DEVELOPMENT OF TECHNOLOGY FOR FUNCTIONAL DODA BURFI

THESIS
SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD
OF THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
DAIRYING

(DAIRY TECHNOLOGY)

BY
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B.Sc (Food Science and Technology) (Hons)
M.Sc (Food Science and Technology)

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Karnal – 132001 (Haryana), India
2010

Regn. No. 1030602

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CERTIFICATE

This is to certify that the thesis entitled "Development of Technology for Functional *doda burfi*" submitted by Ms. Rekha Chawla towards the partial fulfillment of the requirement for the award of the degree of DOCTOR OF PHILOPSOHY IN DAIRYING (DAIRY TECHNOLOGY) of the National Dairy Research Institute (Deemed University), Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

Dated: 17th March' 2010

(Dr. G.R. PATIL)
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Dedicated to
My Heavenly Parents
&
Loving Sisters

Acknowledgement

Foremostly I bow before Almighty, for vesting wisdom to all my wishes and standing by me at very step and helped me to make my parents n dear ones dreams come true.

With abyssal prerogative, I extend my heartfelt gratitude, obligation and indebtedness to my Major Advisor Dr. G.R. Patil, Joint Director (Academics) and Principal Scientist (Dairy Technology Division) for his inspiring guidance, direction and encouragement during the course of my research and studies at the institute. I am unable to express my feelings through words for my advisor because he gave me daughterly care and a protective environment just like a father. His scholarly guidance, constant encouragement, optimistic and determined approach to the research problem, intuitive suggestions and liberal attitude during the entire course of the study. His sincerity and dedication towards work were lessons for life. I am gratefully appreciative for his abiding interest and critical appraisal of the results and organization of the manuscript.

Solemn regards and thanks to members of my Advisory Committee: Dr. R.K. Malik, Principal Scientist and esteemed Directors' Nominee (Dairy Microbiology Division); Dr. I.K. Sawhney, CEO and Principal Scientist, (Dairy Engineering Division); Dr. Ashish Kumar Singh, Senior Scientist (Dairy Technology Division); Dr. R.R.B. Singh, Principal Scientist (Dairy Technology Division); and last but not the least in any case Dr. Sumit Arora, Senior Scientist (Dairy Chemistry Division).

I owe my indebtness to Dr. A.A. Patel, Head of the division for providing me necessary facilities and extending his help and co-operation during the course of this investigation.

Cordial and earnest thanks to Dr. Ashish Kumar Singh, who paved me a way and supported me in every possible way and provided me the necessary facilities needed for carrying out this investigation. I have my sincere thanks to Dr. Sumit Arora, who inspite of his busy schedule always welcomed me *whensoever's I needed help. My words are not at all enough rather I do not have words to express my gratitude to both of them.*

Words are not just enough to couch my deepest sense of reverence to my dearest and loving sisters, Jaya and Rashmi for their love and affection, which fostered the urge to always strive for excellence and continued to enlighten my life. It gives me an immense pleasure to acknowledge in formal manner to my both brother-in- laws- Tarun and Amolak Jiju.

I cannot weight my feelings with words for my friend Ashwani who always been a driving force to perform beyond my capabilities and helped me a lot during the compilation of my thesis. I thank god who gave me a helping and sweetest junior "Rajesh" who always helped me at every possible step to do the best in my thesis. Conducive environment in lab and keen interest made this laborious task a bit easier.

I am thankful to Avneet sir, Arvind sir, the most for guiding me with their valuable suggestions and skills of handling practical equipments, our senior Raju sir, my friends Harsh, Shalini, Sunil and my juniors Sangeeta, Nitika, Alok, Prerna, Pratham, Abdul, Ramesh Modi, and my lab mates, Mrs. Anamika Singh, Swati, Girish sir, Anjuman and Rajat sir and Mritunjay and above all our lab attendant, Sh. Samundar Singh ji for boosting my morale and extending their cooperation in every possible way during this investigation. Also, during the last stage of thesis, I received, warm affection from new lab mates like Sandeep and Ajay.

Last but not the least thanks are due to one and all those who extended the willing help. Finally, I bow my head to Almighty God for having bestowed me with all that I have.

Rekha Chawla

Dated: 17th March' 10

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DEVELOPMENT OF TECHNOLOGY FOR FUNCTIONAL *DODA BURFI*

ABSTRACT

"Doda" or "Dhoda" is immensely popular sweetmeat of northern India. The product is made from germinated wheat flour (*angoori atta*), buffalo milk, sugar along with certain optional ingredients and characterized by pleasant caramelized flavour, dark brown colour and sticky granular body but these traditional dairy products lacks mass acceptability because of high sugar content, which gives high calorific value, no perceived health benefits and many more. Also, since the variability exists in most of the traditional dairy sweets, characterization of these products in terms of physico-chemical, nutritional, compositional, organoleptic and microbiological parameters are urgently required. Keeping these under consideration, market survey of *doda burfi* samples from four renowned cities was carried out. Amongst the four cities surveyed, Ludhiana city was rated "the best" for all compositional and sensory parameters. Application of principal component analysis (PCA) validated the same. The practice of manufacturing varies from place to place, so to optimize a formulation based on milk fat, germinated wheat flour and sugar was carried out employing Box-behnken design of response surface methodology and an optimized formulation was obtained. Targeting the diabetic population, stevia along with other functional ingredients were added to produce a functional product, but the product lack texture and overall acceptability from consumer's point of view so to develop a functional as well as an acceptable product functional ingredient were added in the conventional optimized formulated recipe. Based on the preliminary studies, out of five fibres, oat, wheat, inulin, Microcrystalline cellulose (MCC), and Diesol™; oat fibre and Diesol™ were selected with 2.5 and 0.75% combination to be carried further for the studies. A central composite rotatable design (CCRD) design of 2 factor variable was employed to develop functional *doda burfi* employing Diesol™ at constant value with varying ratios of oat fibre (%) and calcium chloride (mg/100ml). The optimum combination of 1% oat fibre and 50 mg/100 ml of calcium chloride, and 3.5% phytostanol was obtained through response surface methodology. Consumer survey revealed that the product was highly acceptable in terms of sensory profile as 57% consumers rated the product as "excellent" 39% "very good" and 14% "good". To extend the shelf-life of *burfi*, bacteriocin, microgard, potassium sorbate, and sodium EDTA were added and an extended shelf life of 27 days was obtained against 12-15 days of conventional *doda burfi* at 30°C. Costing of an acceptable product came out with much lower and affordable price of 153 Rs./kg against 140-230 Rs./kg of the prevailing price of conventional *doda burfi*.

सारांश

डोडा अथवा ढोडा उत्तरी भारत का एक अत्याधिक सुविख्यात मिष्ठान है। इस उत्पाद को अंकुरित गेहुं के आटे (अंगूरी आटा), भैंस के दूध एवं चीनी के साथ–साथ कुछ वैकल्पिक सामग्री द्वारा बनाया जाता है। यद्यापि यह उत्पाद कैरेमल के विशिष्ट स्वाद, गाढे भूरे रंग एवं चिपचिपे दानेदार संरचना द्वारा वर्णित होता है तथापि इस पांरपरिक दुग्ध उत्पाद का जंनसंघ की स्वीकृति का अभाव है। इसका कारण, डोडा में किसी भी प्रकार के कथित स्वास्थ्य लाभ का अभाव, चीनी की अत्याधिक मात्रा, जोकि उच्च कैलेरी प्रदान करती है एवं इसी प्रकार के कई अन्य कारण है। साथ ही, क्योंकि प्रायः सभी पांरपरिक दुग्ध मिष्ठानों में पृथकता प्रस्तुत है, इसीलिए इन उत्पादों का भौतिक–रसायनिक, पोषण, संयोजन एवं सूक्ष्मजीवविज्ञानी मानकों के संदर्भ में विवरणीकरण की तत्काल आवश्यकता है। इन सब मानकों के विचाराधीन, डोडा बर्फी नमूनों का बाजार सर्वेक्षण चार प्रसिद्ध नगरों में किया गया। चारों नगरों में सर्वेक्षण के अंतर्गत लुधियाना शहर संयोजन एवं संवेदी मापदंडो के लिए सबसे अच्छा था प्रधान घटक विशलेषण के प्रयोग ने भी इसकी पुष्टि करी। क्योंकि डोडा को निर्मित् करने की विधि स्थानाविशेष में भिन्न है इसीलिए दुग्ध वसा, अंकुरित गेहुं के आटे एवं चीनी पर आधारित निर्माण प्रक्रिया का अनुकूलन बॉक्स–बहनकेन स्वरुप (प्रतियुत्तर तल प्रविधि का भाग) द्वारा किया गया एवं अनुकूलित प्रक्रिया की प्राप्ति हुई। मधुमेह रोगियों को ध्यान में रखते हुए स्टीविया के साथ–साथ अन्य कार्यात्मक उत्पाद बनाने हेतू किया गया परन्तु उपभोक्ता की दृष्टि से उत्पाद संरचना एवं समग्र स्वीकार्यता में कम पाया गया। इसीलिए एक कार्यात्मक एवं स्वीकृत उत्पाद बनाने के लिए कार्यात्मक सामग्री को परम्परागत अनुकूलित नुस्खे द्वारा तैयार किया गया। प्रारंभिक अध्ययन के आधार पर, पांच रेशों– जई, गेहुं, इन्युलिन, एमसीसी और डाइज़ोल का चयन कर उन्हें 2.5% और 0.75% के संयोजन में आगे अध्ययन के लिए चुना गया। डाइज़ोल की निश्चित मात्रा के साथ जई रेशा (%) एवं कैल्शियम क्लोरइड (मिलीग्राम/मिलीलीटर) के अनुपात को पृथक करते हुए दो कारक चर वाले सीसीआरडी डिजाइन का प्रयोग कार्यात्मक डोडा बर्फी बनाने के लिए किया गया। प्रतियुत्तर तल प्रविधि द्वारा 1% जई रेशा और 50 मिलीग्राम/100 मिलीलीटर तथा 3.5% फाईटोस्टेनाकल का संयोजन ईष्टतम पाया गया। उपभोक्ता सर्वेक्षण ने उजागर किया कि उत्पाद संवेदी रुपरेखा के मानकों में अत्याधिक स्वीकृत है। 57% उपभोक्ताओं ने उत्पाद को उत्कृष्ट, 39% ने बहुत अच्छा एवं 14% ने अच्छा बताया। बर्फी के परिरक्षण अवधि को बढाने की दृष्टि से बेक्टीरियोसिन, माईक्रोगार्ड, पोटेशियम सोरबेट तथा सोडियम ई.डी.टी.ए. का प्रयोग किया गया एवं 27 दिनों की परिरक्षित अवधि 30°C पर प्राप्त हुई। बिना परिरक्षिणों के उपयोग द्वारा निर्मित बर्फी की परिरक्षित अवधि 12–15 दिन (30°C) पर थी। स्वीकृत उत्पाद की लागत रुपये 153/किलोग्राम पाई गयी जोकि मौजूदा परम्परागत डोडा बर्फी के मूल्य (रुपये 140–230/किलोग्राम) की तुलना में कहीं कम थी।

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LIST OF ABBREVIATIONS

µg	Microgram	g	Gram
µl	Micro liter	GRAS	Generally regarded as safe
ADA	American dietetic association	HMF	Hydroxyl methyl furfural
ADI	Acceptable daily intake	HRV	Human RotaVirus
AIIMS	All India institute of medical science	IS	Indian standards
Anon	Anonymous	kDa	Kilo dalton
ANOVA	Analysis of variance	Kg	Kilogram
AOAC	Association of official analytical chemists	KOH	Potassium hydroxide
a_w	Water activity	LA	Lactic acid
CCRD	Central composite rotatable design	Log	Logarithm
CD	Critical difference	MD	Maltodextrin
cfu	Colony forming units	Mg	Milligram
CVD	Cardio vascular diseases	Min	Minute
df	Degree of freedom	mL	Milliliter
FD&C	Federal Food, Drug, and Cosmetic Act	Ml	Milliliter
FDA	Food and drug administration	MSNF	Milk solids not fat
FDA	Food and Drug Administration	MSS	Mean sum of squares
FDD	Flavored dairy drink	N	Newton
FFAs	Free fatty acids	N.s	Newton second
FOS	Fructo-oligosaccharides	NIH	National Institute of health
FOSHU	Food for Specific Health Use	O.D	Optical density
FSMP	Food Intended for Special Medical Purposes	ºC	Degree centigrade

OTR	Oxygen transmission rate	Sp. gr.	Specific gravity
PARNUT	Foods for Particular Nutritional Purposes	SPC	Standard plate count
PCA	Principal component analysis	TBA	Thiobarbituric acid
PCs	Principal components	TCA	Trichloro acetic acid
PD	Poly dextrose	TCC	Tricalcium citrate
PDA	Poly dextrose agar	TDP	Traditional dairy products
PFA	Prevention of food adulteration	Temp	Temperature
psi	Pounds per square inch	TPA	Texture profile analysis
R^2	Coefficient of determination	TS	Total solids
RDA	Recommended daily intake	USFDA	United States Food and Drug Administration
RSM	Response surface methodology	WDP	Water dispersible protein
s	Seconds	WHO	World Health Organization
SD	Standard deviation	WPC	Whey protein concentrate

CHAPTER -1

INTRODUCTION

INTRODUCTION

In the era of self-care movement, people are becoming more and more aware of their health and thus the demand for health products has shoot up surprisingly high. Though the concerns vary from one age group to another, the basic key factor to be acknowledged in the whole aspect of health is “nutrition”. From a wide variety of food stuffs available to maintain the health of well being, no item till date can achieve the importance comparable to milk. Milk and milk products constitute the second largest gross agricultural produce in the country and conversion of raw milk into wide variety of milk products has been practiced in our country since pre-historic times. Manufacturing of different traditional milk products, especially sweets, not only adds to the range of high-profit products but also increases consumption of milk as they are liked by every segment of population, especially those who don’t relish milk in its natural form. Amongst various categories of Indian milk based sweets available, heat desiccated category presents a pleasing and healthful variety of products compared to others. India has the largest repertoire of milk based sweets and out of them *doda burfi,* simply called as “*Doda*” or “*Dhoda*”, is immensely popular traditional milk-cereal based sweet of northern states of India like Punjab, Haryana, and Western U.P (Singh and Kumar 2006). The product is characterized by dark brown colour, sticky granular texture having glossy surface covered with nuts with intact pleasant caramelized flavour. The attractive and salient feature of the product is the presence of most of the nutrients from cereals in pre-digested form, which also serves as an excellent source of dietary fibre absent in other dairy products. The product also has a longer shelf-life as compared to other milk based sweets.

1.1 CURRENT SCENARIO OF INDIAN TRADITIONAL PRODUCTS’ MARKET

Of the total milk produced, around 50-55% is converted by the traditional sector into variety of milk products (www.indiadairy.com./info_milk_products_ dairyproducts.html). Though exact figures for the production and demand of *doda burfi* are not available, the market for traditional dairy products is valued around Rs. 50,000 crores annually (Aggarwal, 2007). Unfortunately however, at present, the technology for the production of most of the traditional Indian milk products is confined to local sweetmeat makers (*halwais*) and like many other traditional sweets, the practice of *doda* making remains a trade secret till date. Being prepared at small scale, the hygienic conditions are usually not maintained and thus inferior quality products are manufactured and marketed (Patil, 2002). At the same time, due to lack of any prescribed standards laid down by any of the food standards authority, quality in terms of proximate composition, texture and microbiological count varies a lot. Recently, in order to tap the potential of traditional sweet markets, several dairies in private and co-operative sector have started their organized production. Today, a wide range of fresh as well as packaged ethnic milk products and convenience mixes are available in market. These products are also being exported to countries having large Indian ethnic population.

Also, changing scenario offers new opportunities for Indian dairy industry, particularly organized sector, for expanding their product profile. Many of these traditional milk products such as *channa* based sweets, *mishti doi*, *shrikhand*, *basundi* etc. which were manufactured at small scale in specific geographical locations, have been introduced in other parts of the Indian subcontinent. These dairy delicacies have been relished by the consumer as they possess excellent flavour, mouth-feel and exhibit characteristics textural profile. In absence of technical know-how, the large scale production of these region specific milk products in organized sector is a challenging task (Singh and Kumar, 2006), which requires scientific investigations in terms of their characterization, standardization of technological parameters and strategic interventions for ensuring the safety and enhancing the shelf-life.

1.2 NEEDS ASSOCIATED WITH THESE PRODUCTS

The consumers' perception of high calorific value without any apparent health benefit associated with consumption of traditional milk sweets is probably the major obstacle in their acceptability to health conscious strata of consumers. In order to cater this category of consumers, low calorie, probiotic, micronutrient fortified dairy products have been introduced recently in Indian market and demand for such functional and healthy foods is growing day by day. Globally the market for functional dairy products is estimated to be $ 14 billion (seven hundred crores Rs. approx.) with countries like Japan and Europe taking the lead (Anon, 2007). The new ranges of functional ingredients with proven health benefits are available and their incorporation in food products is expected to revolutionize the food industry. In 2004, functional dairy products accounted for 2.6 per cent of the total sector value with sales of $ 68.3 million (thirty four crores Rs. approx) in Poland (Sofroniou, 2005).

Since the variability exists in most of the traditional dairy sweets, characterization of some of these products in terms of physico-chemical, nutritional, compositional, organoleptic and microbiological parameters are urgently required. Recently, manufacture of *"doda burfi"* has been started by many famous sweet-makers; however, there exists lot of variations in manufacturing process, type of ingredients used and quality parameters such as colour, texture and organoleptic characteristics. Therefore, there is need to characterize the product and standardize the unit operations involved in manufacturing of *doda burfi*.

1.3 MODERN HEALTH CONCERNS AND ARISEN AWARENESS

According to WHO (2003), cardiovascular diseases (CVD) and diabetes are the two major diseases prevailing in our country. The diabetic population of the country at present is 180 million and is expected to double by the year 2030 (WHO, 2006). The deaths due to CVD accounts for 15% in India alone. However, the figures are likely to rise if the present scenario prevails (AIIMS, 2002). Modern lifestyle and changes in diet pattern contributed a significant role in increasing these life threatening diseases. To lead a healthful and nutritionally sound life, functional foods presently have become the need as according to World Health Organization (WHO) report, nearly 1 billion adults are overweight, with 300 million estimated to be clinically obese (Montgomery, 2004). Functional foods are available with a wide range of nutraceuticals like fibre enriched, prepared with non-calorie artificial (aspartame, acesulfame-K, sucralose and more) and natural sweeteners (*Stevia rebaudiana*), probiotic, prebiotics, vitamin and mineral fortified foods and many more. These foods are known by different names in different countries like FOSHU (Food for Specific Health Use), PARNUT ((Foods for Particular Nutritional Purposes), FSMP (Food Intended for

Special Medical Purposes), nutraceuticals, etc. The concept of functional food has received a considerable attention both from processors and researchers, in our country as well.

Recently various traditional products such as *rasogolla,* milk cake, *kalakand*, *kheer* and some regional specific products like range of *burfi, channa murki payasam* etc. are being explored and gaining popularity in various countries. Also, with scientific advancement in the field of food science and technology there has been increased availability of novel ingredients like non-nutritive sweeteners, micronutrients, low calorie additives, plant based materials like phytosterols and stanols and fibres, which can be utilized for enhancing the health attributes of traditional sweets. Addition of these ingredients in food products requires clear understanding of mechanisms involved in developing desirable quality characteristics, process modification, selection of suitable packaging material and storage characteristics.

1.4 CONCEPTUAL ASPECTS OF THE PRESENT INVESTIGATION

An attempt has been made in this study to understand the various characteristics of *doda burfi* present in the prevailing market and to standardize a protocol of preparing a product with uniform quality as well as to enhance the health benefits using various health additives so that the health conscious consumers could be benefitted. The present investigation entitled “Development of Technology for Functional *Doda burfi*” was performed with following objectives:

- **Characterization of the physico-chemical, sensory, rheological and microbiological quality of market samples of *doda burfi.***
- **Standardization of technological parameters for manufacture of acceptable quality *doda burfi***
- **Optimization of the level of functional ingredients in standardized *doda burfi* for enhancing the health attributes**
- **Extension of shelf life of developed product through processing and packaging interventions**

CHAPTER -2

Review and literature

REVIEW OF LITERATURE

Earlier consumers prefers the foods that were attractive in appearance and fitted to their palate but with the advancement in food science and technology, now the consumer demands for convenience, nutrition and health benefits. In food, every component has a unique role in our metabolism and thus the previously ignored components like fibre, prebiotics, micronutrients, and vitamins have shaken the "*new consumer*". These functional foods are known by different names in different countries like FOSHU (Food for Specific Health Use), PARNUT ((Foods for Particular Nutritional Purposes), FSMP (Food Intended for Special Medical Purposes), nutraceuticals, etc. The concept of functional food has received a considerable attention both from processors and researchers, in our country as well. Recently many probiotic, low-calorie and fortified foods have been introduced in Indian market and are gaining wider consumer acceptance. Therefore, the product development targeting nutritional and therapeutic attributes in addition to good quality characteristics is urgently required. There is no review of literature available on *doda burfi*. Hence, the related literature pertaining to the related products such as *burfi, kalakand, pera*, etc. is given hereunder. The available literature on the production of *doda burfi*, various dairy products fortified with alternative sweeteners, dietary fibres and nutraceuticals such as phytosterol, and shelf life extenders such as nisaplin and microguard intended for use in the present study, have been reviewed in this section.

The relevant literature pertaining to the related aspects of preparation and enhanced health attributes is reviewed under the following heads:

2.1 *Doda burfi* and other heat desiccated products

- ❖ Definition
- ❖ Role of ingredients
  - o Milk
  - o Germinated wheat
  - o Sugar
- ❖ Method of preparation
  - o Traditional method of manufacturing
  - o Improved method of manufacturing
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    - Moisture
    - Titratable acidity
    - Sugars
    - Fat
- ❖ Other related aspects
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    - Textural studies
- ❖ Preservation
    - Microbiological quality of traditional dairy products
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    - Other anti-bacterial or anti-fungal substance

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- ❖ Definition
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    - Sources of fibre
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        - Oat fibre
        - Inulin
        - Microcrystalline cellulose
        - Gum Acacia
- ❖ Micronutrients
- ❖ Phytosterol esters

2.3 Shelf life of traditional dairy products

2.4 Packaging of processed dairy foods

2.1 *DODA BURFI* AND OTHER HEAT DESICCATED PRODUCTS

2.1.1 Definition: *Doda burfi,* simply called as "*doda*" or "*dhoda*", is a popular sweet in northern India, especially states like Delhi, Punjab and Haryana where it is usually served on special occasions. It has dark brown colour, granular body, thick consistency, chewy mouth-feel and possess pleasant aroma. The product has slightly longer shelf-life than other milk based sweets. It is one of rare sweet traditionally made using a combination of cereal flour and milk solids and hence provides most of the nutrients specially the fibre and micronutrients. At present, "*doda*" is manufactured by sweet-makers (*halwais*), at home and by Haryana Milk Marketing Federation Ltd. on commercial scale but the product do not have any optimized standard recipe which leads to wide variation in quality characteristics.

A resembling product prepared using admixture of wheat flour called "*Ghevar"* is a rare delicacy of northern India. It is a festive disc shaped sweet having yellow to orange brown colour.

Sohan halwa is a wheat based product characterized by extremely chewy texture attributed to wheat gluten. Preparation technology of *Sohan halwa* almost resembles *doda burfi,* using geminated wheat flour addition to milk in ratio 1: 2. However, the product formulation and process description varies from one author to another.

Gulabjamun, a popular *khoa* and *maida* based sweet characterized by round or oval shape and brown color owing to deep fat frying. A standardized recipe of *gulabjamun* consists of buffalo milk *khoa* with wheat flour in the ratio of 80:20, as developed by Rangi *et al.* (1985). Traditionally, *gulabjamun* is prepared from a mixture of *khoa, maida* and baking powder.

2.1.2 Role of ingredients

2.1.2.1 Milk: Milk is one of the major ingredients used in all the sweets. Depending on the texture of the finished product required, milk with different fat and SNF percentages is used. Gothwal and Shukla (1995) used standardized milk of 4.5 % fat and 8.5 % SNF composition for preparation of *burfi* whereas Suresh and Jha (1994)[a] reported that an excellent product (*kalakand*) can be made with 6.0 per cent fat and 9.0- 9.5 per cent SNF. Similarly Gupta *et al.* (2005) used milk of 5.0 per cent fat for carrot milk cake preparation.

Adhikari *et al.* (1994) reported 0.6 as the best fat:SNF ratio for preparation of *gulabjamun* and the same ratio was recommended by Suresh and Jha (1994)[a] for *kalakand*. The best standardized ratio by Varma *et al.* (2005) for milk cake is 0.7 whereas a value of 0.66 has been recommended by Karwasra *et al.* (2001) and Madhava *et al.* (2003) for the same. Palit and Pal (2005) recommended a ratio of 1.5:1 of SNF:fat for *burfi* whereas Narwade *et al.* (2007) reported 0.72 ratio of Fat:SNF best for the preparation of *pedha*.

2.1.2.2 Germinated wheat: Wheat (*Triticum aestivum)* is one of the dominant cereals of world and is rightly known as “King of cereals” or “Golden grain.” In India wheat and wheat products are important components of daily diet. Nutritionally wheat is a rich source of complex carbohydrates, quality proteins, fibre, and it also contains B-vitamins, and myriad of micronutrients- calcium, folacin, iron, magnesium, phosphorus, potassium, zinc, minimal amounts of sodium and other trace elements. Addition of cereals and other grains (i.e. legumes and oilseeds) in the preparation of traditional dairy delicacies is widely practiced in several parts of India. For acceptable product, the *doda burfi* formulation should contain germinated wheat granulates @ approx. 7 per cent as reported by Gajbhiye *et al.* (2006). Germination increases the bioavailability of proteins, carbohydrates, vitamins and minerals. However, the effect of germination on functional property of resulting flour is scanty. Heat treatment followed by germination improves the quality of ultimate product has been reported by Singh *et al.* (1987).

It has been observed that soaking improves colour of the flour made thereof but subsequent germination darkens it. Germination lowers the gluten content, sedimentation value, damaged starch, non-reducing sugars, free lipids and bound lipids but at the same time increases free fatty acids (Leelavathi *et al.*, 1990).

2.1.2.3 Sugar: Sugar plays a vital role in acceptability of the product and a balanced amount of sugar always fetches attraction. Suresh and Jha (1994)[a] studied the best acceptable level of sugar in *kalakand* and reported that the samples prepared with 7.0 per cent sugar level in milk showed best results whereas Gothwal and Shukla (1995) found increase in browning intensity with increased level of added sugar in *kalakand, pera and* milkcake. Similarly, Gupta *et al.* (2005) used 6 per cent sugar for carrot milk cake preparation.

2.1.3 Method of preparation

Doda burfi can be prepared by either of the two methods described below:

2.1.3.1 Traditional method of manufacturing: Traditionally, *doda burfi* is prepared from buffalo milk. In this method, milk is inoculated with curd culture and left for three to four hours so that it develops acidity up to 0.20 per cent. It is then mixed with a mixture of germinated wheat flour and wheat flour (ungerminated) in the ratio of (flour: milk; 1:10) and left undisturbed at room temperature for 1-1.5 hours. The mixture is then cooked over slow flame till whole milk gets coagulated. The temperature is raised thereafter and when after the ¾ water has been evaporated, sugar is added (16%) and the whole mixture is cooked quickly at temperature of 85-90°C with continuous stirring and scrapping. On cooling product is garnished with nuts and cardamom (Singh and Kumar, 2006).

2.1.3.2 Improved method of manufacturing: Jha (2003) has standardized the process of manufacturing of *doda burfi* at laboratory scale. In this, standardized buffalo milk (12% SNF) is acidified using citric acid (50% solution) and left undisturbed for 15- 30 min, which is then blended with desired proportions of flour and malted wheat flour to milk ratio (1:8), and in order to allow proper moistening, the slurry is kept at room temperature for 30 min. Afterwards the slurry is cooked at 60°C with continuous scrapping and stirring so that the products do not get stick at the bottom of vessel. When product approaches to pasty consistency, the temperature is then raised to 80°C with concurrent addition of sugar. Further heating and scraping leads to the development of brown colour and thick consistency product. Heating is continued till free fat oozes out and finally the mixture is poured stainless steel moulds so as to give product a desired shape.

2.1.4 Physico-chemical characteristics

The chemical composition of some indigenous dairy products is summarized in Table 2.1.

2.1.4.1 Moisture/Total solids: *Doda burfi* is similar to *kalakand* and milk cake in appearance and certain other characteristics. The compositions of these products depend upon the severity of heat treatment given during the desiccation. As no legal standards have been made so far, the gross composition of such traditional dairy products depends on composition of raw material used and their processing conditions. Bajwa *et al.* (2005) reported 34.7 per cent of moisture in freshly prepared milk cake and similar values have been reported by Suresh and Jha (1994)[a] for *kalakand*. Mandokhot and Garg (1985) evaluated market samples and reported lower values of moisture that ranged within 9.94-19.37 per cent for *burfi* and 11.26-20.3 per cent for *peda*.

It has been observed that these indigenous products lack uniformity in their composition due to variation from lot to lot but Rammana *et al.* (1983) investigated that large scale production can solve this problem and found a minor difference in moisture from batch to batch in large scale production of *burfi*.

2.1.4.2 Titratable acidity: Bajwa *et al.* (2005) recorded a titratable acidity of 0.41 per cent in carrot milk cake, whereas a bit higher value i.e. 0.5 per cent was recorded by Suresh and Jha (1994)[a] in *kalakand*. Different values were reported by Patel *et al.* (2006) for *peda* and were 0.4 and 0.54 per cent when prepared using traditional and mechanized methods respectively.

2.1.4.3 Sugars: Sugar, apart from giving sweet taste also imparts brown colour to the product by participating in Maillard reaction. Patel *et al.* (2006) reported an average value of 16.0 and 35.0 per cent as reducing and non-reducing sugars respectively in *peda*. Lower values for reducing sugars i.e. 11.30 per cent have been reported by Bajwa *et al.* (2005) for carrot milk cake. Higher values of 20 per cent were recorded in *burfi* by Palit and Pal (2005).

2.1.4.4 Fat: Madhava *et al.* (2003) standardized the process for manufacturing milk cake and observed that increase in fat content in milk beyond 6 per cent decreases its acceptability owing to excessive free fat release and the final product had 16.0 per cent fat content. Suresh and Jha (1994)[a] reported an average of 21 per cent fat content in *kalakand*.

Table 2.1: Chemical composition of some indigenous dairy products

Product	Moisture	Fat	Sugars	Acidity	Reference
Kalam	15.7	22.9	29.9	-	Ghorpade *et al*, 2006
Pedha	14.7	22.1	32.2	-	Ghorpade *et al*, 2006
	(14.4)	(15.3)	(35.0)	(0.5)	Patel *et al,* 2006
Carrot milk cake	34.7	13.2	36	0.29	Bajwa *et al*, 2005
Burfi	9.94-19.37	13.49	29.0	-	Mandokhot and Garg (1985)
Kalakand	34	21.0	-	0.5	Suresh and Jha,1994[a]
Pinni	9.98	22.65	31.97	-	Saxena *et al*, 1996
Gulab jamun	25.0	10.0-15.0	40.0	-	Nath, 1992
Milk cake	15.0	21.32	40.4	-	Kumar, 2005

2.1.5 Other related aspects

2.1.5.1 Development of colour in heat desiccated indigenous dairy products: *Doda burfi* and many other traditional Indian dairy products are darker in colour and owe their brown colour to Maillard and caramelization reactions. The Maillard reaction was first discovered by Louis Camille Maillard. The reaction takes place between carbohydrates (sugar) and proteins and is responsible for changes in color, flavor and nutritive value of food. The melanoidins formed after Maillard reaction and intermittent cyclized products of caramelization reaction impart dark colour to product (Gothwal and Shukla, 1995.

Though browning is not desirable in all traditional sweets but some sweets like *doda burfi,* milk cake and *gulabjamun* are ought to be darker for their acceptance. Addition of cereal components in sweets also enhances Maillard reaction and thence brown colour. Similar results have been reported by Gothwal and Shukla (1995) in *khoa* based products like *pera, burfi* and milk cake with added *maida*, a fraction of milled wheat.

2.1.5.2 Textural studies: With increasing advancement in science and technology, objective evaluation is playing an outstanding role in getting unbiased response. Taking the same object in mind studies carried out by Patil *et al.* (1990) a psychorheological model was developed to find out the interrelationship between sensory and instrumental data of *khoa* and significant correlations were obtained between Instron hardness and sensory firmness, crumbliness, stickiness and smoothness.

Miyani *et al.* (1990) studied the effect of Fat to SNF ratio of milk on rheological and organoleptic quality of *khoa*. Rheological properties of *khoa* such as hardness, chewiness, gumminess, springiness, cohesiveness, brittleness showed a declining trend with increase in Fat: SNF ratio. They concluded on investigation that Fat:SNF ratio of 0.5 (TS 15 %) resulted in the product with most desirable organoleptic quality. The same results were obtained by Gupta *et al.* (1990).

Rajorhia *et al.* (1990) showed increase in crumbliness with increasing total solids, fat, & protein content and decrease in the same with increase in water dispersible protein (WDP). Similarly *peda* made by two different methods namely traditional and mechanized methods was evaluated by Patel *et al.* (2006) but no significant differences were observed between the two. Prabha (2006) observed that increasing fat level and WPC addition had adverse effect on chewiness, resilience, gumminess and springiness of dietetic *burfi*.

2.1.6 Preservation

Preservation not only attracts consumer, retailer, and wholesaler but also to producer. A long shelf life product symbolizes healthy profits over a longer period. Preservation can be done by many ways and thermal treatment in conjunction with sugar is the most acceptable means to preserve the milk based indigenous sweets. Several other methods like drying (freeze drying, vacuum drying), reduction in water activity (through incorporation of sugar and humectants), and addition of preservatives have been attempted for shelf life enhancement. So far thermal treatment and sugar play a vital role in determining the shelf life of sweets. Different products can be queued in this category such as *kalakand,* milk cake, *peda, burfi, kheer, payasam*, etc. sugar alcohols including sorbitol are known to provide bulkiness, improving texture and mouth-feel and thus serve as sugar replacers particularly in products where heat treatment is required.

Use of potassium sorbate @ 0.1 per cent as preservative extends the shelf life of *burfi* by 60 days as reported by Palit and Pal (2005) whereas potassium sorbate added @ 0.20 and 0.25 per cent in *kalakand* enhanced the shelf life of product from 3 to 24 and 15 days stored at 30° C and 37° C respectively (Suresh and Jha, 1994)[b]. Similarly, Ramanna *et al.* (1983) added sorbic acid @ 0.15 per cent to extend the shelf life of *burfi* and reported the shelf life of 90 days instead of 6-8 days as prepared by traditional methods.

Control of moisture content in the processing of foods is an old time practice for the preservation and longer keeping quality of foods. The principle behind this is either lowering its moisture content upon drying or binding water in such a way that it becomes unavailable for the microbial growth. Addition of various polyols like sorbitol, mannitol and other sugar alcohols and humectants has been carried out by many research workers. Sucrose is commonly added in traditional dairy products as a sweetener which acts as humectant to reduce a_w (Erickson, 1982). The a_w of 67 per cent sucrose solution is 0.86. Prajapati *et al.* (1986) reported that addition of 30, 40 and 50 per cent sugar to *khoa* showed water activity of 0.8333, 0.7936, and 0.7800 respectively in comparison to fresh *khoa* having a_w of 0.9254.

2.1.6.1 Bacteriocins: Preservatives of biological origin are called biopreservatives. Now a day consumers are very conscious about their health, and they are reluctant towards foods containing artificial/synthetic food additives. This gives manufacturer no choice to use artificial/synthetic additives in their foods and led them to search for natural preservatives. The legislation in food industry is also very stringent with restricted use of the permitted levels of approved preservatives (Peck, 1997). These problems forced the food industry to think innovative approaches to preserve food (Jeevaratnam *et al.,* 2005). Moreover, preservation by heat, irradiation and other physical methods induces alteration in chemical and sensory qualities of product. Incorporation of certain anti-microbials to milk and milk products like nisin and other bacteriocins, hydrogen peroxide, microgard, diacetyl, etc. is an old and a promising practice. Incorporation of bacteriocins in products is one of the most talked and current hottest issue in food industry of the millennium (Sarkar and Misra, 2001). Bacteriocins by definition are the proteins containing macromolecules, which exert a bacteriostatic mode of action on susceptible bacteria (Tagg *et al.*, 1976). Antagonistic behaviour of lactic acid bacteria may be due to their metabolic end products or via production of low molecular weight compounds, elaborated by lactic acid bacteria (Dodd *et al.*, 1990).

Bactericidal action of bacteriocins attributed to may be due to loss of cellular potassium ions, depolarization of the cytoplasmic membrane of microbial cells, hydrolysis of internal ATP in sensitive cells, affecting the proton motive force (PMF) dependent processes such as amino acid transport and formation of potassium selective channels by the α and β peptides in the bacterial membrane.

2.1.6.2 Classification of bacteriocins: In general bacteriocins are classified into 4 classes depending on their distinguished characteristics.

Class I: These are known as lantibiotics. These are small membrane active peptides (< 5 kDa) (Sablon *et al.,* 2000). In general these have 19 to more than 50 amino acids. These are further divided into linear (Group IA) and globular (Group IB) lantibiotics. Examples of Class I bacteriocins are Nisin, lacticin 3147A , lacticin 3147b, etc.

Class II: This is the largest group of bacteriocins. These are small (< 10 kDa) heat stable, non-modified peptides and can be further subdivided to Class IIA, Class IIB and Class IIC. Examples of Class II bacteriocins are pediocin, sakacin, leucosinA (Class IIA), lactococcin G, lacticin F (Class IIB), divergicin A, acidocin B (Class IIC), etc. (Bhattacharyya and Bhattacharjee, 2007).

Class III: This class of bacteriocins is large in size (> 30 kDa) and is heat labile (Chen and Hoover, 2003). Example is helveticin V (Sharma and Garg, 2005).

Class IV: This class of bacteriocins are composed of proteins plus one or more chemical moieties like lipid and carbohydrates. These chemical moieties are necessary for their activity (Bhattacharyya and Bhattacharjee, 2007).

There are many methods of preservation using bacteriocins. It can be done by inoculating the food with organism which is capable of producing bacteriocin. Direct addition of bacteriocin (purified/semi purified) is another way. The utilization of previously fermented product using bacteriocin producing strain in food processing is also well practiced (Schillinger *et al.,* 1996).

2.1.6.3 Other antibacterial or anti-fungal substance: A range of different other natural products are available at commercial level for preservation of different food products and can be utilized for the same like Nisaplin and Novasin (nisin based), Microgard (fermentate based), Natamax (natamysin based), etc. (Ref.)

2.1.6.4 Microbiological quality of traditional dairy products: Dairy products are rich source of different nutrients and that too available in plenty, serve as a medium for microbial proliferation. Spoilage due to action of microorganisms is one of the main reasons that leads towards the drastic wastage and unacceptability of the product. Sometimes from the appearance one can not predict the extent of spoilage but there the flavor speaks. Presence of bacteria and yeast have been reported by Vijayalakshmi and Murugesan (2001) whereas in a survey conducted over the sweets like *gulabjamun* and *khoa* procured from local market and the Experimental Dairy of National Dairy Research Institute, showed the contamination by coliforms but the count was within acceptable limits (Kumar and Sinha, 1989).

Sachdeva (1980) reported that the total plate count in *burfi* at zero day was 200/gm which increased to 5,400 and 2,80,000/gm on the 5th and 10th day of storage. The yeast and mould count also increased up to total plate count of 1,100 and 1,80,00/gm after 5th and 10th days of storage at 30° C. A survey carried out by Dwarkanath and Srikanta (1977) to assess the market quality of *peda* (Mysore) presented a SPC count i.e. 7.6×10^2 to 5×10^3 /gm. Dietetic burfi prepared by Prabha (2006) had initial TVC 3.9×10^2 /g, which increased to 7.4×10^3 /g after 20 days of storage period whereas the same burfi in vacuum pack had a count of 6.9×10^3 /g after 40 days of storage period. Similarly studies carried out by Londhe (2006) on brown *peda*, showed initial count of 2.40 (log 10 value) which increased during storage to 3.13, after a storage period of 40 days at 30 ±1°C.

2.2 FUNCTIONAL FOODS AND NUTRACEUTICALS

2.2.1 Definition: Functional foods are defined by the Institute of Medicine of the US National Academy of Sciences as “Functional foods are ones in which concentrations of one or more ingredients have been manipulated to enhance their contributions to a healthful diet”. The concept of functional food includes foods or food ingredients that exert a beneficial effect on host health and/or reduce the risk of chronic disease beyond basic nutritional functions (Hugget and Schliter, 1996).

The term “Functional foods” generally include designer foods, pharma foods and other related nomenclature (Chaudhari, 1999). Successful types of functional products that have been designed to reduce the high blood pressure, cholesterol, blood sugar, and osteoporosis, improvement of gut and intestinal health and immune enhancer are available in market. Currently available ingredients for development of functional foods primarily include antioxidants, fibres, herbs, micronutrients, probiotic, prebiotic, fat/calorie replacers, phytosterols, stanols, etc. Functional foods give therapeutic effects and in no case can be regarded as similar to those with the drugs and hence in Japan where functional foods were commercialized first, these are regarded as Foods for Specified Health Use (FOSHU). Presently these have become the need as according to World Health Organization (WHO) report, nearly 1 billion adults are overweight, with 300 million estimated to be clinically obese (Montgomery, 2004) and the diabetic population of 180 million people will be expected to get double by the year 2030 (WHO, 2006). India has the largest diabetic population and one of the highest diabetes prevalence rates in the world.

The interest in the development of functional foods is growing day by day and is because of growing consumer awareness regarding their health, physical status and at the same time satisfying their needs without compromising with the food intake habits. The fear of diabetes and obesity gave inclination towards artificial sweeteners and non-calorie sweeteners. Regarding sales, it is expected that the sale of functional foods in U.S. will reach $ 59.87 billion in 2009 (Sloan, 2006).

Traditional dairy products (TDP) have been developed since time immemorial to serve the Indian palate and to add value to surplus milk. Presently, a range of TDP's are available and approx. 55 per cent of the milk produced in country is converted into a variety of traditional milk products (Patil, 2002). Presence of higher amount of sweeteners like sucrose restricts its consumption among diabetics, obese, heart patients and health conscious people. Therefore, products with fewer calories zero or no sugar are in demand. Sugar substitutes such as artificial sweeteners can be used to replace whole or a part of sugar without compromising organoleptic quality and posing any adverse effect on consumer's health.

2.2.2 Regulations related to functional foods: Functional foods are regulated by the United States Food and Drug Administration (USFDA) under the authority of two laws. The Federal Food, Drug, and Cosmetic Act (FD&C) of 1938 provides for the regulation of all foods and food additives. In India, functional foods come under the supervision of "Food Safety and Standards Act-2006". Under this act functional foods means "foods which are specially processed or formulated to satisfy particular dietary requirements which exist because of a particular physical or physiological condition or specific diseases and disorders and which are presented as such, wherein the composition of these foodstuffs must differ significantly from the composition of ordinary foods of comparable nature, if such ordinary foods exist, and may contain one or more of the following ingredients:

a) Plants or botanicals or their parts

b) Minerals or vitamins or proteins or metals or their compounds or amino acids

c) Substances from animal origin

d) A dietary substance for use by human beings to supplement the diet by increasing the total dietary intake.

Labeling claims of functional foods are regulated by Codex Alimentarius regarding general declaration facts. Functional foods may be categorized as whole foods, enriched foods, fortified foods, or enhanced foods. Labeling claims that are used on functional foods are of two types:

(1) Nutritional claims which refer to the composition of the food and do not inform the consumer directly about the effect the food may have on the body.

(2) Health claims, on the other hand, inform the consumers about the beneficial effect the food has on the body.

2.2.3 Artificial sweeteners

An artificial sweetener belongs to heterogeneous group of additives that are used to provide sweetness to wide range of processed products in place of conventional sugars. Some of them are naturally occurring molecules extracted from plant materials (Stevioside) and some are synthetic ones like Acesulfame–K, Saccharin, Cyclamate, Dulcin, Phyllodulcin, Hernandulcin, Thaumatin and many more. Not only this, some other new artificial sweeteners are also emerging like Neotame, etc. from the artificial sweeteners itself. For example, Neotame, which is derived from Aspartame has sweetness 1000 times more than sugar and does not have undesirable taste characteristics previously known to be present in high intensity sweeteners (Indra *et al.*, 2002).

