. The alkaline water retention capacity of refined wheat flour was observed 69.47±0.37 percent.

Shortening was replaced with different levels of two fat replacers at 40 to 70% with different fat replacement ratios (0:100, 75:25, 50:50, 25:75 and 100:0) in the standardized biscuit formula to develop low fat biscuits. Fat replacers affect sensory properties of biscuits. Therefore, biscuits were evaluated for volume, crust colour and surface character, crumb colour, crumb texture, mouthfeel, taste and aroma and overall acceptability by the panel of 6 experts in 3 replications. The panel was consisting of teachers of P.G. Institute of Food Processing Technology and Bio-energy, Polytechnic in Food Science & Home Economics and SMC College of Dairy Science, Anand Agricultural University, Anand. The following important conclusions were drawn for the different sensory properties.

1. For the volume, none of the biscuits were significantly differ ($p \leq 0.05$) from each other at all the fat replacement levels. Volume of the experimental biscuits gradually increased with increase in amount of WPC. The reason is foaming properties of WPC which helps in producing nicely shaped product and secures a good oven rise.

2. All the experimental biscuits scored higher for crust colour and surface character as compared to control at 40, 50 and 60% fat replacement levels at all the ratios except one. The improved scores for colour and appearance could be due to the Maillard reaction taken place due to maltodextrin and WPC. Crust colour of the biscuit prepared with 100% MD scored higher compared to 100% WPC containing samples at all fat replacement levels. Panel of the judges noted that surface character of the biscuit prepared with the least ratio of WPC was better and further improved with increased amount of WPC.

3. Sensory evaluation showed that the control biscuit had creamy white crumb colour which is significantly differ ($p \leq 0.05$) to all the experimental biscuits at all the ratios but no significant difference ($p \leq 0.05$) was observed among the experimental biscuits themselves.

4. Reduction of fat had most significant effect over crispness of biscuit, as it become hard, give a product with a chewy texture and nontraditional snap characteristics. The bite of the experimental sample was slightly hard as would be expected from elimination of shortening but the product broken down easily during mastication.

5. The values of mouthfeel of the biscuits gradually decreased with decrease in amount of MD at all fat replacement level except the 50:50 ratio at 70% fat replacement levels which was the highest among all the experimental biscuits. Increased amount of WPC in the ratio at each fat replacement level decreased mouthfeel score. These biscuits appeared hard to eat.

6. The values of the taste and aroma of the biscuits gradually decreased with increase in amount of WPC at each level of fat replacement except the 50:50 ratio at 70% fat replacement level which was the highest in among all the biscuits including control. The low scores for the flavour of biscuits incorporated with WPC could be due to its flavour binding capacity.

7. There is no significant effect of fat replacement level on the overall acceptability of the experimental biscuits. Samples with 100% MD were more acceptable than the 100% WPC at all the fat replacement levels. Score of overall acceptability decreased with increased amount of WPC for all the ratios other than 50:50 ratio at all the fat replacement levels. Score for the 50:50 ratio gradually increased with increase in fat replacement level

and scored highest (7.469±0.241) at 70% fat replacement level. Thus that was selected for further research work.

Physical properties of the all the biscuits were analyzed since they help in marketing by placing equal number of biscuits of equal size in predesigned packing medium. It also helps to understand the rheological properties. The conclusions of the same were as under:

1. The experimental biscuit containing 100 % MD scored the highest width among all the experimental biscuits at each level of fat replacement. The width was increased as the level of incorporation of MD was increased in experimental biscuits.

2. Thickness of the biscuits gradually increased with increasing level of WPC through ratio while reversed trend was observed for the MD. Therefore, experimental biscuit prepared with 100 % WPC at maximum level of replacement scored the highest (3.96±0.144) for thickness.

3. A trend was observed that spread ratio of all the experimental biscuits decreased with increase in amount of WPC and reverse trend was observed for MD among all the ratios at each level of fat replacement.

4. Spread factor is defined as the ratio of spread ratio of blend to the spread ratio of control which decreased with increasing amount of WPC in the formulation for each fat replacement level while the spread factor increased with an increase in MD.

5. Weight of most of all the experimental biscuits were more than the control.

The resistance to compression (hardness) and the mean distance compressed before breaking value (fracturability) were analyzed as the rheological characteristics of the biscuits. The following important conclusions were drawn:

1. All the experimental biscuits were significantly ($p \leq 0.05$) different in hardness to the control at different ratios of fat replacement. This is due to decreased shortening action in experimental biscuits. A specific trend was observed that, hardness value of the experimental biscuits containing 100% MD was lower than the experimental biscuits containing 100% WPC at all the levels of fat replacement.

2. Biscuit containing 40 % fat replacement with 50:50::MD:WPC scored the lowest fracturability (0.463±0.038) while with 70 % fat replacement at the ratio of 0:100::MD:WPC scored the highest fracturability (0.637±0.038) among all the experimental biscuits. None of the variations differ significantly between levels.

An experimental biscuit selected, i.e., 50:50::MD:WPC at 70 % fat replacement level, on the basis of sensory evaluation was analyzed along with control biscuit for nutritional composition. The following conclusions were drawn:

1. Results of the present study indicate that fat content of control biscuit was decreased by 68% in the selected experimental biscuit Fat content of experimental biscuit was 4.19%, which meets the standard (i.e., 2.245 g - less than 3.0 g - per 50 g sample) and thus considered as low fat biscuit.

2. Moisture content in the control and experimental biscuits was observed 2.73±0.23 and 3.68±0.14 %, respectively. The reason for higher moisture content in experimental biscuit was that, both fat replacers are hygroscopic in nature.

3. The protein content of the experimental biscuit was higher by 26.76% than that of control due to presence of WPC.

4. Carbohydrate content of control and selected experimental biscuit was 75.56±0.12 and 81.22±0.36 %, respectively.

Observed increase in carbohydrate content was due to decreased fat content and increased MD content.

5. Ash content of the control and selected experimental biscuit was around 1%.

6. Calculated energy for the developed low fat biscuit was 401.43 Kcal/100 g which was more than required for the low calorie food as per the definition. Thus experimental biscuit selected was low fat food but not low calorie food, which is also allowed by definition.

Both biscuits packed in metallised food bag and analyzed for changes in sensory and rheological characteristics at each week during one month storage period. The following important conclusions were drawn:

1. Experimental biscuits scored higher in hardness than the control for all the weeks though they were significantly not differ in all the sensory attributes except mouthfeel as well as taste and aroma.

2. Values for hardness and fracturability of both the biscuits reduced gradually during storage period. Both properties of the control biscuits were significantly differ ($p \leq 0.05$) than the experimental biscuits at all the weeks.

In overall conclusion the experimental biscuits up to 70% fat replacement with 50:50::MD:WPC was found sensorily acceptable. It contains 4.19±0.10 % fat which meets the standard for low fat food. The fat content was reduced by 68%. Thus, the developed low fat biscuit could be beneficial to replace the normal biscuit for the persons suffering from various cardio vascular diseases mainly caused due to excess fat and to maintain normal health for healthy individual. This biscuit can be considered as functional food due to many unique health-enhancing or disease-combating properties of

WPC including protein energy malnutrition, counteracting the wasting syndrome in HIV positive individuals. It contains about 26% more protein as compared to control. The biscuits could be stored safely for one month is an additional benefit to be considered it as ready to eat food with high shelf life. It could be considered economically viable on the bases of their health benefits. This would boost up development of food industry in particular bakery industry, in specialized segment of health food.

CHAPTER VI
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APPENDIX I

SPECIMEN FOR PRODUCT (BISCUIT) EVALUATION SCORE SHEET OF COMPOSITE SCORING TEST

Name : ________________________________ Production Date : __________
Product : ______________ Testing Date : ___________

- Products presented before you are the samples of trail conducted to develop high protein products useful to preschool children.
- Kindly grade the (on the bases of rating given on separate sheet) on a score of 10 for following sensory attributes.

Sensory characteristics	Possible score	Sample score			
		01	02	03	04
Volume	10				
Crust Colour And Surface Character	10				
Crumb Colour	10				
Crumb Texture	20				
Taste And Aroma	30				
Mouth Feel	10				
Overall acceptability	10				

Comments :

Signature

	Volume	Crust character	Crumb colour	Crumb texture	Taste and aroma	Mouth feel	Overall acceptability
Desirable	Appropriate to weight	Golden brown, Smooth	Creamish white	Crisp	Normal, pleasant	No residue in mouth	Rating Excellent 10 Best 9 Very good 8 Good 7 Satisfactory 6 Intermediate 5 Fair 4 Poor 3 Very poor 2 Worst 1
Undesirable	Dense or light to weight	Brownish white or dark, Rough	Brown, dark brown	Soft, hard, brittle	Off flavour, off test	Residual taste formation of dough lump in mouth	

APPENDIX II
Reagents

Boric acid (2 % w/v): Dissolved 2 g of boric acid (H_3BO_3) in hot glass distilled water and diluted to 100 ml (prepared fresh).