2.2.3.1 Stevia: *Stevia rebaudiana* is a perennial Shrub native to the Amambay mountain region of Paraguay and known by different names such as *sweetleaf, sugarleaf, sweet herb, honey leaf,* etc. and belongs to the aster (Asteraceae) or chrysanthemum family of plants. The use of *Stevia rebaudiana* as a food additive is increasing day by day. It is the only naturally occurring non-nutritive sweetener that is permitted in parts of South America, Switzerland and some eastern European countries. Chemically the stevia is glycoside (Stevioside) ($C_{38}H_{60}O_{18}$). 'Rebiana' is the trade name for a stevia-derived sweetener developed jointly by the Coca-Cola company and Cargill with the intent of marketing in several countries and gaining regulatory approval in the US and EU. It has been reported that Stevia isolated from the leaves of *Stevia rebaudiana* is 250-300 times sweeter than sucrose (Hargreaves, 2006). This particular extract has been tested in different recipes since times and it has been concluded that Stevia can be used to replace whole or partial of sugar used in various food preparations and its use is, therefore, need to be enhanced or its potential needs to be explored on a commercial scale (Kerzicnik *et al.*, 1999)**.**

The stevioside is extracted using calcium hydroxide or calcium oxide and then treating extract with $FeCl_3$, followed by desalting, decolouration and evaporation till dryness. It has been pointed out and validated that leaf extract of *Stevia rebaudiana* have beneficial effect on certain physiological systems, such as reducing postprandial blood glucose levels in type 2 diabetic patients, indicating beneficial effects on the glucose metabolism and decreasing hypertension and hyperglycemia (Gregersen *et al.,* 2004) by providing anti-oxidative compounds such as phenols, flavonoids, etc. (Tadhani *et al.*, 2007; Schmandke, 2004). The anti-oxidative activity of stevioside has been found to be higher than sardine oil and linoleic acid (Xi *et al.*, 1998). In a study conducted by Takahashi *et al.* (2001), it has been observed that *Stevia rebaudiana* extract inhibited the replication of all four serotypes of HRV (Human Rota Virus) *in vitro*.

2.2.3.2 The benefits of Stevia as a sweetener

- Stevia balances blood sugar levels; it is calorie-free and is safe for use by both diabetics and hyperglycemic
  - Unlike aspartame, there are no reports of adverse effects from Stevia's use and scientific studies all over the world prove its safety. Also, Stevia reduces the craving for sweets, making it the ideal sweetener for a society desperate to lose weight.
  - Stable when heated up to 200° C
  - Unlike sugar, Stevia reduces cavities by retarding the growth of plaque.
  - Stevia contains antiseptic properties which have proven beneficial in speeding the healing process of skin wounds.

It has been predicted that the market would be $100 million larger if the government allows its use as a sole sweetener (Starling, 2005). Incorporation of *Stevia rebaudiana* powder has been studied in product like yoghurt and it has been found that addition leads to increase in total solids content, increase in the freezing point of frozen yoghurt while decease in Sp. gr. The calorific value of yoghurt decreased by 33.86 per cent when 75 per cent sugar was replaced with Stevia (Salem and Massoud, 2003). Similarly mayonnaise prepared by partially replaced sucrose had same sensory, rheological and microbiological properties. The product showed low calorific value and superior microbiological quality due to anti-microbial effects of Stevia (Zhuravko and Gruzinov, 2004).

2.2.3.3 Safety and stability evaluation of Stevia: In relevance to safety evaluation the study conducted by Geuns (2007) revealed that Stevia and stevioside are safe when used as a sweetener. It is suited for both diabetics, and phenylketonuria patients, as well as for obese persons intending to lose weight by avoiding sugar supplements in the diet. Stability of stevioside has been tested with various other low calorie sweeteners like cyclamate, saccharin, aspartame and acesulfame-K and excellent stability has been assessed under normal conditions of use (Kroyer, 1999). As number of unanswered questions is on the way to judge the safety of Stevia, the FDA has not given green signal to this sweetener yet and its usage has not been taken up at commercial level. However, the Stevia is permitted for use as a "dietary supplement" only (Kroger, 2006).

2.2.4 Dairy products fortified with alternative sweeteners

From the last few decades the consumers needs have prompted the food industry to develop low calorie foods and in this regard dairy industry is no longer behind to adapt itself to trend in the market. A wide range of compounds as intense sweeteners, high potency sweeteners, sugar substitutes are available to meet the requirement of those involved in food product development. The group includes ingredients that provide both physical bulk and sweetness like sugar alcohols (sorbitol, maltitol, xylitol, lactitol, dehydrogenated glucose syrups, trehalose and tagatose). Low-calorie sweeteners are nutritive sweeteners that are relatively less sweet than sucrose and provide energy between 1 to 3 kcal per gram. Low calorie artificial sweetener helps in management of diabetes, dental caries, assist weight control along with creation of variety of tasting foods and beverages (Arora *et al.*, 2001). Many studies have been carried out using different sweeteners in various products.

Verma (2002) had developed ice-cream using artificial sweeteners. A low calorie traditional indigenous frozen dairy delicacy called *kulfi* had been developed by Pandit (2004) using sorbitol (5.5 per cent), maltodextrin (4.26 per cent) and aspartame (742 ppm). Kumar (2000) reported that aspartame at a level of 0.04 per cent was required to replace 6 per cent of crystal sugar for developing artificially sweetened flavored dairy drink. The technology for the production of *rasogolla* was developed by Jayaprakash (2003) using sorbitol (40 per cent) and aspartame (0.08 per cent). Likewise *gulabjamun*, was developed by Chetana, *et al.* (2004) using sorbitol. Acesulfame-K at a level of 0.045 and 0.04 per cent was used as an artificial sweetener in preparation of *burfi*, another *khoa* based sweet delicacy and flavored dairy drink (FDD) respectively. Yarrakula (2006) used acesulfame-K at a level of 0.065 and 0.07 per cent respectively for the preparation of same as above. Muralidhar (2006) prepared FDD using aspartame at a level of 0.07 per cent. Narendra (2006) used saccharin at a level of 0.025 and 0.015 per cent for preparation of *burfi*, and FDD. Singh (2006) prepared the same but used sucralose @ 0.025 per cent in *burfi* and 0.015 per cent in FDD. Likewise, Prabha (2006) used different bulking agents like maltodextrin, sorbitol, etc. at different levels in dietetic *burfi*.

2.2.5 Dietary fibre

In 1953, Hipsley first used dietary fibre to designate non-digestible plant cell wall constituents. Between 1972 and 1976, dietary fibre’s role was expanded to be used in conjunction with a number of health related hypothesis. The dietary fibre hypothesis implies that a high intake of fibre containing foods is directly related to, or is associated with, a low incidence of many disorders and diseases common with western lifestyle (e.g. chronic bowel disease, diabetes, coronary heart disease and colon cancer). Collectively, dietary fibre contributes to a fascinating story about foods nutrition and health. Increased concern about the relationship between diet and chronic disease has led to an increased interest in functional food products (Walker, 1993).

2.2.5.1 Definitions of dietary fibre: Dietary fibre is the term used to describe the total fibre content of the food. It represents the content of substances that cannot be broken down by human digestive enzymes or absorbed by the gastrointestinal tract. It is the non-digestible part of plants that adds bulk to one's diet and acts as a cleanser in the colon. It is found mainly in the cell walls, providing the structure and shape of the plant. Although humans cannot digest dietary fibre, some of it is broken down by gut bacteria to produce gas and acids. Some types of fibre, such as cellulose and hemi cellulose are carbohydrates. Others, such as lignin are not carbohydrates. For years the major information available about food fibre was the crude fibre content, i.e., the residue remaining after the food sample was treated with organic solvents, hot acids and hot alkali. It is much smaller than the actual fibre content of the food.

In the 1970s, dietary fibre was defined as the remnants of edible plant cells including polysaccharides, lignin and associated substances that are resistant to digestion in the alimentary tract of humans. Thus, dietary fibre is a macro constituent of foods, which includes cellulose, hemicellulose, lignin, gums, modified cellulose, mucilages, oligosaccharides pectin and associated minor substances such as waxes, cutin and suberin (DeVries, 1999).

According to the American Association of Cereal Chemists (AACC, 2001), dietary fibre are the remnants of the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. It includes polysaccharides, oligosaccharides, lignin and associated plant substances. Further, dietary fibres exhibit one or more of three major physiological impacts: laxation (fecal bulking and softening, increased frequency and/or regularity), blood cholesterol attenuation, and blood glucose attenuation. Analogous to dietary fibres are those materials, which are not necessarily intrinsic to plants as consumed, but exhibit the digestion and fermentation properties of fibre (e.g. oligosaccharides). Oligosaccharides includes fructan, neosugar, raffinose, stachyose, 4^1-galactosyl-lactose, 6^1-galactosyl-lactose, xylo-oligosaccharides, maltiton, lactitol, palatinit, lactulose, oligofructose and inulin while associated plant substances include phytate, lectins, non-polymeric polyphenols, etc. AOAC (1995) recommended that:

- Resistant oligosaccharides, resistant starch, non-starch polysaccharides and lignin be included in the term dietary fibre, and
- The sum of dietary fibre and analytically measured starch values be considered as complex carbohydrates (implying that total carbohydrates minus sugar minus available oligosaccharides could be used to derive complex carbohydrates concentration).

Non-digestible oligosaccharides are complex carbohydrates which are resistant to hydrolysis by acid and enzymes in the human digestive tract due to the configuration of their osidic bonds e.g., fructo-oligosaccharides (FOS) including inulin and oligofructose; palatinose condensates (PC) including polydextrose, pyrodextrins, sol-oligosaccharides and xylo-oligosaccharides (Roberfroid, 1999). Fructo-oligosaccharides are naturally found in foods such as garlic, onion, leek, asparagus, banana and wheat. If the degree of polymerization (DP) is less that 9, then they are termed as oligofructose, and with an average DP between 12 and 60 are termed as inulin. They are available under the product names of RAFTLOSE® (oligofructose) and RAFTLINE® (inulin). Inulin is obtained by hot water extraction of chicory root and is sweet in taste (one-third of the sweetness of sugar). Inulin is one on the GRAS list.

2.2.5.2 Classification of dietary fibres: Dietary fibre can be classified into two major groups depending on their solubility in water, as insoluble and soluble forms. Insoluble fibres shorten bowel transit time, increase fecal bulk and render feces softer. Insoluble fibres make stools heavier and speed up their passage through the gut. Wheat bran and whole grain as well as the skins of many fruits and vegetables and seeds are rich sources of insoluble fibre. Soluble fibres delay gastric emptying, slow glucose absorption; enhance immune function and lower serum cholesterol level. Soluble fibres include plant gums (such as guar), β-glucans, some hemicelluloses and other compounds, and are found in oats, legumes (peas, kidney beans, lentils), some seeds, brown rice, barley, fruits (such as apple), green vegetables (such as broccoli) and potatoes (Pilch, 1987).

A soluble fibres breaks down as it passes through the digestive tract, forming a gel that traps some substances related to high cholesterol. There are several evidences that soluble fibres may lessen heart disease risk by reducing the absorption of cholesterol into the blood stream.

FDA allows food specific claims for oats and psyllium, which are rich in soluble fibre. Food products must contain at least 0.75g of soluble fibre from edible oat products (oat bran, rolled oats or whole oat flour) or 1.75g of soluble fibre from psyllium husk per serving (Vetter, 1993). Most of the dietary fibres remain undigested; the energy value of dietary fibre is 2.0 kcal/g; although energy value of fibre of particular polysaccharide can vary from 0-4 kcal/g (Wisker and Feldhein, 1992). Majority of the water-soluble fibres have hypocholesterolemic property, while insoluble fibres have little or no effect on cholesterol metabolism (Kritchestvsky, 1999). The possible mechanisms by which a soluble fibre may affect serum cholesterol are inhibition of fat digestion and absorption, increased loss of bile acid and cholesterol, and inhibition of cholesterol synthesis in the liver by propionate or other bacterial products (Truswell and Beyen, 1992).

With increasing knowledge of the importance of fibre rich diets in human nutrition, dietary changes has led pathway towards fibre incorporation. However, recent reports (Truswell, 1993) and recommendations have recognized that dietary fibre should be included in the diet as it lowers blood lipids. Apart from this, dietary fibre also absorbs many organic molecules such as bile acids, cholesterol, steroids, hormones and toxic compounds.

2.2.5.3 Sources of fibre: Most dietary fibres are derived from plant products such as fruits, vegetables, legumes, nuts and whole grains (Table 2.2). But being high water content, fruits and vegetables provide less dietary fibre than the whole grains. The most concentrated sources of dietary fibre are whole grains and cereal brans. Dietary fibre can be classified into two broad categories, namely soluble and insoluble fibre. The most common form of dietary fibre is insoluble fibre comprises of cellulose, lignin and some hemicellulose. Wheat bran, oat husks and corn bran are sources of fibre with high bulking effect. In addition to cereal brans and whole grain cereals, other good sources of insoluble fibre are dried beans, peas, vegetables and nuts. Although soluble sources are less abundant than insoluble fibre, good sources of soluble fibre are whole grain oats and barley, oat bran, some fruits, dried beans and other legumes (Dreher, 2001). Dietary fibre intake should range from 20-35 g/day but the usual intake is half than the required in developing countries. From a nutritional point of view it is recommended to increase the intake of DF, particularly of soluble DF which is considered to be too low in most cereal-based products. It has been reported by Nayak *et al.* (2000), that insoluble dietary fibre sources are known for their preventive action against development of colon cancer while soluble fraction is associated with tumor enhancement.

2.2.5.4 Types of dietary fibre

Soluble dietary fibre: Soluble fibre aids in lowering blood cholesterol level. It slows down the process of absorption of glucose, thereby helps in keeping the blood glucose level in control. A large number of research workers have proved that soluble fibre lowers total and LDL blood cholesterol with less or no effect on HDL cholesterol and triglycerides (Judd and Truswell, 1985). The psyllium holds a valuable position in soluble dietary fibre category and from ancient times it has been used as a laxative (Leeds, 1995). The soluble dietary fibre contribution from various sources is summarized in Table 2.3.

Insoluble dietary fibre: Insoluble fibre ensures smooth bowel movements and thus helps in easily flushing out waste products from the body. But intake should not exceed more than 50 to 60 grams per day, otherwise can pose hurdles for the absorption of vitamins and minerals.

The traditional method for preparation of *doda burfi* includes addition of germinated wheat, which adds fibre to the product prepared thereof through means of wheat bran. It has been pointed out and recorded that some phenolic compounds present in wheat bran contributes to anti-oxidative action (Esposito *et al.*, 2005). Normal wheat i.e. *Triticum aestivum* provides mainly insoluble dietary fibre and has role in stool bulking effect and has a ratio of 1:4.8 crude fibre to total dietary fibre and for bran the ratio is and 1:19.9 (Cubadda and Galli,1989).

Table 2.2: Dietary fibres: sources and physiological functions

Type of fibre	Sources	Physiological function
Insoluble fibre	Wheat bran, corn bran, fruit skins	May contribute to maintenance of a healthy digestive tract; may reduce the risk of some types of cancer
Whole grains	Cereal grains, whole wheat bread, oatmeal, brown rice	May reduce risk of CHD and some types of cancer; may contribute to maintenance of healthy blood glucose levels
Soluble fibre	Psyllium seed husk, peas, beans, apples, citrus fruit	May reduce risk of CHD and some types of cancer
Beta glucan	Oat bran, oatmeal, oat flour, barley, rye	May reduce risk of coronary heart disease (CHD)
Prebiotics		
Inulin, Fructo-oligosaccharides (FOS), Polydextrose	Whole grains, onions, some fruits, garlic, honey, leeks, fortified foods and beverages	May improve gastrointestinal health; may improve calcium absorption

(Source: Mehta and Kaur,1992)

Table 2.3: Soluble dietary fibre from various sources

Source	Soluble fibre content (g/100g)
Psyllium	85.0
Beans, rye, barley	7.8
Oat bran	7.2
Oatmeal	5.0

(Source: Dreher, 2001)

2.2.5.4 (a) Wheat bran fibre: Wheat bran is a component of whole wheat, a food used by humans for millennia. Wheat bran consists of the outer coats of the wheat grain, which can be separated from the germ and various grades of flour during the milling process. The bran amounts to approximately 12–15 per cent of the grain. The estimated amount of dietary fibre in wheat bran ranges between 35-40 per cent (Huang *et al.*, 1978); out of which it contains 40.3 and 2.1 g/100 g edible portion of insoluble and soluble dietary fibre respectively (Dreher, 2001). Various researches have been conducted on wheat bran and it has been proved that it helps in reducing the risk of some type of cancers, obesity, hypertension, constipation and diabetes (Susan and Clark, 2001). Wheat fibre is a component of whole wheat, a food used by humans for millennia. Many clinical studies have shown that wheat fibre is useful in prevention and treatment of constipation. Constipation becomes more prevalent with increasing age. The benefits include reducing transit time, increasing the bowel movement and reducing or eliminating the need for laxative (Read *et al.*, 1995). Clinical studies suggest that wheat fibre is an effective inhibitor of experimentally induced carcinogenesis (Kritchestvsky, 1999).

2.2.5.4 (b) Oat fibre: Oats have been used as human food since ancient times and have been a staple food in many countries. Today oats are one of the richest and most economical sources of soluble fibre, which also contains insoluble fibre. Oat bran provides 22.2 per cent total dietary fibre and 10.5 per cent of soluble fraction (Dreher, 2001). It has also been proved that oat bran have serum cholesterol lowering effects associated with viscous soluble β–glucans (soluble fibre) (Asp *et al.,* 1992). By tradition, oats have been used as human food. Reduction of serum total cholesterol and LDL-cholesterol are the most known physiological effect of oat soluble fibre. It is unanimous that the principal causative component is oat β-Glucan (Kritchestvsky, 1999).

2.2.5.4 (c) Inulin: Inulins are a group of oligosaccharides occurring naturally in many plants. They belong to a class of carbohydrates known as fructans. Inulin is advantageous because it contains 25-30 per cent the food energy of sugar or other carbohydrates and 10-15 per cent the food energy of fat and provides calorific value of 4.0 kJ/g. In a study conducted it has been proved that intake of inulin increase calcium absorption from the diet. High absorption of calcium increases peak bone mass, and therefore, has been proved beneficial to postpone delay of osteoporosis (Bosscher *et al.*, 2006). Inulin is a carbohydrate belonging to a class of compounds known as fructans. It is composed mainly of linear chains of fructose units linked to a terminal sucrose molecule and varies in length from 12 to 60 fructose moieties (Gibson *et al.*, 1994). Some 36,000 plants from wide varieties of genera contain inulin as an energy reserve or as an osmoregulator assuring cold resistance. Among these plants, several have been consumed by mankind for centuries. Examples of commonly consumed foods containing inulin include wheat, onions, garlic, banana, leeks (Van Loo *et al.,* 1995). Because inulin is resistant to digestion in the upper gastrointestinal tract (Knudsen and Hessov, 1995) it reaches the large intestine essentially intact, where it is fermented by indigenous bacteria. Thus, it may be classified as a soluble dietary fibre (Roberfriod, 1993). Non-digestible fructans, especially chicory fructans are known to play a major role in stimulating the growth of beneficial bacteria in the colon, thereby acting as prebiotics.

2.2.5.4 (d) Micro-crystalline cellulose: Microcrystalline cellulose (MCC) plays a vital role in fat free applications also and is been used widely presently. Microcrystalline cellulose is a mechanically disintegrated level of cellulose. It comprises of purified, de-polymerised, microcrystalline sub micron size colloidal particles. It is produced by treating alfa cellulose obtained as a pulp from fibrous plants with minerals acid. Microcrystalline cellulose is offered in ordinary non spray dried and spray dried directly compressible forms under the brand name "***LIBRAWCEL***" (www.//en.wikipedia.org/wiki/Talk:Microcrystalline_cellulose). It is white, odourless, tasteless, extra free flowing powder which is relatively free from organic and non-organic contaminants. It is metabolically inert and has excellent water absorptive, swelling and dispersion properties, and is insoluble in water (http://www.nbent.com/microcryst.htm). It is a non-fibrous form of cellulose produced by acid hydrolysis of the cellulose pulp (Humphreys, 1996). Powdered forms of MCC are commonly used as an inert fibre source and non-calorie bulking agent. It imparts the fat like mouth feel of full fat product (Humphreys, 1996). It is used as fat replacer in salad dressing, bakery and dairy products.

A number of advantages are associated with the MCC, which are:

- forms a soft creamy gel with a typical setup of viscosity, and
- rheologically similar to o/w emulsion (Penichter and Ginley, 1991).

2.2.5.4 (e) Gum acacia: Gum Acacia is a completely soluble dietary fibre prepared from a blend of species and is popular with a trade name "DIESOL". As it is bland in taste and an odourless fibre, it can be used in all the food/pharma/nutraceutical applications without having an effect on the end product (http://drytechindia.com/new01.htm). It is the right choice for nutritional/health/dietetic food products as it has a low calorific value. The major advantages of Diesol include:

- ✓ Minimum 80 per cent soluble fibre (AOAC on dry basis).
- ✓ Stable at a wide range of pH.
- ✓ Bland in taste and flavourless.
- ✓ High level of incorporation up to 10 per cent in products without affecting their viscosity to a major extent.
- ✓ Cold water soluble and heat stable

2.2.5.5 Effects of dietary fibre on mineral availability: Substances found in fibre or associated with fibre may bind to nutritionally important minerals, thus decreasing their availability. Toma and Curtis (1986) found that the addition of 15-20g of fibre from a variety of sources like wheat bran, neutral detergent fibre (NDF), soybean fibre and cellulose did not significantly affect the calcium bioavailability. Kansal and Deodhar (1978) studied the effect of incorporating skim milk powder to leafy vegetables (bengal gram, spinach, fenugreek, mustard and raddish leaves) and cereals (wheat, rice and bajra). They found an increase in the utilization of calcium for all combinations. Takahara *et al.* (2000) found that soluble oligosaccharides such as inulin and fructo-oligosaccharides showed enhanced calcium and magnesium absorption. It is quantifiable by increased femoral bone volume and mineral concentration in rats. Bosscher *et al.* (2003) studied calcium availability from dairy infant formulae supplemented with soluble dietary fibres and modified starches *in vitro*. They found that calcium availability from standard formula was elevated by 30 per cent after inulin supplementation (17.2 per cent), whereas locust bean gum (11.9 %) and high esterified pectin (11.7 %) reduced availability by approximately 10 per cent.

2.2.5.6 Food uses of fibre: Fibre fortification of dairy products has currently generated a great deal of interest in reducing the fat and calorie content besides improving the visual appearance of the product. Milk products such as reduced fat evaporated, condensed and chocolate milks also benefit from the addition of hydrocolloids/gums, which suspend solids as well as provide body. Hennelly *et al.* (2004) successfully incorporated Oatrim (a branded oat fibre) into the limitation cheese matrix at level of 3.44 g/100g cheese. At this level, it directly replaced 63 per cent of the total fat in the formulation without any significant effect on the melting characteristics.

Delaney *et al.* (2003) observed that cholesterol-reducing potency of β-glucans is approximately identical whether its origin was from oats or barley. Carmen *et al.* (2004) investigated the potential use of barley β-glucans in cheese production. Information gained from the rheology and textural characteristics of β-glucans-fat-casein network illustrate that the inclusion of β-glucans has positive effects in the production of low fat dairy products. They observed that there is possibility of use of β-glucans in low fat milk formulations as fat replacer to create low fat curd structure that resembles those of the full fat products. They also observed that coagulation time of the milk was reduced significantly with the incorporation of β-glucans, besides increasing the curd yield. Lario *et al.*, (2004) prepared fibre enriched yoghurt by adding dehydrated orange fibre (from juice by-product) to milk at 1 per cent before pasteurization. They observed that orange fibre fortification enhanced yoghurt acidity during fermentation and did not modify the overall composition.

Costmagns and Rossi (1977) developed yoghurt using dietary fibre in the form of wheat bran added at the rate of 15 per cent to the milk before inoculation with 4 per cent yoghurt culture. They found that wheat bran addition did not impair growth of *Sterptococcus thermophilus* and appeared to have positive effects on the growth of *Lactobacillus bulgaricus* in the incubation phase.

Staffolo *et al.* (2004) reported various sensory and rheological properties of yoghurt added with commercial fibres from apple, wheat, bamboo as well as inulin. The yoghurts were reported to be acceptable at 1.3 per cent fibre level and were stable for 21 days at refrigeration temperature. Yoghurt fortified with inulin scored highest for flavour attributes while the yoghurt with wheat fibre scored highest for textural attributes followed by yoghurt with bamboo fibre.

2.2.6 Dairy products fortified with fibre

Various studies have been conducted with reference to fortification with dietary fibres. In India, some traditional dairy products are manufactured that contain significant quantities of fibre e.g., *gajarella* (carrot milk cake), *giya-ka-halwa* (bottle gourd *halwa*), *doda-burfi*, and *kaju-burfi*. Some cereal based puddings or desserts like *kheer, payasam* and *dalia* are other dairy food sources of dietary fibre in Indian diets (Patel and Arora, 2005). Recently, *dahi* (Chandrakant, 2002), *lassi* and other dairy products have been fortified with fruits and commercial dietary fibres to give the benefits of dietary fibre. Dietary fibre enriched bread suitable for diabetics have been developed by Golob *et al.* (1995). Similarly inulin in bread formulations has been reported to improve dough processing properties and roasted bread rolls had soft texture, good flavour and prolonged shelf life with enhanced nutritional properties (Codina and Bilan, 2006). A low fat paneer using soy fibre and inulin has been prepared and reported to have texture similar to that prepared from full cream milk (6 % fat) in respect to sensory quality (Kantha, 2005).

2.2.7 Phytosterol and phytosterol esters as novel food ingredients

Phytosterols (including plant sterols and stanols) are natural components of edible vegetable oils such as sunflower seed oil and as such are natural constituents of the human diet. Plant sterols have a role in plants similar to that of cholesterol in mammals, e.g. forming cell membrane structures. Plant sterols fall into one of three categories: 4-desmethylsterols (no methyl groups); 4-monomethysterols (one methyl group) and 4, 4-dimethylsterols (two methyl groups). Plant stanols belong to the group of 4-desmethylsterols. Plant stanols are hydrogenation products of the respective plant sterols, e.g. campestanol/campesterol and sitostanol/sitosterol are found in nature at very low levels and structurally these are very similar to cholesterol. Consequently, the normal dietary intake of plant stanols is much less than that of plant sterols. When edible oils undergo normal refining, plant sterols are partially extracted together with some tocopherols (in the process of natural vitamin E production). It is estimated that 2500 tones of vegetable oil needs to be refined to produce 1 tonne of plant sterols (IFST, 2005).

There are over 40 phytosterols, but β-sitosterol is the most abundant one, comprising about 50 per cent of dietary phytosterols. The next most abundant phytosterols are campesterol (about 33%) and stigmasterol (about 2 to 5%). Other phytosterols found in the diet include brassicasterol, α-7-stigmasterol and α-7-acenasterol. β-sitosterol differs from cholesterol by the presence of an ethyl group at the 24th carbon position of the side chain. In the case of campesterol, a methyl group occupies the position. Chemically, the phytosterols are classified as 4-desmethylsterols of the cholestane series.

In the case of most of these products, phytosterols are found esterified with long-chain fatty acids. These phytosterols are derived from soybean oil and are mainly β-sitosterol, campesterol and stigma sterol. Phytosterol esters are hydrolyzed by pancreatic cholesterol esterases (SCF, 2000) and are similar to free phytosterols in the ability to lower cholesterol absorption in rats, suggesting that the liberated phytosterols are the active moieties of the esters (Mattson *et al.*, 1977). Therefore, the available toxicological data on phytosterol esters are considered relevant for the evaluation of the free phytosterols as well.

2.2.7.1 Nutritional aspects: Phytosterols lower serum cholesterol by decreasing cholesterol absorption in the small intestine with a consequential increase in fecal excretion of cholesterol. The principal mechanism of serum cholesterol lowering is considered to be competition between cholesterol and the phytosterol for micellar solubilisation in the small intestine. The first studies demonstrating the cholesterol lowering effect of phytosterols in humans were reported by Pollok in the early 1950's. Since then, a large number of clinical studies with phytosterols have been conducted. Two studies reported recently, investigate the effect of spreads containing phytostanol esters and phytosterol esters when used in conjunction with cholesterol lowering drugs i.e. Statins and Fibreates. These studies showed that phytosterol esters and phytostanol esters can be used safely, to provide an additional cholesterol lowering effect to that of the medication alone (Neil *et al.,* 2001; Nigon *et al.*, 2001).

Another study was carried out in a group of children with familial hypercholesterolemia. Significant cholesterol-lowering effect was seen in these children during the study period (Amundsen *et al.*, 2001 and 2002). This effect was maintained during a further six-month open label follow up period. The spreads were well tolerated by the children with no adverse effects reported. The study confirmed potential benefit of their use by children with familial hypercholesterolemia.

Yellow fat spreads and soft cheese products, supplemented with plant sterols and plant stanols, have appeared on the market. The consumption of average amounts of spreads per day (approximately 20 g in Western Europe) supplemented with 8-10 per cent plant sterol lowers serum cholesterol and LDL-cholesterol by 8-13 per cent (Weststrate and Meijer, 1998; Hendriks *et al.*, 1999). This is equivalent to the consumption of 1.6 - 2.0 g plant sterol per day.

In an efficacy study on plant stanols, a daily consumption of 24 g of spread, containing 2-3 g of plant stanol esters resulted in total serum cholesterol and LDL cholesterol being lowered by up to 6.4 per cent and 10.1 per cent respectively (Nguyen, 1999). A dose dependent decrease in total and LDL cholesterol was observed with increasing levels of plant stanol esters up to a level of 1.6 g per day. However, increasing the dose from 2.4 g to 3.2 g did not provide any clinically important additional effect (Hallikainen *et al.,* 2000a; 2000b). Plant stanol and sterols appear to be equally effective in lowering plasma total and LDL cholesterol (Weststrate and Meijer, 1998; Law, 2000) and in a study with ileostomy subjects, cholesterol absorption was found to be inhibited equally by esterified plant sterol and β-sistostanol esters (Normen, 2000).

Since novel foods approval in the year 2000, Unilever launched yellow fat spreads containing phytosterol esters in 12 EU countries. The recommended intake is 2-3 servings per day from the range of foods containing phytosterol esters. This will be equivalent to a daily intake of 2-3g free phytosterol. The currently available phytosterol added products on the market in the EU include: yellow fat spreads (fat content from 32-63%), yoghurts (natural, vanilla, toffee and fruit flavours including strawberry, raspberry, apricot and black cherry), semi-skimmed milk, chicken balls, chicken gratin (chicken dish with potatoes and cream), sausages, salads (potato and beet root), mayonnaise, cereal bars (chocolate chip, raisin nut and apricot), and soft cream cheeses (natural, garlic and herbs).

2.2.7.2 Effect of phytosterol on cholesterol-lowering effect: The focus of recent scientific literature has been on the cholesterol lowering effect of phytosterol esters and phytostanol esters in yellow fat spreads. However, their effectiveness in lowering both total and LDL-cholesterol in human subjects when incorporated to other foods matrices (yoghurt, milk, salad dressing and lean ground beef) has also been successfully demonstrated (Volpe *et al.*, 2001; Davidson *et al.*, 2001; Judd *et al.*, 2002; Carr *et al.*, 2001; Matvienko *et al.*, 2002). In summary, the food matrix does not influence the cholesterol lowering effect of phytosterol esters.

2.2.7.3 Sources of phytosterol: The major sources of phytosterols for current foods and dietary supplements are tall oil and vegetable oil deodorizer distillate (Table 2.4). Tall oil is a by-product of the kraft pulping of wood to make paper. Tall oil contains a mixture of phytosterols and phytostanols.

Another major source of phytosterols is the deodorizer distillate fraction from vegetable oil refining. A process for the production of phytosterols and tocopherols from deodorizer distillates was developed by Eastman and patented in 1995 (Sumner *et al.*, 1995).

2.2.8 Micronutrients

The term enrichment and fortification are in use synonymy in today's life. The term signifies the act of adding the nutrients that are lost in processing; in such a way that nutritive value corresponds to that of the unprocessed material. Fortification thus refers specifically to the addition of nutrients to a food above the level present in natural food (Richardson, 1990). With respect to all enrichments and fortifications, calcium and iron enrichment is gaining popularity and when it comes to milk calcium fortification, it cannot be overlooked. Due to increased awareness about higher calcium intakes and its role in bone forming material, calcium has become the vital salt in fortification.

Table 2.4: Current and future sources of phytosterols for functional foods

Source	Concentration of phytosterols (Wt %)	Types of phytosterols	Reference
Tall oil	10-20	Free phytosterols (Sitosterol) Free phytostanols (Sitostanol)	Robinson *et al.* (2000)
Deodorizer distillate from soybean oil and from other vegetable oils (palm, rape, peanut, etc.)	15-30	Free phytosterols (Sitosterol)	Sumner *et al.* (1995)
Corn fibre oil	10-15	Free phytosterols (Sitosterol) Phytostanyl fatty acyl ester Phytosteryl fatty acyl ester Phytostanyl ferulate ester	Hicks and Moreu (2001)
Rice bran oil	1-2	Free phytosterols (Sitosterol) Phytostanyl ferulate ester	Hoffpauer and Wright (2002)

According to National Osteoporosis Foundation (1998), 28 million people are suffering from osteoporosis, a bone disorder, and figure may increase to 41 million by 2015. The rapidly changing lifestyles and increasing reliance on more highly processed foods has necessitated the addition of nutrients to an expanding range of foods in order to ensure nutritional adequacy of the diet. The main driving forces behind calcium fortification are:

- The recommended daily intake (RDI) of calcium ranges from 600-1000 mg/day in most countries, which is much more than the actual intake.
- Whenever calcium consumption is low, the human body reacts by pulling calcium away from the bones in order to maintain the functionality of the bio-chemical processes.
- Continuous shortage of calcium leads to brittle bones or osteoporosis.

- As the population ages, the risk increases, especially for women after the menopause and osteoporosis is better prevented than treated.

Calcium from milk is easily absorbed by the intestine in comparison to the calcium from vegetables and cereals. Phytate (present in cereals, beans, pulses), oxalates (present in leafy vegetables), long chain saturated fatty acids and dietary fibre can reduce the bioavailability of calcium by forming insoluble calcium complexes. Cow milk contains 125-150 mg calcium/100g which is much less compared to 180-200 mg/100g in buffalo milk (Pyne, 1962; Sindhu and Sharma, 1986). High intake of calcium will only be possible through formulating calcium enriched stable dairy products, since calcium absorption form other sources is insignificant.

2.2.8.1 Calcium and chronic diseases: Adequate calcium intake has been associated with reduced risk of osteoporosis, hypertension, colon cancer, kidney stone, obesity and lead absorption. A high intake of calcium is not normally regarded as harmful. The precise homeostatic control mechanism ensures that even when excess calcium is consumed; it does not accumulate in the body. However, a failure of these mechanisms can result in hypocalcaemia, which can be life threatening (NIH, 1994).

2.2.8.2 Selection of calcium fortificant source: The choice of calcium fortificant may be made more on technological grounds, since there is little variation in the bioavailability of the calcium from different compounds (Couzy, 1996). The ideal calcium source for fortification of foods should be highly absorbable and available to enhance bone mass, inexpensive and safe for human consumption. The ultimate nutritional benefit of the calcium source must aim at increasing peak bone mass during growth and to reduce bone loss later in life (Weaver, 1998). Several commercial calcium salts are available to the food manufacturers for use in food and dietary supplement e.g. inorganic salts like calcium carbonate ($CaCO_3$), calcium chloride ($CaCl_2$), calcium phosphate and organic salts like calcium citrate, calcium lactate and calcium gluconate. The selection from the wide range of calcium sources available is based upon various selection criteria including solubility, bioavailability and sensory profile (taste and mouthfeel), calcium content, marketing advantages based on natural image, etc. The salt best suited for fortification should possess a high nutritional value and low interference with the absorption of other nutrients besides its cost effectiveness and minimal effect on consistency, mouthfeel and taste of the product (Witte, 2000).

2.2.8.3 Bioavailability: In general, organic salts of calcium ensure more bioavailability than the inorganic salts (Goldscher and Edelstein, 1996). As calcium is ingested, the acid in the stomach begins to break down the molecule into ionic components. The higher the bioavailability, the more calcium ion can travel into the body. In addition, the faster the supplement attains the ionic state, the sooner it becomes available for absorption.

Calcium citrate for supplementation, especially for older individuals where absorption can be a limiting factor due to reduced gastric acid production has been recommended (Avioli, 1988; NIH, 1994). Tricalcium citrate (TCC) is particularly recommended in acidic applications like juices and fruits preparations, and also in various dairy products such as yoghurt and cheese.

As one of the most readily assimilated forms of calcium in human metabolism, calcium gluconate is used widely in both human and veterinary pharmaceuticals to treat calcium deficiency. It has been widely used for fortification of fruit juices, bread, cookies and bakery products, dairy products such as cheese and yoghurt and sport drinks.

2.2.8.4 Sensory profile: Generally, high levels of calcium, particularly insoluble forms like carbonates and phosphates tend to produce a chalky mouthfeel and may promote astringency or bitter notes. Tricalcium citrate, calcium lactate and calcium gluconate are considered to be the most neutral calcium salts, and therefore, are ideal for application in delicately flavoured beverages (Wie, 2002).

2.2.8.5 Technology for fortification: Milk and milk-based beverages have been fortified with calcium. Calcium fortification may also require addition of stabilizers and emulsifiers to maintain calcium in suspension so as to improve mouthfeel and appearance of the product (FAO, 1995). The important processing steps involve the careful control of final pH followed by the addition of calcium salts. Upon addition of calcium salts, there is a marked drop in the pH, which will render the system unstable unless it is corrected to near neutrality by the addition of a food grade base.

2.2.8.6 Calcium salts: A number of factors are responsible which determines the absorption of calcium like effect of meal, solubility, presence of inhibitors and enhancers, etc. When fortification becomes an issue, the solubility characteristics and stability are the ones, which are of prime importance. Organic salts such as calcium lactate, calcium gluconate, tri-calcium citrate and calcium gluconate lactate are more soluble than inorganic salts but suffer with a defect of lesser calcium content than inorganic salts (Ranjan, 2005). Inorganic salts like calcium phosphate and calcium carbonate on the other hand have very poor solubility (Table 2.5). Inadequate calcium intake has been associated with risks of osteoporosis, high blood pressure, hypertension, colon cancer, kidney stones and various other related problems. Calcium fortified foods offer an important avenue for individuals to increase their calcium consumption. Dietary calcium influences the skeletal stores reflected in bone mineral content and helps in building peak bone mass and protect the loss of bone thereafter (Weaver, 1998).

2.2.8.6 (a) Tricalcium citrate: Tricalcium citrate is supplied as a white, odourless, crystalline powder or fine powder with a chemical formula $(C_6H_5O_7)_2Ca_3 \cdot 4H_2O$. It is slightly soluble in water and freely soluble in diluted hydrochloric acid. In contrary to other salts, tricalcium citrate is better soluble at lower temperatures (so-called "inverse solubility"). It is practically insoluble in alcohol. During heat treatment, it is less reactive than other calcium salts due to its inverse solubility. With regard to its significantly better bioavailability, tricalcium citrate is preferred over inorganic calcium sources such as calcium carbonate or calcium phosphate. Among the organic salts with better solubility and more neutral taste profiles, TCC clearly stands out as the most economic option for calcium addition. It is produced by complete neutralization of citric acid with a high purity calcium source such as calcium hydroxide or calcium carbonate. Is a highly bioavailable calcium source in pharmaceuticals, foods and beverages. With its high calcium content of 21 per cent, tricalcium citrate is the most economic calcium salt among the commonly used organic calcium salts. The US Food and Drug Administration (FDA) affirmed tricalcium citrate as generally recognized as safe (GRAS). Its main characteristics are good solubility under cold processing conditions and at low pH values, neutral taste and flavor profile, high calcium content and excellent bioavailability.

Table 2.5: Calcium content and solubility of different calcium salts

Product	Calcium content (%)	Solubility (g/L water)
Calcium lactate	13	66
Calcium gluconate	9.0	35
Calcium chloride	36	-
Tricalcium citrate	21	0.9

(Source: Goldscher and Edelstein, 1996)

2.2.8.6 (b) Calcium carbonate: Calcium carbonate is the active ingredient in agricultural lime, and is usually the principal cause of hard water with a chemical formula $CaCO_3$ bearing molecular weight of 100.09. It is commonly used medicinally as a calcium supplement or as an antacid, but excessive consumption can be hazardous. The main use of calcium carbonate is in the construction industry, either as a building material in its own right (e.g. marble) or limestone aggregate for road building or as an ingredient of cement or as the starting material for the preparation of builder's lime by burning in a kiln. Calcium carbonate is widely used medicinally as an inexpensive dietary calcium supplement or antacid. It may be used as a phosphate binder for the treatment of hyperphosphatemia. Excess calcium from supplements, fortified food and high-calcium diets, can cause the "milk alkali syndrome," which has serious toxicity and can be fatal (http://en.wikipedia.org/wiki/Calcium_carbonate). Calcium has been added to over-the-counter products, which contributes to inadvertent excessive intake. Excessive calcium intake can lead to hypocalcaemia, complications of which include vomiting, abdominal pain and altered mental status (Gabriely *et al.* 2008).

2.2.8.6 (c) Calcium phosphate: Unlike most other compounds calcium phosphate is increasingly insoluble at higher temperatures. Thus heating causes precipitation. In milk it is found in higher concentrations than would be possible at the normal pH because it exists in a colloidal form in micelles bound to casein protein with magnesium, zinc and citrate collectively referred to as colloidal calcium phosphate (CCP) (Huppertz *et al*., 2006).

2.2.8.6 (d) Calcium gluconate lactate: When uniformly labeled ^{14}C, ^{3}H-sodium gluconate was administered intraperitoneally to normal rats for three successive days, 37% of the administered ^{14}C was excreted unchanged in the urine, 14% appeared in expired carbon dioxide, and a fraction (gluconate carbon) was recovered as urinary saccharate. When labeled gluconate was administered to phlorizinized rats, about 10% of the total ^{14}C label appeared in expired carbon dioxide. Urinary glucose from the phlorizinized rats and liver glycogen from the normal rats was uniformly labeled with ^{14}C. When 5 g (84 mg/kg bw) were given orally, none was recovered in the urine. The largest dose given was 30 g, equivalent to 500 mg/kg bw (Chenoweth *et al.,* 1941).

2.2.8.7 Calcium fortified dairy products: Calcium enrichment of food and dairy products has gained interest with the increased awareness about the importance of higher calcium intake. Dairy products are an excellent source of dietary calcium, which can be further fortified with calcium salts to achieve higher calcium intake per serving.

Calcium enriched UHT skim milk was prepared by addition of sodium citrate as stabilizer to milk adjusted to pH 7.3 and then addition of calcium lactate followed by indirect UHT treatment at 139^{o}C for 4 seconds. A stable product $\leq$ 50 per cent calcium enrichment (approximately 160 mg calcium/100 mL milk) could be produced by this method (Guamis *et al.*, 1996). Buffalo milk was fortified with calcium at the rate of 50 mg calcium per 100 mL milk using calcium chloride, calcium lactate and calcium gluconate, and the resultant decrease in pH was restored to its original value by adding disodium phosphate. The maximum heat stability of calcium fortified buffalo milk remained slightly lower than that of unfortified milk. Calcium gluconate-fortified milk had highest heat stability, bioavailability of calcium, partitioning of calcium in the dissolved state and viscosity and the least curd tension compared to other fortified milk, without any adverse effect on sensory properties (Ranjan *et al.*, 2005).

A novel strategy was developed for the calcium fortification of liquid milk and calcium fortified milk powder based on the results obtained in liquid milk system. The process involves adding a combination of orthophosphates with a soluble calcium salt to milk, subjecting the milk to low heat (72^{o}C for 30 sec) or high heat (90^{o}C for 10 min) treatment, concentrating the milk to approximately 450 g TS/kg and spray drying the concentrate to produce a powder with approximately 50 g/kg moisture. These powders were easily reconstituted in water to give skim milk solution (100 g TS/kg) with total calcium content between 2000 and 2300 mg calcium per liter (Wilum *et al.*, 2005).

Calcium fortified cottage cheese was prepared by partially replacing cottage cheese dressing (10-30%) by hydrocolloid in solution (2.5% low viscosity guar gum) and fortified with calcium salts (such as calcium chloride, calcium phosphate, calcium lactate and calcium citrate) at 117 mg added calcium/100 g (Pushpitasari *et al.*, 1991).

Singh *et al.*, (2005), prepared *dahi* from milk fortified with calcium @ 50 mg/100 mL and found that among the three salts namely calcium gluconate, calcium chloride and calcium lactate, dahi from calcium gluconate was better among the all. A research study conducted by Arora *et al.* (2005) on heat stability of fortified milk revealed that addition of calcium salts namely calcium chloride, calcium lactate and calcium gluconate lowers pH and causes destabilization of milk. Addition of disodium phosphate restores its pH and gives more stability than originally it had. The study also stated that organic salts namely calcium lactate and calcium gluconate have more bioavailability than inorganic salts and may be due to more calcium present in aqueous phase. But in another study, it has been pointed out that calcium interacts with dietary fibre specially cellulose and its bioavailability decreases (Goel *et al.*, 1996). Weaver *et al.* (1996) studied the mineral interaction with dietary fibre in humans and concluded that a range of fibre such as lignin, wheat bran, etc. strongly binds minerals such as calcium, cupper, zinc and magnesium.

2.3 SHELF LIFE OF TRADITIONAL DAIRY PRODUCTS

Kinetics of any reaction depends upon several factors and water in this all play an important role in it. To be more precise, water has a critical influencing role over chemical reactivity of all reactions. It may hasten the rate of reaction by acting as a reactant, or may act as a moderator and boost the reaction, or may exert a dilution affect and decrease the rate of reaction. Overall the reaction kinetics gives the idea about shelf life and predicting shelf life with the help of several computerized models is one important aspect under this category. Only packing the food should not be the aim of the ultimate producer rather enhancing the shelf life with the help of selecting suitable packaging material should be the aim and is decisive factor while being purchased by the consumer.