Bromophenol blue (0.4 g%) (W/V): 400 mg bromophenol blue was dissolved into distilled water and volume made upto 100 ml.

Digestion mixture: Copper sulphate ($CuSO_4.5H_2O$) and potassium sulphate (K_2SO_4) was mixed in equal parts (1:1).

Isopropyl alcohol : Lactic acid : distilled water mixture :: 20:8:62 (V/V): 18 ml 25 % lactic acid refluxed, 20 ml isopropyl alcohol and distilled water was mixed and volume made up to 100 ml. The mixture should be of 0.5 N. Prepared 48 hrs before use. Protected against evaporation.

Lactic acid (25%) (V/V): Diluted 25 ml lactic acid into distilled water and volume was made up to 100 ml. Refluxed 6 hrs without loss of volume. This gives a solution of about 2.78%N.

Mixed Indicator: Dissolved 25 mg of bromocresol green ($C_{21}H_{13}Br_4O_5SNa$) and 75 mg of methyl red ($C_{15}H_{15}N_3O_2$) in ethanol (C_2H_5OH) and diluted to 100 ml.

Sodium bicarbonate (0.1 N): Dissolved 0.84 g of sodium bicarbonate in glass distilled water and diluted to 100 ml.

Sodium hydroxide (50 % w/v): Dissolved 50 g of sodium hydroxide (NaOH) in glass distilled water and diluted to 100 ml.

Sulphuric acid (0.02N): 0.2 ml of conc. sulphuric acid (H_2SO_4) was diluted to 360 ml with distilled water.

Sulphuric acid (Concentrated): Sulphuric acid was used as AR grade commercially available.

Table 4.20 Cost estimation of control and selected experimental biscuits

Ingredients Price	Quantity (%)	Price (Rs /Kg)	Amount (Rs)[6]	
			Control	Experimental biscuit
Refined wheat flour	100	16	16.00	16.00
Margarine	20/6*	74	14.80	4.44
Maltodextrin	0/7$	50	0.00	3.50
Whey protein concentrate	0/7$	250	0.00	17.50
Lecithin	0/0.5☺	80	0.00	0.40
Sugar	30	34	10.20	10.20
Dextrose syrup	2	40	0.80	0.80
Non fat dry milk powder	2	102	2.04	2.04
Vanilla essence	1.25	500	6.25	6.25
Salt	1	8.00	0.08	0.08
Ammonium bicarbonate	1.5	15.00	0.22	0.22
Sodium bicarbonate	0.4	20.00	0.08	0.08
Total cost of 1.500 Kg biscuits produced from 1Kg RWF			50.47	61.51
Production cost of 1 Kg biscuit			33.65	41.00
Cost price &			50.48	61.50
Selling price @			63.10	83.03
Extra percent (%) as compared to control			-	31.58%

[6] Amount based on 1 Kg RWF
* 20% for control biscuit and 6% for low-fat biscuit
$ 0% for control biscuit and 7% for low-fat biscuit
☺ 0% for control biscuit and 0.5% for low-fat biscuit
& Cost Price = Production cost of 1 kg biscuit + 50% of production cost
@ Selling price = Cost price + 25 or 35% of cost price #
# 25% for control biscuit and 35% for experimental biscuit

Table 4.18 Sensory evaluation of control and selected experimental biscuit during storage