Date and Bhatia (1955) reported that *burfi* with moisture content ranging from 5.0-9.0 % could be stored for 6 months. Sachdeva and Rajorhia (1982) observed the beneficial aspects of hot packaging of *burfi* in tin cans. *Burfi* packaged in tins and stored at 5±1° C kept well for more than 105 days whereas *burfi* packed in parchment paper stored at 30° C and 5 ±1° C was acceptable for 15 and 50 days respectively.

Use of BHA and BHT treated polyethylene films reduced the rate of peroxidation during storage. Rate of deterioration was reported to be lowest at water activity of 0.33. Shelf life studies carried out by Palit and Pal (2005) on *burfi* using preservative and vacuum packaging reported shelf life of 60 days at 30° C without any noticeable sign of spoilage.

2.3.1 Nisin and its significance in in-package processed foods: Nisin represents several closely related polypeptide antimicrobial substances produced by certain strain of *Sterptococcus lactis.* FAO/WHO expert committee on food additives evaluated nisin as a food preservative and recommended that its addition should be considered acceptable. Nisin has inhibitory effect on *Streptococci, Staphylococci, Micrococci,* and vegetative cells and spores of *Bacillus* species (Naquib *et al.*, 1985). The vegetative cells and spores of almost all *Clostridium* species are completely inhibited with nisin levels of 500-1000 IU/mL. The most remarkable property of nisin is its ability to prevent the outgrowth of bacterial spores, which are significant in a wide variety of in-package heat processed foods.

2.3.2 Nisin as a thermal adjuvant in canned foods: The thermal treatments employed during the processing of different canned foods are always aimed to eliminate the microorganisms responsible for the spoilage and toxin production. In the normal course, these canned foods are thermally processed far in excess of the condition required, which results in greater loss of sensory, textural and nutritional qualities (Heinemann *et al.*, 1965). These quality attributes are vital from the consumer acceptability point of view and could be retained to a larger extent by utilizing a sterilization aid, which meets the requirements of being non-toxic, heat stable, economical and active against all types of spores that are likely to survive normal sterilization temperatures. Nisin fulfills the above criteria as thermal adjuvant or in permitting the reduction of time used or the temperature necessary to obtain the same sterilization effect, particularly in canned foods for achieving greater shelf-life. Nisin has also been successfully used in some traditional dairy products at varying levels viz. 200 IU/g in *kheer* (Singh *et al.,* 1987); 100-200 IU/g in *khoa* and 85-100 IU/g in yoghurt (Gupta *et al.,* 1989). Rao (1990) found that initial level of at least 500 IU/g nisin is required for attaining a residual value of around 50 IU/g following thermal processing of in-package sterilized *paneer* at 118^{o}C for 5 minutes.

2.4 PACKAGING OF PROCESSED DAIRY FOODS

Packaging always plays a major role in enhancing shelf life of product. Not only preservation, packaging also boosts the sale of a product by appealing consumer to buy the product. Packaging deserves adequate attention in food manufacturing systems with a view to protect the product from contamination with environmental pollutants and to prolong the shelf-life of products. Sweets like *kalakand*, milk-cake and other sweets have been packaged in paper cartons or duplex boards boxes as reported by Goyal and Rajorhia (1991). Sharma *et al.* (1992) evaluated polyethylene, polypropylene, paper, polyethylene laminate and BHA and BHT treated polyethylene film packaging materials for *besan burfi* and found that at room temperature polyethylene treated with BHA and BHT protected the *burfi* against spoilage for a longer time and the rate of peroxidation was slower in BHT than BHA treated films. Bajwa *et al.* (2005) determined suitability of different packaging material for carrot milk cake and among the different packaging materials i.e. laminated pouches, polyethylene pouches and cardboard box, laminated pouches were found best to preserve the product. Sachdeva and Rajorhia (1982) observed that hot packing of *burfi* in tin containers was beneficial in terms of its keeping quality. It was concluded from the study that addition of 0.15 per cent saffron to *burfi* improved its microbiological quality.

2.5 DESCRIPTIVE SENSORY ANALYSIS

Descriptive analysis is the tool of choice for qualitatively and quantatively differentiating foods and for exploring and defining the relationships between sensory and instrumental perception. Descriptive analysis of any food requires a descriptive technique and a lexicon or language to describe the sensory properties. There are several valid approaches to descriptive analysis (Drake and Civille, 2003). These approaches include Flavour Profile method, Quantitative Descriptive Analysis, the Spectrum technique, and several others. Sensory languages can be identified for any dairy food of interest using any of these approaches. Panelist selection, scale usage and training are the critical parts of any descriptive analysis approach. These specifics are reviewed elsewhere (Drake and Civille, 2003; Meilgaard *et al.,* 1999). A panel or group of individuals (generally 8 to 12 trained panelists are employed for descriptive sensory analysis). A panel of individuals is used as factors such as age, saliva flow and onset of fatigue vary between panelists. Panelists also vary in sensitivity to particular stimuli, and it is highly probable that they also vary in their concentration-response functions. Thus, a group or panel is used rather than 1 or 2 individuals for consistent results. A good descriptive analysis panel will require training by an experienced panel leader. The number of hours of training varies with the food, the number of items, and the modalities used. For instance, texture generally requires less training than flavour. Once trained, a descriptive panel operates as an instrument, as data can be reproduced with any instrument (Lawless and Heymann, 1998) where panelists are units of the instrument, and each panelist should replicate each sample.

Descriptive sensory analysis has been applied as a tool to interpret a myriad of research objectives in dairy foods research in recent years as well as product development goals. Thus, descriptive analysis techniques involve the detection and description of both qualitative and quantitative sensory attributes of consumer products by trained panel of judges (Meilgaard *et al.*, 1999). Academic researchers have used descriptive analysis or specific attribute analysis (sensory characterization) to study aging and various processing parameters in cheese (Drake *et al.*, 2001), for whey and soy proteins (Drake *et al.,* 2007), emollients (Parente *et al*., 2008), malbec wines (Goldner and Zamora, 2007), chocolate milks (Thomson *et al.*, 2004), and beverage (Kappes *et al.*, 2006).

2.6 STATISTICAL TOOL

2.6.1 Principal components analysis: Principal components analysis (PCA) (Shlens, 2005) is a mainstay of modern data analysis- a black box that is widely used but poorly understood. PCA has been called one of the most valuable results from applied linear algebra. PCA is used abundantly in all forms of analysis, from neuroscience to computer graphics, because it is a simple, non parametric method of extracting relevant information from confusing data sets. With minimal additional effort PCA provides a roadmap for how to reduce a complex data set to a lower dimension to reveal the sometimes hidden, simplified structure that often underline it. PCA is a statistical technique applied to a single set of variables to discover which sets of variables in the set form coherent subsets that are relatively independent of one another. Variables that are correlated with one another which are also largely independent of other subsets of variables are combined into factors. Factors which are generated are thought to be representative of the underlying processes that have created the correlations among variables. PCA can be exploratory in nature, is used as a tool in attempts to reduce a large set of variables to a more meaningful, smaller set of variables. PCA is sensitive to the magnitude of correlations robust comparisons must be made to ensure the quality of the analysis. Accordingly, PCA is sensitive to outliers, missing data, and poor correlations between variables due to poorly distributed variables. As a result data transformations have a large impact upon PCA. Correlation coefficients tend to be less reliable when estimated from small sample sizes. In general, it is a minimum to have at least five cases for each observed variable. Missing data need be dealt with to provide for the best possible relationships between variables. Normality provides for an enhanced solution, but some inference may still be derived from non-normal data. Multivariate normality also implies that the relationships between variables are linear. Linearity is required to ensure that correlation coefficients are generated form appropriate data, meeting the assumptions necessary for the use of the general linear model. Univariate and multivariate outliers need to be screened out due to a heavy influence upon the calculation of correlation coefficients, which in turn has a strong influence on the calculation of factors. In PCA, multi-collinearity is not a problem as matrix inversion is not required. If the determinant of R and eigen values associated with some factors approach zero, multi-collinearity or singularity may be present. Deletion of singular or multi-collinear variables is required. PCA is directly used in identification of groups of inter-related variables and reduction of number of variables. PCA is indirectly used as a method of transforming data. Transformation of data through rewriting the data with properties the original data did not have. The data may be efficiently simplified prior to a classification while also removing artifacts such as multi-collinearity. Its goal is to replace the original (numerical) variables with new numerical variables called "principal components" that have the following properties.(a) They can be ranked by decreasing order of "importance" (this term can be given a precise meaning). The first few most "important" principal components account for most of the information in the data. In other words, one may then discard the original data set, and replace it with a new data set with the same observations, but fewer variables, without throwing away too much information. (b) These new variables are uncorrelated. Therefore, PCA can be perceived as a technique for dimensionality reduction. Its original goal (and its main use up to now) is to generate 2D representations of variables and observations for the purpose of visual examination. Any such representation (or "projection") can be obtained in the plane generated by any 2 among the most important principal components. Indeed, conventional use of PCA emphasizes the interpretation of such 2D diagrams. Interpreting correctly PCA diagrams require a good deal of experience. The plane generated by the first two principal components can be interpreted as the plane that "fits best" the cloud of observations. This somewhat vague but intuitive concept can be given a precise meaning. The expectations from PCA is an interpretation of the first principal components in terms that make sense in the context of the data, that is, giving meaningful names to the first principal components and to give the user a chance to detect visually meaningful clusters in the data. Principal component analysis involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called principal components. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. Thus the objectives of principal component analysis are to discover or to reduce the dimensionality of the data set and to identify new meaningful underlying variables.

2.6.1.1 Application of PCA: The PCA has been used in dairy science in studies of sensory characterization of ultrapasteurized milk (Chapman *et al.,* 2001), chocolate milks (Thompson *et al.,* 2004), Cheddar cheese (Drake *et al.,* 2001; Young *et al.,* 2003, Hannon *et al.,* 2007, Kilcawley *et al.,* 2007), Mozzarella cheese (Mauriello *et al.,* 2002), Ricotta cheese (Sinelli *et al*., 2005), acidified milk drinks (Salomonsen *et al.,* 2007), fermented whey (Gallardo-Escamilla *et al.,* 2007).

2.6.2 Response surface methodology (RSM): RSM is a statistical technique, which consists of a group of mathematical and statistical procedures that uses quantitative sensory data to determine and simultaneously solve multivariate equations that specify the optimum product for a specified set of factors.

This considers interaction among the test factors and can be used to determine how the product changes in the factor level (Thompson, 1982; Desai *et al.,* 1992).

2.6.3 Consideration in use of RSM: Before adopting RSM, the researcher should know that the technique is dependent on the following assumptions:

a) The factors, which are critical to the product, are known.

b) The regions of interest where the factor levels influence the product are known.

c) The factors vary continuously through out the experimental range tested.

d) There exists a mathematical function, which relates the factors to the measured response

e) The response that is defined by this function is a smooth surface (Desai *et al.,* 1992).

2.6.4 Steps in RSM

RSM is a four–step process:

a) **Identify factors:** The first step is to identify factors, which are critical and account for most of the variation in the quality of the product under study.

b) **Define factors levels**: The second step is to define a range of factor levels which encircle the product quality. These factors have to be carefully selected keeping in view feasibility, cost and government regulations, if any.

c) **Select test samples:** The third step is to select pertinent samples, from among all the possible combinations to be tested using proper statistical design. The experimental design specifies only those samples which are close to the midpoints of these ranges, thereby decreasing the total number samples to be tested. After selecting the samples, experiments are conducted and related data for example quantitative sensory data on the product quality are obtained and subjected to appropriate statistical analysis.

d) **Data analysis:** The data so obtained are analyzed by an appropriate computer programme, which are further interpreted collectively by a statistician, experimenter, sensory panelists and others, who have participated in the data collection.

2.6.5 Application of RSM: Most of the RSM applications come from area such as optimal sensory quality, setting sensory standards, new/modified products and chemical or engineering processes, industrial research and biological investigations, which emphasis is on optimizing a process or system (Thompson,1982). Henika (1982) described response surface analysis as a useful tool for analyzing experimental data from food products to optimize the physical properties of food products using different levels of ingredients.

The response surface analysis has been used in dairy science in studies of ice cream (Tong *et al.,* 1984; Wittinger and Smith, 1996, and Guinard *et al.,* 1996), whey protein gel (Cooper et al., 1977), whipped topping (Min and Thomas, 1980), long life paneer (Rao, 1983), yoghurt cultures (Torriani *et al .,*1996), cream (Ahn and Kwak, 1999) and milk coagulation (Sbodio *et al.,* 2002).

CHAPTER -3

Material & Methods

MATERIALS AND METHODS

This chapter is composed of experimental details employed in the present investigation, including information pertaining to the raw materials utilized along with methodologies adopted and analytical procedures (sensory, physico-chemical microbiological and statistical), equipments and instruments.

The present investigation is divided into preliminary process of market survey of four renowned cities, characterization and standardization of technology at laboratory scale, and incorporation of functional ingredients for the preparation of functional *doda burfi*, storage study, and consumer survey. Plant trials for the manufacture of *doda burfi* were carried out in the Dairy Technology Division of the National Dairy Research Institute (NDRI), Karnal. The methodologies followed in technological and analytical aspects are delineated hereunder.

3.1 RAW MATERIALS AND INGREDIENTS

The particulars regarding the materials used in the present investigation are delineated hereunder:

3.1.1 Selection of ingredients: Selection of good quality ingredients is without doubt the most important factor for successful investigation. Hence, good quality raw materials were selected for use in the present investigation.

3.1.2 Milk: Freshly pooled buffalo milk obtained from Institutes' cattle yard was received from Experimental Dairy of the Institute in clean, sanitized and dry aluminum can. The milk was standardized to different fat and SNF levels as per the design of Response Surface Methodology (RSM).

3.1.3 Skim milk: The Skim milk (0.02% fat) was obtained after preheating to 40-45°C for effective separation of cream and was then taken in sanitized milk cans directly from the separator and pasteurized at 63°C for 30 min in a steam chest and cooled to room temperature.

3.1.4 Skim milk powder: The skim milk powder (roller dried) containing 1.5% fat, 5.0% moisture and 93.5% SNF was procured from Experimental Dairy of this Institute.

3.1.5 Table sugar: Food grade sugar was obtained from the local market.

3.1.6 Wheat: Wheat of variety PB 343 was procured from local market on the basis of plumpness of kernels. The same wheat was further used after germination for the preparation of product.

3.1.7 Citric acid: Commercial grade (AR) citric acid, monohydrate (SQ grade, Qualigens Fine chemicals, Mumbai) was used for adjusting pH for coagulation of milk.

3.1.8 Cow Ghee: Cow ghee was procured from the Experimental Dairy of this Institute.

3.1.9 Nuts (almond, cashew nut, coconut): High quality nuts like almond, cashew nut and coconut were procured from the local market. Almonds and cashew nut were used after cutting them into smaller pieces while coconut was used after grating.

3.1.10 Tricalcium citrate: Free sample of high grade tricalcium citrate (Batch Number A09TCC0019) was supplied by M/s Sujata Chemicals, Baroda, Gujrat, India.

3.1.11 Calcium chloride: Commercial grade (AR) calcium chloride (SQ grade, Qualigens Fine chemicals, Mumbai) was used for enrichment of *doda burfi*.

3.1.12 Calcium carbonate: Commercial grade (AR) calcium carbonate, (SQ grade, Qualigens Fine chemicals, Mumbai) was used for enrichment of *doda burfi*.

3.3.13 Food grade chocolate brown colour: Chocolate brown colour with brand name "*Ajanta*" was procured from the local market.

3.1.14 Potassium sorbate: Commercial grade (AR) potassium sorbate (Batch Number BO7Z/3606/1704/13) was used as preservative and was procured from SD Fine-chemicals limited.

3.1.15 Dahi culture NCDC 167: Freeze dried lactic cultures NCDC-167 (*Lactococcus lactis* ssp. *diacitilactis*, *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis*) was procured from the National Collection of Dairy Cultures (NCDC), Dairy Microbiology Division, NDRI, Karnal.

3.1.16 Inoculation with culture NCDC-167: The pasteurized skim milk was inoculated with NCDC-167 (*Lactococcus lactis* ssp. *diacitilactis*, *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis*) @ 1.5 per cent and incubated at 25°C for 8 hours. Thereafter, cultured skim milk was blended with the help of blender and kept in refrigerator till admixing with buffalo milk.

3.1.17 Media for microbiological analysis: The media for different microbiological analysis were procured from M/s Himedia laboratories Ltd., Mumbai.

3.1.18 Packaging material: The packaging material (5-layer Natural PFP, 20x20 cm size) was procured from Hitkari Industries Limited, Parwanoo (Solan) Himachal Pradesh.

3.1.19 Bulking agents

a) **Maltodextrin:** Maltodextrin powder (Batch Number 79/B3899) of DE 12-15 was supplied by M/s Sukhjit Starch and Chemicals Limited, Phagwara (Pb), India.

b) **Sorbitol:** Free sample of sorbitol was provided by M/s Sukhjit Starch and Chemicals Limited, Phagwara (Pb), India.

c) **Corn syrup:** Free sample of corn syrup was procured from M/s. Bharat Starch Industries limited, Yamunanagar (Haryana).

d) **Glucose syrup:** Free sample of liquid glucose was obtained from M/s. Bharat Starch Industries limited, Yamunanagar (Haryana).

3.1.20 Dietary fibres

a) **Diesol (Hydrolyzed gum acacia):** Free sample of hydrolyzed gum acacia (Batch Number 75060) available with a brand name Diesol™, was supplied by M/s Drytech processes (I) Pvt. Ltd, India.

b) **Wheat bran:** Wheat bran was availed from M/s S.A. Pharmachem, Chemicals Limited, Mumbai (MH), India.

c) **Oat fibre:** Oat bran (300-33) (Batch Number CA8 259) was procured from M/s S.A. Pharmachem Chemicals Limited, Mumbai (MH), India.

d) **Microcrystalline cellulose (MCC):** Microcrystalline cellulose pure grade 2037 (Batch Number 2037) was procured from M/s Vikaash chemicals, Chennai (TN), India.

e) **Simplesse:** Simplesse ® *1*00 (Batch Number 2J2281F) was provided by M/s CP Kelco, San Diego, United States.

f) **Inulin:** Inulin (Batch Number RTDCW8 DCW8) with brand name *Beneo ST* was procured from M/s DPO Food Internationals Pvt. Limited, Mumbai (MH), India.

g) **Gum acacia:** Commercial grade gum acacia was procured from M/s Himedia laboratories Ltd., Mumbai (Lot: 0000032888).

3.1.21 Stevia powder (Natural High potency sweetener): Natural artificial sweetener *Stevia rebaudiana* was procured from M/s Harboveda (Batch number SW100655), Noida, India.

3.1.22 Microbial metabolites for shelf life enhancement

a) **Microgard:** Free sample of microgard, was supplied by M/s Danisco India Pvt. ltd., Gurgoan.

b) **Nisin:** Free sample of nisin, available with a brand name nisaplin™ was supplied by M/s Danisco India Pvt. ltd., Gurgoan.

3.1.23 Saturated Phytosterol esters: Free sample of Phytostanol esters was supplied by M/s Raisio, Finland.

3.2 METHOD OF MANUFACTURE

3.2.1 Method of manufacture of *doda burfi:* The method of manufacture of *doda burfi* is depicted in Fig. 3.1. The milk was first filtered/clarified and then standardized for adjustment of fat levels as per RSM design. Buffalo milk was utilized along with *dahi* (similar to western yogurt) for coagulation purpose. Germinated wheat flour (GWF) was mixed with *dahi* and mixed with milk of standardized formulation with respect to fat and SNF followed by mixing of both and in order to allow proper moistening of the slurry where it was kept at room temperature for 30 min. Afterwards the slurry was cooked slowly with half of the added total sugar required with continuous scrapping and stirring so that the products did not stick at the bottom of vessel. When product approached to pasty consistency, the temperature is then raised further with concurrent addition of sugar. Product was further heated and scrapped which led to the development of brown colour and thick consistency product. Heating was continued till free fat oozed out and finally the mixture was poured in stainless steel moulds so as to give product a desired shape.

3.2.1.1 Optimization of fat, germinated wheat flour (GWF) and sugar levels for standardization of technology for the manufacture of *doda burfi:* The influence of different combinations of three factors viz. fat level (% of milk), GWF (% of milk) and sugar (% of milk) on quality of *doda burfi* was studied. The product was evaluated for colour, texture, sweetness, flavour and overall acceptability. Though physical parameters and textural attributes were also studied but were not taken under consideration for optimization studies. The experiment was carried using 3-factor Box-Behnken of Response Surface Methodology (Tables 3.1 and 3.2).

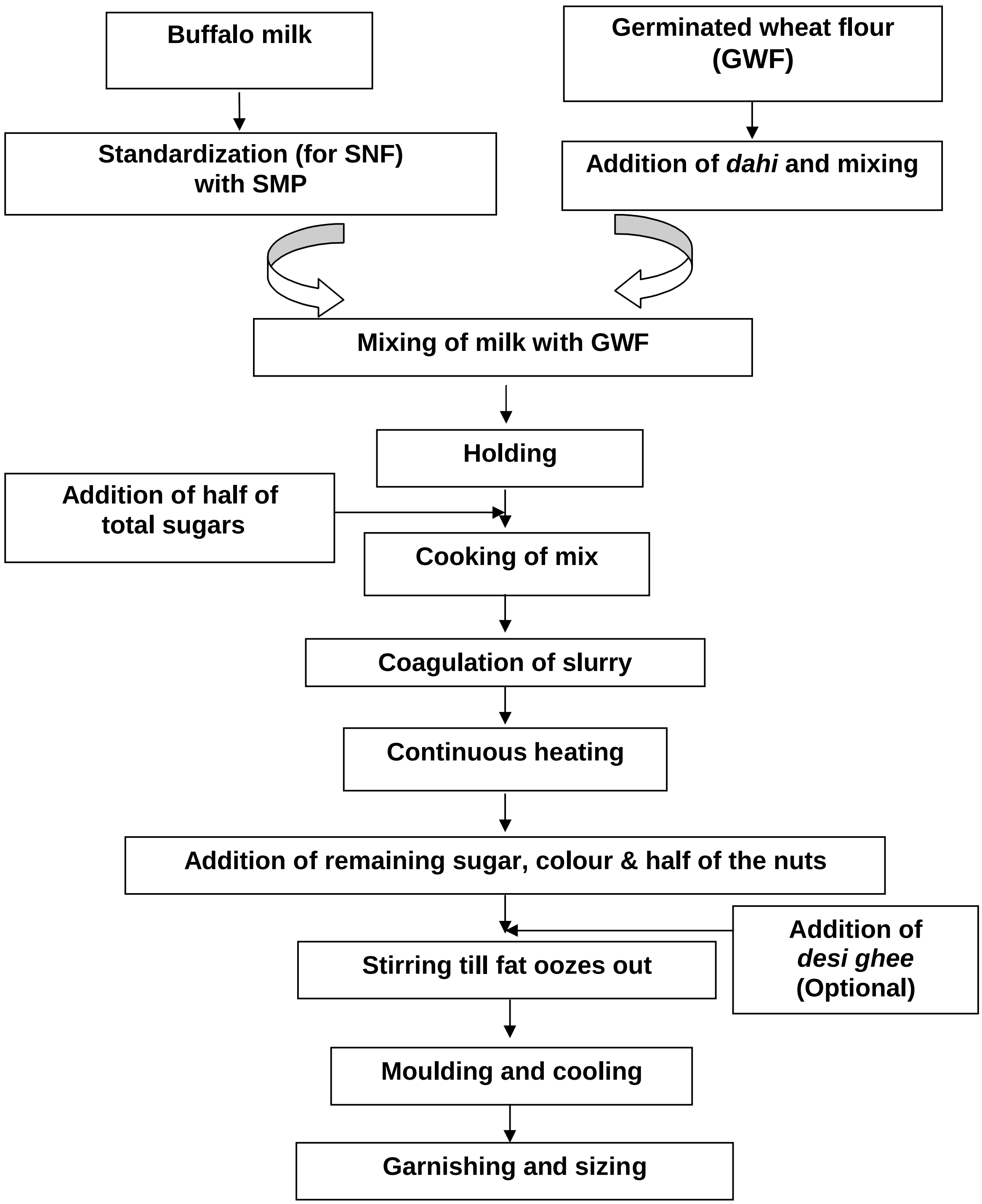


Fig. 1: Flow diagram of a standardized process for manufacture of *doda burfi*

Table 3.1: Level of variables used for Box-Behnken design

Variable	Code	levels tested		
		-1	**0**	**+1**
Fat levels (%) in buffalo milk	**X1**	3	4.5	6
Germinated wheat flour (GWF) (% of milk)	**X2**	2.5	4	5.5
Sugar (% of milk)	**X2**	14	16	18

Table 3.2: RSM design using culture NCDC-167 (3-factors) for optimization

Standard order	X1	X2	X3
1	-1	-1	0
2	1	-1	0
3	-1	1	0
4	1	1	0
5	-1	0	-1
6	1	0	-1
7	-1	0	1
8	1	0	1
9	0	-1	-1
10	0	1	-1
11	0	-1	1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0
16	0	0	0
17	0	0	0

3.3 METHOD OF MANUFACTURE OF DIETETIC DODA BURFI

3.3.1 Optimization of oat fibre, Diesol (hydrolyzed gum acacia, stevia and sugar levels for manufacture of dietetic *doda burfi:* The influence of different combinations of three factors viz. oat fibre (% of milk), diesol (% of milk), stevia (% of milk) and sugar (% of milk) on quality of *doda burfi* was studied. The product was evaluated for colour, texture, sweetness, flavour and overall acceptability. Though physical parameters and textural attributes were also studied but were not taken under consideration for optimization studies. The experiment was carried using 4-factor CCRD of Response Surface Methodology (Tables 3.3 and 3.4).

Table 3.3: Level of variables used for CCRD design

Variable	Code	levels tested				
		-2	-1	0	+1	+2
Oat fibre (%)	X1	1	1.50	2.00	2.50	3
Diesol (%)	X2	0.375	0.50	0.625	0.75	0.875
Stevia (%)	X3	0.14	0.19	0.24	0.290	0.34
Sugar (%)	X4	2.4	3.2	4.0	4.8	5.6

Table 3.4: RSM design using functional ingredients (4-factors) for optimization

Standard order	Oat fibre (%)	Diesol (%)	Stevia (%)	Sugar (%)
1	-1	-1	-1	-1
2	1	-1	-1	-1
3	-1	1	-1	-1
4	1	1	-1	-1
5	-1	-1	1	-1
6	1	-1	1	-1
7	-1	1	1	-1
8	1	1	1	-1
9	-1	-1	-1	1
10	1	-1	-1	1
11	-1	1	-1	1
12	1	1	-1	1
13	-1	-1	1	1
14	1	-1	1	1
15	-1	1	1	1
16	1	1	1	1
17	-2	0	0	0
18	2	0	0	0
19	0	-2	0	0
20	0	2	0	0
21	0	0	-2	0
22	0	0	2	0
23	0	0	0	-2
24	0	0	0	2
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0

28	0	0	0	0
29	0	0	0	0
30	0	0	0	0

3.4 METHOD OF MANUFACTURE OF FUNCTIONAL DODA BURFI

3.4.1 Optimization of oat fibre, and calcium levels for manufacture of functional *doda burfi:* The influence of different combinations of two factors viz. oat fibre (% of milk), and calcium (mg/100mL milk) on quality of functional *doda burfi* was studied. The product was evaluated for colour, texture, sweetness, flavour and overall acceptability. Though physical parameters and textural attributes were also studied but were not taken under consideration for optimization studies. The experiment was carried using 2-factor CCRD of Response Surface Methodology (Tables 3.5 and 3.6).

Table 3.5: Coded and real levels of functional *doda burfi* in the two factor CCRD Experiment

Variable	Code	levels tested		
		-1	**0**	**+1**
Oat fibre (%)	**X1**	0.59	2	3.41
Calcium (mg/100mL)	**X2**	39.64	75	110.35

Table 3.6: The CCCRD design for manufacture of *doda burfi* using culture NCDC 167

Standard order	Factor-1	Factor-2
	Oat fibre %	Calcium (mg/100mL)
1	-1	-1
2	1	-1
3	-1	1
4	1	1
5	-1.414	0
6	1.414	0
7	0	-1.414
8	0	1.414
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0

3.5 ANALYTICAL PROCEDURES

3.5.1 Physico-chemical analysis: The brief description of different methods used to examine physico-chemical properties of *doda burfi* is given below.

3.5.1Moisture: The moisture content in *doda burfi* was determined by standard gravimetric method as described in IS (1981).

3.5.2 Fat: Fat was determined with the help of Mojonnier fat extraction apparatus as specified in IS (1967).

Five g of *doda burfi* sample was accurately weighed into a small beaker and made to paste with an equal amount of warm (65^{o}C) distilled water. Two mL of concentrated ammonia solution (sp.gr. 0.88) and 9 mL of distilled water were added. The contents were swirled gently, cooled and 10 mL ethyl alcohol (95% v/v) was added.

The contents were then completely transferred to Mojonnier fat extraction tube and swirled for 2 min. Twenty five mL each of diethyl ether (sp. gr. 0.721; peroxide free) and petroleum ether (40^{o}-60^{o}C BP) were added and the tube was closed with a wet cork and shake vigorously for 1 min. The tube was centrifuged for 1 min to separate the ethereal layer from the aqueous layer and the supernatant liquid was decanted in clean, dry previously weighed aluminum dish. Extractions were repeated twice using 15 mL of diethyl and petroleum ether each time. The solvents were distilled off and the dish heated in the oven at 98–100°C, cooled and weighed. Heating and weighing were repeated till weighing did not show a loss in weight by more than 1 mg. Fat was calculated by weight difference method.

3.5.3 Protein: The protein content of *doda burfi* was determined by micro Kjeldahl method (Menefee and Overman, 1940).

Accurately weighed 200 mg of *doda burfi* sample was transferred to a Kjeldahl digestion tube followed by addition of 2 g of digestion mixture comprising of K_2SO_4, $CuSO_4$ and SeO_2 (in ratio 100:10:1) and 15 mL concentrated H_2SO_4. The contents were then digested to a transparent clear fluid. The digested material was transferred to a 100 mL volumetric flask and distilled water was added to make the volume upto the mark. An aliquot (10 mL) of digest was distilled with 50% sodium hydroxide and the librated ammonia was collected in 10 mL saturated boric acid containing 2-3 drops of mixed indicator (prepared by dissolving 100 mg methyl red and 30 mg methylene blue in 60 mL of 95% ethyl alcohol and then making up the volume to 100 mL with distilled water).

Approximately 65 to 70 mL of distillate was collected in a 100 mL conical flask. The contents of the flask were titrated against 0.02N HCl. A blank determination using distilled water in place of sample was also carried out. The total nitrogen and percent protein were calculated as follows:

$$\text{Per cent Nitrogen} = \frac{14.007(\text{S-B}) \times \text{Normality of HCl} \times \text{dilution factor} \times 100}{1000 \times \text{Weight of sample}}$$

where,

X = mL of HCl required for sample

Y= mL of HCl required for blank

N= Normality of HCl used, and

W= Weight of the sample in mg.

Percent Total Protein = Percent total nitrogen x 6.38

3.5.4 Reducing and total sugars: Sugars were determined following the method prescribed by IS (1981). Accurately weighed 40 g of sample was taken in 100 mL beaker to which 50 mL of hot water (80-90°C) was added and after thorough mixing, contents were transferred to 250 mL volumetric flask. Final volume was made up to about 120-150 mL. The contents in the volumetric flask were then mixed and cooled to room temperature followed by addition of 5 mL of 10 per cent dilute ammonia. After 15 min, 5 mL of 10 per cent dilute acetic acid was added to neutralize the added ammonia. To this, 12.5 mL zinc acetate was added, followed by 12.5 mL of potassium ferrocyanide solution and mixed again. Finally content was made to 250 mL volume using distilled water and further filtered through Whatman no. 1 filter paper. The filtrate thus obtained was marked as B_1. From $B_{1,}$ 25 mL was taken into a 100 mL volumetric flask and 5 mL of conc. HCl was added followed by heating at 68°C for 5 min. The same was cooled and neutralized with 50 per cent NaOH and made up to 100 mL with distilled water and was marked as A_1. The solution marked as A_1 was diluted 20 times while B_1 was diluted 4 times and were marked as A_2 and B_2 respectively. Both the solutions were taken into a burette and titrated against the mixture of 5 mL each of Fehling A and Fehling B solutions added with a mixed indicator. Similarly, standard lactose and sucrose were taken and titrated. For calculation, the following formula was used:

10 mL of Fehling (A+B) solution= V_1 mL of standard invert sugar solution of concentration C_1 mg/L (i.e. C_1=2.5 mg/mL)

10 mL of Fehling (A+B) solution= V_2 mL of sample solution before inversion of concentration C_2 mg/mL

10 mL of Fehling (A+B) solution= V_3 mL of inverted sample filtrate having concentration C_3 mg/mL

$$V_1C_1 = V_2C_2$$

$$\therefore C_2 = \frac{V_1C_1}{V_2}\frac{mg}{mL}$$

$$C_2 = \frac{V_1C_1}{V_2 * 1000} * \frac{250}{40} * \frac{100}{25} * 100\ \%$$

$$C_3 = \frac{V_1C_1}{V_3 * 1000} * \frac{250}{40} * \frac{100}{25} * \frac{100}{25} * 100\ \%$$

3.5.5 Titratable acidity (% lactic acid): The titratable acidity was determined by adopting the method as described by AOAC (1975). Two g of sample was taken in a porcelain dish and mixed homogenously by adding 20 mL hot distilled water (65°C). This was followed by addition of 10 mL of 0.1 N sodium hydroxide and 1 mL of 0.5% phenolphthalein indicator. The mixture was titrated against 0.1 N hydrochloric acid with continuous stirring till the pink color disappeared completely. Acidity was expressed as per cent lactic acid.

3.5.6 Ash: Ash was determined by adopting the procedure given by AOAC (1975). About 2 g of the product was weighed accurately in a silica dish and ignited on a Bunsen burner with final incineration in a muffle furnace at 550±5°C for 2 h. Constant weight of ash was considered to have reached when the difference in the two consecutive weighings after repeated ignition was less than 0.2 mg. Ash content was expressed as percent of the gross product.

3.5.7 pH: Ten g of grated *doda burfi* sample was weighed in a 50 mL beaker and made into paste by addition of 10 mL of glass distilled water. The pH was measured by LabIndia pH-analyzer.

3.5.8 Dietary fibre: Total dietary fibre content in the product was determined using 'Total Dietary Fibre Assay Kit' supplied by Sigma Aldrich Inc. (TDF-100A). It uses a combination of enzymatic and gravimetric methods which is based on AOAC (2000). The principle involves gelatinization of samples with heat-stable α-amylase and enzymatic digestion with protease and amyloglucosidase to remove the protein and starch present in the sample. Ethanol precipitates the soluble dietary fibre. The filtered residue is washed with ethanol and acetone and weighed after drying. Half of the residue is analyzed for protein and the other half for ash. Total dietary fibre is the weight of the residue less the weight of the protein and ash.

Fritted crucibles of porosity #2 (coarse 40-60μ) were washed and heated in a muffle furnace at 525°C for one h and cooled. They were then soaked and washed in water followed by air drying. Celite (0.5 g) was added to each crucible and dried at 130°C in a hot air oven to constant weight (for one h). The crucibles were then cooled in a desiccators and weighed to the nearest 0.1 mg. This was recorded as ‘W_1’ (celite + crucible weight). These crucibles were stored in the desiccators until needed.

Samples and blanks to be tested were run in quadruplicate so that duplicate protein and ash values were available for improved accuracy. One g samples of defatted *doda burfi* were weighed into tall form beakers in such a way that sample weights did not differ by more than 20 mg. The weights were recorded to 0.1 mg. 50 mL of phosphate buffer (pH 6.0) was added to each beaker. Using a micropipette 0.10 ml α-amylase (Product code A3306) was dispensed into each beaker and mixed well. Beakers were covered with aluminum foil and placed in a water bath. Contents were incubated for 15 min after the internal temperature reached 95°C and beakers were agitated gently at 5 min intervals. They were then allowed to cool to room temperature. The pH of the solutions was adjusted to 7.5±0.2 by adding 10 ml of 0.275 N NaOH. Again pH was checked and adjusted using NaOH or HCl. 50 mg/ml solution of protease (Product code P3910) was prepared immediately before use in phosphate buffer (0.08 M, pH 6.0). Using a micropipette accurately 0.1mL of this solution (5 mg protease) was added into each beaker. After covering with aluminum foil, the beakers were kept in a 60°C water bath. They were incubated for 30 min after the internal temperature of the beakers reached 60°C with continuous agitation. After cooling the solutions to room temperature, the pH was adjusted to 4.0 using 0.325 M HCl (~10 ml). Again 0.1 mL of amyloglucosidase (Product code A9913) was added to each beaker with a micropipette. After covering the beakers were again kept for incubation in a water bath at 60°C for 30 min after the internal temperature reached 60°C. Four volumes of 95 per cent ethanol was added to each beaker and were kept overnight at room temperature to allow complete precipitation.

The bed of celite was wetted and redistributed in each crucible using 78 per cent ethanol. Using gentle suction the celite was drawn onto the frit as an even mat and maintaining that suction the precipitate and suspension were transferred from each beaker to its respective crucible. The residue was washed with three 20 ml portions of 78 per cent ethanol, two 10 mL portions of 95 per cent ethanol and two 10 mL portions of acetone. The crucibles containing residues were dried overnight in an oven set at 105°C. The crucibles were cooled in a desiccator, weighed to nearest 0.1 mg. This weight was recorded as 'W_2' (residue + celite + crucible Weight).

The residues from two samples and two blanks were analyzed for protein by Kjeldahl nitrogen analysis. The residue in the crucible from the remaining two samples and two blanks were ignited for 5 hours at 525°C in a muffle furnace. After cooling in a desiccator, they were weighed to nearest 0.1 mg and the weight recorded as 'W_3' (ash + celite + crucible weight).

Calculations:

$$Residue\ weight = W_2 - W_1$$

$$Ash\ weight = W_3 - W_1$$

$$B = [R_{BLANK} - P_{BLANK} - A_{BLANK}]$$

$$\%\ TDF = \left[{(R_{SAMPLE} - P_{SAMPLE} - A_{SAMPLE} - B)}/_{SW}\right] \times 100$$

Where,

TDF= total dietary fibre

R= average residue weight (mg)

P = average protein weight (mg)

SW = average sample weight (mg)

3.5.9 Free fatty acid: The method prescribed by Deeth *et al*. (1975) was used to estimate the FFA content of *doda burfi*. The method consisted of accurate weighing of 5 g of *doda burfi* sample into a 60 mL stoppered test tube. Ten mL of extraction mixture (Isopropanol: petroleum ether: 4N H_2SO_4 in the ratio of (40:10:1) was added and mixed thoroughly. This was followed by the addition of 6 mL petroleum ether and 4 mL distilled water. The test tube was stoppered and tempered at 40^oC for 10 min the contents were vigorously shaken for 20 sec. The two layers were allowed to separate for 10-15 min and an aliquot of the upper layer (5-8 mL) was withdrawn and titrated against 0.02 N methanolic KOH solution using 1 percent methanolic phenolphthalein indicator. The FFA content of *doda burfi* was obtained from the following formula:

$$\text{FFA } (\mu \text{ eq/g}) = \frac{\mathbf{TN}}{\mathbf{PW}} \times 1000$$

Where,

T = mL of 0.02 N KOH used

N = Normality of methanolic KOH solution

P = Proportion of upper layer of aliquot titrated/total aliquot

W = Weight of sample (g)

3.5.10 Hydroxy methyl furfural (HMF): Total HMF in *doda burfi* (stored) was determined by the method recommended by Keeney and Bassette (1959) with slight modification.

Three g of *doda burfi* was thoroughly mixed with 7 mL distilled water. Five mL of 0.3 N oxalic acid was added and the tube was kept in boiling water bath for 60 min. The contents of the tube were cooled and 5 mL of 40 percent trichloroacetic acid solution was added. The precipitated mixture was filtered through Whatman No. 42 filter paper. 0.5 mL of the filtrate was pipetted out into a 5 mL test tube and added with 3.5 mL of distilled water and 1 mL of 0.05 M Thiobarbituric acid solution (aq) and mixed well. The tubes were kept in water bath at 40°C for 50 min. After cooling to room temperature, absorbance was measured at 443 nm. A blank test was carried out in the same manner as above substituting distilled water for *doda burfi*.

Total HMF (μ mol/litre) = (Absorbance – 0.055) x 87.5 x 0.4

3.5.11 Tyrosine value: The tyrosine content as a measure of proteolysis in *doda burfi* was calorimetrically determined according to the method of Hull (1947).

Five grams of *doda burfi* was exactly weighed and taken in a clean dry test tube, added with 10 mL of distilled water followed by addition of 10 mL of 0.72 N TCA (117.64 g TCA 1000 mL^{-1} soln.). The test tubes were then stoppered shaken vigorously and incubated at 27°C for 10 min. The precipitated proteins were filtered through Whatman No. 42 filter paper and the tyrosine content in the filtrate was determined calorimetrically. To 5.0 mL of this TCA soluble filtrate, 2.0 mL of distilled water, 10 mL of sodium carbonate reagent (75.0 g anhydrous Na_2CO_3 and 10.0 g sodium tetra phosphate dissolved in glass distilled water and diluted to 500 mL) followed by 3.0 mL of the Folin's phenol reagent (Folin's phenol reagent diluted 1: 2 with distilled water) was added, mixed well and incubated again at 27°C for 10 min for colour development. The intensity of blue colour so developed was measured at 650 nm using spectrophotometer.

Developing regression equation

A stock solution of L-tyrosine was prepared by dissolving 100 mg in a minimum quantity of 0.1 N sodium hydroxide and the volume was made up to 100 mL with distilled water. Twenty five mL of this solution was then made up to 250 mL with distilled water to give a tyrosine concentration of 100 μg 5 mL^{-1}. A series of dilution was prepared from this working solution to have standards with tyrosine concentration ranging from 10-100 μg 5 mL^{-1}. Five mL aliquots of the tyrosine standards were treated as previous for developing the color. The standard curve is given in Fig 3.1. The following regression equation was obtained using the absorbance of the standard solutions against their tyrosine concentration (μg 5 mL^{-1}).

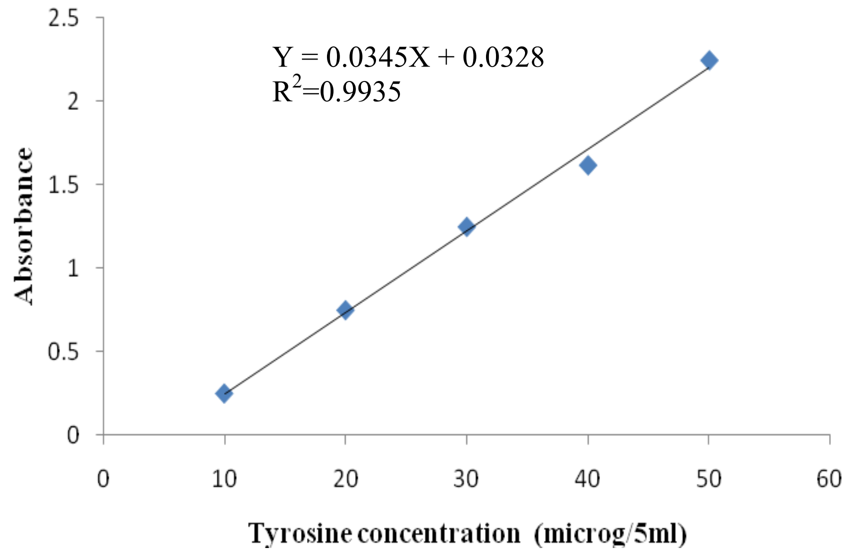


Fig. 3.1 Linear (0.0345X + 0.0328, R^2=0.9935) calibration curve for tyrosine in the range between 10 to 50 μg/5 ml of water

Where,

X = Concentration of tyrosine in μg 5 mL^{-1}

Y = absorbance at the respective tyrosine concentration.

3.5.12 Mineral analysis: One gram sample of *doda burfi* was digested in Kjeldhal tube at very low heat initially by adding 15 mL of tri acid mixture (HNO_3: $HCLO_4$:H_2SO_4 = 3:1:1) and at a higher temperature till the content were clear and perchloric acid fumes ceased to come out. The final volume after digestion was made to 25 mL and filtered and through ash free filter paper to remove silicates and other insoluble material.

Estimation of Ca, Fe and Zn: The Ca, Fe, and Zn content were estimated with the help of Hitachi Z- 5000 Polarized Zeeman Atomic Absorption Spectrophotometer (AAS) using acetylene as fuel and air as oxidant. Specific hollow cathode lamp was used to determination of three elements. The procedure was described in AAS manual (2003) were followed:

In the estimation of calcium the elements such as aluminum, beryllium, phosphorous, silicon, titanium and mineral acids are known to mask the response of calcium in air acetylene flame (AAS manual, 2003). Therefore, strontium as strontium chloride was added as a damasking agent in the process of dilution of acid extract so that a concentration of 0.2 per cent strontium was achieved in the sample as well as in the standards as recommended in the AAS manual.

3.6 SENSORY EVALUATION OF *DODA BURFI*

The sensory evaluation of fresh and stored samples of *doda burfi* was conducted by a panel of seven selected judges. Seven individuals from academia (from the faculty of Dairy Technology Division) participated in a roundtable discussion to generate descriptive terms related to colour & appearance, flavour and texture. Prior to sensory evaluation, sensory panel was trained and briefed about the terminology used to judge the product involving a descriptive intensity scale of 100 points for describing major sensory attributes. Market samples were analyzed on descriptive scale using twenty attributes for color, texture, flavour and overall acceptability whereas standardization of recipe was carried out using 9-point Hedonic scale.

3.6.1 Descriptive sensory analysis: The descriptive sensory analysis of *doda burfi* was done as per the method suggested by (Delahunty and Drake, 2004). Market samples of *doda burfi* were evaluated sensorily by a trained panel of seven judges. A descriptive language was developed by judges for various attributes and *doda burfi* was presented to panel for validation with the identified lexicon (Appendix I). A 10 cm linear scale for each attribute was drawn with corresponding marking of 0, 25, 50, 75 and 100 and panelists were asked to draw a vertical line across the horizontal line. The panelist rated the intensity of a given stimulus by making a mark on a horizontal line which corresponds to the amount of perceived intensity of the attribute in question. The marks from line scale were converted to numbers by manually measuring the position of each mark on each scale using a scale. The panelists evaluated products one at a time in separate booths to reduce distraction and panelist's interaction to give unbiased rating. The panel accounted for the attributes (qualitative) of a sample, and the intensity of each attribute (quantitative), detected differences among products in the order in which certain parameters manifest themselves. Five panelists were selected from Dairy Technology Division, NDRI according to their ability to discriminate difference in sensory properties among samples and consistency in sensory analysis. The panelists were free to develop their own approach to scoring. The results of a quantitative descriptive analysis (QDA) test were analyzed statistically and the graphic representation of the data was presented in the form of spider web (Radar graphs) with a branch or spoke from a central point for each attribute. The score card used for judging the market samples is presented in Appendix - II.

3.6.2 Sensory evaluation of laboratory made standardized *Doda burfi* samples: The samples prepared by standard procedure were evaluated sensorily by a trained panel of judges in order to assess the quality of the fresh product on a nine point Hedonic scale. Stored *doda burfi* samples were evaluated sensorily after a certain interval during storage studies by a trained panel of five judges to determine the period of its acceptability. The score card used for judging the product is presented in Appendix - III.

3.7 TEXTURAL ANALYSIS

Various textural properties such as hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness were studied using TA-XT2i (Stable Micro Systems, UK) Texture Analyzer fitted with a 25 kg load cell.

The samples of cylindrical shape of height 1 cm and 1 cm diameter were subjected to mono-axial compression of 80 per cent of the initial sample height which were already tempered to 25°C. The force distance curve was obtained for a two-bite compression cycle employing a cross head speed of 2.5 mm/sec (Fig 3.1). The pre settings test conditions maintained were as under:

Test mode and option	:	TPA
Pre-test speed	:	5 mm/sec
Test speed	:	2.5 mm/sec
Post–test speed	:	2.5 mm/sec
Strain	:	80 per cent
Trigger type	:	Auto
Probe	:	P75 Compression Platen

Temperature : 25^oC

The typical graphs obtained were analyzed using Texture Expert Exceed Software supplied along with the instrument. The following texture profile parameters were obtained.

Hardness: The peak force of the first compression cycle (first bite) expressed in Newton (H_1).

Adhesiveness: It is indicated by the area of negative peak following the first peak (H_2) (N.s).

Springiness: The height that the sample recovers during force relaxation time between first and second compression cycle (Dimensionless)

Cohesiveness: The ratio of positive force area during the second compression to that during the first compression (A_2/A_1) (Dimensionless)

Gumminess: Gumminess is related to primary parameters of hardness and cohesiveness and is obtained by multiplication of these two parameters (N).

Chewiness: The energy required to masticate food into a state ready for swallowing and is a product of gumminess and springiness (N).

3.8 COLOUR MEASUREMENT

The colour of the *doda burfi* was measured using a Colorflex colorimeter supplied by Hundterlab (Hunter Associated Laboratory, Inc., VA, USA) along with the software (version 4.10). Before the test, the instrument was calibrated with standard black and white tile as specified by the manufacturer. The light source was dual beam xenon flash lamp. Data was received through the software in terms of L* (lightness), ranging from zero (black) to 100 (White), a* (Redness), ranging from +60 (red) to -60(green) and b* (yellowness), ranging from +60 (yellow) to -60 (blue) values of the international colour system.

The finely ground sample is placed in the transmission port of the optical unit of a color difference meter. Light energy from a controlled source is directed through the specimen into an integrating sphere. Phototubes are positioned at the top of the sphere to view the interior of the sphere. Electrical signals proportional to light quantities present are directed by cable to the measuring unit, where they are read directly as L*, a* and b* colour values.

3.9 WATER ACTIVITY (a_w)

Water activity was measured using water activity meter Aqua Lab (Model Series 3 TE) supplied by Decagon Devices, WA, USA.

3.10 MICROBIOLOGICAL ANALYSIS

3.10.1 Preparation of dilution blanks: The dilution blank consisted of 0.08 per cent (w/v) sterile sodium chloride solution (AR grade, procured from M/S S D-Fine Chem. Ltd., Mumbai) in 99 mL and 9 mL portions in screw capped dilution test tubes. These were autoclaved at a pressure (121°C) for 20 minutes. The dilution blanks were warmed to 45°C before use for preparation of samples.

3.10.2 Preparations of dilutions: One gram of *doda burfi* was weighed in sterile aluminum dish, in the balance using a sterile spatula. The contents of the aluminum dish were then transferred to a sterile dilution blank of 9 mL. This gives a dilution of 1:10 from this initial dilution further dilutions were prepared by transferring 1 mL into 9 mL blanks.

3.10.3 Total plate count: Plate count agar (Hi-media) was used to enumerate the total plate counts in the *doda burfi* sample.

To rehydrate this medium 23.5 g of the dry medium was suspended in 1000 mL distilled water. The mixture was then boiled to dissolve the medium completely. It was then filled in conical flask and the mouth of the conical flask was closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15 psi pressure (121^{o}C) for 15 min.

One mL of the diluted sample (suitable dilution) was transferred in each of the triplicate petri dishes. Fifteen to twenty milliliter of the melted agar (at 45^{o}C) was then poured, and the contents were mixed well by rotating in a clock wise and anti-clockwise position slowly. The contents were allowed to solidify at room temperature. The plates were then inverted and incubated at 37^{o}C for 24-48 hrs.

3.10.4 Yeast and mould count: Potato dextrose agar (Hi-Media) was used to enumerate yeast and mould counts in the *doda burfi* samples.

To rehydrate this medium 39 g of PDA powder was suspended in 1000 mL distilled water and then boiled to dissolve the medium completely. It was then filled in conical flasks and then mouths of the flasks were closed with cotton plugs. The flasks were then sterilized by autoclaving at 15 psi pressure (121^{o}C). The pH of the media was adjusted to 3.5 at the time of plating by using 10% sterile tartaric acid solution.

One mL of the diluted sample (suitable dilution) was added to each of the triplicate sterile petri dishes and 10-15 mL of the melted Potato dextrose agar (at 45^{o}C) was added to each petri dish. The contents of the petri dishes were mixed by rotating the plates in horizontal position placing them on a table. The media was allowed to solidify and then inverted and incubated at 25±2^{o}C for 3 to 4 days.

3.10.5 Coliform count: Violet red bile agar (Hi-media) was used to enumerate the coliform counts in *doda burfi* samples.

To rehydrate this medium 41.5 g of VRB agar powder was suspended in 1000 mL distilled water. Then the mixture was brought to boil to dissolve completely. The media was then cooled to 45^{o}C and poured into conical flasks (150 mL). This media was not autoclaved.

To each of the three sterile petri dishes, was added 1 mL of 1:10 dilution of the sample. To each of these petri dishes, 10-15 mL of melted (45^{o}C) violet red bile agar was added and the contents of petri dishes were mixed well by rotating the plates by placing them horizontally on a table. The media was then allowed to solidify and then a second layer of agar was made by adding 5-10 mL of melted agar. The media was allowed to solidify and then incubated after inverting the plates at 37±1^{o}C for 2 days.

3.11 STORAGE STUDIES

The optimized product was prepared and packed in multilayer laminates. The product was packed in multilayer laminates under non vacuum conditions at 30^{o}C. The samples were analyzed at a regular interval of three days for the sensory, chemical, and microbiological attributes to determine the shelf life of the product.

3.12 COST ESTIMATION

The cost of manufacture of *Doda burfi* was estimated as per the guidelines of Narnaware (2000) with some modifications. In order to arrive at a realistic cost of processing and that of the end product, certain assumptions were also made.

3.13 CONSUMER ACCEPTANCE TRIALS

For consumer acceptance trial, the product was made and packed separately in multilayer laminates. It was distributed to about 110 probable consumers (Alvensleben and Schrader, 1998). The response was elicited by a consumer survey form (Appendix IX).

3.14 STATISTICAL ANALYSIS

The data obtained during the present investigation was analyzed by employing suitable statistical designs. The quality of market samples in terms of sensory, chemical, textural, colour and microbiological terms was assessed by employing analysis of variance (ANOVA). Descriptive scale used while evaluating the market samples was analyzed using Principal component analysis (PCA) and was done by using Unscrambler software (unscrambler version 7.5 programme, Camo A/S, Oslo, Norway 2000) procured from U.S. CAMO, (www.camo.com). The data was statistically analyzed as per the methods described by Snedecor and Cocharan (1994) and Evanston (1990) with the application of SYSTAT software, version 6.0.1 1996, SPSS INC and also by Microsoft® Excel StatPro™ (Palaside Corporation, Newfield, NY). Response surface methodology (RSM) was also used to optimize the various production parameters such as, milk fat level (%), per cent GWF and per cent sugar. While storage data was analyzed by Least Square Design using SYSTAT, version 10.2.05 procured from SYSTAT Software Inc, 501 Canal Boulevard, Suit F1 Richman CA 98408-2028.

chapter-4

plan of work

PLAN of work

4.1 MARKET SURVEY FOR PRODUCT CHARACTERIZATION

Several samples of *Doda burfi* manufactured by traditional method were procured from different markets of renowned cities of Northern India for analysis at NDRI, Karnal. Cities were chosen on the basis of their excellence in their manufacturing practices and market reputation with good brand image like Delhi, Ludhiana, Jalandhar and Karnal.

4.1.1 Documentation of the information collected about *Doda burfi*

Proximate parameters and technical details collected following the market survey have been documented.

4.1.2 Market areas: number of samples

a) Delhi 4

b) Ludhiana 4

c) Jalandhar 4

d) Karnal 4

4.1.3 Analysis for physico-chemical properties

4.1.3.1 Proximate composition

a) Moisture (IS: 1964)

b) Fat (IS: 1981)

c) Protein (Menefee and Overman, 1940)

d) Sugars (Total and Reducing sugars) (IS: 1981)

e) Ash (AOAC,1975)

f) Acidity (AOAC,1975)

4.1.3.2 Colour (Hunterlab, U.S.A)

4.1.3.3 Water activity (Aqua lab, U.S.A)

4.1.3.4 pH (LABINDIA pH Analyser)

4.1.3.5 Dietary Fibre (AOAC, 2000)

a) Insoluble fibre

b) Soluble fibre

4.1.3.6 Textural properties

Texture profile analysis (TA-XT2i)

4.1.4 Microbiological Analysis

a) Total plate count

b) Yeast and mould count

c) Coliform count

4.1.5 Descriptive sensory analysis

4.1.5.1 Selection of panelists (5-7) for descriptive sensory evaluation

4.1.5.2 Training of the panelists for developing descriptive terms and product attribute scale

4.1.5.3 Generation of data and application of principal component analysis for sensory characterization of *doda burfi*

4.2 PROCESS STANDARDIZATION FOR COMMERCIAL MANUFACTURE OF *DODA BURFI*

4.2.1 Optimization of Technological Parameters

Technological parameters were optimized by employing response surface methodology (RSM) (3-factor Box-behnken).

4.2.1.1 Fat level in mixed milk (%)

a) 3.0

b) 4.5

c) 5.5

d) 6.5

4.2.1.2 Level of wheat granules (germinated wheat flour) (% in milk)

a) 2.5

b) 3.5

c) 4.5

d) 5.5

4.2.1.3 Level of sugar) (% in milk)

a) 14

b) 15

c) 16

d) 18

These were the best acceptable levels, which were selected after preliminary trials and market survey, after conducting physico-chemical tests and sensory evaluation and were taken for further optimization studies of variables.

4.2.2 Evaluation of the standardized product

4.2.2.1 Physico-chemical properties

Proximate composition (vide 3.1.3.1)

4.2.2.2 Colour (Hunterlab, U.S.A)

4.2.2.3 Water activity (Aqua lab, U.S.A)

4.2.2.4 pH (LABINDIA pH Analyser)

4.2.2.5 Dietary Fibre (AOAC, 2000)

a) Insoluble fibre

b) Soluble fibre

4.2.2.6 Textural properties (vide 3.1.3.6)

4.2.3 Microbiological analysis (vide 3.1.4)

4.2.4 Sensory profile analysis (9-point Hedonic scale)

4.3 INCORPORATION OF FUNCTIONAL INGREDIENTS*

To enhance functionality of the product, following ingredients were added.

a) Replacement of sugar with Stevia * (0.02- 0.07%)

b) Addition of calcium salts *

Tri-calcium citrate (50 and 75 mg/100 mL milk)

c) Addition of fibre**

a. Wheat bran

b. Oat fibre

c. Inulin

d. Hydrolyzed gum acacia (Diesol)

e. Micro-crystalline cellulose

d) Addition of bacteriocins# (On % yield basis)

Pediocin (2000 AU)

a) Addition of potassium sorbate (antibacterial) and Microguard 100 (anti-fungal substance) (0.5 – 1.0 %)# (On % yield basis)

**The two best fibres were screened on the basis of sensory evaluation and were used further along with other best technological parameters, to be optimized using RSM.

* Best Levels were optimized using RSM (4-factor, CCRD).

#Addition of bacteriocins and any other antifungal or anti-bacterial substance for shelf life enhancement studies was taken up over the final optimized product, resulted through RSM.

4.4 PREPARATION OF FUNCTIONAL *DODA BURFI* WITHOUT REPLACEMENT OF SUGAR

To enhance functionality of the product, following ingredients were added.

a) Addition of calcium salts *

Calcium carbonate or calcium chloride (50 and 75 mg/100 mL of milk)

b) Addition of fibre

   a) Oat fibre* (1.0-3.0% of milk)

   b) Hydrolyzed gum acacia (Diesol) (0.5% constant for all the RSM trails)

   c) Addition of phytosterol (On % yield basis)

   d) Addition of bacteriocins# (On % yield basis)

   Pediocin (2000 AU)

   b) Addition of potassium sorbate (antibacterial) and Microguard 100 (anti-fungal substance) (0.5 – 1.0 %)# (On % yield basis)

* Best Levels were optimized using RSM (2-factor, CCRD).

Addition of bacteriocins and any other antifungal or anti-bacterial substance for shelf life enhancement studies was taken up over the final optimized product, resulted through RSM.

4.4.1 Analysis of functional *doda burfi*

- Total dietary fibre

   a) Insoluble fibre

   b) Soluble fibre

- ❖ Determination of added calcium content (Atomic Absorption Spectra)

4.5 SHELF LIFE EVALUATION

For storage study, multilayer laminates were used for the packaging of the product.

4.6 STORAGE STUDIES

Storage temperature was conducted at 30°C and the following parameters were analyzed during storage-

- ❖ TBA
- ❖ HMF
- ❖ FFA
- ❖ pH (LABINDIA pH Analyser)
- ❖ Water activity (Aqua lab, U.S.A)
- ❖ Colour (Hunterlab, U.S.A)
- ❖ Texture profile analysis (vide 3.1.3.6)
- ❖ Microbiological studies (vide 3.1.4)

4.7 STATISTICAL ANALYSIS OF DATA

4.8 COST ANALYSIS

4.9 CONSUMER SURVEY

chapter-5

Results and discussion

Results and discussion

The present research work was undertaken to unveil the *doda burfi* from almost every aspect. The foremost part was to characterize *doda burfi*, which is a popular traditional sweetmeat of northern India within states like Punjab, Haryana, U.P and Rajasthan. Standardization of recipe and improvement in functionality was also undertaken with the incorporation of certain health benefitting nutraceuticals and levels were optimized using response surface methodology (RSM). The first phase of study dealt with analysis of market samples bought from diverse regions of northern India. A process was also standardized for manufacture of *doda burfi* using response surface methodology for optimization of various ingredients for different responses. With an objective to produce a healthful sweetmeat, various nutraceuticals such as soluble dietary fibre, calcium and phytostanol were tried and the product was developed. The optimized product obtained thereafter, was studied for storage studies in terms of sensory, physico-chemical, microbiological and instrumental textural changes at 30°C storage temperature with 5-ply polyethylene pouches. The last part of the study was related with consumer response studies of the developed product and cost analysis for the manufacture of the product to evaluate the techno-economic feasibility of the technology. The results obtained during these studies are presented and discussed in this chapter.

5.1 CHARACTERIZATION OF MARKET SAMPLES OF *DODA BURFI*

Doda burfi, a traditional and a popular sweetmeat considered as a delicacy on several occasions is a hitherto undocumented traditional dairy product of northern India with little or almost negligible information available about the product composition or its characteristics. Product is prepared by coagulating milk and cereal based slurry using *dahi* culture and little amount of citric acid. The manufacturing of the product is still limited to certain specific areas of northern population and lacks scientific basis, which is necessary for the manufacture of the product on commercial scale and its mechanization. This study was, therefore, planned with the objectives of characterizing the market samples of *doda burfi*. Sixteen lots of fresh *doda burfi* samples, four each from four cities viz., Delhi, Jalandhar, Ludhiana and Karnal; the four renowned cities for manufacturing of *doda burfi* in northern India, were brought to Dairy Technology Division, NDRI Karnal. The samples for microbiological studies were collected in sterilized pouches (multilayer laminate) so that count is least affected with transportation and handling. All the samples were subjected to descriptive sensory analysis, detailed physico-chemical, microbiological, instrumental colour and texture profile analysis.

5.1.1 Descriptive sensory analysis of market samples of *doda burfi*

Descriptive sensory analysis has been applied as a tool to interpret a myriad of research objectives in dairy foods research in recent years as well as in product development goals. It has been applied to a wide variety of cheeses, wines, flavored milks, etc. In the present study, market samples were evaluated subjectively by a trained panel of seven judges. The panelists were selected from Dairy Technology Division of NDRI Karnal according to their ability to discriminate difference in sensory properties among samples. The panel accounted for the quantitative attributes of a sample, the intensity of each attribute detected, and differences among products in the order in which certain parameters manifest themselves. The panelists were free to develop their own approach to scoring. A descriptive language was developed by the panel of judges for various attributes. *Doda burfi* samples were presented to panel for validation with the identified lexicon (Appendix I). Prior to sensory evaluation, sensory panel was trained and briefed about the terminology used to judge the product. Descriptive sensory analysis (DSA) technique was employed to carry out the evaluation of samples and total of nineteen attributes of colour, flavour and texture characteristics were generated which could fully describe the *doda burfi.* An additional attribute to judge the preferability/liking of product (overall acceptability) was added along with other descriptive attributes. Descriptive intensity scale of 100 points was used for describing the attributes. A line of 10 cm ranging from 0 to 100 points was drawn with marking interval of 25 points for evaluation and reporting sensory results (Appendix II). Panelists were asked to mark a vertical linear mark indicating the sample number along the horizontal linear scale for a particular attribute. The main descriptors used for surface appearance were colour and glossy character while the flavour was best described using caramelized, unclean, off-flavour, rancid, nutty, doughy, oxidized, stale, bitter and acidic flavour whereas texture descriptors used in the study were crumbliness, granularity, firmness, chewiness, cohesiveness and stickiness. The marks from line scale were converted to numbers by manually measuring the position of each mark on each scale using a scale. The panelists evaluated the products one at a time in separate booths to reduce distraction and panelist's interactions. The results of a sensory test were analyzed statistically. A graphic representation of the data in the form of spider web with a branch or spoke from a central point for each attribute is given in Fig. 5.1.

The mean sensory scores for colour and appearance, texture and flavor are listed in Table 5.1 and its corresponding statistical analysis is presented in Table 5.2. The variation in descriptive sensory profile of colour and appearance, texture and flavour in different cities are shown in Fig 5.2 and 5.3.

5.1.1.1 Colour and appearance

The attributes for colour and appearance of *doda burfi* are important sensory attributes as far as consumer is concerned. The data relating to different sensory attributes of colour and appearance obtained from market of Karnal, Ludhiana, Delhi and Jalandhar and used for principal component analysis (to maintain the uniformity in data, results obtained from minimum of 4 panelists are taken) is presented in appendices III and IV.

(a) Glossiness: Wide variations were observed in glossiness score of market samples with range from 13 to 83, 0 to 87, 43 to 83 and 25 to 77 for Karnal, Delhi, Ludhiana and Jalandhar cities, respectively. The maximum mean score (Table 5.1) was obtained for samples procured form Ludhiana market (65.25 ± 2.95) followed by Delhi (64.56 ± 5.37), Karnal (58.25 ± 4.70) and the lowest was recorded in Jalandhar samples (52.56 ± 3.30). There were not significant differences ($p<0.05$) for the glossy character between samples of different market areas (Table 5.2).

(b) Surface colour: Colour is an important rather a deciding parameter of the product which persuades a consumer to buy a product. The colour of *doda burfi* ranged from amber yellow to chocolate brown with mid values corresponding to moderately brown colour of the product. The maximum dark colour was observed in samples procured from Ludhiana city (69.18 ± 2.95) followed by Karnal (63.62 ± 4.00), Jalandhar (61.75 ± 4.21) and the colour was least in samples of Delhi market (60.00 ± 4.40) (Table 5.1). There were not statistical differences ($p<0.05$) for the score found between the markets and samples of various cities surveyed (Table 5.2).

5.1.1.2 Flavour

(a) Caramelized: Caramelized flavor is an important attributes of heat desiccated products and is considered an important parameter from its acceptability point of view by its ultimate user. The maximum mean score (Table 5.1) of Karnal samples was (63.75 ± 2.65) followed by Delhi (63.50 ± 4.08), Ludhiana (62.93 ± 4.65) and was least in Jalandhar samples (48.00 ± 6.01). The corresponding range of the same attribute was 41 to 80, 25 to 86, 25 to 86 and 0 to 88 for Karnal, Delhi, Ludhiana and Jalandhar cities, respectively. There were significant differences ($p<0.05$) between the various cities surveyed for the *doda burfi* (Table 5.2). Though the differences found were very minute in the cities amongst Karnal, Delhi and Ludhiana, but the Jalandhar city had significantly less caramelized samples obtained from different shops.

(b) Doughy: Doughy flavour of the product comes from the more intense flavour produced from the incorporation of more of the cereal component. In *doda burfi* the same flavour could be due to germinated wheat and is considered undesirable in extreme cases. Doughy character was found highest in Karnal samples (52.75 ± 3.47), followed by Delhi (50.06 ± 4.34), Ludhiana (45.31 ± 4.07), and was least in Jalandhar samples (39.37 ± 6.40) (Table 5.1). There were no significant differences found for doughy flavour ($p<0.05$) between *doda burfi* market areas (Table 5.2). The corresponding range was 29 to 76, 9 to 74, 25 to 82 and 0 to 80 respectively for Karnal, Delhi, Ludhiana and Jalandhar cities.

(c) Nutty: Nutty flavour of the product comes from the incorporation of nuts added during the processing and at the final stages for garnishing purposes. Various nuts like cashew nut, almond and coconut in grated form can be added which gives pleasing flavor to the product. Nutty character (Table 5.1) was found highest in Karnal samples (60.12 ± 4.64), followed by Delhi (58.56 ± 4.70), Ludhiana (58.25 ± 3.75), and was least in Jalandhar samples (42.18 ± 5.80). There were significant differences found for nutty flavour (p<0.05) between *doda burfi* market areas (Table 5.2).

(d) Unclean: The minimum mean score (Table 5.1) for unclean attribute was found in Karnal samples (3.06 ± 1.13), followed by Ludhiana (4.00 ± 3.13), Jalandhar (5.37 ± 2.68), and was maximum in Delhi samples (5.56 ± 3.47). The range for unclean attribute varied from 0 to 14, 0 to 50, 0 to 50, 0 to 36, for Karnal, Delhi, Ludhiana and Jalandhar samples respectively. There were no significant differences found for unclean flavour (p<0.05) between *doda burfi* market areas (Table 5.2).

(e) Acidic: Acidic character is an indication of developed acidity in the product. Though the fresh product retains some of the inherent acidity but developed acidity was not liked by the trained panelists. The maximum acidity was observed (Table 5.1) in samples collected from Karnal city (7.93 ± 2.80) followed by Delhi (6.62 ± 3.35), Ludhiana (5.31 ± 3.14) and was least in samples of Jalandhar market (3.43 ± 1.64). There were no statistical significant differences found (p<0.05) between different market areas (Table 5.2).

(f) Rancid: The rancid flavour is also undesirable in *doda burfi* from consumer's point of view. The maximum mean score (Table 5.1) of rancid attribute was found in Karnal samples (7.93 ± 3.35) followed by Jalandhar (4.87 ± 2.13) and Delhi (3.93 ± 1.44) while it was minimum in (2.31 ± 0.94) samples procured from Ludhiana. The corresponding range was 0 to 46, 0 to 16, 0 to 11 and 0 to 25, for samples procured from Karnal, Delhi, Ludhiana and Jalandhar markets respectively. There were no significant differences (p≤0.05) for this sensory attribute between market areas (Table 5.2).

(g) Stale: The maximum mean score (Table 5.1) of stale attribute was found in Jalandhar samples (4.50 ± 2.16) followed by Karnal (3.06 ± 1.03) and Ludhiana (1.25 ± 0.48) while it was minimum in (1.00 ± 0.44) samples procured from Delhi. The corresponding range was 0 to 15, 0 to 4, 0 to 5, and 0 to 25, for samples procured from Karnal, Delhi, Ludhiana and Jalandhar markets respectively. There were no significant differences (p≤0.05) for this sensory attribute between market areas (Table 5.2).

(h) Bitter: The maximum score (Table 5.1) for bitter flavour was obtained from the samples obtained from Karnal region (4.00± 1.55) followed by Ludhiana (0.62± 0.30) and Delhi (0.50 ± 0.22) while it was least recorded in Jalandhar (0.43 ± 0.32) samples. The score of samples varied in the range from 0 to 20, 0 to 2, 0 to 4 and 0 to 5, for Karnal, Delhi, Ludhiana and Jalandhar markets, respectively. There were significant differences ($p \leq 0.05$) for this sensory attribute between cities (Table 5.2). Samples of Karnal varied significantly in this attribute compared to different cities surveyed and were found to be highly bitter amongst different cities.

(i) Off-flavour: The maximum mean score (Table 5.1) of this attribute was found in Karnal samples (1.68 ± 1.00), followed by Jalandhar (1.56 ± 0.76), Ludhiana (1.12 ± 0.87) and Delhi (0.12 ± 0.12) with similar mean values. The corresponding range was 0 to 15, 0 to 2, 0 to 14, and 0 to 9, for samples procured from Karnal, Delhi, Ludhiana and Jalandhar markets respectively. There were no significant differences ($p \leq 0.05$) for this sensory attribute between market areas (Table 5.2).

(j) Oxidized: The oxidized flavour is also undesirable in *doda burfi* from consumer's point of view and is probably because of oxidation of fat with its interaction with air under aerobic conditions. The maximum mean score (Table 5.1) of oxidized attribute was found in Karnal samples (9.37 ± 3.30) followed by Ludhiana (6.81 ± 3.01) and Delhi (6.25 ± 2.79) while it was minimum in Jalandhar (4.56 ± 3.11) samples. The corresponding range was 0 to 37, 0 to 25, 0 to 37 and 0 to 50, for samples procured from Karnal, Delhi, Ludhiana and Jalandhar markets respectively. There were no significant differences ($p \leq 0.05$) for this sensory attribute between market areas (Table 5.2).

5.1.1.3 Texture

The mean score for texture attributes of markets samples is presented in Table 5.1. The data relating to different texture attributes of samples collected from market areas of Karnal, Delhi, Ludhiana and Jalandhar are presented in Table 5.1 and 5.2 respectively. The texture attributes specially in products like *doda burfi* carries an immense importance from the consumer's acceptability point of view and are discussed here under.

(a) Granularity: Granularity is important texture attribute of *doda burfi* and largely determines the acceptability of the product. The score for granularity varied from 17 to 85, 14 to 100, 39 to 85, and 39 to 86 for Karnal, Delhi, Ludhiana and Jalandhar markets respectively. The maximum score (Table 5.1) was found in samples from Ludhiana markets (63.37 ± 3.75) followed by Jalandhar (62.25 ± 3.90) with little difference from Delhi samples (62.12 ± 5.02) and was least recorded in Karnal samples (58.93 ± 4.98). The texture score for this attribute revealed non-significant differences ($p \leq 0.05$) between market areas (Table 5.2).

(b) Cohesiveness: Consumers prefer a cohesive texture of *doda burfi* and any openness is considered as a defect. The highest score (Table 5.1) was found in samples obtained from Delhi (73.18 ± 3.31) followed by Ludhiana (68.18 ± 3.02) and Karnal (63.81 ± 4.12) and least in Jalandhar samples (58.06 ± 4.89). The cohesiveness score varied from 29 to 92, 50 to 100, 50 to 82 and 25 to 89, for Karnal, Delhi, Ludhiana and Jalandhar city, respectively. The texture score for this attribute revealed highly significant differences ($p \leq 0.05$) between *doda burfi* samples procured from different market areas (Table 5.2).

(c) Firmness: Firmness scores varied from 32 to 72, 39 to 75, 43 to 72, 17 to 87 for samples procured from Karnal, Delhi, Ludhiana and Jalandhar markets, respectively. The maximum mean score (Table 5.1) was found in Delhi market samples (57.12 ± 2.80), followed by Ludhiana (54.62 ± 2.12) and Jalandhar (54.19± 3.76) whereas it was minimum in Karnal (50.31 ± 2.95). The texture score for this attribute revealed non-significant differences ($p \leq 0.05$) between samples procured from different cities and shops ($p \leq 0.05$) of the same market (Table 5.2).

(d) Chewiness: Chewiness scores varied from 16 to 89, 25 to 79, 29 to 77, and 41 to 85 for samples procured from Karnal, Delhi, Ludhiana and Jalandhar markets, respectively. Almost similar chewiness values were recorded in samples from the cities surveyed except Karnal where variation was higher compared to others. The maximum mean score (Table 5.1) was found in Delhi market samples (58.93± 3.57), followed by Ludhiana (58.56 ± 3.14) and Jalandhar (58.06 ± 3.38) whereas it was minimum in samples from Karnal (55.12

± 5.33). The texture score for this attribute revealed non-significant differences ($p \leq 0.05$) between samples procured from different cities and shops ($p \leq 0.05$) of the same city (Table 5.2).

(e) Stickiness: High degree of stickiness is desirable texture attribute of *doda burfi* from consumer's point of view and is attributed to the concentration of soluble constituents and sugar at high temperature employed at the last stages of the product manufacturing. This attribute varied though non-significantly ($p \leq 0.05$) between the samples procured from different market areas (Table 5.2). The corresponding range for this texture attribute varied from 15 to 92, 18 to 82, 18 to 80 and 25 to 89 for samples procured from Karnal, Delhi, Ludhiana and Jalandhar markets respectively. The highest mean score (Table 5.1) was found in samples procured from Jalandhar region (54.87 ± 5.09) followed by Karnal (54.87 ± 5.80) and Ludhiana (49.50 ± 4.30) while the same was minimum in samples from Delhi (45.00 ± 5.10). The texture score for this attribute revealed non-significant differences ($p \leq 0.05$) between *doda burfi* samples procured from different market areas (Table 5.2).

(f) Crumbliness: Crumbliness is an important texture attribute of *doda burfi* and a product with low crumbliness is desirable attribute liked by the consumers. The maximum mean score (Table 5.1) was found in samples procured from Karnal markets (33.81 ± 5.00) followed by Delhi (33.18 ± 7.53) and with least and incomparable scores (20.62 ± 2.64) and (17.18 ± 4.41) in Jalandhar and Ludhiana samples, respectively. The range varied from 12 to 81, 3 to 83, 4 to 75 and 11 to 53 for samples procured from Karnal, Delhi, Ludhiana and Jalandhar markets respectively. The sensory score for this attribute revealed no significant differences ($p \leq 0.05$) between different samples procured from different cities and different shops of same city ($p \leq 0.05$) (Table 5.2).

(g) Gumminess: Gumminess is an important texture attribute of *doda burfi* and significant gumminess attributes to the presence of sugar in the product. The maximum mean score (Table 5.1) was found in samples procured from Jalandhar markets (55.81 ± 5.04) followed by Delhi (55.43 ± 4.14) Karnal (53.37 ± 5.83) and Ludhiana (49.12 ± 4.58), respectively. The range varied from 9 to 92, 13 to 75, 0 to 76 and 0 to 92 for samples procured from Karnal, Delhi, Ludhiana and Jalandhar markets respectively. The sensory score for this attribute revealed non-significant differences ($p \leq 0.05$) between different samples procured from different cities and different shops of same city ($p \leq 0.05$) (Table 5.2).

5.1.1.4 Overall acceptability

Overall acceptability is the main deciding criteria which determines the acceptability of the product and is based on the collective impression of the attributes inherited by the product. The range varied from 24 to 100, 25 to 92, 50 to 100 and 28 to 100 for samples procured from Karnal, Delhi, Ludhiana and Jalandhar markets respectively. The minimum and maximum value depicts the acceptability of the product procured from Ludhiana by most of the panelists. The minimum acceptable value was 50 in case of least acceptable Ludhiana samples. The maximum mean score (Table 5.1) was found in samples procured from Ludhiana markets (76.31 ± 4.01) followed by Karnal (72.43± 5.17) and Delhi (70.93 ± 4.41) and the sample from Jalandhar was least acceptable (65.25 ± 5.43). The texture score for this attribute revealed non-significant differences ($p \leq 0.05$) between different samples procured from different market areas and between samples ($p \leq 0.05$) (Table 5.2).

Table 5.1: Colour, Flavour and Texture profile scores of market samples of *doda burfi**

Characteristics	Attributes	Market areas				CD 5%
		Karnal	Delhi	Ludhiana	Jalandhar	
Surface appearance	**Glossiness**	58.25 ± 4.70	64.56 ± 5.37	65.25 ± 2.95	52.56 ± 3.30	NS
	Surface colour	63.62 ± 4.00	60.00 ± 4.40	69.18 ± 2.95	61.75 ± 4.21	NS
Flavour	**Caramelized**	$63.75^{a} \pm 2.65$	$63.50^{a} \pm 4.08$	$62.93^{ab} \pm 4.65$	$48.00^{b} \pm 6.01$	14.50
	Doughy	52.75 ± 3.47	50.06 ± 4.34	45.31 ± 4.07	39.37 ± 6.40	NS
	Nutty	$60.12^{a} \pm 4.64$	$58.56^{a} \pm 4.70$	$58.25^{ab} \pm 3.75$	$42.18^{b} \pm 5.80$	15.91
	Unclean	3.06 ± 1.13	5.56 ± 3.47	4.00 ± 3.13	5.37 ± 2.68	NS
	Acidic	7.93 ± 2.80	6.62 ± 3.35	5.31 ± 3.14	3.43 ± 1.64	NS
	Rancid	7.93 ± 3.35	3.93 ± 1.44	2.31 ± 0.94	4.87 ± 2.13	NS
	Stale	3.06 ± 1.03	1.00 ± 0.44	1.25 ± 0.48	4.50 ± 2.16	NS
	Off-flavour	1.68 ± 1.00	0.12 ± 0.12	1.12 ± 0.87	1.56 ± 0.76	NS
	Bitter	$4.00^{a} \pm 1.55$	$0.50^{b} \pm 0.22$	$0.62^{b} \pm 0.30$	$0.43^{b} \pm 0.32$	2.72

	Oxidized	9.37 ± 3.30	6.25 ± 2.79	6.81 ± 3.01	4.56 ± 3.11	NS
Texture	**Granularity**	58.93 ± 4.98	62.12 ± 5.02	63.37 ± 3.75	62.25 ± 3.90	NS
	Cohesiveness	63.81 ± 4.12	73.18 ± 3.31	68.18 ± 3.02	58.06 ± 4.89	NS
	Firmness	50.31 ± 2.95	57.12 ± 2.80	54.62 ± 2.12	54.19 ± 3.76	NS
	Chewiness	55.12 ± 5.33	58.93 ± 3.57	58.56 ± 3.14	58.06 ± 3.38	NS
	Stickiness	54.87 ± 5.80	45.00 ± 5.10	49.50 ± 4.30	54.87 ± 5.09	NS
	Crumbliness	33.81 ± 5.00	33.18 ± 7.53	17.18 ± 4.41	20.62 ± 2.64	NS
	Gumminess	53.37 ± 5.83	55.43 ± 4.14	49.12 ± 4.58	55.81 ± 5.04	NS
	Overall acceptability	72.43 ± 5.17	70.93 ± 4.41	76.31 ±4.01	65.25 ± 5.43	NS

Intensity scored on a 100-point line scale
Values are the Mean ± S.E
* Values in corresponding row with different superscripts are significantly different ($p<0.05$)
NS Non Significant

Table 5.2: ANOVA for sensory quality of market samples of *doda burfi*

Characteristics	Attributes	F-ratio	
		Cities	Shops
Colour and appearance of surface	Glossiness	2.18	1.48
	Surface colour	1.14	0.38
Flavour	Caramelized	2.92*	0.45
	Doughy	1.36	0.11
	Nutty	3.19*	1.46
	Unclean	0.19	0.31
	Acidic	0.53	0.44
	Rancid	1.05	0.22
	Stale	1.61	0.71
	off flavor	0.86	0.94
	Bitter	4.13**	0.59
	Oxidized	0.37	0.60
Texture	Granularity	0.17	0.51
	Cohesiveness	2.50	0.16
	Firmness	0.81	0.38
	Chewiness	0.19	0.50
	Stickiness	0.88	1.22
	Crumbliness	2.31	0.30
	Gumminess	1.03	0.17
	Overall acceptability	1.32	1.78

* Significant at $p<0.05$, ** significant at $p<0.01$

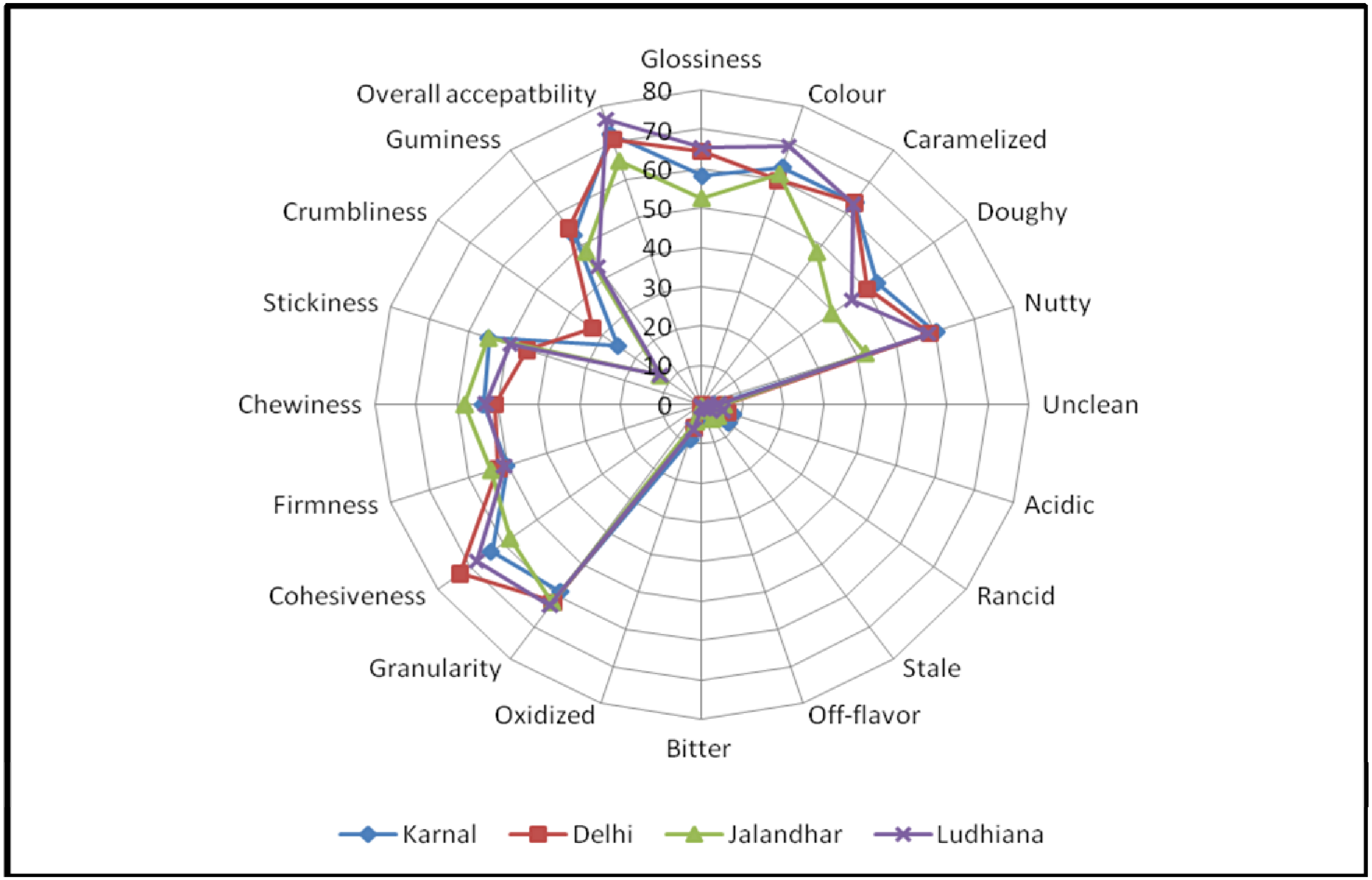


Fig. 5.1 Descriptive sensory profile of market samples of *Doda burfi* from four cities (scores are average of 4 samples from each city) measured on 100 point scale

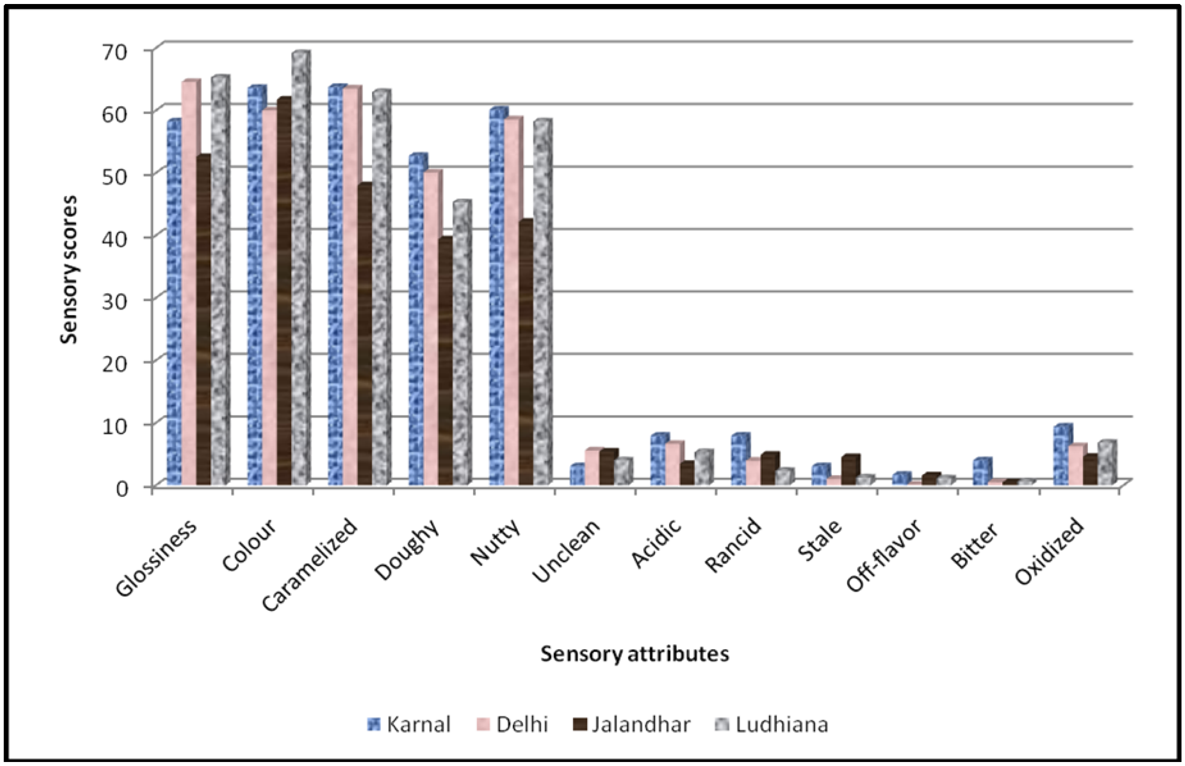


Fig. 5.2 Variation recorded in colour and flavour attributes of market samples of *doda burfi* as collected from various cities

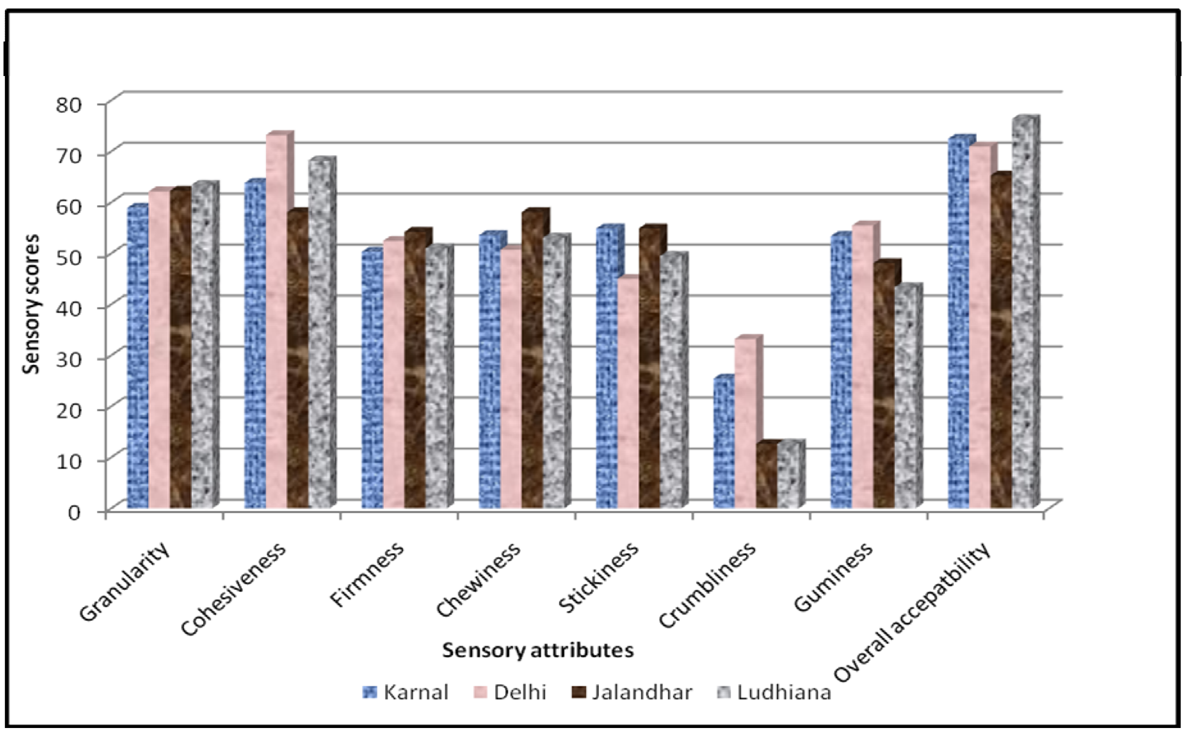


Fig. 5.3 Variation recorded in textural attributes of market samples of *doda burfi* as collected from various cities

5.1.2 Principal component analysis for sensory characterization of market samples of *doda burfi*

The mean sensory score of the market samples of *doda burfi* from four markets was compared and correlated by principal component analysis (PCA). The PCA was performed on all samples and variables. The PCA distinguished and segregated market areas on the basis of sensory characteristics. The statistical parameters pertaining to the PCA of samples are presented in Table 5.3 whereas for attributes pertaining to colour & appearance of surface, flavour and texture are presented in Tables 5.4 and 5.5 respectively. The statistical parameters pertaining to the PCA of explained variance plot, score plot, influence and loading plot are shown in Tables 5.6, 5.7, 5.8 and 5.9, respectively.