Week	Biscuit	Volume (10)*	Crust colour and surface character (10)*	Crumb colour (10)*	Crumb texture (20)*	Mouthfeel (10)*	Taste and aroma (30)*	Overall Acceptability (10)*
Week 0	Control	7.34±0.09	8.25±0.25	7.92±0.27	15.68±0.31	8.25±0.25	23.76±0.33	7.67±0.25
	Experimental biscuit	7.50±0.08	8.34±0.21	8.25±0.25	16.00±0.26	7.91±0.27	23.40±0.22	8.00±0.39
	P value	0.22	0.80	0.38	0.68	0.38	0.76	0.48
Week 1	Control	7.28±0.32	7.92±0.37	7.75±0.17	15.58±0.29	7.84±0.30	23.52±0.17	7.54±0.24
	Experimental biscuit	7.42±0.23	8.17±0.30	7.84±0.10	15.84±0.27	7.58±0.45	23.01±0.21	7.71±0.34
	P value	0.73	0.61	0.68	0.76	0.65	0.54	0.70
Week 2	Control	7.25±0.26	7.84±0.30	7.58±0.20	15.24±0.26	7.50±0.43	22.98±0.21	7.41±0.20
	Experimental biscuit	7.34±0.09	8.08±0.32	7.67±0.17	15.50±0.25	7.34±0.21	22.62±0.20	7.70±0.19
	P value	0.77	0.58	0.75	0.73	0.73	0.68	0.31
Week 3	Control	7.05±0.10	7.67±0.31	7.25±0.17	15.00±0.43	7.42±0.49	22.74±0.42	7.75±0.36
	Experimental biscuit	7.27±0.10	7.83±0.27	7.42±0.20	15.34±0.47	7.17±0.48	22.26±0.32	7.46±0.24
	P value	0.15	0.69	0.54	0.80	0.72	0.75	0.52
Week 4	Control	7.00±0.20	7.25±0.44	7.17±0.40	14.50±0.17	7.00±0.36	21.75±0.44	7.34±0.40
	Experimental biscuit	7.25±0.26	7.41±0.37	7.34±0.33	14.66±0.42	6.84±0.40	21.48±0.38	7.38±0.46
	P value	0.47	0.77	0.75	0.85	0.76	0.89	0.94

Values are mean score of a composite scoring test by a panel of 6 judges x 3 replications