The terms generated at roundtable discussion served as a basis for the descriptive sensory analysis (DSA). Many of the terms though sighted as synonym to untrained panel but were quiet different and were helpful in discriminating between the two or more samples. Consensus discussion among the panel refined the list of different lexicons used in evaluation to describe the range of *doda burfi* (Table 5.10). Many of these attributes have been used to characterize the flavour attributes of soymilks (Keast *et al.,* 2006). Similar work has been carried out by Drake *et al.* (2007) and Parente *et al.* (2008). Data obtained after sensory analysis was analyzed using principal component analysis (PCA) and multivariate analysis of variance for 20 different attributes. Principal component analysis also indicated non-redundant terms that clearly differentiated *doda burfi.*

Factor analysis was applied on the correlation matrix of different sensory attributes of *doda burfi* means, in addition to principal component analysis in order to clarify groups of sensory characteristics of *doda burfi.* The PCA distinguished and segregated different sensory attributes on the basis of their eigen values. Variables with eigen value over one were taken to explain the total variance. The mean sensory scores of market samples of *doda burfi* from four cities was analyzed using principal component analysis wherein, PCA differentiated cities in four different quadrants (Fig.5.4). Similar results were observed *by* Sabbe *et al.* (2009) in case of fruit juices with different concentrations of Acai, where juices with same characteristics combined in few quadrants while others with different characteristics remained segregated. The results revealed that on the basis of eigenvector loadings, first principal component with an eigen value of 9.814 was able to explain 49.07% of the total variation whereas second PC with an eigen value 6.287 explained 31.34% of the variation and both the PC's (PC1 and PC2) explained 80.50% of the total variation (Fig. 5.5). In PC1, among all the attributes pertaining to colour, few textural and flavour attributes along with and overall acceptability were positively loaded in PC1. Similarly in PC2, majority of the flavour attributes like doughy, nutty, acidic, gummy, bitter, oxidized and rancid were negatively loaded whereas unclean, granularity and firmness were loaded positively in PC2 (Fig. 5.6). The loadings plot depicts the distance between the location of any two attributes which is proportional to the degree of difference or similarity between them. In loadings graph (Fig.5.7). Varimax rotation was applied in order to clarify groups of sensory characteristics of *doda burfi* (Fig. 5.8).

Factor analysis revealed, component 1 positively and significantly loaded with glossiness (0.978), firmness (0.895), cohesiveness (0.791), chewiness (0.869), whereas found negatively loaded with stale, off-flavour and stickiness. Similarly, caramelized, doughy, nutty and bitter flavours were found positive with values (0.909), (0.926), (0.929) and (0.901) (Table 5.11). The characteristics of prime importance were found loaded in Ludhiana city whereas doughy, nutty, acidic, gummy, bitter, oxidized were dominating characters in Jalandhar samples. Granularity the identifying and important attribute was found highest in Karnal samples.

Table 5.3: Sample statistics for principal component analysis of market samples of *doda burfi*

Market areas	Minimum	Maximum	Mean	SD
Karnal	0.85	72.44	35.39	25.21
Delhi	0.48	73.19	37.78	28.36
Ludhiana	0.40	76.31	36.86	28.04
Jalandhar	2.25	67.75	38.71	26.40

Table 5.4: Variable statistics for principal component analysis of market samples of *doda burfi* for colour and appearance attribute

	Glossiness	Colour	Caramelized	Doughy	Nutty	Unclean	Acidic	Rancid	Stale	Off-flavour	Bitter	Oxidized
Min.	52.73	60.96	48.73	34.65	39.15	2.64	2.61	2.60	0.80	0.72	0.40	5.15
Max.	64.45	69.84	65.83	50.50	61.25	5.10	10.58	9.42	6.27	2.25	5.17	7.38
Mean	59.61	65.39	58.15	41.73	50.39	4.14	5.48	5.07	2.89	1.53	1.72	6.45
SD	5.52	4.11	7.15	6.99	9.39	1.08	3.58	3.09	2.539	0.69	2.30	1.09

Table 5.5: Variable statistics for principal component analysis of market samples of *doda burfi for* textural attributes

Textural attributes	Minimum	Maximum	Mean	S.D
Granularity	58.94	63.37	61.67	1.91
Cohesiveness	58.06	73.19	65.81	6.43
Firmness	50.31	57.12	54.06	2.81
Chewiness	55.12	58.94	57.67	1.73
Stickiness	45.00	54.87	51.06	4.77
Crumbliness	17.19	33.81	26.20	8.55
Gumminess	49.12	55.81	53.44	3.07
Overall acceptability	65.25	76.31	71.23	4.59

Table 5.6: Numericals for explained variance plot

Principal components(PC)	ExpX CalTot (PCs)
PC_00	0
PC_01	43.47
PC_02	81.65

Table 5.7: Numericals for score plot

Market areas	PC_01 (Samples)	PC_02 (Samples)
Karnal	-0.51	-0.69
Delhi	-0.29	0.59
Ludhiana	0.001	0.34
Jalandhar	0.81	-0.23

Table 5.8: Numericals for influence plot

Market areas	PC_01 (Samples)	PC_01 (Samples)
Karnal	0.57	11.03
Delhi	0.31	9.63
Ludhiana	0.25	9.89
Jalandhar	0.86	0.90

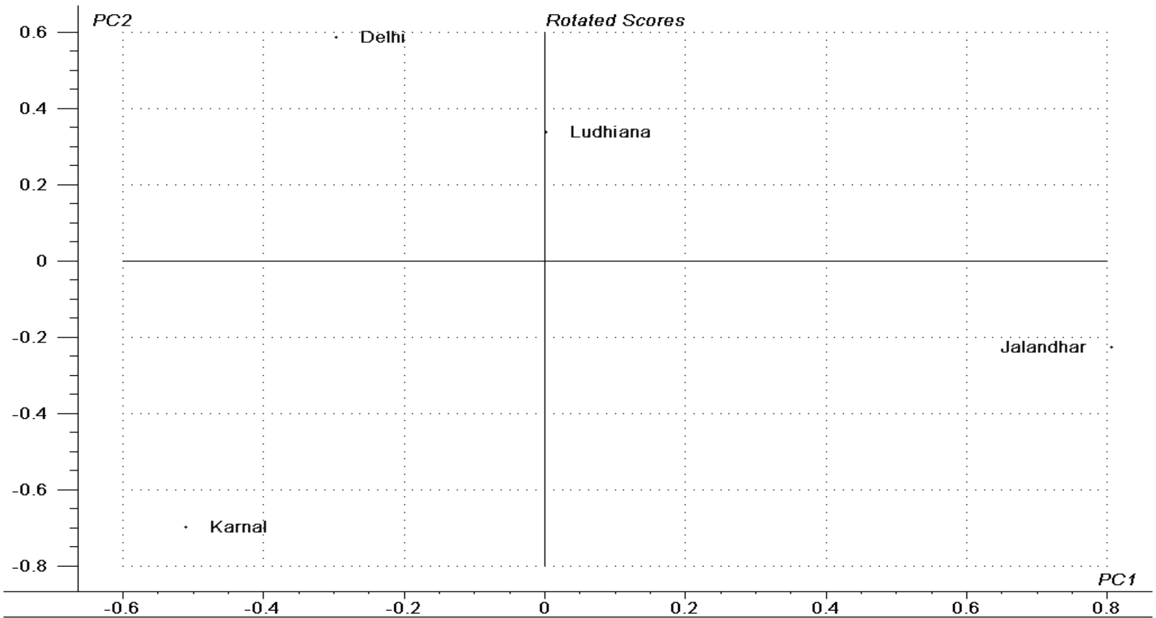


Fig. 5.4 Internal preference mapping of *doda burfi* collected from four cities using principal component analysis

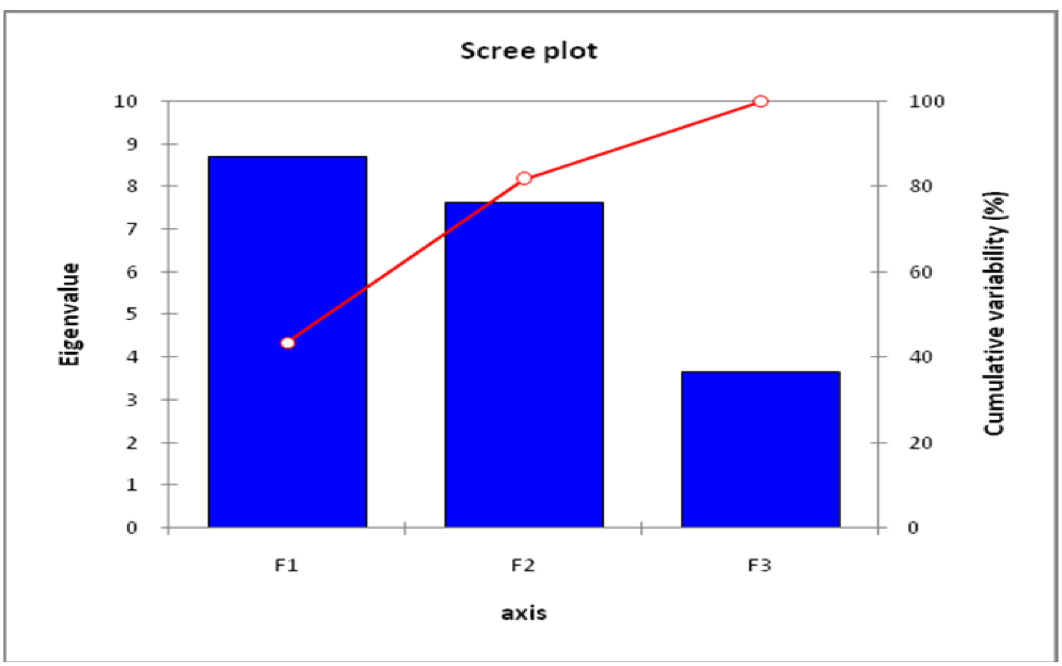


Fig. 5.5 Explained variance plot using principal component analysis

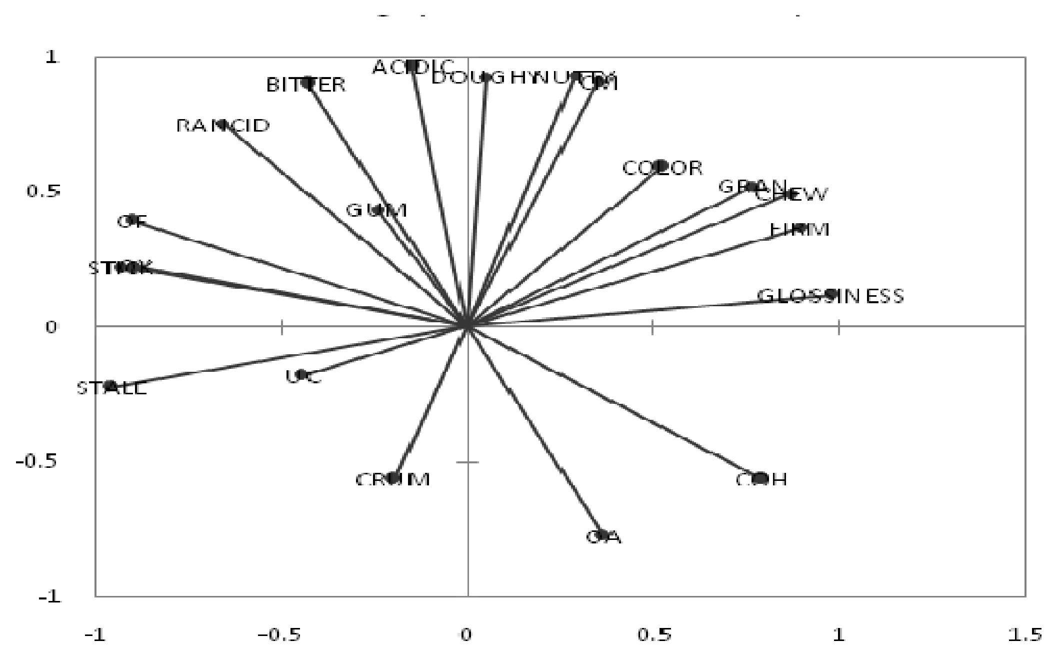


Fig. 5.6 Biplots from the 1st and 2nd principal components of PCA: Sensory attributes

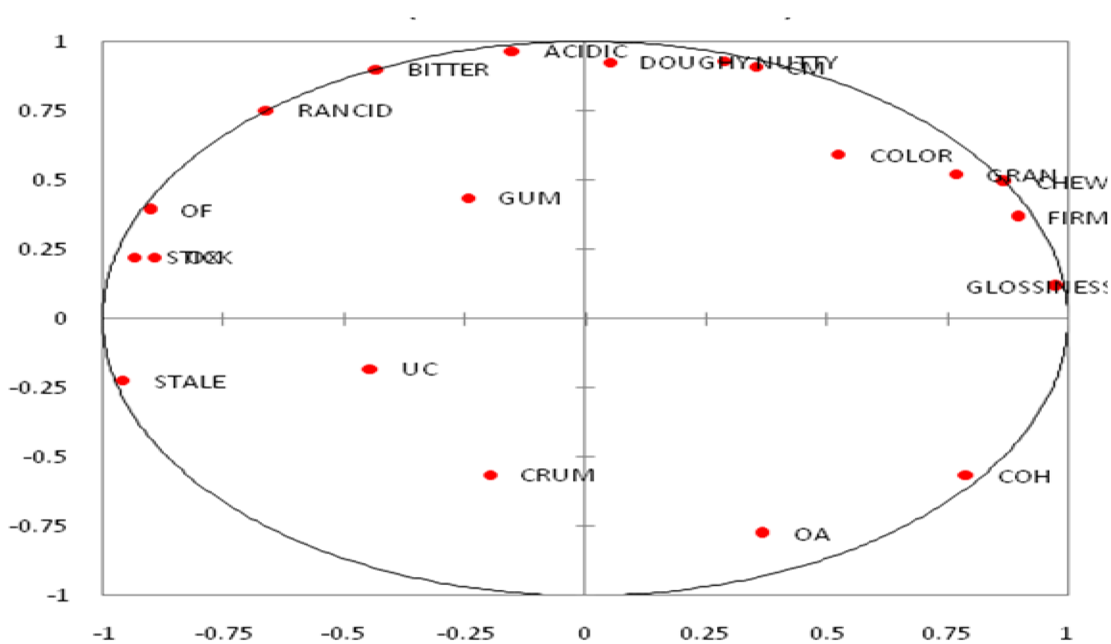


Fig. 5.7 Correalation loading graph obtained using Principal Component Analysis

Where, CM=caramelized; UC=unclean; OF=off-flavour; OX=oxidized; OX=oxidized; GRAN=Granularity; COH=cohesiveness; FIRM= firmness; CHEW=chewiness; STICK=stickiness; CRUM=crumbliness; GUM=gumminess; OA=overall acceptability

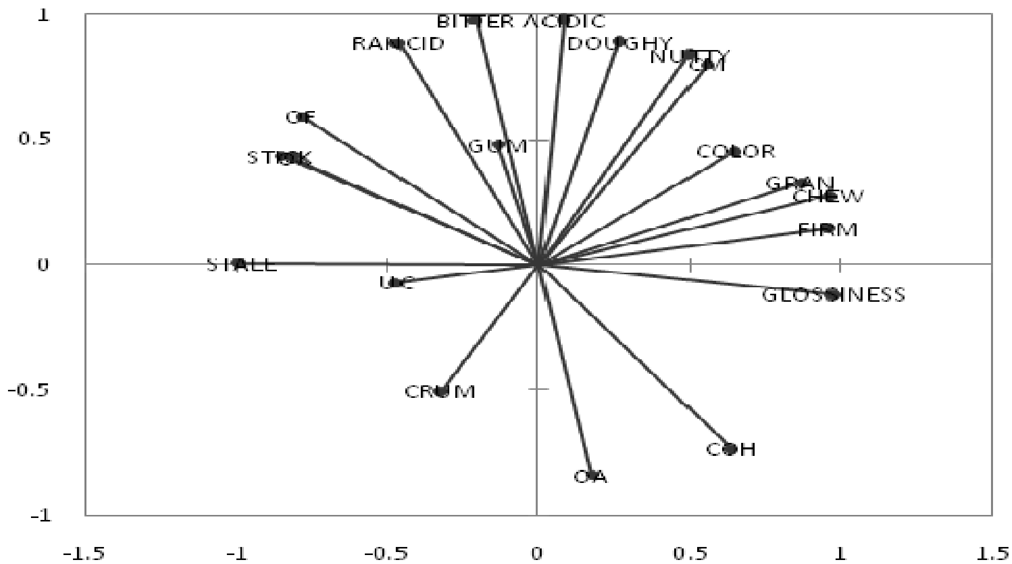


Fig. 5.8 Biplots from the 1st and 2nd principal components of PCA: sensory variables after varimax rotation

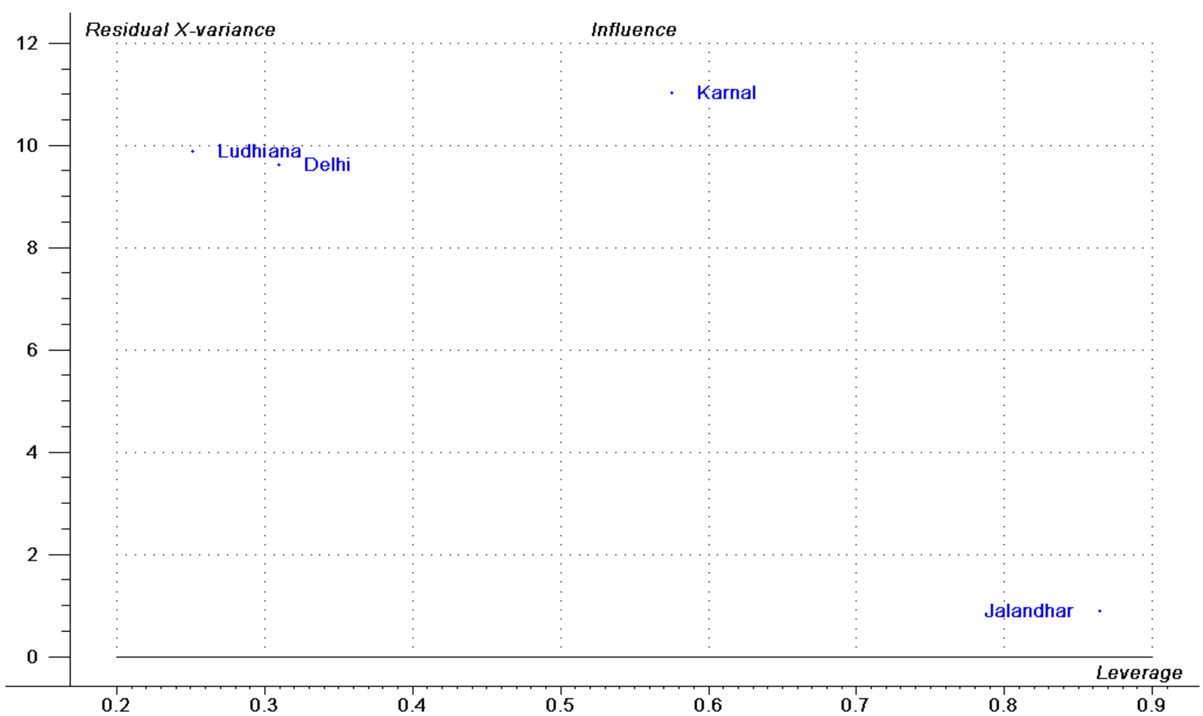


Fig. 5.9 Influence plot from the 1st and 2nd principal components of PCS: sensory variables

Table 5.9: Numericals for factor pattern coefficients

Sensory attributes	Factor 1	Factor 2	Factor 3
Glossiness	0.112	0.015	0.048
Surface colour	0.060	0.078	-0.167
Caramelized	0.041	0.119	0.060
Doughy	0.006	0.121	0.102
Nutty	0.033	0.122	-0.063
Unclean	-0.051	-0.024	0.239
Acidic	-0.017	0.127	0.058
Rancid	-0.076	0.098	0.002
Stale	-0.111	-0.029	-0.044
off flavor	-0.104	0.051	0.049
Bitter	-0.050	0.118	-0.014
Oxidized	-0.103	0.029	-0.106
Granularity	0.089	0.068	0.100
Cohesiveness	0.091	-0.074	-0.064
Firmness	0.103	0.048	-0.069
Chewiness	0.100	0.065	0.006
Stickiness	-0.107	0.029	0.077
Crumbliness	-0.023	-0.074	-0.218
Gumminess	-0.028	0.057	-0.236
Overall acceptability	0.042	-0.102	0.140

Table 5.10: Developed language used for sensory assessment of *doda burfi*

Attribute name	Attribute definition
Glossiness	Having a smooth, shiny, lustrous surface
Colour (towards brown)	The intensity of brown color from light to dark colour
Caramelized	Aromatics associated with caramelized milk products
Cereal like/ doughy	Textural property resembling an unbaked water and flour mixture; pasty, soft and heavy
Nutty	A light, brown, slightly musty aromatic associated with nuts and certain whole grain
Unclean	Covered or stained with or as if with dirt or other impurities
Acidic	Taste associated with dilute aqueous solutions of acids
Rancid	Having the disagreeable odor or taste of decomposing oils or fats
Stale	Not fresh, rapid from age
Off-flavour	abnormal unappetizing flavor.
Bitter	Unpleasant biting taste perceived at back of tongue characterized by aqueous solution of quinine
Oxidized	Painty aroma associated with aged oils.
Granularity	Geometrical textural attribute relating to perception of size and shape of particles
Cohesiveness	Property of unconsolidated fine-grained sediments by which the particles stick together by surface forces
Firmness	Stress/force necessary to attain a given recoverable viscoelastic deformation.
Chewiness	Mechanical textural attribute related to cohesiveness and to length of time or the number of chews required to masticate a solid product into a state ready for swollowing
Stickiness	Having the property of adhering or sticking to a surface
Crumbliness	The consistency of product that breaks under pressure.
Gumminess	Effort required to disintegrate the product to be state ready for swallowing
Overall acceptability	Adequate to satisfy a need, requirement, or standard

Table 5.11: Factor loadings for sensory analysis of *doda burfi* employing all attributes

Sensory attributes	Factor 1	Factor 2	Factor 3
Glossiness	**0.978**	0.117	0.175
Surface colour	0.523	0.592	**-0.613**
Caramelized	0.354	**0.909**	0.220
Doughy	0.050	**0.926**	0.375
Nutty	0.289	**0.929**	-0.230
Unclean	-0.444	-0.180	**0.878**
Acidic	-0.150	**0.966**	0.211
Rancid	-0.660	**0.752**	0.007
Stale	**-0.961**	-0.224	-0.162
off flavor	**-0.902**	0.392	0.182
Bitter	-0.431	**0.901**	-0.051
Oxidized	**-0.893**	0.223	-0.390
Granularity	**0.770**	0.521	0.369
Cohesiveness	**0.791**	-0.565	-0.235
Firmness	**0.895**	0.367	-0.253
Chewiness	**0.869**	0.494	0.022
Stickiness	**-0.934**	0.222	0.281
Crumbliness	-0.196	-0.564	**-0.802**
Gumminess	-0.241	0.435	**-0.867**
Overall acceptability	0.367	**-0.776**	0.513

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

Table 5.12: Matrix showing correlation between different attributes

Variables	GLOSS	COLOR	CM	DOUGHY	NUTTY	UC	ACIDIC	RANCID	STALE	OF	BITTER	OX	GRAN	COH	FIRM	CHEW	STICK	CRUM	GUM	OA
GLOSS	**1**	0.473	0.491	0.222	0.351	-0.301	0.003	-0.556	**-0.994**	**-0.804**	-0.325	**-0.916**	**0.878**	**0.666**	**0.874**	**0.911**	**-0.838**	-0.397	-0.337	0.358
COLOR		**1**	**0.589**	0.345	**0.842**	**-0.877**	0.364	0.096	**-0.536**	-0.351	0.339	-0.096	0.485	0.223	**0.841**	**0.734**	**-0.529**	0.055	**0.663**	**-0.582**
CM			**1**	**0.942**	**0.896**	-0.128	**0.871**	0.451	**-0.580**	0.077	**0.655**	-0.200	**0.828**	-0.285	0.594	**0.761**	-0.067	**-0.759**	0.119	-0.462
DOUGHY				**1**	**0.789**	0.140	**0.966**	**0.666**	-0.317	0.386	**0.793**	0.015	**0.659**	**-0.571**	0.289	0.509	0.264	**-0.833**	0.065	-0.508
NUTTY					**1**	-0.497	**0.806**	0.506	-0.449	0.062	**0.725**	0.039	**0.622**	-0.243	0.657	**0.705**	-0.128	-0.397	**0.534**	**-0.733**
UC						**1**	0.078	0.163	0.324	0.489	-0.016	0.014	-0.112	-0.455	-0.686	-0.456	**0.621**	**-0.515**	**-0.733**	0.427
ACIDIC							**1**	**0.826**	-0.107	**0.552**	**0.924**	0.266	0.466	**-0.714**	0.166	0.351	0.414	**-0.685**	0.273	**-0.696**
RANCID								**1**	0.464	**0.891**	**0.961**	**0.754**	-0.113	**-0.948**	-0.317	-0.202	**0.785**	-0.301	0.480	**-0.822**
STALE									**1**	**0.749**	0.220	**0.872**	**-0.916**	**-0.595**	**-0.901**	**-0.950**	**0.802**	0.445	0.275	-0.262
OF										**1**	**0.733**	**0.822**	-0.423	**-0.978**	**-0.710**	**-0.586**	**0.980**	-0.190	0.231	**-0.542**
BITTER											**1**	**0.606**	0.119	**-0.838**	-0.043	0.069	**0.588**	-0.383	**0.540**	**-0.883**
OX												**1**	**-0.715**	**-0.741**	**-0.619**	**-0.675**	**0.774**	0.362	**0.651**	**-0.701**
GRAN													**1**	0.228	**0.787**	**0.934**	-0.499	**-0.741**	-0.279	0.067
COH														**1**	**0.561**	0.404	**-0.930**	0.352	-0.233	**0.608**
FIRM															**1**	**0.954**	**-0.826**	-0.179	0.163	-0.086
CHEW																**1**	**-0.696**	-0.466	-0.014	-0.053
STICK																	**1**	-0.168	0.078	-0.371
CRUM																		**1**	0.497	-0.045
GUM																			**1**	**0.871**
OA																				**1**

Where, GLOSS= Glossiness; CM=caramelized; DGHY=doughy; NUT=nutty; UC=unclean; ACID= Acidic; RNCD=rancid; OF=off-flavour; BITR=bitter; OX=oxidized; OX=oxidized; GRAN=Granularity; COH=cohesiveness; FIRM= firmness; CHEW=chewiness; STICK=stickiness; CRUM=crumbliness; GUM=gumminess; OA=overall acceptability

Numbers in bold represent significant differences *p<0.05

A weaker correlation was observed after recalculating for stickiness and off-flavour and acidic and doughy flavour attributes (Fig. 5.8). This analysis was performed to illustrate graphically the correlations between ratings given to the different descriptors. Principal component analysis, factor analysis and correlation analysis all indicated relationship between terms (Table 5.12). Removal of unclean, rancid, off-flavour, bitter terms from data set and firmness from textural set did not result in a loss of explained variability by PCA, suggesting that these aroma and textural terms could be removed from the sensory language without a loss of product differentiation. The results obtained after analysis of variance validated the same hence the attributes were omitted from the analysis and data was reanalyzed depicting PC1 and PC2 explaining 42.60 and 37.91 per cent of the variation, respectively.

Correlation matrix (Table 5.12) revealed many expected correlations and was conducted to determine if linear relationships existed between individual terms of sensory attributes. Bitterness was positively correlated with oxidized flavour whereas found negatively associated with overall acceptability. A good degree of correlation was also observed between many other attributes. Texture depictors such as “chewiness” “firmness” were correlated to each other. Though these texture descriptors are distinct from each other, but the both were predominate in good quality *doda burfi.* Colour showed a positive degree of correlation with caramelized, nutty, firmness, chewiness and gumminess while the same has been found to be negatively correlated with unclean, stale, and stickiness. A positive correlation was observed for gumminess and cohesiveness with overall acceptability whereas negatively correlated with oxidized, bitter, off-flavour, rancid and acid flavour attributes (Table 5.12). These results suggest that high glossy colour, nutty flavour and cohesive character necessarily drive the consumer liking. Statistically, location specific analysis indicates (Table 5.2) that three attributes namely caramelized, nutty and bitter were found significantly different from others ($p<0.05$). Fig. 5.9 shows the influence plot of four cities surveyed.

5.1.3 Chemical quality of market samples of *doda burfi*

The chemical composition explains the richness of the product with respect to different constituents. The mean chemical composition of market samples is listed in Table 5.13 and analysis of variance in Table 5.14. The data for proximate composition of samples from Karnal, Delhi, Ludhiana and Jalandhar markets is given in Appendix V.

5.1.3.1 Moisture: Moisture is an important constituent of *doda burfi* and wide variation was recorded within the sample also which could be attributed to mixing up of various lots after cutting into pieces. Variations in the moisture content of *Doda burfi* are likely to cause not only alterations in the sensory, chemical and rheological characteristics of the product but also in the microbiological profile which ultimately influence the shelf life of the product. The highest moisture percentage was observed (Table 5.13) in samples procured from Jalandhar market (14.62 ± 0.61 %) followed by Karnal (12.59 ± 0.37%). The least mean moisture content was recorded in samples procured from Ludhiana (10.40 ± 0.98) followed by Delhi city (11.86 ± 0.98) samples. The lesser moisture content may be an attribute of longer shelf life of Ludhiana market samples. The moisture percentage varied in the range from 10.34 to 14.45, 6.75 to 16.94, 5.28 to 15.68 and 11.01 to 17.95 % for Karnal, Delhi, Ludhiana and Jalandhar market samples respectively. The statistical analysis revealed highly significant ($p \leq 0.01$) differences for moisture content between *doda burfi* samples procured from different shops of same city as well as among different cities (Table 5.14). Similar results were reported by Saxena *et al.* (1996) for *pinni* (a traditional sweetmeat prepared by using *khoa* and roasted ground pulses); Ray *et al.* (2002) and Patel *et al.* (2006) for *peda* (*khoa*- based delicacy).

5.1.3.2 Fat: Fat is an important constituent in *doda burfi*. It not only influences flavour and texture characteristics of *doda burfi* but also contributes significantly to its nutritional and calorific value. Though fat rich dairy products always attract consumers owing to glossy surface-appearance and good flavor but are susceptible to oxidation at higher storage temperature. The highest fat percentage was observed (Table 5.13) in samples procured from Delhi market (22.42 ± 1.18%) followed by Karnal (20.26 ± 3.77%). This may be due to use of high fat milk for manufacture of *doda burfi* and added ghee during its manufacturing which gives product a nutritional and rich flavour appeal. The fat percentage was low in samples from Ludhiana city (17.70 ±1.73%) and Jalandhar market (18.44 ± 3.46%). The fat percentage varied in the range from 8.85 to 43.40, 15.70 to 27.90, 9.40 to 24.80 and 4.10 to 37.40% for Karnal, Delhi, Ludhiana and Jalandhar market samples, respectively. The statistical analysis revealed highly significant ($p \leq 0.01$) differences for fat content between *doda burfi* samples procured from different shops of same city as well as among different cities (Table 5.14). Wide variability in fat has also been reported by Sharma and Zariwala (1978) and Patel (1996) in market samples of plain *peda*.

5.1.3.3 Protein: The protein content is another important constituent which exerts direct as well as indirect influence on colour and texture and on rheological properties of *doda burfi*. Comparatively lesser variation was observed in protein percentage of samples procured from Karnal, Delhi, Ludhiana and Jalandhar market areas with range of 3.64 to 10.91, 6.99 to 11.12, 9.27 to 15.69 and 8.98 to 14.56 % for Karnal, Delhi, Ludhiana and Jalandhar market samples, respectively (Table 5.13). Maximum protein percentage was observed in samples procured from Jalandhar market (12.88 ± 0.64%) followed by Ludhiana (11.86 ± 0.63%). The protein content of samples from Delhi market (9.05 ± 0.44 %) and Karnal (7.51 ± 0.74%) was slightly lower. It could be observed from Table 5.14 that protein content of *doda burfi* from different market areas and shops varied significantly ($p \leq 0.01$). Similar results of market samples have been observed in *pinni* by Saxena *et al.* (1996).

5.1.3.4 Reducing sugars: It could be observed from Table 5.14 that reducing sugars content of *doda burfi* samples procured from different market areas differed significantly ($p \leq 0.05$). The maximum reducing sugars content was found in samples from Ludhiana market (15.74± 1.05%) followed by Delhi (12.85 ± 0.80%), Jalandhar (12.72 ± 0.36%) and Karnal (10.77 ± 0.65 %) market samples. The corresponding range was 8.17 to 13.70, 9.34 to 16.97, 11.27 to 21.52 and 11.00 to 14.62, for samples from Karnal, Delhi, Ludhiana and Jalandhar market samples, respectively (Table 5.13). Reducing sugars are an indicator of hydrolysis of sugar with time and mainly represents lactose and other monosaccharide sugars components. It could be observed from Table 5.14 that reducing sugar content of *doda burfi* from different market areas varied significantly ($p \leq 0.01$).

5.1.3.5 Total sugars: Total sugar varied from 23.75 ± 0.85, 22.70 ± 0.97, 22.01 ± 1.16 and 21.31± 0.51 for markets of Ludhiana, Delhi, Karnal and Jalandhar city, respectively. The corresponding range was 16.02 to 26.35, 17.49 to 26.83, 18.52 to 26.75 and 18.59 to 23.71 %, respectively for Karnal, Delhi, Ludhiana and Jalandhar market samples (Table 5.13). As high as 40.16% of total sugars and traces of reducing sugars were reported in *pinni* (Saxena *et al.,* 1996). The statistical analysis revealed highly significant ($p \leq 0.01$) differences for total sugars content between *doda burfi* samples procured from different shops of same city as well as among different cities (Table 5.14).

5.1.3.6 Ash: Ash content in samples from markets varied by 1.49 to 2.40, 1.65 to 3.10, 1.52 to 2.28 and 0.35 to 2.13%, respectively. Samples collected from Delhi city market had maximum (2.26 ± 0.15%) ash content followed by samples from Ludhiana (1.95 ± 0.07%), Karnal (1.92 ± 0.08%) and Jalandhar (1.41 ± 0.16%) samples (Table 5.13). Highly significant ($p \leq 0.01$) differences were (Table 5.15) observed in ash content of *doda burfi* samples procured from different cities and different shops. *Kalakand* and Milk cake, which are very much similar products (by appearance) have also been reported to contain 2.78 and 2.28% of ash, respectively (Rao and Goyal, 2007; Varma *et al.,* 2005).

5.1.3.7 pH: The pH values of *doda burfi* varied from 5.67 to 7.92, 5.90 to 6.19, 5.00 to 5.21 and 5.57 to 6.02 in the samples from Karnal, Delhi, Ludhiana and Jalandhar markets, respectively. The maximum pH (6.44 ± 0.25) was found in samples from Karnal market followed by Delhi (6.05 ± 0.03) and Jalandhar samples (5.80 ± 0.04) and minimum pH was observed in Ludhiana (5.11 ± 0.02) samples (Table 5.13). The statistical analysis revealed highly significant ($p \leq 0.01$) differences for pH content between *doda burfi* samples procured from different shops of same city as well as among different cities (Table 5.14).

5.1.3.8 Acidity: The highest acidity (in terms of lactic acid) was found (Table 5.13) in samples from Ludhiana (2.15 ± 0.70%) and minimum in Delhi market (1.22 ± 0.12%) (in per cent). The corresponding range for Karnal, Delhi, Ludhiana and Jalandhar market was 1.38 to 1.64, 0.77 to 1.70, 1.85 to 2.54 and 0.08 to 2.11%, respectively. From Table 5.14 it could be observed that acidity of samples differed significantly ($p \leq 0.01$) between market areas and between samples.

5.1.3.9 Soluble dietary fibre (SDF): Dietary fibre is an important constituent of *doda burfi.* In other milk based sweets this constituent is almost absent. In case of *doda burfi* germinated wheat is a major contributor of soluble and insoluble dietary fibre. The soluble dietary fibre was recorded highest in Jalandhar samples (4.18 ± 0.24%), followed by Delhi (3.61 ± 0. 31%), Ludhiana (3.51 ± 0.19%) and was lowest in Karnal (2.32 ± 0.14%) samples. The corresponding range was 1.51 to 2.85, 2.43 to 5.45, 2.24 to 4.25 and 3.00 to 5.82% for Karnal, Delhi, Ludhiana and Jalandhar samples, respectively. Highly significant ($p \leq 0.01$) differences in soluble dietary fibre content (SDF) in *doda burfi* samples procured from different market areas and samples (Table 5.14) were observed.

5.1.3.10 Insoluble dietary fibre (IDF): The insoluble dietary fibre was recorded highest in Ludhiana samples (10.95 ± 1.34%), followed by Jalandhar (9.37 ± 0.48%), Karnal (9.00 ± 0.55%) and was lowest in Delhi (8.67 ± 0.61%) samples. The corresponding range was 7.26 to 12.20, 6.43 to 12.04, 2.89 to 14.87 and 7.58 to 12.34% for Karnal, Delhi, Ludhiana and Jalandhar samples, respectively. Significant ($p \leq 0.01$) differences in insoluble dietary fibre (IDF) in *doda burfi* samples procured from different market areas (Table 5.14) were observed.

5.1.3.11 Water activity (a_w): The a_w values varied from 0.67 to 0.72, 0.65 to 0.76, 0.65 to 0.77 and 0.66 to 0.74 for Karnal, Delhi, Ludhiana and Jalandhar markets, respectively. The maximum a_w (0.71 ± 0.01) was found in samples from Jalandhar market followed by Delhi (0.70 ± 0.01) and Ludhiana samples (0.70 ± 0.01) and minimum a_w was observed in Karnal (0.69 ± 0.01) samples (Table 5.13). Amongst the surveyed cities, the water activity of *doda burfi* from different cities as well among different shops of same city exhibited significant difference ($p > 0.01$) (Table 5.14). The chemical composition of the samples collected from different shops of a same city were expected to show variation, but it was strange to experience variability even within the samples collected from different shops of same city. Hence, all the variables under investigation were found statistically significant for samples ($p<0.01$). Another reason for variability could be attributed to the use of variable amount of nuts in grated form at intermittent stages of preparation, for garnishing and flavoring purpose.

The average composition obtained is quiet similar with the one obtained in milk cake (Madhava *et al.,* 2003; Varma *et al.,* 2005), *Peda* (Pal, 1997; Ray *et al.,* 2002), *burfi* (Prabha, 2006), brown *Peda* (Londhe, 2006) and *Kalakand* (Moulick *et al.,* 1996). The wide variability among different compositional attributes could be attributed to difference in manufacturing practices, varying ingredient quantity and quality and the storage temperature of the final product. This even corroborates with the market survey findings of Londhe (2006) and Punoo (2008).