Data analyzed by t-Test

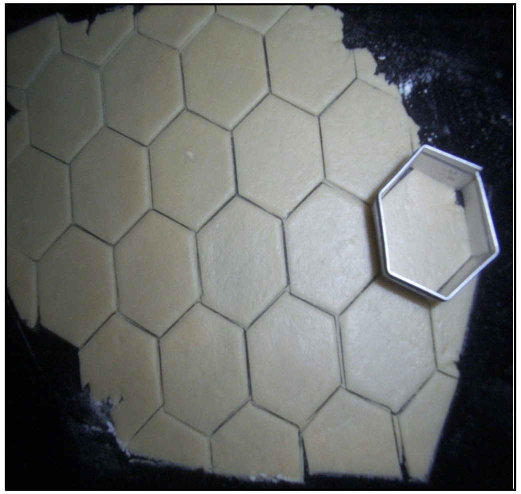
* maximum score

Baker's fat

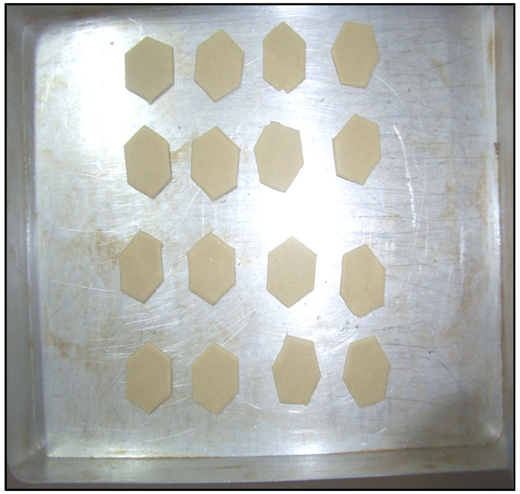
Creaming

Dough

Plate 3.1: Biscuit preparation - I



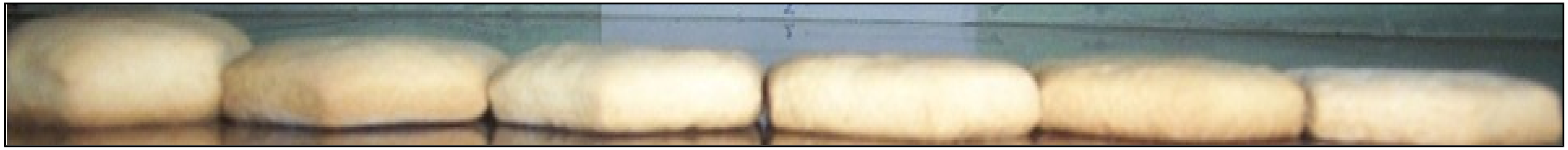
Cutting



Biscuits in tray

Baked biscuits

Plate 3.2: Biscuit preparation- II



0:100 25:75 50:50 75:25 100:0 Control

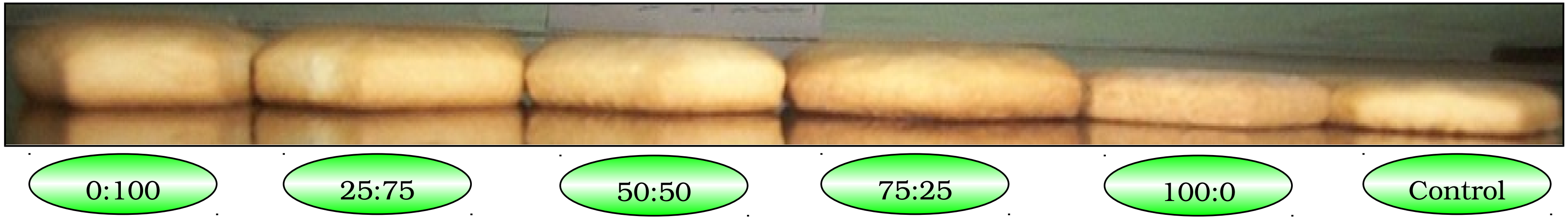
Biscuits with 40% fat replacement level

0:100 25:75 50:50 75:25 100:0 Control

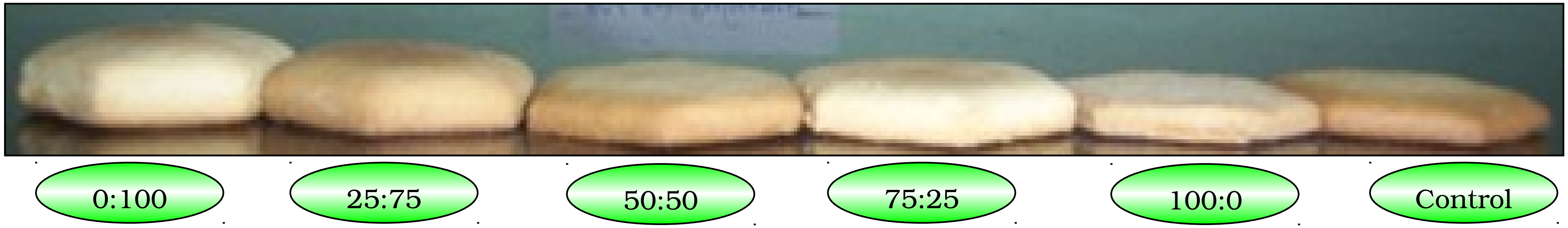
Biscuits with 50% fat replacement level

Ratios are MD:WPC

Plate 4.1: Volume of biscuits - I



Biscuits with 60% fat replacement level

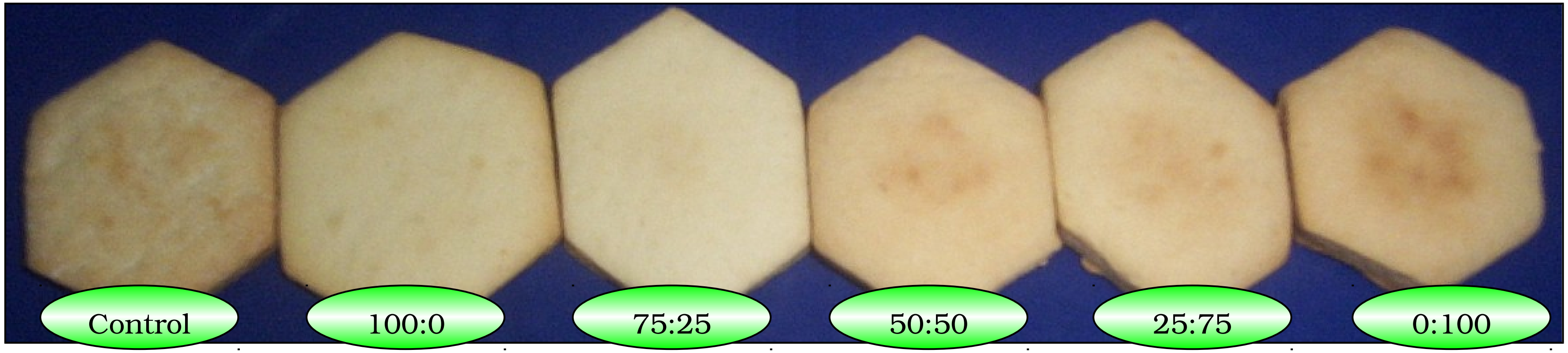


Biscuits with 70% fat replacement level

Ratios are MD:WPC

Plate 4.2: Volume of biscuits - II

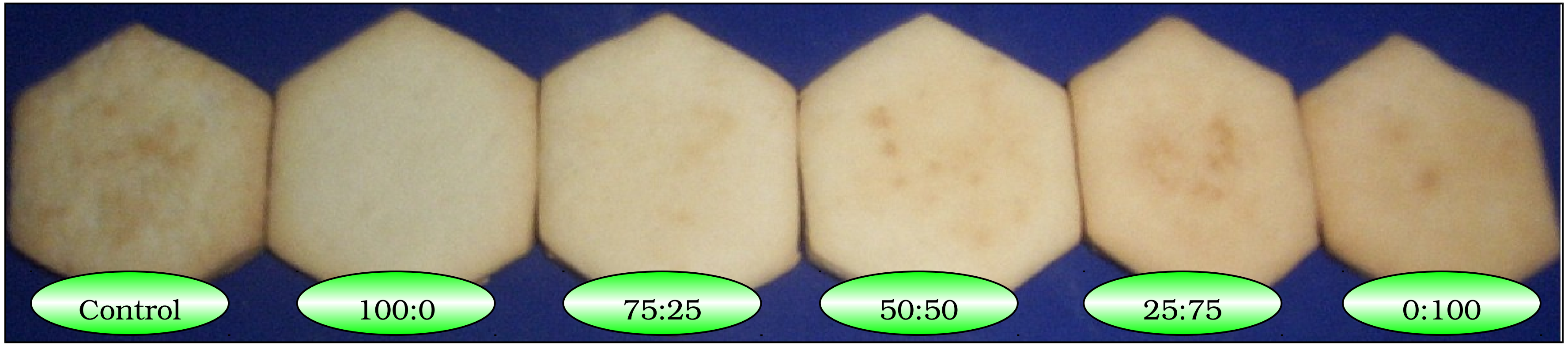
Biscuits with 40% fat replacement level



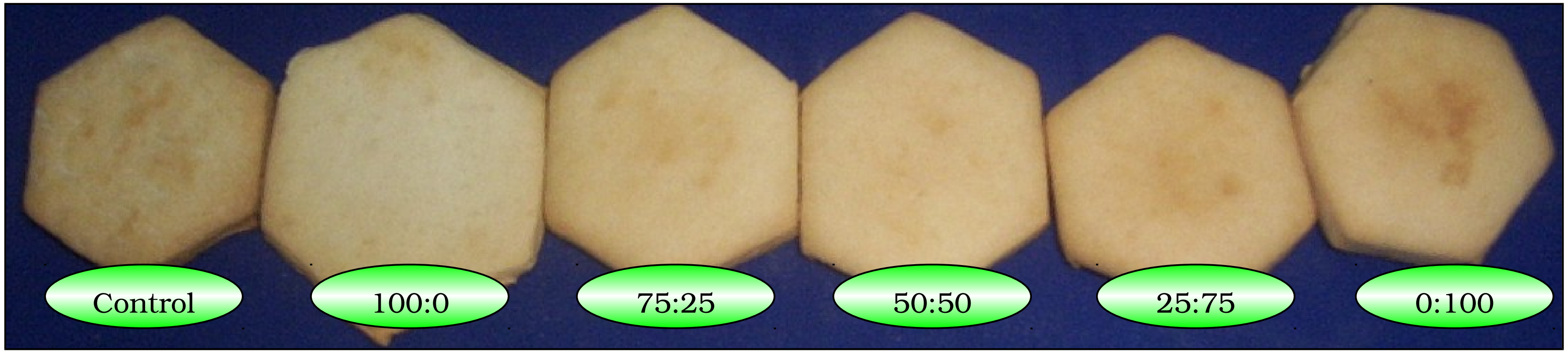
Biscuits with 50% fat replacement level

Ratios are MD:WPC

Plate 4.3: Crust colour and surface character of biscuits - I



Biscuits with 60% fat replacement level



Biscuits with 70% fat replacement level

Ratios are MD:WPC

Plate 4.4: Crust colour and surface character of biscuits - II

Plate 4.5: Texture analyzer and its action

Auto soxhlet equipment

Extracted oil from samples

Plate 4.6: Fat extraction equipment and extracted oil samples