Table 5.13: Physico-chemical characteristics of *doda burfi* as collected from different cities of India (n=4)*

Attributes	***Shops***	***Cities***			
		Karnal	**Delhi**	**Ludhiana**	**Jalandhar**
Moisture, %	**Shop 1**	12.31 ± 0.52	10.83 ± 0.24	10.38 ± 0.09	11.86 ± 0.68
	Shop 2	14.19 ± 0.17	7.60 ± 0.53	6.77 ± 0.79	14.56 ± 0.13
	Shop 3	12.79 ± 0.38	16.51 ± 0.38	9.00 ± 0.13	17.31 ±0.31
	Shop 4	11.08 ± 042	12.49 ± 0.35	15.45 ± 0.21	14.77 ± 0.26
	Average	**12.59 ± 0.37 (10.34-14.45)**	**11.86 ± 0.98 (6.75-16.94)**	**10.40 ± 0.98 (5.28-15.68)**	**14.62 ± 0.61 (11.01-17.95)**
Fat, %	**Shop 1**	12.23 ± 0.26	23.04 ± 0.20	12.16 ± 0.17	21.86 ± 0.68
	Shop 2	41.05 ± 1.33	16.11 ± 0.32	12.10 ± 1.55	35.09 ± 02.00
	Shop 3	9.11 ± 0.24	23.83 ± 0.08	24.66 ± 0.08	4.70 ± 0.32
	Shop 4	18.66 ± 0.26	26.68 ± 0.66	21.86 ± 0.26	12.10 ± 0.26
	Average	**20.26 ± 3.77 (8.85-43.40)**	**22.42 ± 1.18 (15.70-27.90)**	**17.70 ± 1.73 (9.40-24.80)**	**18.44 ± 3.46 (4.10-37.40)**
Protein, %	**Shop 1**	8.56 ± 0.08	7.04 ± 0.02	10.31 ±0.14	14.01 ± 0.04
	Shop 2	4.31 ± 0.64	10.42 ± 0.01	15.09 ± 0.42	14.01 ± 0.10
	Shop 3	6.39 ± 035	10.14 ± 0.53	10.00 ± 0.41	14.25 ± 0.16
	Shop 4	10.79 ± 0.08	8.60 ± 0.51	12.06 ± 0.42	9.23 ± 0.15
	Average	**7.51 ± 0.74 (3.64-10.91)**	**9.05 ± 0.44 (6.99-11.12)**	**11.86 ± 0.63 (9.27-15.69)**	**12.88 ± 0.64 (8.98-14.56)**
Reducing sugars, %	**Shop 1**	11.34 ± 0.92	14.89 ± 1.85	16.01 ± 0.81	13.95 ± 0.35
	Shop 2	8.82 ± 0.13	9.47 ± 0.13	13.80 ± 0.23	12.25 ± 0.52
	Shop 3	8.52 ± 0.23	11.90 ± 0.36	11.51 ± 0.16	11.18 ± 0.09
	Shop 4	13.49 ± 0.14	13.30 ± 0.42	20.81 ± 0.40	13.19 ± 0.19
	Average	**10.77 ± 0.65 (8.17-13.70)**	**12.85 ± 0.80 (9.34-16.97)**	**15.74 ± 1.05 (11.27-21.52)**	**12.72 ± 0.36 (11.00-14.62)**
Total sugars, %	**Shop 1**	26.34 ± 0.00	26.52 ± 0.26	26.59 ±0.08	23.36 ± 0.22
	Shop 2	20.67 ± 0.17	24.82 ± 0.03	19.05 ± 0.27	21.45 ± 0.23
	Shop 3	16.34 ± 0.25	18.34 ± 0.42	24.44 ± 0.30	18.65 ± 0.06
	Shop 4	24.69 ± 0.12	21.14 ± 0.32	24.72 ± 0.10	21.76 ± 0.16
	Average	**22.01 ± 1.16 (16.02-26.35)**	**22.70 ± 0.97 (17.49-26.83)**	**23.75 ± 0.85 (18.52-26.75)**	**21.31 ± 0.51 (18.59-23.71)**

Ash, %	**Shop 1**	1.61 ± 0.06	1.77 ± 0.06	1.78 ± 0.08	1.84 ± 0.17
	Shop 2	1.74 ± 0.09	1.98 ± 0.15	2.00 ± 0.24	0.56 ± 0.15
	Shop 3	2.18 ± 0.11	2.76 ± 0.20	1.85 ± 0.16	1.60 ± 0.15
	Shop 4	2.16 ± 0.00	2.59 ± 0.26	2.17 ± 0.02	1.65 ± 0.12
	Average	**1.92 ± 0.08 (1.49-2.40)**	**2.26 ± 0.15 (1.65-3.10)**	**1.95 ± 0.07 (1.52-2.28)**	**1.41 ± 0.16 (0.35-2.13)**
pH	**Shop 1**	6.04 ± 0.03	6.16 ± 0.00	5.16 ± 0.01	5.71 ± 0.00
	Shop 2	5.81 ± 0.07	6.18 ± 0.00	5.04 ± 0.02	5.62 ± 0.04
	Shop 3	6.02 ± 0.01	5.92 ± 0.01	5.19 ± 0.00	5.99 ± 0.01
	Shop 4	7.88 ± 0.03	5.96 ± 0.01	5.05 ± 0.03	5.89 ± 0.05
	Average	**6.44 ± 0.25 (5.67-7.92)**	**6.05 ± 0.03 (5.90-6.19)**	**5.11 ± 0.02 (5.00-5.21)**	**5.80 ± 0.04 (5.57-6.02)**
Acidity,% LA	**Shop 1**	1.56 ± 0.02	1.68 ± 0.00	2.18 ± 0.13	0.09 ± 0.00
	Shop 2	1.53 ± 0.07	1.61 ± 0.02	1.85 ± 0.00	1.37 ± 0.09
	Shop 3	1.58 ± 0.03	0.82 ± 0.00	2.09 ± 0.05	2.09 ± 0.00
	Shop 4	1.52 ± 0.01	0.78 ± 0.01	0.48 ± 0.03	1.85 ± 0.04
	Average	**1.55 ± 0.02 (1.38-1.64)**	**1.22 ± 0.12 (0.77-1.70)**	**2.15 ± 0.70 (1.85-2.54)**	**1.35 ± 0.23 (0.08-2.11)**
Dietary fibre SDF, %	**Shop 1**	2.50 ± 0.02	3.16 ± 0.01	3.58 ± 0.25	3.50 ± 0.27
	Shop 2	1.55 ± 0.02	3.47 ± 0.12	3.86 ± 0.23	3.48 ± 0.11
	Shop 3	2.65 ± 0.10	2.55 ± 0.06	2.60 ± 0.22	4.85 ± 0.10
	Shop 4	2.60 ± 0.12	5.28 ± 0.13	3.91 ±0.12	4.91± 0.45
	Average	**2.32 ± 0.14 (1.51-2.85)**	**3.61 ± 0.31 (2.43-5.45)**	**3.51 ± 0.19 (2.24-4.25)**	**4.18 ± 0.24 (3.0-5.82)**
IDF, %	**Shop 1**	12.07 ± 0.06	7.46 ± 0.24	3.56 ± 0.42	7.86 ± 0.14
	Shop 2	7.85 ± 0.00	11.92 ± 0.07	14.66 ± 0.11	8.83 ± 0.14
	Shop 3	7.36 ± 0.08	8.57 ± 0.26	11.42 ± 0.16	8.76 ± 0.07
	Shop 4	8.73 ± 0.02	6.73 ± 0.27	14.15 ± 0.26	12.04 ± 0.25
	Average	**9.00 ± 0.55 (7.26-12.20)**	**8.67 ± 0.61 (6.43-12.04)**	**10.95 ± 1.34 (2.89-14.87)**	**9.37 ± 0.48 (7.58-12.34)**
Water activity	**Shop 1**	0.67 ± 0.00	0.69 ± 0.00	0.71 ± 0.00	0.74 ± 0.00
	Shop 2	0.69 ± 0.00	0.65 ± 0.00	0.65 ± 0.00	0.71 ± 0.00

Shop 3	0.67 ± 0.00	0.75 ± 0.00	0.66 ± 0.00	0.73 ± 0.00
Shop 4	0.72 ± 0.00	0.71 ± 0.00	0.77 ± 0.00	0.66 ± 0.00
Average	**0.69 ± 0.01 (0.67-0.72)**	**0.70 ± 0.01 (0.65-0.76)**	**0.70 ± 0.01 (0.65-0.77)**	**0.71 ± 0.01 (0.66-0.74)**

* Values presented in parentheses is range
Mean ± S.E

Table 5.14: ANOVA for proximate composition of market samples of *doda burfi*

Attributes		**MS**	**df**	**F**
Moisture, %	Among cities	37.10	3	75.51**
	Among samples	28.38	3	57.76**
Fat, %	Among cities	53.22	3	28.99**
	Among samples	253.80	3	138.25**
Protein, %	Among cities	73.61	3	226.82**
	Among samples	2.24	3	6.92**
Reducing sugars, %	Among cities	50.13	3	251.26**
	Among samples	70.19	3	351.80**
Total sugars, %	Among cities	12.52	3	83.36**
	Among samples	83.75	3	557.60**
Ash, %	Among cities	1.47	3	22.45**
	Among samples	0.95	3	14.53**
pH	Among cities	3.74	3	1256.98**
	Among samples	0.66	3	223.03**
Acidity, % LA	Among cities	2.00	3	245.96**
	Among samples	0.20	3	25.29**
Dietary fibre SDF, %	Among cities	7.29	3	69.38**
	Among samples	3.21	3	30.57**
IDF, %	Among cities	12.18	3	104.82**
	Among samples	23.59	3	203.04**
Water activity	Among cities	0.001	3	440.32**
	Among samples	0.004	3	1344.73**

** Significant at p<0.01

5.1.4 Microbiological quality of market samples of *doda burfi*

The mean microbiological quality parameter of market samples is listed in Table 5.15 and analysis of variance in Table 5.16. The data for microbiological quality parameter for samples of Karnal, Delhi, Ludhiana and Jalandhar markets is given in Appendix VI.

5.1.4.1 Standard plate count: Wide variations were observed in SPC count of market samples of Karnal, Delhi, Ludhiana and Jalandhar market which were 1.66 to 3.73, 1.66 to 5.49, 0 to 3.43 and 3.43 to 5.89 log cfu/g, respectively. The highest SPC count was found (Table 5.15) in Jalandhar market samples with mean (5.08 ± 0.47 log cfu/g) followed by samples from Delhi (3.55 ± 0.66log cfu/g), and Karnal samples (3.01 ± 0.77 log cfu/g). The Ludhiana market samples had lower mean SPC count (2.13 ± 0.76 log cfu/g) compared to other market samples. It could be observed from Table 5.16 that significant ($p \leq 0.05$) differences in standard plate count of *doda burfi* were found between different market areas whereas between the shops significant differences were not found.

5.1.4.2 Yeast ad mould count: The yeast and mould count for market samples (Table 5.15) of Karnal, Delhi, Ludhiana and Jalandhar ranged from 3.56 to 3.83, 3.50 to 4.51, 1.26 to 4.06 and 2.53 to 4.27 log cfu/g, respectively. The highest yeast and mould count was found in Delhi market samples (Table 5.15) with mean 3.95 ± 0.14 log cfu/g followed by samples from Karnal 3.71 ± 0.05 log cfu/g and Jalandhar samples (3.62 ± 0.34 log cfu/g). The Ludhiana market samples had lower mean yeast and mould count (2.83 ± 0.49 log cfu/g) compared to other market samples. Non-significant differences were observed in yeast and mould count of *doda burfi* samples procured from different shops whereas among different cities the count was observed to be significant ($p<0.05$) (Table 5.16). Lower SPC and yeast and mould count in Ludhiana depicts good manufacturing practices employed during and after the processing and the way the renowned brands has been established and known for their products.

5.1.4.3 Coliform count: The highest coliform count was (Table 5.15) found in Ludhiana market samples with mean of 2.00 ± 0.27 log cfu/g followed by samples from Delhi (1.68 ± 0.24 log cfu/g), and Jalandhar samples (0.95 ± 0.34 log cfu / g). The Karnal market samples had lower mean coliform count (0.33 ± 0.22 log cfu / g) compared to other market samples. The corresponding range for coliform count for market samples of Karnal, Delhi, Ludhiana and Jalandhar markets was 0 to 0.66, 1.43 to 2.23, 0.82 to 2.49, 0.66 to 1.49 log cfu/g respectively. The coliform count showed highly significant differences ($p \leq 0.01$) between market areas whereas the coliform count in samples from different shops of the same city were found to differ non-significantly ($p \leq 0.05$) (Table 5.16). Although very scanty information is available with regard to the incidence of coliforms in Indian traditional milk products, the results obtained in the present study are in compliance with those obtained in some *khoa* based sweets (Viajayalakshmi & Murugesan, 2001). Ghodekar *et al.,* (1974) also reported similar observations in market samples of *khoa* and *khoa* based sweets.

5.1.5 Instrumental texture quality of market samples of *doda burfi*

Texture is an important attribute of *doda burfi* and contributes a lot towards its final acceptability by the consumers. Textural parameters in terms of texture profile analysis (TPA) comprising hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness were measured and presented in Table 5.17, and its analysis of variance in Table 5.18. The data for textural parameters for Karnal, Delhi, Ludhiana and Jalandhar market samples of *doda burfi* are given in Appendix VII.

Table 5.15: Microbiological count (log cfu/g) of *doda burfi* collected from different cities (n=3)

Parameters	Shops	Cities			
		Karnal	**Delhi**	**Ludhiana**	**Jalandhar**
SPC	**Shop 1**	3.73 ± 1.87	5.49 ± 0.25	3.43 ± 1.72	5.42 ± 0.06
	Shop 2	3.33 ± 1.66	3.42 ± 0.06	3.43 ± 1.72	3.43 ± 1.72
	Shop 3	1.66 ± 1.66	3.65 ± 1.82	0.00 ± 0.00	5.59 ± 0.15
	Shop 4	3.33 ± 1.66	1.66 ± 1.67	1.67 ± 1.67	5.89 ± 0.11
	Average	**3.01 ± 0.77 (1.66-3.73)**	**3.55 ± 0.66 (1.66-5.49)**	**2.13 ± 0.76 (0-3.43)**	**5.08 ± 0.47 (3.43-5.89)**
Yeast and mould	**Shop 1**	3.87 ± 0.11	3.52 ± 0.14	2.40 ± 1.200	4.27 ± 0.11
	Shop 2	3.56 ± 0.04	4.51 ± 0.04	3.63 ± 0.032	2.53 ± 1.27
	Shop 3	3.83 ± 0.07	4.28 ± 0.19	1.26 ± 1.26	3.56 ± 0.041
	Shop 4	3.59 ± 0.06	3.50 ± 0.10	4.06 ± 0.088	4.11 ± 0.02
	Average	**3.71 ± 0.05 (3.56-3.88)**	**3.95 ± 0.14 (3.501-4.51)**	**2.83 ± 0.49 (1.26-4.062)**	**3.62 ± 0.34 (2.53-4.28)**
Coliform	**Shop 1**	0.67 ± 0.66	1.43 ± 0.72	2.46 ± 0.08	0.67 ± 0.67
	Shop 2	0.00 ± 0.00	1.65 ± 0.09	0.82 ± 0.82	0.89 ± 0.89
	Shop 3	0.67 ± 0.67	1.43 ± 0.72	2.26 ± 0.14	0.77 ± 0.77
	Shop 4	0.00 ± 0.00	2.23 ± 0.23	2.49 ± 0.25	1.50 ± 0.76
	Average	**0.33 ± 0.22 (0-0.66)**	**1.68 ± 0.24 (1.43-2.23)**	**2.00 ± 0.27 (0.82-2.49)**	**0.95 ± 0.34 (0.66-1.49)**

(figures in parenthesis indicate range)

Table 5.16: ANOVA for microbiological quality of market samples of *doda burfi*

Attributes		MSS	df	F
Standard plate count	Among cities	18.41	3	3.31*
	Among samples	7.06	3	1.27^{ns}
Yeast and mould count	Among cities	0.69	3	3.13*
	Among samples	2.79	3	0.77^{ns}
Coliform count	Among cities	6.78	3	6.98**
	Among samples	1.05	3	1.07^{ns}

*Significant at p<0.05, ** Significant at p<0.01, ns Non-significant

5.1.5.1 Hardness: Wide range of hardness was observed in all samples of *doda burfi* collected from different markets. The samples from Ludhiana market had higher hardness (Table 5.17) (122.66 ± 8.51 N) with range between 71.31 to 171.81N, followed by samples from Delhi (96.72 ± 9.85 N) with range between 56.12 to 160.24 N, and from Karnal (81.89 ± 6.68 N) (Appendix VII) with range between 49.03 to 133.53N. The samples from Jalandhar market had least hardness with mean value of 72.00 ± 8.16 N with range between 33.68 to 111.61N. From Table 5.18 it could be observed that hardness of *doda burfi* differed significantly between market areas and shops ($p<0.01$).

The variation in hardness may be due to the variation in moisture, fat and total sugar content of the samples. Samples of Ludhiana city contained least amount of moisture and maximum amount of total sugars amongst others and could probably be correlated with maximum hardness. Adhikari *et al*. (1994), Gupta *et al.* (1990) and Patil *et al.* (1990) also reported a direct negative relationship between moisture content (TS) and hardness for *khoa*.

5.1.5.2 Adhesiveness: Adhesiveness is related to the sensory stickiness and indicated by negative peak following the first peak. The adhesiveness for samples from Karnal, Delhi, Ludhiana and Jalandhar varied between 0.55 to 3.74, 0.51 to 2.69 , 0.24 to 1.51, and 0.49 to 6.03 N.s (values are in negative) with an average of 2.14 ± 0.32, 1.34 ± 0.19, 0.93 ± 0.14 and 2.90 ± 0.53 N.s respectively (Table 5.17). Significant differences were observed for samples collected from different shops and market areas ($p<0.01$) (Table 5.18).

5.1.5.3 Springiness: The mean values for springiness for Karnal, Delhi, Ludhiana and Jalandhar markets were 0.22 ± 0.03, 0.20 ± 0.04, 0.18 ± 0.02 and 0.20 ± 0.01 (Table 5.17) with range between 0.13 to 0.42, 0.07 to 0.41, 0.12 to 0.38 and 0.11 to 0.82 respectively. Significant differences were found ($p<0.01$) for the springiness between samples of *doda burfi* from different market areas and shops ($P<0.05$) (Table 5.18).

5.1.5.4 Cohesiveness: The mean cohesiveness values for springiness for Karnal, Delhi, Ludhiana and Jalandhar markets were 0.19 ± 0.01, 0.16 ± 0.01, 0.18 ± 0.02, 0.20 ± 0.01 (Table 5.17) with range between 0.18 to 0.25, 0.02 to

0.22, 0.17 to 0.21 and 0.16 to 0.29, respectively. The cohesiveness of *doda burfi* differed significantly ($p \leq 0.05$) between market areas (Table 5.18) as well as between the samples from different shops ($p<0.01$).

5.1.5.5 Gumminess: Gumminess is a secondary parameter obtained by multiplying primary parameters such as hardness and cohesiveness. The highest gumminess values (Table 5.17) was obtained for samples procured from Ludhiana region (23.31 ±1.73 N) followed by samples from Delhi (16.86 ±1.51 N) and Karnal (16.26 ± 1.31 N) whereas gumminess in Jalandhar samples (14.81 ± 1.80 N) was lowest. The gumminess for samples of Karnal, Delhi, Ludhiana and Jalandhar markets showed wide variation, the range being 8.92 to 27.06, 8.94 to 24.40, 13.49 to 34.39 and 0.59 to 23.04 N respectively. Almost similar value has been reported (28.6N) in plain *peda* prepared by traditional method (Patel *et al*., 2006). The gumminess of *doda burfi* samples differed significantly amongst market areas as well as amongst shops (Table 5.18).

5.1.5.6 Chewiness: Wide variations were observed in chewiness values of samples procured from Karnal, Delhi, Ludhiana and Jalandhar markets, the range being 1.96 to 6.70, 1.12 to 5.67, 1.95 to 8.08 and 0.75 to 17.97 N respectively. The corresponding mean values for chewiness from Karnal, Delhi, Ludhiana and Jalandhar markets were 3.54 ± 0.41, 3.05 ± 0.46, 4.26 ± 0.52 and 6.07 ± 1.62 N respectively (Table 5.18). The chewiness of *doda burfi* differed significantly between market areas and shops ($p<0.05$) (Table 5.18).

5.1.6 Instrumental colour quality of market samples of *doda burfi*

The mean instrumental colour (Hunter values) parameters of market samples are listed in Table 5.19 and analysis of variance in Table 5.20. The data for instrumental colour (Hunter values) parameter for Karnal, Delhi, Ludhiana and Jalandhar market samples is given in Appendix VI.

5.1.6.1 Colour (L*): Color of the product plays an important part in the overall acceptability of the product and hence instrumental colour was measured using Hunter lab meter, in terms of L*, a* and b* values, where ‘a*’ signifies colour hue from redness to greenness and b corresponds hue from yellowness to blueness of the product. The darker the product, the lower will be the L* value of the product. Highest darker colour (lowest L* value) was recorded in samples from Ludhiana samples (31.64 ± 0.37), followed by Delhi (33.76 ± 0.43), Jalandhar (33.40 ±0.62) and was lowest in Karnal (33.47 ± 1.04) samples (Table 5.20). The corresponding range was 30.59 to 40.38, 31.62 to 35.95, 30.22 to 33.97 and 30.73 to 37.00 for Karnal, Delhi, Ludhiana and Jalandhar samples respectively. Highly significant ($p \leq 0.01$) differences in (colour) lightness L* in *doda burfi* was observed in samples procured from different cities as well as from samples of different shops (Table 5.20). Surface color of many of the similar Indian sweetmeats, like *burfi* (Prabha, 2006), brown *peda* (Londhe, 2006), has been determined instrumentally.

The lower L* values of *doda burfi* could be due to added caramel and intense heat treatment given, heat interactions between sugar (both milk sugar and added cane sugar) and milk constituents, leading to well-known Maillard reaction (Coulter *et al.,* 1949; Holsinger *et al.,* 1973; Labuza and Saltmarch, 1982; Gothwal and Shukla, 1995; Pintea, 2008).

5.1.6.2 Colour (a*): Highest 'a*' value, indicating more redness, colour was recorded in samples from Ludhiana samples (13.22 ± 0.24), followed by Jalandhar (12.90 ± 0.55), Delhi (12.26 ±0.13) and was lowest in Karnal (12.14 ± 0.11) samples (Table 5.19). The corresponding range was 11.34 to 12.65, 11.40 to 12.88, 11.99 to 14.49 and 10.96 to 15.81 for Karnal, Delhi, Ludhiana and Jalandhar samples respectively. Highly significant ($p<0.01$) differences in 'a*' value of *doda burfi* samples procured from different cities (Table 5.20) were observed.

Table 5.17: Texture profile parameters recorded using texture expert*

Parameters	Shops	Cities			
		Karnal	Delhi	Ludhiana	Jalandhar
Hardness, N	Shop 1	107.07 ± 15.58	97.68 ± 9.57	161.02 ± 9.75	93.91± 1.72
	Shop 2	53.90 ± 2.80	87.30 ± 3.54	88.47 ± 8.65	56.11 ± 12.61
	Shop 3	79.85 ± 2.41	143.72 ± 11.56	124.02 ± 8.41	40.47 ± 3.34
	Shop 4	86.74± 1.94	58.20 ± 1.41	117.15 ± 3.76	97.53 ± 10.15
Average		**81.89 ± 6.68 (49.03-133.53)**	**96.72 ± 9.85 (56.12-160.24)**	**122.66± 8.51 (71.31-171.81)**	**72.00± 8.16 (33.68-111.61)**
Adhesiveness, N.s	Shop 1	3.43 ± 0.23	1.71 ± 0.20	0.63 ± 0.37	4.11 ± 0.42
	Shop 2	2.70 ± 0.20	0.77 ± 0.13	1.36 ± 0.14	4.50 ± 0.86
	Shop 3	1.62 ± 0.19	2.05 ± 0.31	0.87 ± 0.17	0.92 ± 0.22
	Shop 4	0.80 ± 0.21	0.84 ± 0.02	0.86 ± 0.30	2.06 ± 0.89
Average		**2.14± 0.32 (3.74-0.55)**	**1.34 ± 0.19 (2.69-0.51)**	**0.93 ± 0.14 (1.51-0.24)**	**2.90 ± 0.53 (6.03-0.49)**
Springiness	Shop 1	0.16 ± 0.01	0.24 ± 0.05	0.17 ± 0.03	0.56 ± 0.14
	Shop 2	0.34 ± 0.02	0.09 ± 0.01	0.18 ± 0.02	0.57 ± 0.14
	Shop 3	0.27 ± 0.07	0.09 ± 0.01	0.22 ± 0.08	0.15 ± 0.04
	Shop 4	0.15 ± 0.02	0.39 ± 0.01	0.17 ± 0.01	0.17 ± 0.02
Average		**0.22 ± 0.03 (0.13-0.42)**	**0.20 ± 0.04 (0.07-0.41)**	**0.18 ± 0.02 (0.12-0.38)**	**0.20 ± 0.01 (0.11-0.82)**
Cohesiveness	Shop 1	0.18 ± 0.01	0.21 ±0.01	0.19 ± 0.004	0.19 ± 0.01
	Shop 2	0.21 ± 0.02	0.16 ± 0.004	0.18 ± 0.002	0.28 ± 0.02
	Shop 3	0.19 ± 0.00	0.12 ± 0.05	0.19 ± 0.002	0.16 ± 0.002
	Shop 4	0.20 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.19 ± 0.004
Average		**0.19 ± 0.01 (0.18-0.25)**	**0.16 ± 0.01 (0.02-0.22)**	**0.18 ± 0.02 (0.17-0.21)**	**0.20 ± 0.013 (0.16-0.29)**
Gumminess, N	Shop 1	20.09 ± 3.75	20.37 ± 1.82	31.29 ± 1.83	18.32 ± 1.42
	Shop 2	11.66 ± 1.71	13.98 ± 0.37	16.30 ± 1.41	15.96 ± 4.37
	Shop 3	15.73 ± 0.51	22.44 ± 1.01	23.32 ± 1.79	6.63 ± 0.56
	Shop 4	17.56 ± 1.26	10.63 ± 0.85	22.32 ± 0.81	18.32 ± 1.95
Average		**16.26 ± 1.31 (8.92-27.06)**	**16.86 ± 1.51 (8.94-24.40)**	**23.31 ± 1.73 (13.49-34.39)**	**14.81 ± 1.80 (0.59-23.04)**
Chewiness, N	Shop 1	3.23 ± 0.77	4.66 ± 0.50	5.41 ± 1.34	9.95 ± 1.86
	Shop 2	4.01 ± 0.69	1.31 ± 0.19	2.99 ± 0.52	10.25 ± 4.49
	Shop 3	4.29 ± 1.20	2.07 ± 0.43	4.83 ± 1.37	0.99 ± 0.19
	Shop 4	2.62 ± 0.49	4.14 ± 0.47	3.81 ± 0.42	3.10 ± 0.60

Average	**3.54 ± 0.41 (1.96-6.70)**	**3.05 ± 0.46 (1.12-5.67)**	**4.26 ± 0.52 (1.95-8.08)**	**6.07 ± 1.62 (0.75-17.98)**

**Values for adhesiveness attribute are negative

*Each value is a mean of 4 different samples, analyzed 5 times each

Table 5.18: ANOVA for textural attributes of market samples of *doda burfi*

Attributes		MSS	df	F
Hardness (N)	Among cities	5830.50	3	30.22**
	Among samples	3881.83	3	20.12**
Adhesiveness (N.s)	Among cities	9.15	3	20.21**
	Among samples	5.40	3	11.94**
Springiness	Among cities	0.08	3	6.87**
	samples	0.03	3	2.95*
Cohesiveness	Among cities	0.01	3	4.15*
	Among samples	0.01	3	4.80**
Gumminess (N)	Among cities	170.20	3	12.50**
	Among samples	137.01	3	15.52**
Chewiness (N)	Among cities	21.12	3	3.56*
	Among samples	18.94	3	3.192*

*Significant at p<0.05, ** Significant at p<0.01

Table 5.19: Instrumental colour quality of market samples of *doda burfi*

Parameters	Shops	Cities			
		Karnal	Delhi	Ludhiana	Jalandhar
Lightness (L*)	Shop 1	40.31 ± 0.09	31.75 ± 0.09	32.18 ± 0.09	36.89 ± 0.056
	Shop 2	30.77 ± 0.09	33.18 ± 0.025	30.45 ± 0.14	31.32 ± 0.29
	Shop 3	36.55 ± 0.10	35.45 ± 0.261	33.37 ± 0.37	34.16 ± 0.13
	Shop 4	34.26 ± 0.16	34.68 ± 0.13	30.59 ± 0.19	35.24 ± 0.14
	Average	**35.47 ± 1.04 (30.59-40.38)**	**33.76 ± 0.43 (31.62-35.95)**	**31.64 ± 0. 37 (30.22-33.97)**	**34.40 ± 0.62 (30.73-37.00)**
Redness (a*)	Shop 1	11.84 ± 0.31	12.58 ± 0.09	14.26 ± 0.135	15.69 ± 0.06
	Shop 2	12.29 ± 0.11	11.61 ± 0.10	13.67 ± 0.008	13.35 ± 0.05
	Shop 3	12.09 ± 0.21	12.19 ± 0.11	12.37 ± 0.257	11.42 ± 0.16
	Shop 4	12.34 ± 0.17	12.68 ± 0.10	12.61 ± 0.114	11.13 ± 0.10
	Average	**12.14 ± 0. 11 (11.34-12.65)**	**12.26 ± 0.13 (11.40-12.88)**	**13.22 ± 0.24 (11.99-14.49)**	**12.90 ± 0.55 (10.96-15.81)**
Yellowness (b*)	Shop 1	24.99 ± 0.11	24.19 ± 0.13	23.39 ± 0.13	24.04 ± 0.05
	Shop 2	19.45 ± 0.06	20.47 ± 0.14	20.79 ± 0.29	20.23 ± 0.20
	Shop 3	22.28 ± 0.23	22.18 ± 0.25	19.62 ± 0.36	23.86 ± 0.38
	Shop 4	23.04 ± 0.05	22.22 ± 0.17	20.67 ± 0.21	23.53 ± 0.17
	Average	**22.44 ± 0.60 (19.37-25.19)**	**22.26 ± 0.40 (20.32-24.35)**	**22.12 ± 0.43 (18.98-23.64)**	**22.91 ± 0.48 (19.83-24.45)**

Table 5.20: ANOVA for instrumental colour quality of market samples of *doda burfi*

Parameters		MSS	df	F
Lightness (L*)	Among cities	31.21	3	355.34**
	Among samples	36.03	3	410.24**
Redness (a*)	Among cities	3.19	3	47.02**
	Among samples	5.99	3	88.40**
Yellowness (b*)	Among cities	6.96	3	54.11**
	Among samples	31.07	3	241.35**

** Significant at $p<0.01$

5.1.6.3 Colour yellowness (b*): Highest 'b*' value, indicating higher yellowness, was recorded in samples from Jalandhar samples (22.91 ± 0.48), followed by Karnal (22.44 ± 0.60), Delhi (22.26 ± 0.40) and was lowest in Ludhiana (22.12 ± 0.43) samples. The corresponding range was 19.37 to 25.19, 20.32 to 24.35, 18.98 to 23.64 and 19.83 to 24.45 for Karnal, Delhi, Ludhiana and Jalandhar samples respectively. Highly significant ($p<0.01$) differences in 'b*' of *doda burfi* samples procured from different cities (Table 5.20) were observed.

5.2 PRODUCT DEVELOPMENT

In the initial stages of the present study, several preliminary trials were conducted to select levels of various ingredients and a tentative protocol for the manufacture of *doda burfi*. The product development essentially comprised of optimization of levels of milk fat (%), germinated wheat flour (GWF) and sugar (%). The process of preparing GWF used in the development of *doda burfi* has been depicted in Photo plate I. In this study, individually three RSM were employed, one for standardizing the product with sugar and other without added sugar i.e. using artificial sweetener "stevia" and finally product was developed using conventional standardized recipe with added nutraceuticals components. The product development for the standardization of recipe using sugar is presented under the following sections.

5.2.1 Optimization of fat, germinated wheat flour and sugar in milk using RSM

Preliminary trials were conducted to narrow down the levels of various ingredients used for optimization study. Thus, in the present study after preliminary experiments range of each of three factors were employed for standardization process viz. fat, germinated wheat flour and sugar with values ranging from 3-6, 2.5-5.5 and 14-18% in milk, respectively. The level of milk solid-not-fat (MSNF) was kept constant at 12% in all the formulations based on the results recorded by Jha (2003). In total seventeen trials were conducted based on the formulations provided by Box-behnken design using response surface methodology (RSM). The optimization was carried out using sensory scores (colour, texture, sweetness, flavour and overall acceptability) measured on 9-point hedonic scale (Appendix VIII) to reach at optimum product formulation. Table 5.21 and 5.22 represents coded and real values of various ingredients used for optimization.

5.2.1.1 Effect on color and appearance score: Score for colour and appearance of *doda burfi* varied from 7.2 to 8.5 (Table 5.23). The minimum score was obtained for doda burfi consisting of 3% fat, 4.0% GWF and 14% sugar in the product whereas maximum score was observed for experiment no.17 consisting of 4.5, 4 and 16% fat, GWF and sugar, respectively.

The regression analysis of the data is presented in Table 5.26. It reveals that the coefficient of determination (R^2) was 0.87 and indicated that the model is statistically significant. Further the statistical analysis indicated that the model fits the data well (model F-value: 5.40). The adequate precision value (APV) was found to be 7.25, which is more than the minimum desirable (4.00) for high prediction ability. It was found that the model can be used to navigate the design.

The coefficient estimates of colour and appearance in terms of linear order, second order and interaction of all the three ingredients as presented in Table 5.26 reveal that fat ($p<0.01$) and sugar at quadratic levels had significant effect on the colour and appearance score of *doda burfi* ($p<0.05$). Sugar at linear level also showed a significant effect ($p<0.01$). The interactive effect between the fat and sugar and sugar and GWF and fat and GWF, were found non-significant.

Multiple regression equation generated to predict the colour and appearance score as affected by different factors in terms of actual factors is as follows:

$$\begin{aligned}\textbf{Colour and appearance} = & -17.134 + 1.614^{*}\,\text{Fat} + 0.283^{*}\text{GWF} + 2.502^{*}\text{Sugar} + \\ & 0.0233^{*}\,\text{Fat}^{*}\text{GWF} - 0.034^{*}\text{Fat}^{*}\text{Sugar} - 0.014^{*}\text{GWF}^{*}\text{Sugar} - \\ & 0.129^{*}\text{Fat}^2 - 0.015^{*}\text{GWF}^2 - 0.067\text{Sugar}^2\end{aligned}$$

Interaction among variables and their effect on colour and appearance of *doda burfi* is depicted in form of three dimensional graphs (Fig 5.10). It shows that when fat was kept at center point (4.5%), the colour values increased with increase in sugar content whereas no significant colour development occurred with GWF addition.

Table 5.21: Coded and real levels of milk fat, germinated wheat flour and sugar in the three factor Box-Behnken experiment using culture NCDC 167

Factor	Lower limit	Center ordinate	Upper limit
	Coded levels		
	-1	0	+1
Milk fat level (%)	3	4.5	6
Germinated wheat flour (%)	2.5	4.0	5.5
Sugar (%)	14	16	18

Keeping sugar at central point, fat showed the increasing trend when increased upto 4.5% which decreased afterwards whereas GWF showed no significant or null effect on colour development score values. Similar trend was observed with the increase in sugar and fat content keeping GWF constant wherein fat though showed increasing trend but the effect of sugar was quiet significant and a steep increasing curve was obtained.

5.2.1.2 Effect on texture score: A wide variation in texture score of *doda burfi* was observed and ranged from 6.71 to 8.28 (Table 5.23). The minimum score obtained was in the experiment consisted of 3.0% fat, 4.0% GWF and 14% sugar in the product whereas maximum score was observed from experiment no.17, consisting of 4.5, 4 and 16% fat, GWF and sugar, respectively.

The regression analysis of the data is presented in Table 5.26. It reveals that the coefficient of determination (R^2) was 0.83 indicating that the model developed is satisfactory. Further the statistical analysis indicated that the model fit well with the data (model F-value: 3.70). The calculated adequate precision value (APV) was found to be 6.34 and hence the model can be used to navigate the design.

Table 5.22: The Box-Behnken design for manufacture of *doda burfi* using culture NCDC 167

Standard order	Factor-1	Factor-2	Factor-3
	Milk fat %	Germinated wheat flour (%)	Sugar (%)
1	3.0	2.5	16
2	6.0	2.5	16
3	3.0	5.5	16
4	6.0	5.5	16
5	3.0	4.0	14
6	6.0	4.0	14
7	3.0	4.0	18
8	6.0	4.0	18
9	4.5	2.5	14
10	4.5	5.5	14
11	4.5	2.5	18
12	4.5	5.5	18
13	4.5	4.0	16
14	4.5	4..0	16
15	4.5	4.0	16
16	4.5	4.0	16
17	4.5	4.0	16

The coefficient estimates of texture in terms of linear order, second order and interaction of all the three ingredients as presented in Table 5.26 reveal that GWF and sugar at quadratic level had significant effect on the texture of *doda burfi* ($p<0.05$). None of the variable showed significant response on texture score at linear or interactive level.Multiple regression equation generated to predict the texture as affected by different factors in terms of actual factors is as follows:

$$\textbf{Texture} = -13.483 + 2.745*Fat + 1.589*GWF + 1.503*Sugar + 0.062*Fat*GWF - 0.080*Fat*Sugar - 0.018*GWF*Sugar - 0.176*Fat^2 - 0.216*GWF^2 - 0.032*Sugar^2$$

Interaction among variables and their effect on texture of *doda burfi* is depicted in form of three dimensional graphs (Fig 5.11). It shows that when sugar was kept at center point (16%), texture showed a slight increasing trend with increase in GWF whereas fat showed steep increase in texture score when increased upto a level of 4.5% but further decreased when increased beyond this level. In case of GWF at center point (4.0%), fat and sugar both showed a drastic increase in texture score. The same has been proven by statistical analysis indicating them to be significant at quadratic level. Sugar showed the same increasing trend for texture score when used along with GWF keeping fat at centre point (4.5%), whereas with GWF addition, score first increased and thereafter decreased beyond 4 per cent of its addition.

5.2.1.3 Effect on sweetness score: A wide variation in sweetness score of *doda burfi* was observed and ranged from 6.4 to 8.35 (Table 5.23). The minimum score obtained was in the experiment consisted of 6.0% fat, 2.5% GWF and 16% sugar in the product whereas maximum score was observed from run no. 11, consisted of 4.5, 4 and 16% fat, GWF and sugar, respectively.

The regression analysis of the data is presented in Table 5.26. It reveals that the coefficient of determination (R^2) for sweetness score was 0.89 indicating that the model developed was satisfactory. Further the statistical analysis indicated that the model fit well with the data (model F-value: 6.13). The calculated adequate precision value (APV) was found to be 6.31, which is more the minimum desired value (4) and hence the model can be reliably and satisfactorily used to navigate the design.

The coefficient estimates of sweetness in terms of linear order, second order and interaction of all the three ingredients as presented in Table 5.26 reveal that quadratic effect of fat and GWF had significant effect on the sweetness of *doda burfi* ($p<0.01$) and ($p<0.05$). Multiple regression equation generated to predict the texture as affected by different factors in terms of actual factors is as follows:

$$\textbf{Sweetness} = -15.053 + 1.712*Fat + 0.937*GWF + 1.994*Sugar + 0.015*Fat*GWF - 0.003*Fat*Sugar + 0.016*GWF*Sugar - 0.183*Fat^2 - 0.141*GWF^2 - 0.057Sugar^2$$

Interaction among variables and their effect on sweetness of *doda burfi* is depicted in form of three dimensional graphs (Fig 5.12). It shows that when sugar was kept at center point (16%), sweetness score showed an increase trend with respect to GWF and fat addition wherein score for sweetness increased between 3.0- 4.5% but further decreased when added beyond this level. In case of GWF at center point (4.0%), fat and sugar both showed a distinct increase in sweetness score but the increase seen in sweetness was distinct from 14 to 18%. Sugar showed the same increasing trend for sweetness score when used along with GWF keeping fat at centre point (4.5%), but not much change in score was observed with increase in GWF values.

5.2.1.4 Effect on flavour score: Flavour is an important criterion for deciding the quality of the product, which in turn determines its ultimate acceptability. The sensory scores for the flavour of *doda burfi* ranged between 7.1 - 8.37 (Table 5.23). The maximum score was given to the product prepared from 4.5% fat, 4.0% germinated wheat flour (GWF) and 16% sugar, while minimum was obtained to that made with 6, 4.0 and 14% fat, GWF and sugar, respectively. This clearly indicates that flavour score increased with decrease in fat percentage up to 4.5% in milk but decreased thereafter.

As indicated by model statistics, the R-square for flavour was 0.95, which is far more than the minimum value prescribed by Henika (1982) (0.80) for developing a model for sensory data. A value of 1.0 represents the ideal case at which 100 per cent of the variation in the observed values can be explained by the chosen model.

Fig 5.13 is a response surface graph depicting interactive effect among variables on flavour and has been presented in the form of three dimensional graphs. The coefficients estimates of the flavour model (Table 5.26) show that the level of fat and GWF at interactive level had significant ($p<0.01$) effect on flavour. The positive sign indicates that there is an increase in score for flavour response with an increase in level of ingredients used, while negative sign indicates the reciprocal of the same. Therefore, with the increased level of fat in milk, there was a significant increase ($p<0.05$) in flavour score when sugar was kept at centre (16%). The trend followed well till the fat is increased upto 4.5% but decreased afterwards. Increasing the amount of GWF upto 4.5% showed the same trend for increase in score and decreased thereafter. The quadratic effect of fat and GWF also had a great significant effect ($p<0.01$) on the flavour score of *doda burfi*. Keeping GWF level at centre point (4%), a slight increase in score has been observed with increase in sugar concentration from 14 to 18% but drastic increase was observed in flavour score when fat was increased up to a level of 4.5%.

Milk fat has been known to provide rich and mellow flavour to the dairy products from the time immemorial. Similar observations was reported by Reddy (1985) and Londhe (2006), and found an increase in flavour score of plain *peda* and brown *peda*, respectively; with an increase in fat content.

Multiple regression equation generated to predict the firmness as affected by different factors in terms of actual factors is as follows:

$$\textbf{Flavour} = -9.690 + 0.419*\text{Fat} + 1.907*\text{GWF} + 1.607*\text{Sugar} + 0.225*\text{Fat}*\text{GWF} + 0.040*\text{Fat}*\text{Sugar} - 0.036\text{GWF}*\text{Sugar} - 0.222*\text{Fat}^2 - 0.296*\text{GWF}^2 - 0.049\text{Sugar}^2$$

5.2.1.5 Effect on overall acceptability score: Overall acceptability is the most active and deciding parameter of sensory quality of the product in totality and consists of colour and appearance, flavor, body and textural characteristics. The sensory score for overall acceptability of *doda burfi* ranged from 6.43-8.28 (Table 5.23). Overall acceptability is an important criterion for deciding the quality of the ultimate product, which in turn determines its final acceptability by the consumer. The maximum score was given to the product prepared from 4.5% fat, 4.0% germinated wheat flour (GWF) and 16% sugar, while minimum was obtained to that made with 3, 4 and 18% fat, GWF and sugar, respectively. This clearly indicated that overall acceptability score increased with increase in fat and lowered sugar addition.

As indicated by model statistics (Table 5.26), the R-square for overall acceptability was 0.92, which is far more than a minimal value prescribed by Henika (1982) for developing a model for sensory data (0.85). A value of 1.0

represents the ideal case at which 100 per cent of the variation in the observed values can be explained by the chosen model. Value of adequate precision (APV) as 8.26, indicates model could be used to navigate the design. The model F-value of 9.49 implies the model is highly significant.

Fig 5.14 is a response surface graph depicting interactive effect among variables on overall acceptability and has been presented in the form of three dimensional graphs. The coefficients estimates of the overall acceptability model (Table 5.26) reveal fat to be significant at linear ($p<0.05$) level. The positive sign indicates that there is an increase in score for overall acceptability with an increase in level of ingredients used, while negative sign indicates the reciprocal of the same. Therefore, with the increased level of fat in milk, there was a significant increase ($p<0.05$) in overall acceptability when sugar was kept at centre (16%). Increased amount of GWF showed the same trend but not much increase was observed. At quadratic level each attribute under consideration e.g. fat, sugar and GWF showed a significant effect. The former of the two were found highly significant ($p<0.01$), while the later was found significant ($p<0.05$). Keeping GWF level at centre point (4%), a slight increase in overall acceptability score was observed with increase in sugar concentration from 14 to 17%, which decreased thereafter but drastic increase in fat up to a level of 5.25% fat was observed. With fat at centre (4.5%), increase in overall acceptability was observed with increase in sugar and GWF.

Multiple regression equation generated to predict the overall acceptability as affected by different factors in terms of actual factors is as follows:

Overall acceptability = -33.082 + 4.421*Fat + 0.704*GWF + 3.701*Sugar - 0.0344*Fat*GWF - 0.039*Fat*Sugar + 0.051GWF*Sugar - 0.383*Fat^2 - 0.166*GWF^2 - 0.117Sugar^2

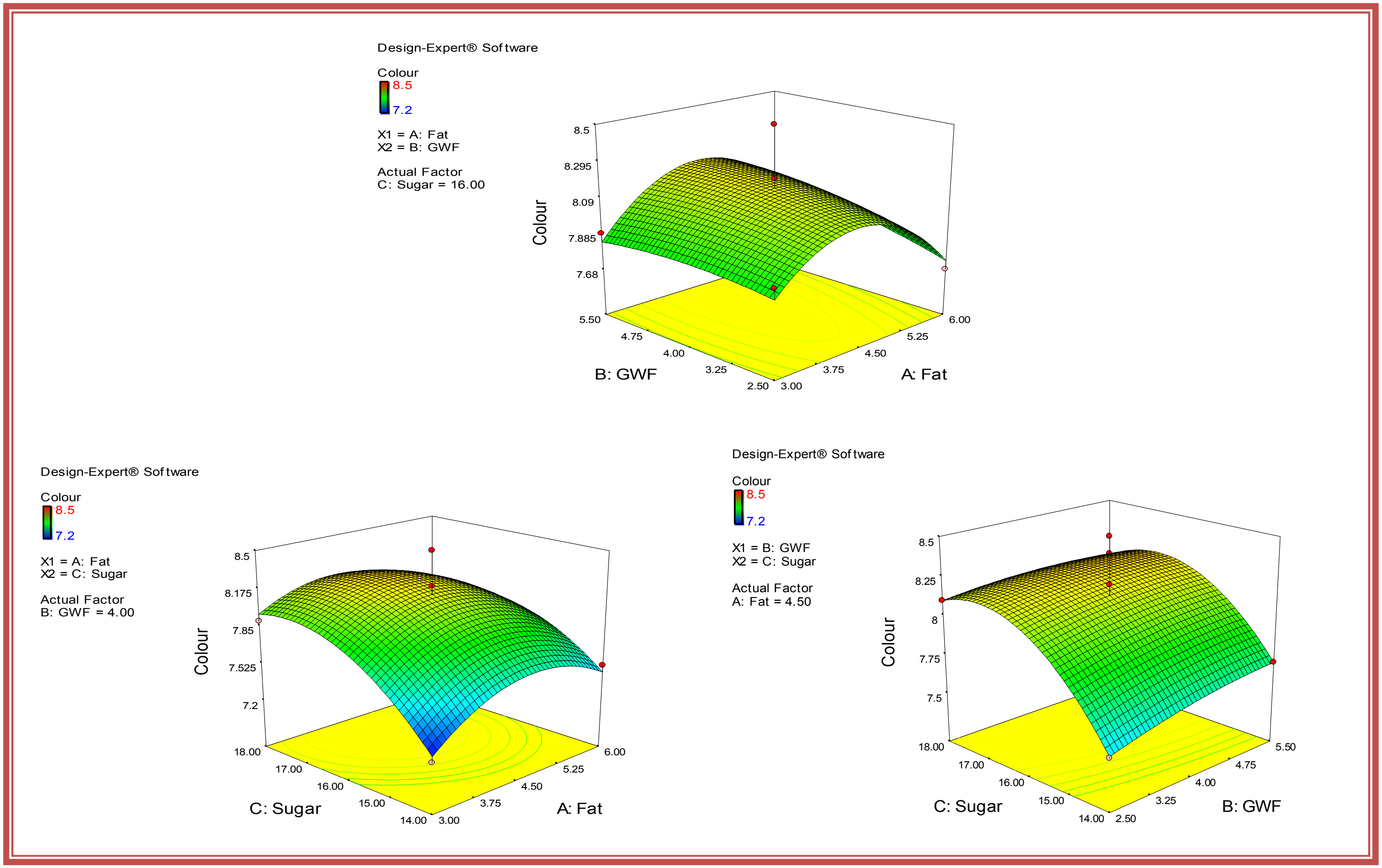


Fig. 5.10 Response surface graph of colour of *doda burfi* as influenced by different levels of ingredients

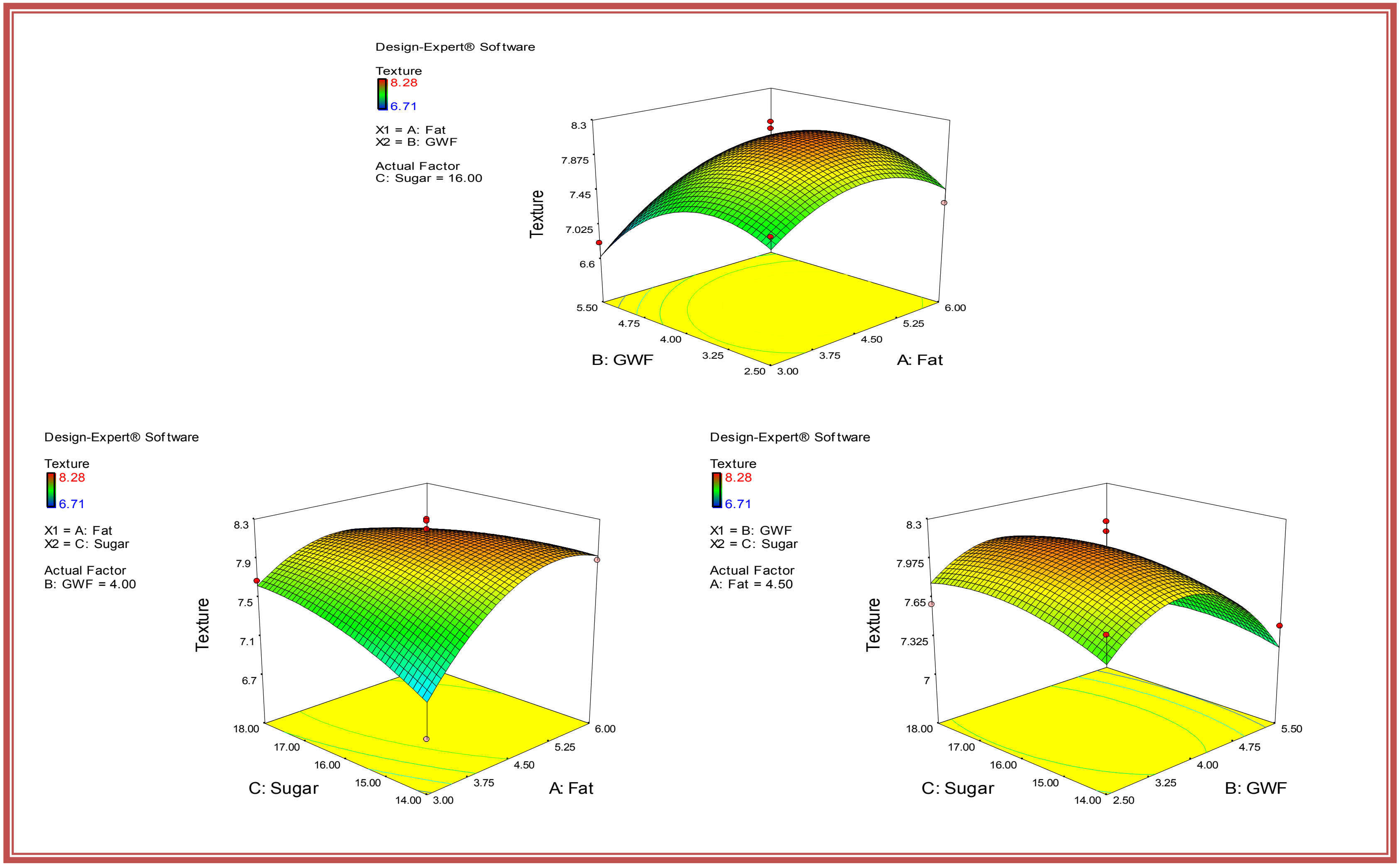


Fig 5.11 Response surface graph of texture of *doda burfi* as influenced by different levels of ingredients

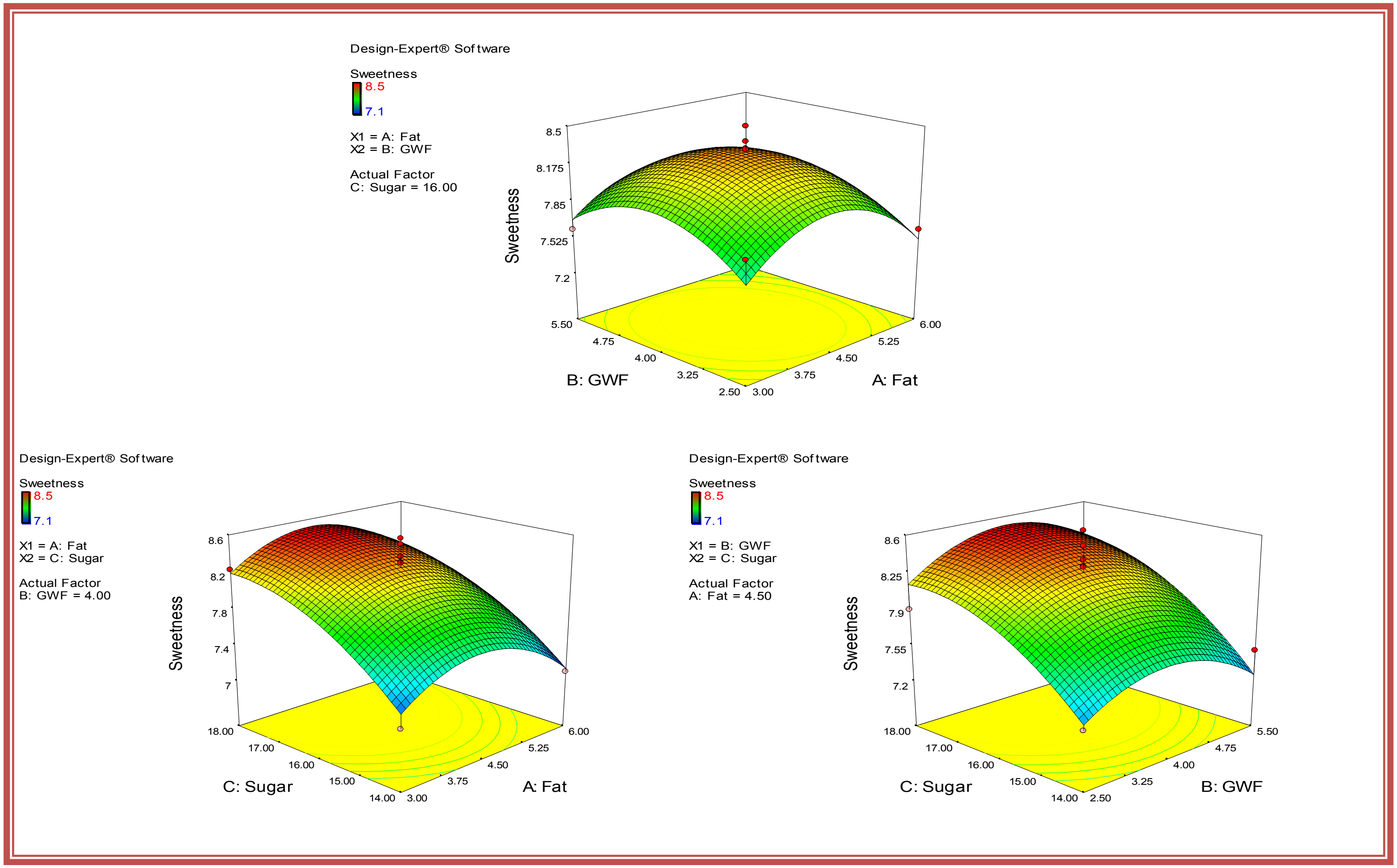


Fig 5.12 Response surface graph of sweetness of *doda burfi* as influenced by different levels of ingredients

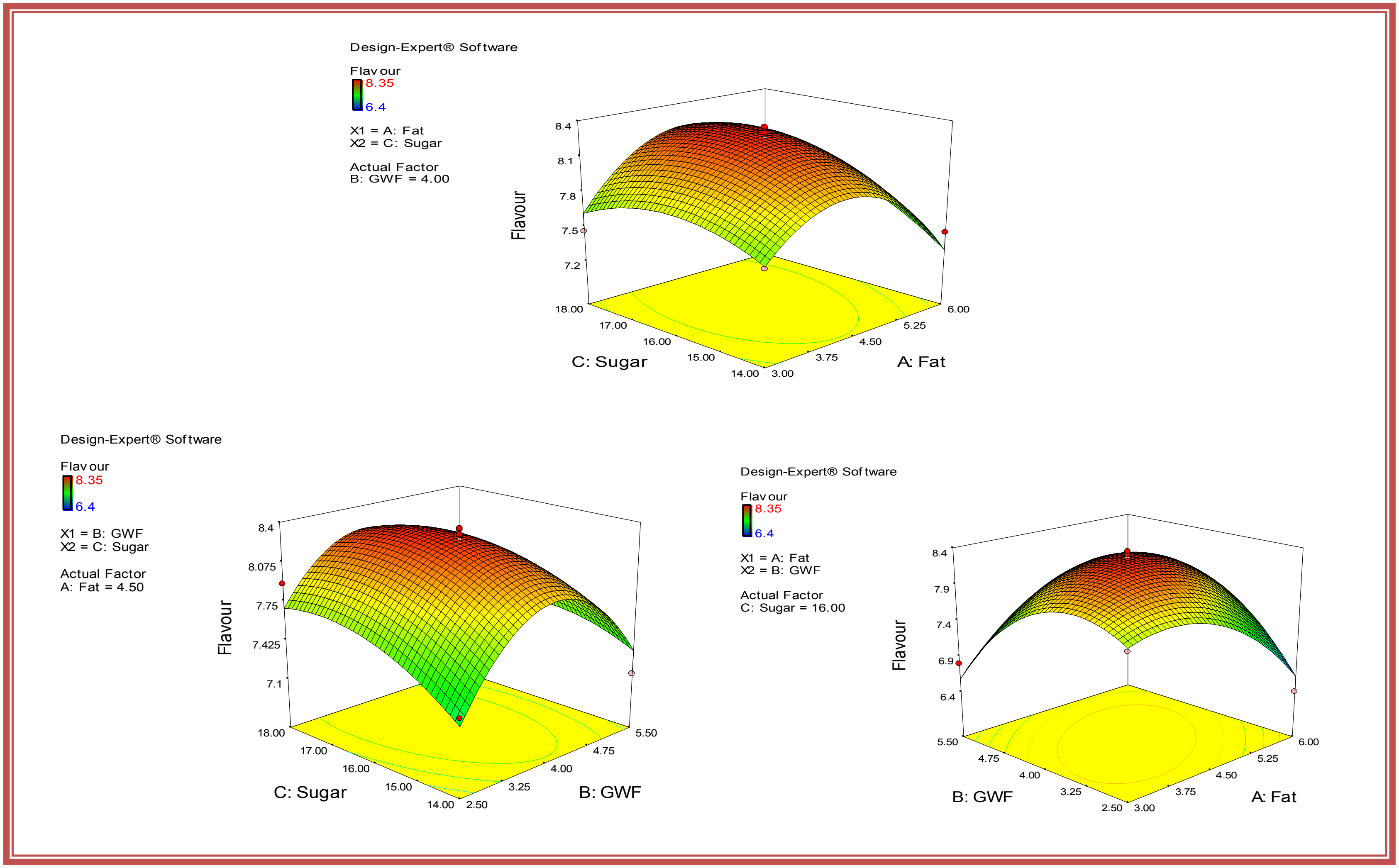


Fig 5.13 Response surface graph of flavour of *doda burfi* as influenced by different levels of ingredients

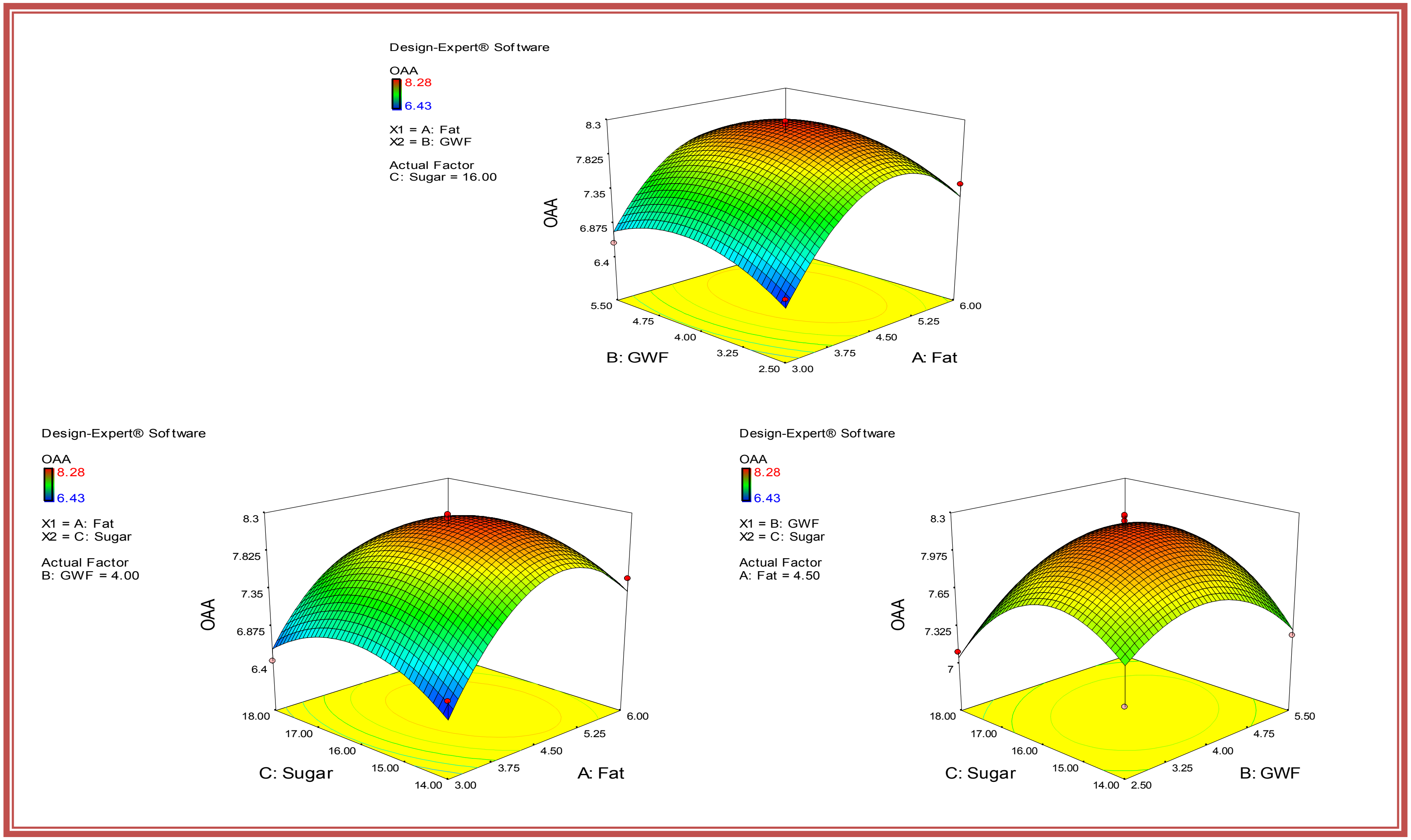


Fig 5.14 Response Surface graph of overall acceptability of *doda burfi* as influenced by different levels of ingredients

Table 5.23: Sensory attributes of *doda burfi* manufactured using culture NCDC 167

Standard order	Fat (%)	GWF (%)	Sugar (%)	Colour	Texture	Flavour	Sweetness	Overall acceptability
1	3.0	2.5	16	7.9	7.5	7.81	7.67	6.6
2	6.0	2.5	16	7.68	7.3	7.6	6.4	7.43
3	3.0	5.5	16	7.89	6.8	7.6	6.8	6.6
4	6.0	5.5	16	7.88	7.16	7.25	7.56	7.12
5	3.0	4.0	14	7.2	6.71	7.13	7.6	6.7
6	6.0	4.0	14	7.51	7.89	7.1	7.45	7.49
7	3.0	4.0	18	7.9	7.68	8.23	7.46	6.43
8	6.0	4.0	18	7.8	7.9	8.16	7.8	6.75
9	4.5	2.5	14	7.5	7.8	7.3	7.3	7.16
10	4.5	5.5	14	7.7	7.42	7.5	7.14	7.25
11	4.5	2.5	18	8.1	7.6	7.9	7.9	7.1
12	4.5	5.5	18	8.13	7.0	8.3	7.3	7.81
13	4.5	4.0	16	8.03	7.98	8.0	8.34	8.05
14	4.5	4.0	16	8.2	8.0	8.31	8.14	8.05
15	4.5	4.0	16	8.0	7.9	8.3	8.25	8.23
16	4.5	4.0	16	8.09	8.2	8.37	8.3	8.27
17	4.5	4.0	16	8.5	8.28	8.5	8.35	8.28

Table 5.24: Instrumental colour and water activity values of *doda burfi*

Standard order	Fat (%)	Malt (%)	Sugar (%)	L*	a*	b*	Water activity
1	3.0	2.5	16	35.11	12.79	23.68	0.68
2	6.0	2.5	16	30.12	12.50	17.08	0.79
3	3.0	5.5	16	28.85	11.28	15.92	0.62
4	6.0	5.5	16	28.56	12.86	18.63	0.79
5	3.0	4.0	14	27.56	15.24	17.24	0.76
6	6.0	4.0	14	27.91	13.63	14.93	0.69
7	3.0	4.0	18	28.06	9.23	16.72	0.73
8	6.0	4.0	18	29.31	14.83	16.01	0.86
9	4.5	2.5	14	27.45	12.95	15.29	0.77
10	4.5	5.5	14	27.28	13.77	20.32	0.74
11	4.5	2.5	18	29.33	14.72	19.49	0.74
12	4.5	5.5	18	28.40	12.43	15.21	0.67
13	4.5	4.0	16	25.09	15.42	15.79	0.71
14	4.5	4.0	16	25.98	14.99	15.90	0.78
15	4.5	4.0	16	30.82	11.33	19.62	0.80
16	4.5	4.0	16	28.15	14.11	19.08	0.78
17	4.5	4.0	16	29.11	13.64	18.94	0.85

Table 5.25: Textural attributes of *doda burfi* manufactured using culture NCDC 167

Standard order	Fat (%)	GWF (%)	Sugar (%)	Hardness (N)	Adhesiveness (N.s)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)
1	3.0	2.5	16	120.26	-2.18	0.23	0.17	20.80	4.85
2	6.0	2.5	16	89.16	-1.28	0.34	0.18	15.96	5.49
3	3.0	5.5	16	96.71	-1.48	0.27	0.25	23.81	6.33
4	6.0	5.5	16	116.88	-2.15	0.40	0.23	26.53	10.69
5	3.0	4.0	14	86.96	-0.88	0.34	0.27	23.74	8.00
6	6.0	4.0	14	87.05	-0.89	0.37	0.19	16.19	6.07
7	3.0	4.0	18	110.19	-2.88	0.31	0.24	26.56	8.21
8	6.0	4.0	18	160.26	-2.49	0.39	0.20	31.41	12.37
9	4.5	2.5	14	72.24	-2.57	0.24	0.12	8.53	2.09
10	4.5	5.5	14	87.19	-1.66	0.35	0.22	19.36	6.75
11	4.5	2.5	18	99.61	-2.36	0.31	0.19	18.57	5.71
12	4.5	5.5	18	116.99	-2.35	0.27	0.19	22.46	6.17
13	4.5	4.0	16	87.96	-2.86	0.43	0.21	18.83	8.04
14	4.5	4.0	16	93.11	-1.01	0.42	0.27	24.86	10.34
15	4.5	4.0	16	84.52	-1.18	0.26	0.17	14.62	3.87
16	4.5	4.0	16	102.20	-2.17	0.21	0.18	19.01	3.94
17	4.5	4.0	16	81.67	-3.31	0.35	0.17	14.13	4.89

Table 5.26: Coefficient of full second order polynomial model for sensory responses of *doda burfi* using NCDC -167

Factor	Colour	Texture	Flavour	Sweetness	Overall acceptability
Intercept	-17.13	-13.48	-9.69	-15.05	-33.08
A- Fat	1.61	2.75	0.42	1.71	4.421*
B- Germinated wheat flour (GWF)	0.28	1.59	1.91	0.937**	0.70
C- Sugar	2.50**	1.50	1.61	1.99**	3.70
A*B	0.02	0.06	-0.22**	-0.015*	-0.03
A*C	-0.03	-0.08	0.04	0.00	-0.04
B*C	-0.01	-0.02	-0.04	0.02	0.05
A^2	-0.13**	-0.18	-0.22**	-0.18**	-0.38**
B^2	-0.02	-0.216*	-0.296**	-0.141*	-0.166*
C^2	-0.07*	-0.03*	-0.05	-0.06	-0.12**
R^2	0.87	0.83	0.95	0.89	0.92
PRESS	0.65	8.40	4.48	3.84	7.72
APV	7.25	6.34	11.33	6.31	8.26
Model 'F' value	5.40*	3.70*	13.67**	6.13*	9.49**

*Significant at p<0.05
**Significant at p<0.01

5.2.2 Effect of GWF, fat and sugar on instrumental texture attributes

5.2.2.1 Effect on hardness: The hardness values of *doda burfi* ranged from 72.24 to 160.26 N (Table 5.25). The maximum hardness (160.26N) was obtained for the product prepared from 6.0% fat, 4% GWF and 18.0% sugar concentration whereas the minimum value was obtained in the product with 4.5, 2.5 and 14.0% of fat, GWF and sugar, respectively.

As indicated by model statistics (Table 5.27), the R-square for hardness was 0.85. Value greater than 0.80 represents and indicates that the model developed as satisfactory, being greater than the minimum desirable value (0.80). Value of adequate precision (APV) as 8.29, indicates model could be used to navigate the design. The model F-value of 4.47 implies the model is statistically significant.

The coefficient estimates of hardness in terms of linear order, second order and interaction of all the three ingredients as presented in Table 5.27 reveal that quadratic effect of fat ($p<0.05$) and sugar ($p<0.01$) at linear level had significant effect on the hardness of *doda burfi.* Multiple regression equation generated to predict the hardness as affected by different factors in terms of actual factors is as follows:

$$\textbf{Hardness} = -771.391 - 152.078*Fat - 23.597*GWF - 47.796*Sugar + 5.688*Fat*GWF + 4.163*Fat*Sugar + 0.201*GWF*Sugar + 7.329*Fat^2 - 0.272*GWF^2 + 1.182Sugar^2$$

Interaction among variables and their effect on hardness of *doda burfi* is depicted in form of three dimensional graphs (Fig 5.15). It shows that when sugar was kept at center point (16.0%), hardness score showed first a decreasing trend till 4.50% fat then increasing afterwards with the increasing fat content, whereas GWF had no significant effect on the hardness value. In case of GWF at center point (4.0%), increasing sugar content showed a distinct increase in hardness score and the steep was markedly sharp for 6.0% fat. Sugar showed the same increasing trend for hardness score when used along with GWF keeping fat at centre point (4.5%), but not much change in hardness was observed with increase in GWF values.

5.2.2.2 Effect on adhesiveness: The values for adhesiveness of *doda burfi* have been given in Table 5.25. As indicated by model statistics (Table 5.27), the R-square for adhesiveness was 0.40. Value lesser than 0.80 represents values less than desirable, indicating that the model developed was unsatisfactory. Value of adequate precision (APV) as 2.39, indicates model could not be used to navigate the design. The model F-value of 0.53 implies the model is non-significant.

The coefficient estimates of adhesiveness in terms of linear order, second order and interaction of all the three ingredients as presented in Table 5.27 reveal that none of the variable at any level showed significant effect for the attribute studied.

Interaction among variables and their effect on adhesiveness of *doda burfi* is depicted in form of three dimensional graphs (Fig 5.16). It shows that when sugar was kept at center point (16.0%), value for the adhesiveness showed an increase trend with respect to GWF and fat addition. In case of GWF at center point (4.0%), increasing fat increased adhesiveness score whereas increasing sugar decreased it. Sugar showed the non-significant effect on adhesiveness when used along with GWF keeping fat at centre point (4.5%).

5.2.2.3 Effect on springiness: The values for springiness of *doda burfi* have been given in Table 5.25 and there was least difference observed between the values of the different trials conducted. Values varied from 0.21 to 0.43 and owe the difference to sugar concentration.

As indicated by model statistics (Table 5.27), the R-square for springiness was 0.48. Value lesser than 0.80 represents values less than desirable, indicating that the model developed was unsatisfactory. Value of adequate precision (APV) as 3.00, indicates model could not be used to navigate the design. The model F-value of 0.72 implies the model is statistically non-significant.

The coefficient estimates of springiness in terms of linear order, second order and interaction of all the three ingredients as presented in Table 5.27 reveal that none of the variable at any level showed significant effect for the attribute studied.

Interaction among variables and their effect on springiness of *doda burfi* is depicted in form of three dimensional graphs (Fig 5.17). It shows that when sugar was kept at center point (16.0%), the springiness increased with increasing fat content whereas with increase in GWF content, the springiness first increased slightly and then decreased. In case of GWF at center point (4.0%), fat and sugar both showed an increase in springiness score and out of both, fat showed distinct increase in springiness value. Sugar showed the significant increasing effect trend for springiness when used along with GWF keeping fat at centre point (4.5%). Though GWF showed the same trend but attained a parabola path for the same.

5.2.2.4 Effect on cohesiveness: The values for cohesiveness of *doda burfi* have been given in Table 5.25 and there was narrow difference (0.12-0.27) observed between the values of the different trials conducted. As indicated by model statistics (Table 5.27), the R-square for cohesiveness was 0.66. Value lesser than 0.80 represents values less than desirable, indicating that the model developed was unsatisfactory. Value of adequate precision (APV) as 5.05, indicates model could be used to navigate the design. The model F-value of 1.49 implies the model is non-significant.

Fig 5.18 is a response surface graph depicting interactive effect among variables on cohesiveness and has been presented in the form of three dimensional graphs. The graph shows with the increase in fat content, cohesiveness decreased whereas with increase in GWF, score increased rapidly. In case of GWF at center point (4.0%), cohesiveness decreased with increasing fat content whereas sugar had insignificant effect on the cohesiveness. Sugar showed the significant increasing effect on cohesiveness when used along with GWF keeping fat at centre point (4.5%) but marked increase in cohesiveness was observed with GWF increase.

5.2.2.5 Effect on gumminess: The values for gumminess of *doda burfi* have been presented in Table 5.25 and the minimum value obtained for the same was as low as 8.53N whereas the maximum attained was 31.41N.

As indicated by model statistics (Table 5.27), the R-square for gumminess was 0.84 indicating that the model developed was quiet satisfactory. Value of adequate precision (APV) as 8.91, which indicates model could be used to navigate the design. The model F-value of 4.25 implies the model is statistically significant.

Fig 5.19 is a response surface graph depicting interactive effect among variables on gumminess and has been presented in the form of three dimensional graphs. The graph shows with the increase in fat and GWF content, gumminess increased. In case of GWF at center point (4.0%), gumminess showed a first decreasing and then increasing trend with increase in fat whereas sugar had insignificant effect on the gumminess. GWF showed the significant increasing effect on gumminess when used along with sugar keeping fat at centre point (4.5%), though sugar showed the same trend but marked increased was observed with GWF increase on the gumminess value.

Multiple regression equation generated to predict the gumminess as affected by different factors in terms of actual factors is as follows:

$$\textbf{Gumminess} = 119.275 - 41.786{*}Fat + 14.477{*}GWF - 6.914{*}Sugar + 0.840{*}Fat{*}GWF + 1.033{*}Fat{*}Sugar - 0.574{*}GWF{*}Sugar + 2.387{*}Fat^2 - 0.837{*}GWF^2 + 0.2034\ Sugar^2$$

5.2.2.6 Effect on chewiness: The chewiness values of *doda burfi* have been given in Table 5.25 and the minimum value obtained for the same was as low as 2.09 whereas the maximum attained was 12.37 N.

As indicated by model statistics (Table 5.27), the R-square for chewiness was 0.69. Value lesser than 0.80 represents values less than desirable, indicating that the model developed was unsatisfactory. However, value of adequate precision (APV) was 6.31, which indicates model could be used to navigate the design. The model F-value of 1.74 implies the model is statistically non-significant.

Fig 5.20 is a response surface graph depicting interactive effect among variables on chewiness. The graph shows with the increase in fat and GWF content, chewiness increased, keeping sugar at central point (16.0%). In case of GWF at center point (4.0%), lower fat and sugar levels showed high chewiness value, which decreased with increasing levels of fat and sugar. GWF showed the significant increasing effect for chewiness when used along with sugar keeping fat at centre point (4.5%), though sugar showed the same trend but marked increased was observed with increasing level of GWF.

5.2.3 Effect of GWF, fat and sugar on Hunterlab color values (CIE LAB) and water activity

5.2.3.1 Effect on lightness value (L*): The instrumental lightness values of *doda burfi* have been given in Table 5.24 and the minimum value obtained for the same was as low as 25.09 whereas the maximum attained was 35.11. The differences in the colour lightness values may be due to the differences in sugar concentration, which in turn is responsible for the maillard reaction for the development of colour in the ultimate product.

As indicated by model statistics (Table 5.27), the R-square for lightness was 0.56, indicating that the model developed was unsatisfactory. However, the model could be used to navigate the design as the value of adequate precision (APV) was 4.088. The model F-value of 0.98 implies the model is statistically non-significant. Fig 5.21 is a response surface three dimensional graphs depicting interactive effect of variables on lightness.

5.2.3.2 Effect on redness (a*): The instrumental colour scores for redness of *doda burfi* have been presented in Table 5.24 and the minimum value obtained for the same was as low as 9.23 whereas the maximum attained was 15.42. As indicated by model statistics (Table 5.27), the R-square for redness was 0.66, indicating that the model developed was less satisfactory. However, value of adequate precision (APV) as 4.45, indicates model could be used to navigate the design. The model F-value of 1.52 implies the model is statistically non-significant. Fig 5.22 is a response surface graph depicting interactive effect among variables on redness and has been presented in the form of three dimensional graphs.

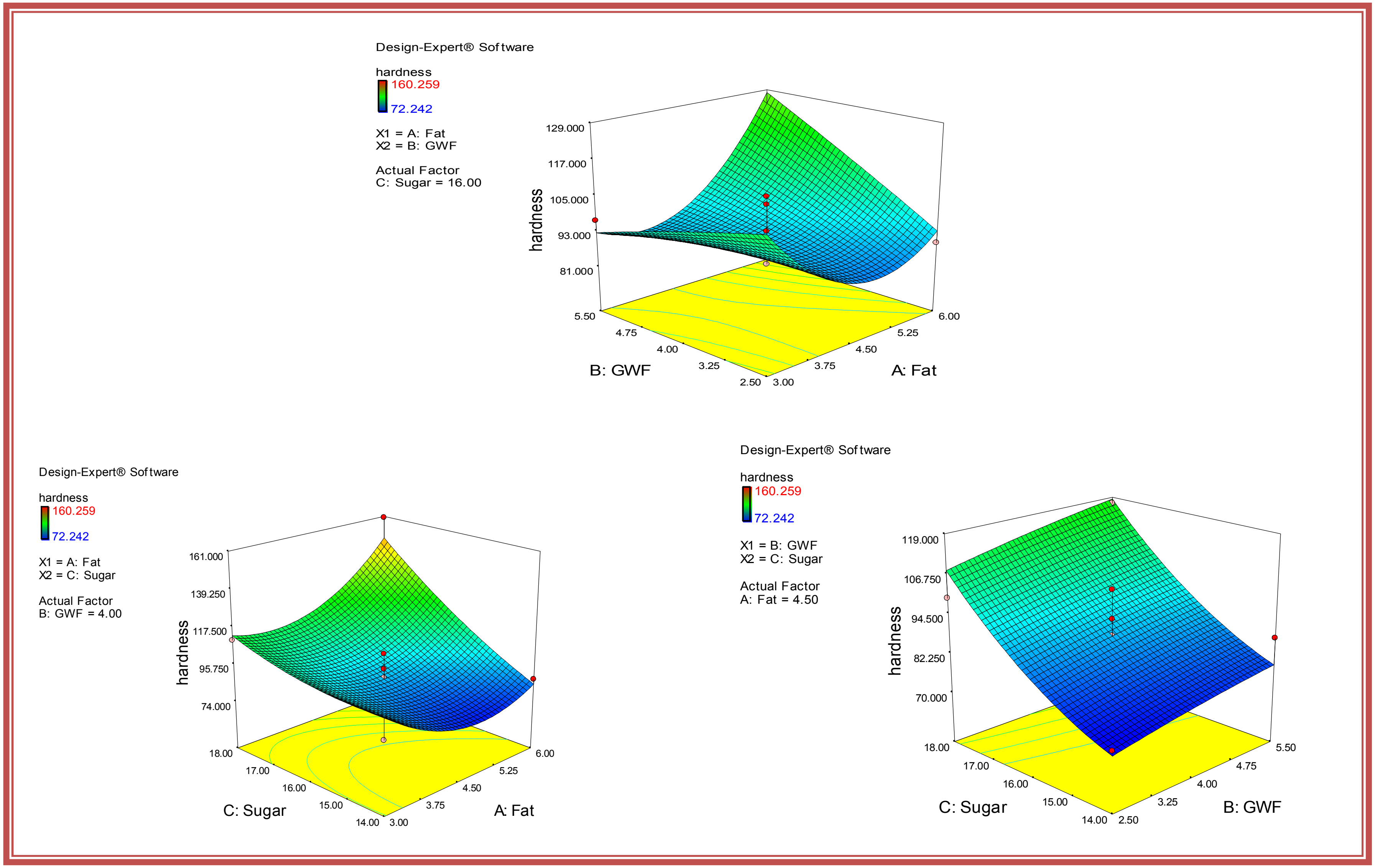


Fig. 5.15 Response surface graph of hardness of *doda burfi* as influenced by different levels of ingredients

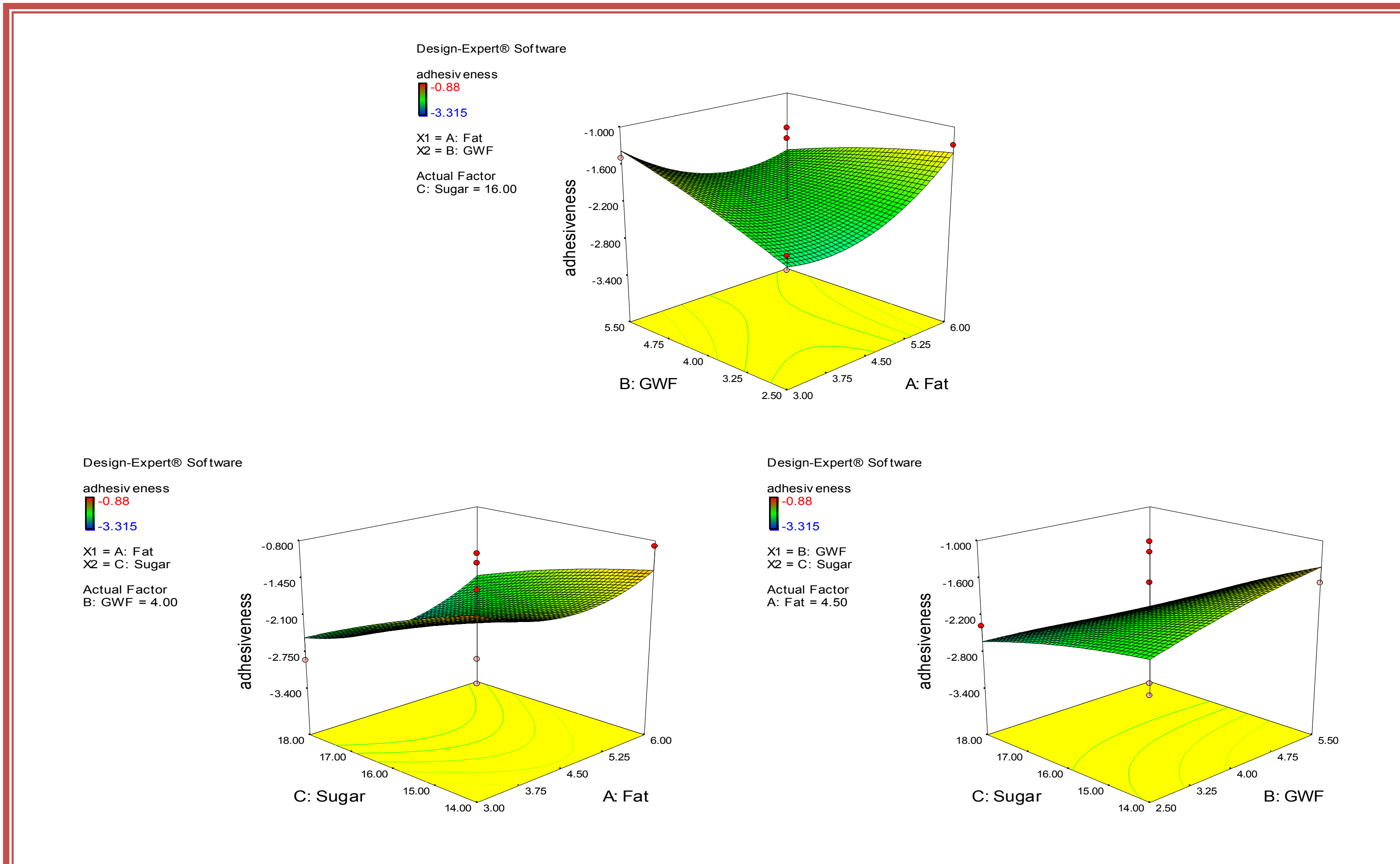


Fig 5.16 Response surface graph of adhesiveness of *doda burfi* as influenced by different levels of ingredients

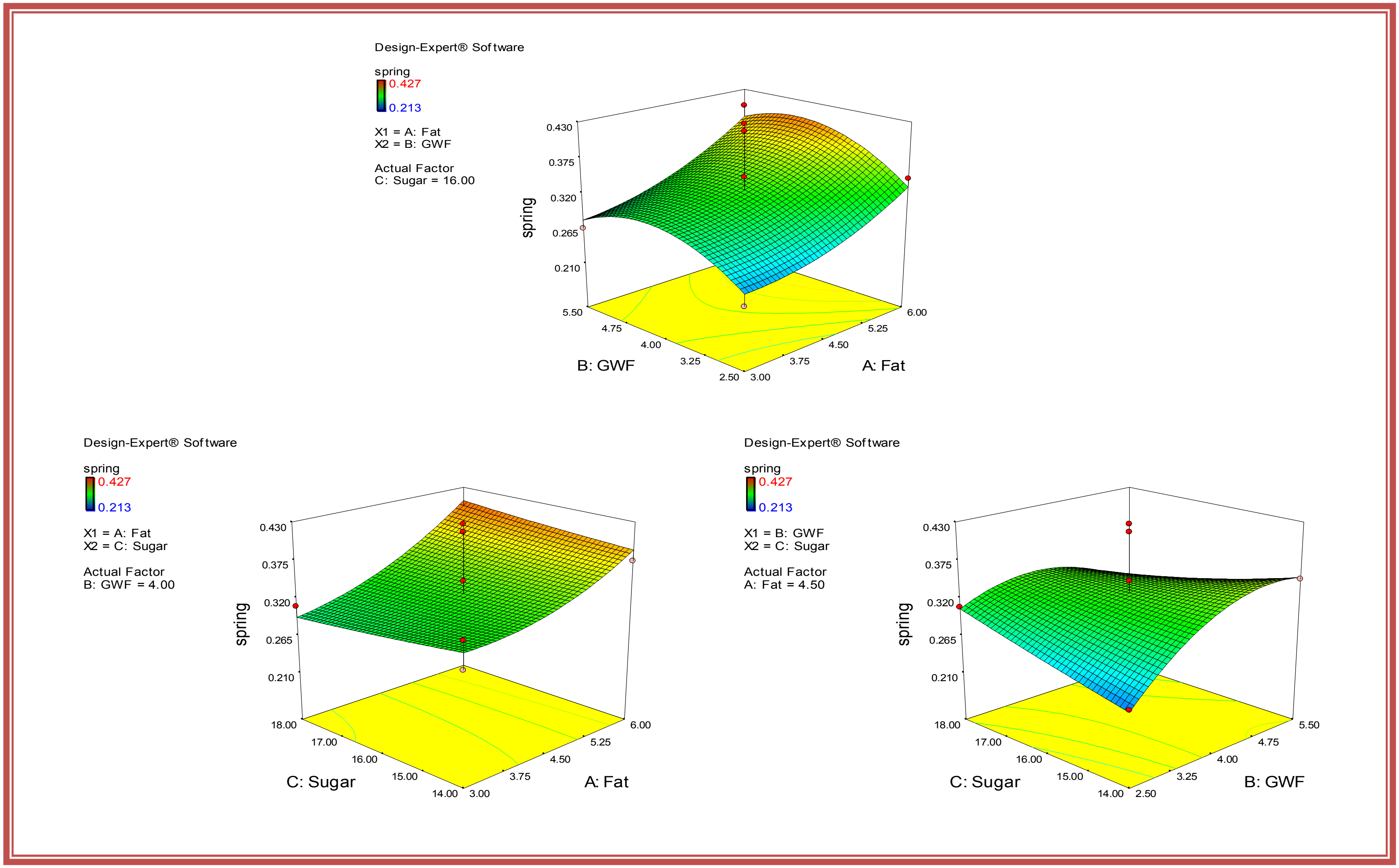


Fig 5.17 Response surface graph of springiness of *doda burfi* as influenced by different levels of ingredients

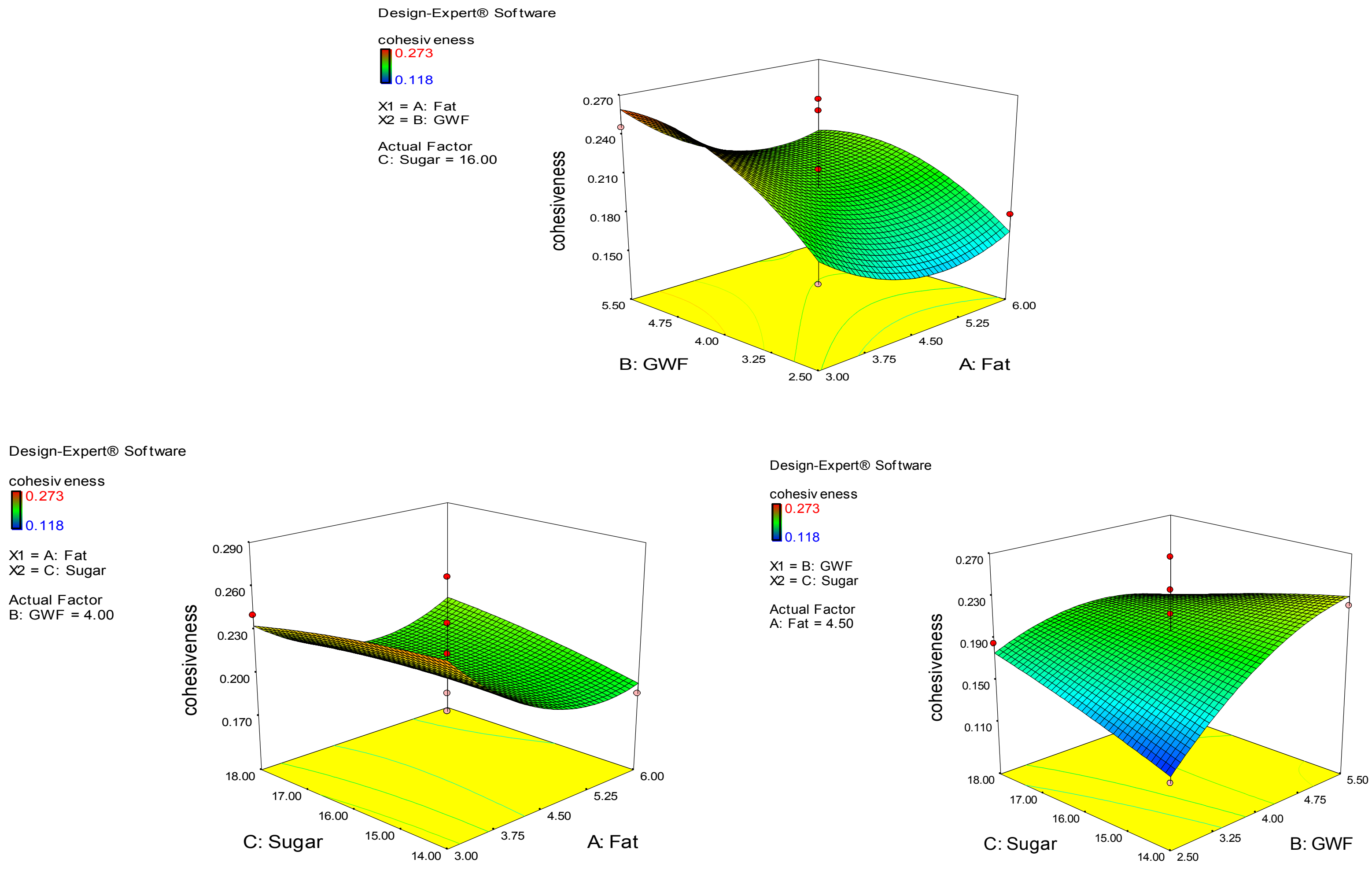


Fig 5.18 Response surface graph of cohesiveness of *doda burfi* as influenced by different levels of ingredients

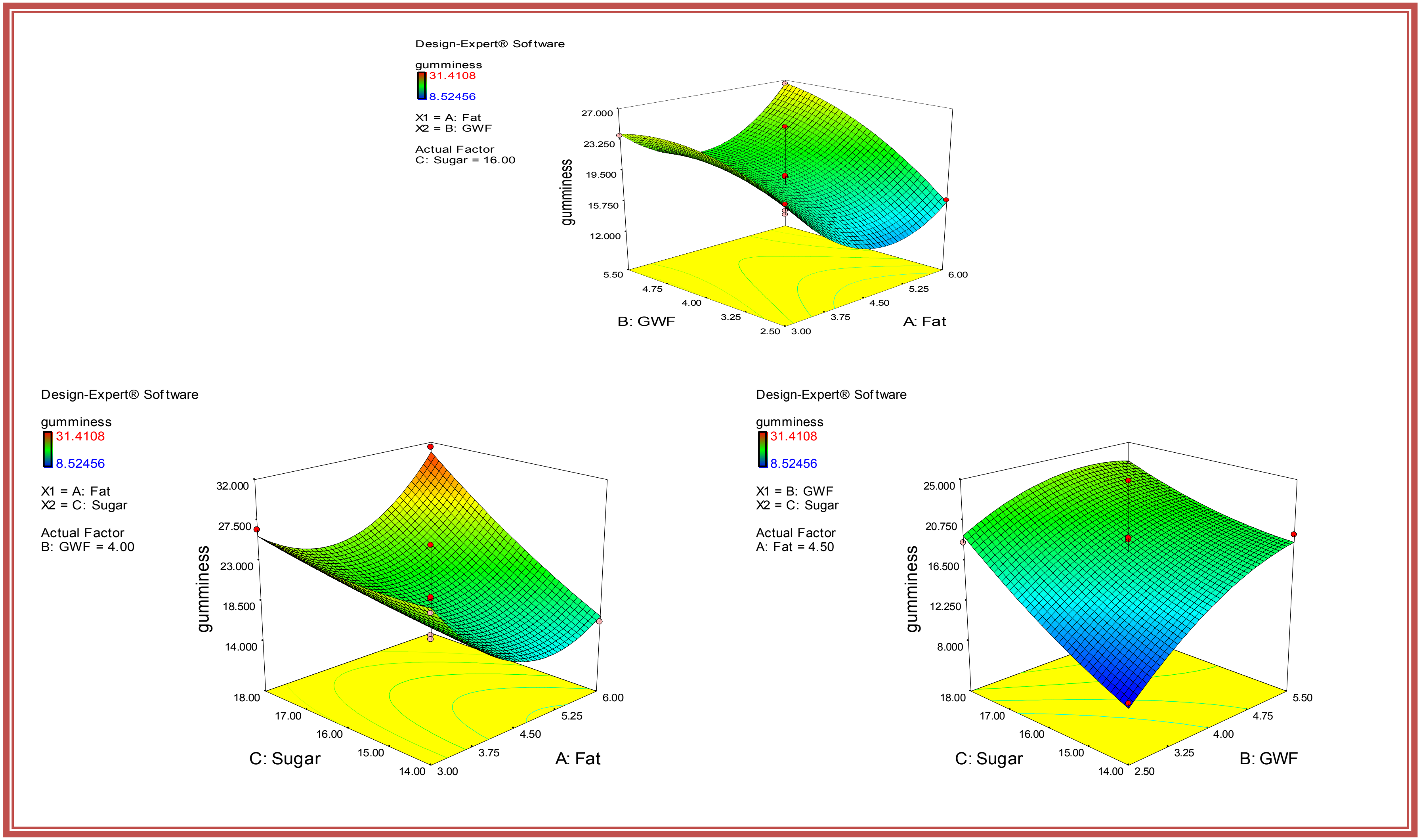


Fig 5.19 Response Surface graph of gumminess of *doda burfi* as influenced by different levels of ingredients

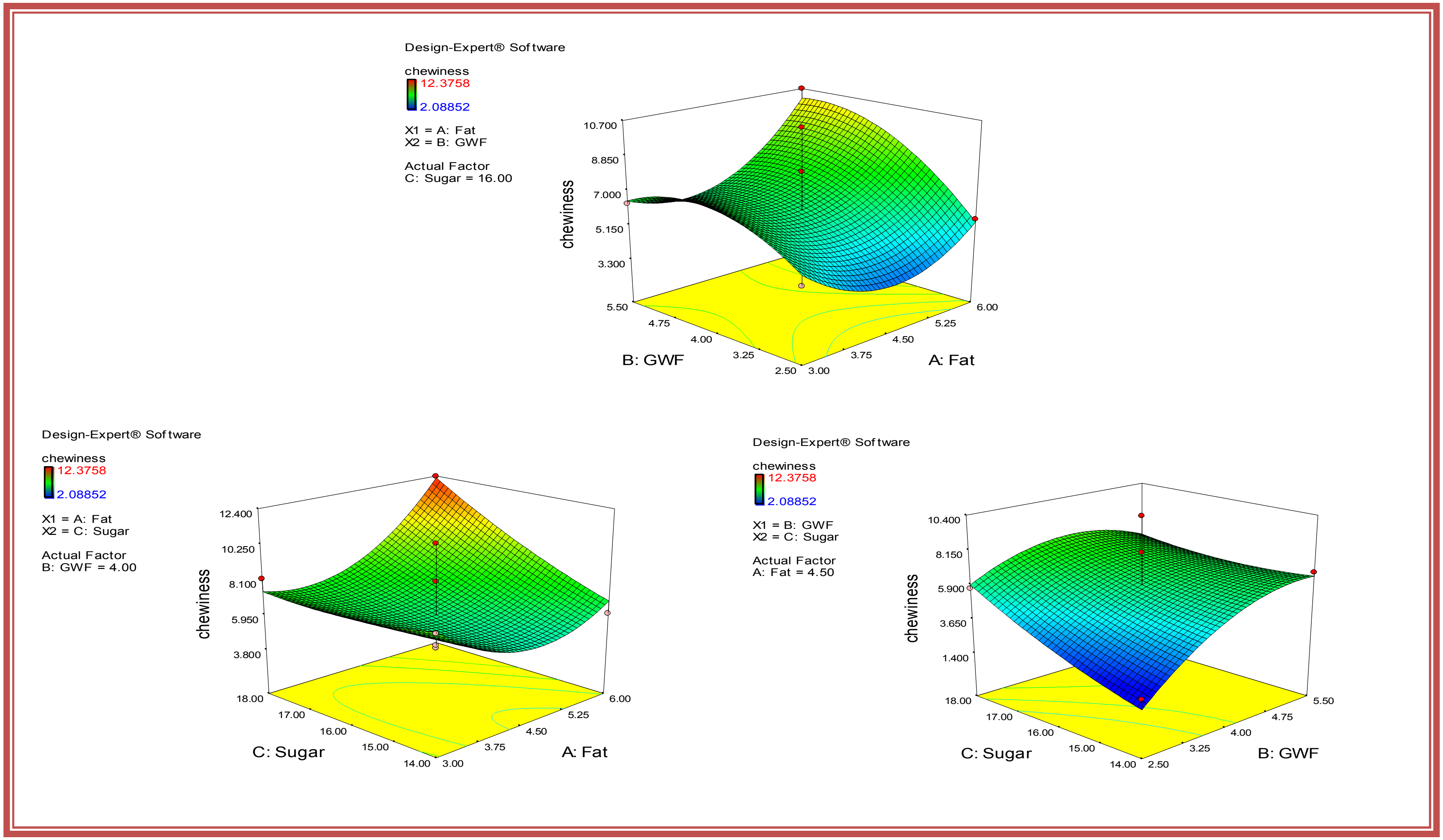


Fig 5.20 Response Surface graph of chewiness of *doda burfi* as influenced by different levels of ingredients

5.2.3.3 Effect on yellowness (b*): The instrumental colour scores for yellowness of *doda burfi* have been given in Table 5.24. As indicated by model statistics (Table 5.27), the R-square for yellowness was 0.77. Value lesser than 0.80 represents values less than desirable, indicating that the model developed was unsatisfactory. Value of adequate precision (APV) as 6.25, indicates model could be used to navigate the design. The model F-value of 2.59 implies the model is statistically non-significant. Fig 5.23 is a response surface graph depicting interactive effect among variables on yellowness and has been presented in the form of three dimensional graphs.

5.2.3.4 Water activity: The instrumental water activity values for functional *doda burfi* have been shown in Table 5.24. The water activity values of all the combinations studied were in intermediate moisture range of water activity, the minimum value being 0.62 whereas the maximum was 0.86. The variation could be attributed to difference in the percentage of sugar and GWF added during processing.

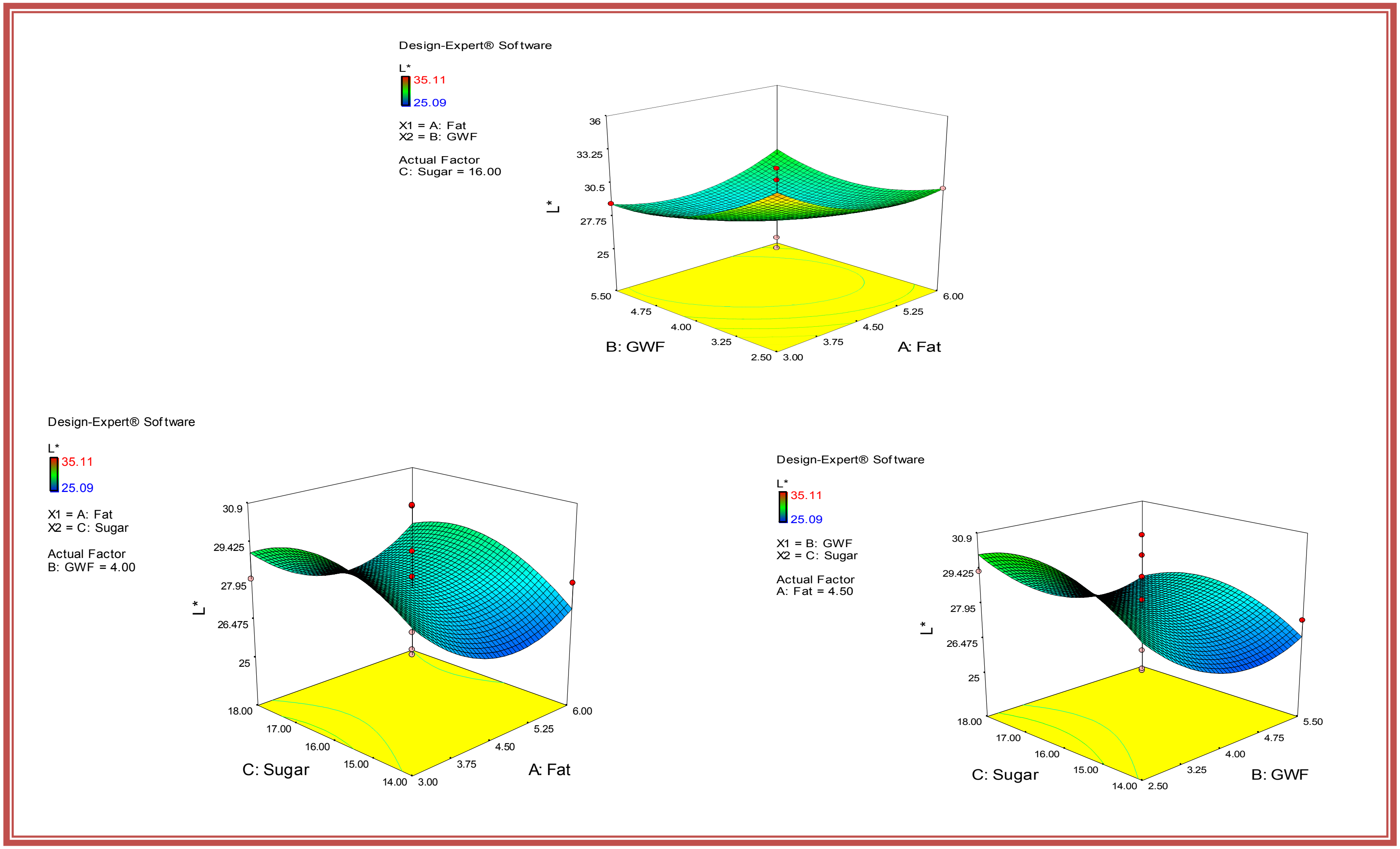


Fig. 5.21 Response surface graph of Lightness (L*) of *doda burfi* as influenced by different levels of ingredients

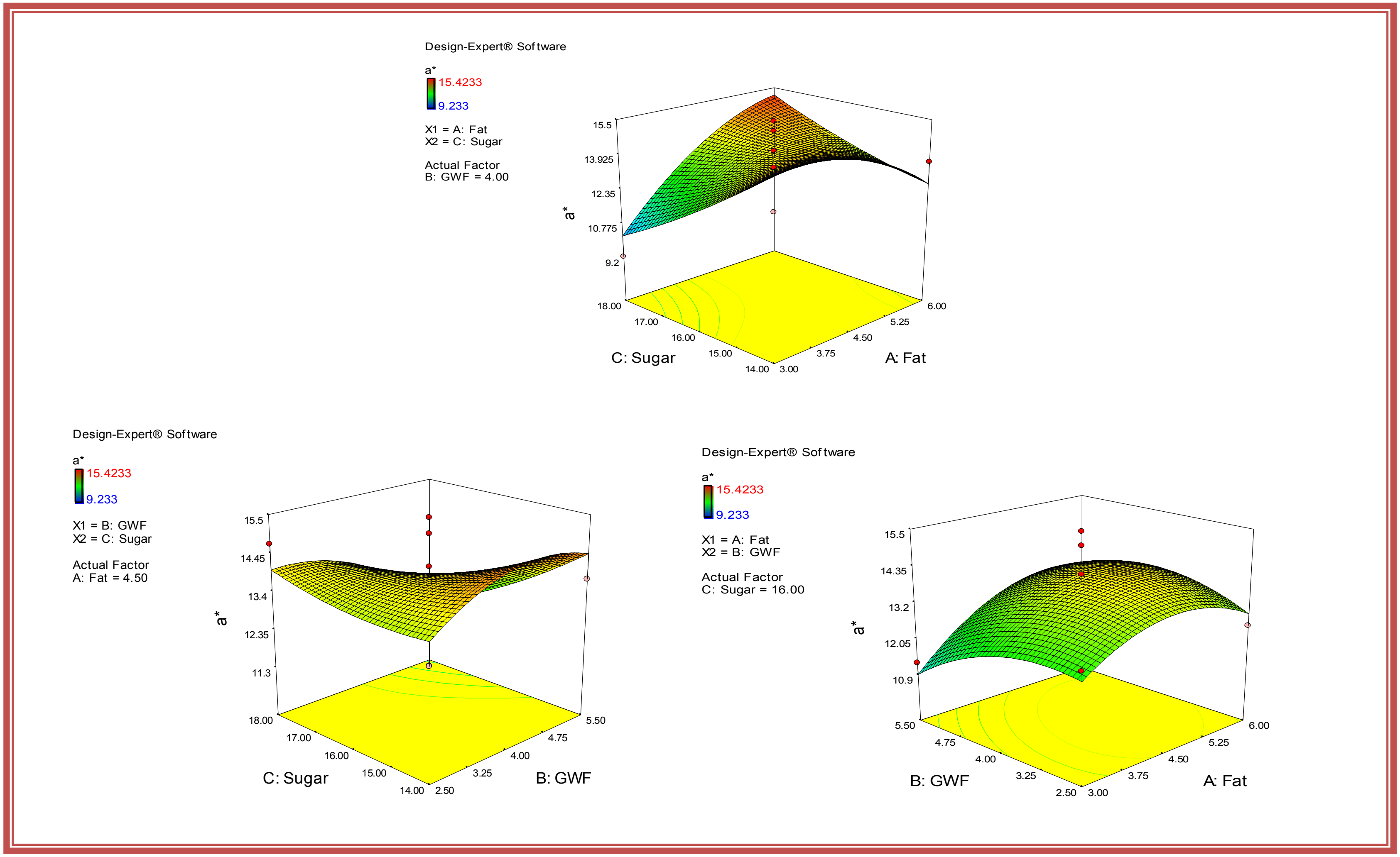


Fig 5.22 Response surface graph of redness (a*) of *doda burfi* as influenced by different levels of ingredients

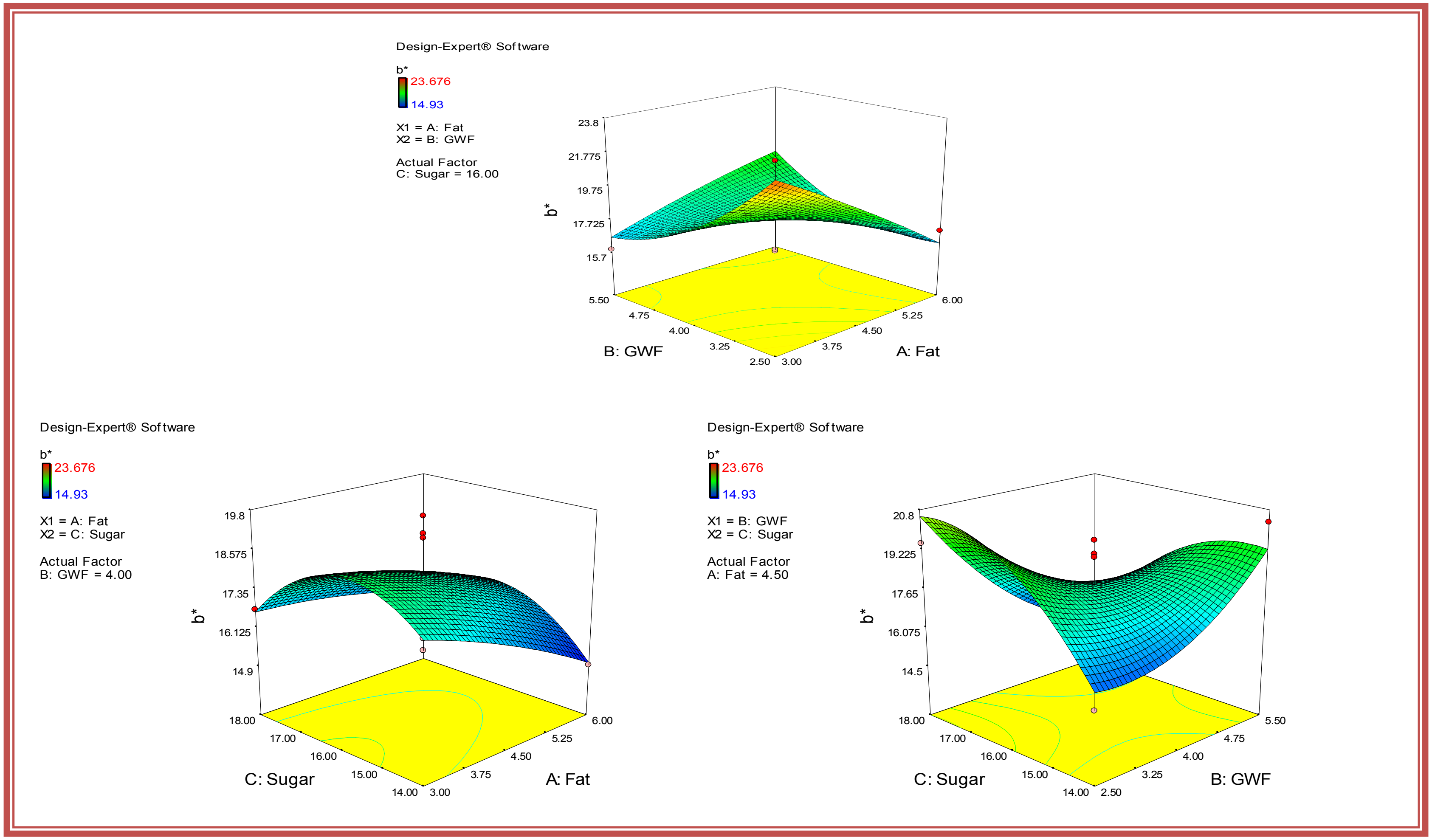


Fig 5.23 Response surface graph of yellowness (b*) of *doda burfi* as influenced by different levels of ingredients

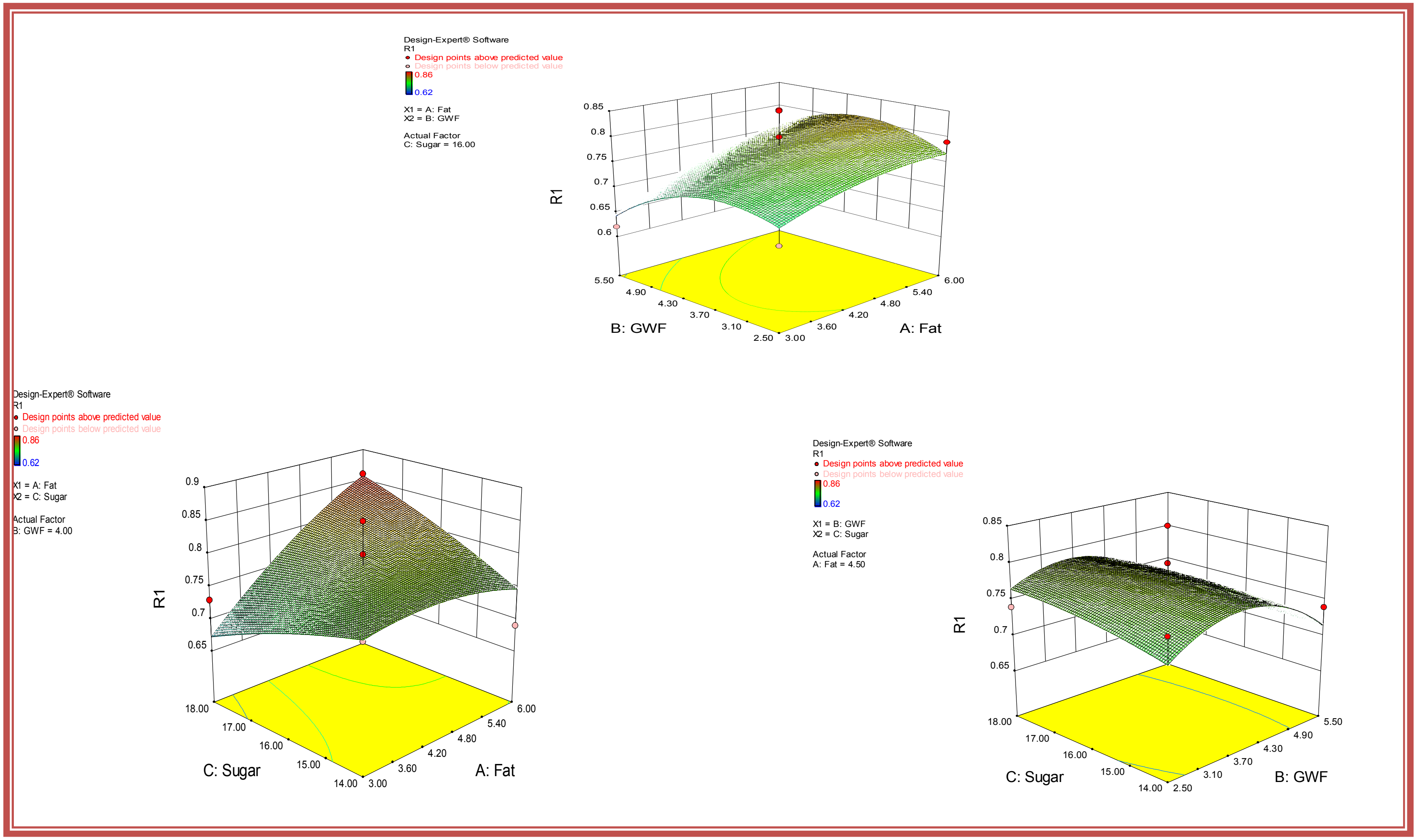


Fig 5.24 Response surface graph of water activity of *doda burfi* as influenced by different levels of ingredients

As indicated by model statistics (Table 5.27), the R-square for water activity was 0.63, indicating that the model developed was unsatisfactory. Value of adequate precision (APV) as 4.83, indicates model could be used to navigate the design. The model F-value of 1.34 implies the model is statistically non-significant. Fig 5.24 is a response surface graph depicting interactive effect among variables on water activity and has been presented in the form of three dimensional graphs.

5.2.4 Optimized solutions and their validation

Since only sensory parameters affect the acceptability of the finished product to a great extent, the optimized solutions based on sensory parameters were obtained. The goals set for the three factors and fifteen responses are presented in Table 5.28. Thus using the sensory data generated through RSM experiments, four best solutions were obtained as shown in Table 5.29. The predicted values for sensory responses for the four solutions are presented in Table 5.29. It was noticed that all the first four solutions had similar desirability value (0.931), whereas only last solution had a desirability of 0.908.

The solution no. 1 (milk fat level 4.72 %, GWF 4.01 and 15.94 % sugar had a fairly high desirability (0.931) indicating reliability of predicted values. The predicted values of four solutions ranged from 8.13 to 8.17 for colour, 8.08 to 8.12 for texture, 8.23 to 8.32, 8.23 to 8.35 for flavour, 8.21 to 8.23 for overall acceptability. The differences between different solutions were so small that it could be neglected and also on practical basis could not be maintained. So, keeping these all in mind, solution number 1 was tried for the verification purposes.

5.2.5 Verification of the optimum formulations

The optimized product was prepared using variables given by solution No. 1 and culture NCDC 167 (Photo plate II) and the resultant product was studied for all the response of sensory, physico-chemical, microbiological, textural profile analysis and instrumental color analysis. The average of triplicate values of the same for the optimized product is given in Table 5.30. The t-test (Table 5.31) was applied upon the predicted and observed values of solution number 1 for all the responses obtained against colour and appearance of surface, texture, sweetness, flavour and overall acceptability, which showed that there were no significant differences for predicted score and observed score for the attributes studied.

Table 5.27: Coefficient of full second order polynomial model for instrumental textural and colour responses of *doda burfi* using NCDC -167

Factor	Hardness (N)	Adhesiveness (N.s)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)	L*	a*	b*
Intercept	-771.39	-5.25	-0.26	-0.26	119.27	44.33	-8.43	49.23	84.82
A- Fat	-152.08	-1.36	-0.12	-0.15	-41.79	-17.34	-9.45	-6.44	-6.06
B- (GWF)	-23.59	2.25	0.33	0.23	14.47*	9.84	-6.95	5.31	3.21
C- Sugar	-47.79**	0.44	0.01	0.04	-6.91*	-3.35	8.88	-3.73	14.04
A*B	5.69	0.17	0.00	0.00	0.84	0.41	0.52	0.21	1.03*
A*C	4.16	0.03	0.00	0.00	1.03	0.51	0.07	0.60*	0.13
B*C	0.20	-0.07	-0.01	-0.01	-0.57	-0.35	-0.06	-0.26	-0.77*
A^2	7.33*	0.17	0.01	0.01	2.39*	0.91	0.65	-0.40	-0.09
B^2	-0.27	-0.02	-0.02	-0.01	-0.83	-0.64	0.61	-0.29	0.51
C^2	1.18	– 0.01	0.00	0.00	0.20	0.09	-0.27	0.06	-0.36
R^2	0.85	0.40	0.48	0.65	0.84	0.69	0.56	0.66	0.77
PRESS	12555.22	28.32	0.08	0.05	168.93	94.58	220.72	85.34	124.71
APV	8.29	2.39	3.00	5.05	8.91	6.31	4.09	4.45	6.25
Model 'F' value	4.47*	0.53	0.72	1.49	4.25*	1.74	0.98	1.52	2.59

Table 5.28: Goals set for constraints to optimize the technology using culture NCDC 167

Constraints name	Goal set for optimized product	Lower limit	Upper limit	Lower weight	Upper weight	Importance
Fat	is in range	3	6	1	1	3
GWF	is in range	2.5	5.5	1	1	3
Sugar	is in range	14	18	1	1	3
Colour	is in range	7.9	8.5	1	1	3
Texture	is in range	7.6	8.28	1	1	3
Sweetness	is in range	8.2	8.5	1	1	3
Flavour	is in range	7.9	8.35	1	1	3
OAA	Maximize	7.5	8.28	1	1	3

TABLE 5.29: Predicted values of different responses for the four suggested solutions vide from RSM using culture NCDC 167

Solutions number	Fat (%)	GWF %)	Sugar (%)	Colour	Texture	Sweetness	Flavour	OAA	Desirability
1	4.72	4.01	15.94	8.17	8.12	8.32	8.35	8.23	0.931
2	4.71	4.00	15.94	8.17	8.12	8.32	8.35	8.23	0.931
3	4.72	3.98	15.96	8.17	8.12	8.32	8.35	8.22	0.931
4	4.77	4.07	15.83	8.13	8.08	8.23	8.23	8.21	0.908

Table 5.30: Characteristics of the optimized product made from solution no.1 using culture NCDC 167

Property	Attribute	Value
Sensory	**Colour and appearance**	8.06 ± 0.09
	Texture	8.25 ± 0.18
	Sweetness	8.26 ± 0.64
	Flavour	8.15 ± 0.14
	Overall acceptability	8.00 ± 0.57
Physico-chemical	**Moisture, %**	15.28 ± 1.23
	Fat, %	18.07 ± 1.54
	Protein, %	15.31 ± 0.98
	Reducing sugars, %	13.68 ± 0.65
	Total sugars, %	15.27 ± 0.49
	Ash, %	2.43 ± 0.09
	pH	5.61 ± 0.05
	Acidity, % LA	1.15 ±0.06
	Soluble dietary fibre (SDF %)	6.02 ± 0.81
	Insoluble dietary fibre (IDF %)	12.95 ± 0.90
	Water activity (a_w)	0.76 ± 0.01
Textural attributes	**Hardness (N)**	66.84 ± 1.06
	Adhesiveness (N.s)	-3.24 ± 0.16
	Springiness	0.27 ± 0.29
	Cohesiveness	0.06 ± 0.32
	Gumminess (N)	4.03 ± 0.52
	Chewiness (N)	3.24 ± 0.24
Instrumental colour values	**Lightness (L*)**	27.97 ± 0.52
	Redness (a*)	10.69 ± 0.23
	Yellowness (b*)	19.56 ± 0.29
Microbiological count	**Standard plate count (log cfu/g)**	$1.2X10^2$
	Yeast and mould count (log cfu/g)	nil
	Coliform count (log cfu/g)	nil

Table 5.31: Comparison of predicted and observed average values of the optimized product using culture NCDC 167

Parameter	Sensory attributes				
	Colour & appearance	**Texture**	**Flavour**	**Sweetness**	**0verall acceptability**
Predicted values	8.171	8.120	8.325	8.350	8.226
Observed values#	8.06 ± 0.09	8.25 ± 0.18	8.15 ± 0.14	8.26 ± 0.64	8.00 ± 0.57
t-value	0.65^{ns}	-0.75^{ns}	0.62^{ns}	0.58^{ns}	0.68^{ns}

Figures are the Mean ± SE of three replications

ns not significant (p<0.05)

5.3 DIETETIC PRODUCT DEVELOPMENT

To enhance the functional aspects of conventional sweetmeat, various health ingredients can be incorporated and the product hence made can be termed as functional product, with enhanced health benefits, something which provides extra that a normal food provides to us. Keeping the same under consideration, *doda burfi* was prepared with added fibre, calcium, artificial sweetener and bulking agent. Various preliminary trails were conducted to choose from the wide availability of bulking agents and fibres and there ranges thereof to be carried forward for the response surface methodology.

5.3.1 Selection of fibre and its desirable acceptable value: In the initial stages of the present study, several preliminary trials were conducted to select levels of various functional ingredients and a tentative protocol for the manufacture of functional d*oda burfi*. The levels of best two fibres amongst five different fibres studied namely wheat fibre, oat fibre, Diesol™ (hydrolyzed gum acacia), microcrystalline cellulose (MCC) and inulin were selected on the basis of sensory studies. In the earlier phase of this study, several preliminary trails were conducted to optimize the maximum level of various fibres that could be incorporated in the preparation of functional *doda burfi*. The minimum values were decided to reach at a point so that product could be labeled as "fortified with dietary fibres". Various minimum and maximum values thus obtained for different fibres were used in the preparation of product, so that fibre along with its desirable level could be used further in the preparation of functional *doda burfi.* The maximum range for various fibres except Diesol was taken as 5% whereas the minimum was 2.5% to enrich and label the product as "fortified with dietary fibres" whereas the minimum and maximum limits for Diesol worked out as 0.5 and 0.75 % respectively. In the screening process, oat fibre and Diesol at levels of 2.5 and 0.75 per cent, respectively were adjudicated as best to be used further in the study. A comparative data is tabulated in Table 5.32.

5.3.2 Selection of level of sweetener: In the earlier phase of this study whole sugar was replaced with natural sweetener "stevia" taking into consideration its perceivable intensity starting from 0.15%, but the product was very much similar to cake owing to complete replacement of sugar with stevia, lacking the granular texture of traditional *doda burfi* and hence the results obtained were unacceptable from sensory point of view.

Table 5.32: Sensory scores of various fibres used for screening in the preparation of *doda burfi*

Fibre	MCC (%)		Wheat fibre (%)		Inulin (%)		Oat fibre (%)		Diesol (%)	
Level used	**2.5**	**5.0**	**2.5**	**5.0**	**2.5**	**5.0**	**2.5**	**5.0**	**0.5**	**0.75**
Colour	6.6	6.9	5.6	6.0	6.5	6.3	6.7	6.4	5.8	6.5
Texture	5.7	5.6	5.8	6.3	5.9	5.4	6.7	5.7	6.0	6.7
Sweetness	5.8	5.7	5.9	6.3	7.0	6.8	6.8	6.3	6.9	7.1
Flavour	5.9	6.0	4.9	5.6	5.9	5.8	6.4	5.6	6.7	6.9
OA	**5.7**	**5.7**	**5.1**	**5.6**	**5.8**	**5.5**	**6.6**	**5.7**	**6.5**	**6.9**

Thus, replacement was then thought to be made on minimum sugar basis and few preliminary trials consisting of sugar and stevia in the ratio 25:75, 50:50, 75:25, 100:0 (control) were conducted for standardizing the level of sugar and stevia (Table 5.33). The results obtained from the product made from 75:25 sugar to stevia ratio were better compared to others than the product developed from the same using their ratios 25:75 but to prepare a product with maximum health benefits the later ratios were chosen sacrificing a little texture of traditional *doda burfi*.

5.3.3 Development of dietetic *doda burfi*

The product development essentially comprised of using the optimized recipe of standardized *doda burfi* along with selected levels of oat fibre, Diesol™, stevia, and sugar using response surface methodology. The coded and real values for the design and its output for sensory scores and instrumental colour data obtained after conducting the experimental trials has been presented in Tables 5.34, 5.35, 5.36 and its regression analysis has been presented in Table 5.37. As can be seen from Table 5.35, none of the combinations was sensorily acceptable as it lacked typical granularity associated with the traditional *doda burfi*. Hence, it was concluded that the sugar is an essential component of *doda burfi* as it is required for proper body and texture as well as for colour development, and therefore, it is not possible to replace it with artificial sweeteners. Hence, the idea of developing a dietetic product was abandoned and the study on development of functional *doda burfi* using functional ingredients such as dietary fibre, calcium and phytostanol was pursued further.

Table 5.33: Sensory scoring of *doda burfi* prepared using a blend of table sugar and artificial sweetener in different ratios

Attributes	A	B	C	D
Colour	6.4	6.9	7.1	7.3
Texture	5.9	6.6	6.6	7.1
Sweetness	6.3	6.9	6.6	7.3
Flavour	5.6	6.8	6.4	7.1
OA	5.7	6.7	6.6	7.1

Where, A: 50% each
B: 25% Stevia and 75% sugar
C: 75% Stevia and 25 % sugar
D: control 100 % sugar

Table 5.34: Coded and real levels of oat fibre, Diesol, stevia and sugar in the four factor CCRD

Factors	- α value	Lower limit	Center ordinate	Upper limit	+ α value
			Coded level		
	-2	-1	0	+1	+2
A:Oat fibre	1	1.50	2.00	2.50	3
B: Diesol™ (hydrolyzed gum acacia)	0.37	0.50	0.62	0.75	0.87
C: Stevia	0.14	0.19	0.24	0.290	0.34
D: Sugar	2.4	3.2	4.0	4.8	5.6

5.4 DEVELOPMENT OF FUNCTIONAL *DODA BURFI*

In the present study, a functional *doda burfi* containing functional ingredients such as calcium, soluble fibres, and phytostanol was attempted. In the initial stages of the present study, several preliminary trials were conducted to select levels of phytostanol whereas the levels for the addition of fibres were taken as those finalized previously with the *doda burfi* containing artificial sweetener. The product development essentially comprised of optimization of levels of oat fibre (%), and calcium (mg/100ml) keeping the other ingredients like sugar (16%), GWF (4.0%), Diesol (0.75%) as constant. The product development for the standardization of functional recipe with sugar is presented under the following sections.

5.4.1 Optimization of oat fibre (%) and calcium in milk (mg/100 ml) using RSM

Preliminary trials were conducted to narrow down the levels of functional ingredients used for optimization study. Thus, in the present study after preliminary experiments range of each of two factors were employed for standardization of functional ingredients viz. oat fibre (%) ranging from 1 to 3 % and calcium, ranging from 50 to 100mg/100 ml of milk. The level of milk solid-not-fat (MSNF) at 12% and Diesol at 0.75% (as per the desirable results recorded using sensory evaluation) was kept constant in all the formulations based on the results obtained in the optimization study (vide section 5.2). In total thirteen trials were conducted based on the formulations provided by central composite rotatable design (CCRD) using response surface methodology. Table 5.38 and 5.39 represents coded and real values of various ingredients used for optimization.

5.4.1.1 Effect of oat fibre and calcium on sensory quality of *doda burfi*

Sensory attributes play an important role in deciding the overall acceptability of the product. The sensory parameters chosen to assess the quality of functional *doda burfi* were colour, flavour, texture, sweetness and overall acceptability of the product.

(a) Effect on color score: Score for colour and appearance of *doda burfi* varied non-significantly ranging from 7 to 7.5 with the constant addition of artificial colour in the product @ 1% (Table 5.40). Though the values obtained were almost similar in many of the experiments conducted and a little variation was observed between the scores obtained.

The regression analysis of the data is presented in Table 5.41. It reveals that the coefficient of determination (R^2) was 0.48 and indicated that the model is statistically non-significant (F-value: 1.32). The adequate precision value (APV) was found to be 4.28, which is more than the minimum desirable (4.00) for high prediction ability. It was found that the model can be used to navigate the design.

The coefficient estimates of colour in terms of linear order, second order and interaction of all the three ingredients as presented in Table 5.41 reveal that none of the attribute were found significant at any of these levels ($p<0.05$). Multiple regression equation generated to predict the colour as affected by different factors in terms of actual factors is as follows:

Colour = 8.367 - 0.384*Oat fibre - 0.021*Calcium + 0.008*Oat fibre*Calcium – 0.049*Oat fibre^2 + 0.00006*Calcium^2

Interaction among variables and their effect on colour of *doda burfi* is depicted in form of three dimensional graphs {(Fig 5.25 (a)}. It shows that when calcium and oat fibre at lower values had significant desirable values in terms of colour scores whereas the increase in both of the variable led to lower scores values thereafter.

(b) Effect on texture score: A wide variation in texture score of *doda burfi* was observed and ranged from 6.66 to 8.26 (Table 5.40). The regression analysis of the data is presented in Table 5.41. It reveals that the coefficient of determination (R^2) was 0.95 indicating that the developed model is satisfactory. Further the statistical analysis indicated that the model fit well with the data (model F-value: 24.53). The calculated adequate precision value (APV) was found to be 15.03 and hence the model can be used to navigate the design.

Table 5.35: Sensory attributes of Dietetic *doda burfi* manufactured using functional ingredients

Standard order	Oat fiber %	Diesol %	Stevia %	Sugar %	Colour	Texture	Sweetness	Flavour	Overall acceptability
1	1.5	0.5	0.19	3.2	6.76	7.19	7.1	5.833	5.87
2	2.5	0.5	0.19	3.2	7.19	7.12	6.93	6.56	6.31
3	1.5	0.75	0.19	3.2	6.61	7.25	7.54	6.0	6.31
4	2.5	0.75	0.19	3.2	7.26	6.61	7.08	6.52	6.13
5	1.5	0.5	0.29	3.2	6.71	7.16	7.46	5.68	5.87
6	2.5	0.5	0.29	3.2	7.26	6.68	6.76	6.41	6.41
7	1.5	0.75	0.29	3.2	6.71	7.10	7.31	6.83	6.25
8	2.5	0.75	0.29	3.2	7.16	6.5	6.91	6.0	5.89
9	1.5	0.5	0.19	4.8	6.75	7.28	7.63	6.0	6.12
10	2.5	0.5	0.19	4.8	7.12	7.0	7.1	6.78	6.45
11	1.5	0.75	0.19	4.8	6.82	7.26	7.75	6.72	6.4
12	2.5	0.75	0.19	4.8	7.22	6.833	7.29	6.67	6.33
13	1.5	0.5	0.29	4.8	6.87	7.21	7.65	6.56	6.01
14	2.5	0.5	0.29	4.8	6.96	6.87	7.166	6.69	5.93
15	1.5	0.75	0.29	4.8	6.86	7.21	7.67	7.166	6.31
16	2.5	0.75	0.29	4.8	7.21	6.66	6.38	6.13	6.0

17	1.0	0.63	0.24	4.0	5.75	6.917	7.16	6.54	6.0
18	3.0	0.63	0.24	4.0	6.25	6.25	7.0	5.93	5.67
19	2.0	0.38	0.24	4.0	6.54	7.28	7.41	7.23	6.77
20	2.0	0.88	2.4	4.0	6.42	7.11	6.82	6.0	6.43
21	2.0	0.63	0.14	4.0	6.92	7.49	7.166	7.41	6.83
22	2.0	0.63	0.34	4.0	6.92	6.92	6.18	7.01	6.0
23	2.0	0.63	0.24	2.4	6.81	7.12	6.81	7.12	6.61
24	2.0	0.63	0.24	5.6	7.67	7.67	7.85	7.56	6.92
25	2.0	0.63	0.24	4.0	7.25	7.25	7.0	7.33	6.6
26	2.0	0.63	0.24	4.0	7.16	7.31	7.16	7.32	6.43
27	2.0	0.63	0.24	4.0	6.79	7.41	7.12	7.26	6.50
28	2.0	0.63	0.24	4.0	6.91	7.31	7.06	7.36	6.50
29	2.0	0.63	0.24	4.0	6.92	7.36	7.0	7.11	6.60
30	2.0	0.63	0.24	4.0	7.0	7.26	7.09	7.21	6.53

Table 5.36: Instrumental colour and water activity of dietetic *doda burfi* manufactured using functional ingredients

Standard order	Oat fibre (%)	Diesol (%)	Stevia (%)	Sugar (%)	Water activity (a_w)	L*	a*	b*
1	1.5	0.5	0.19	3.2	0.946	24.88	11.28	12.13
2	2.5	0.5	0.19	3.2	0.934	23.12	8.53	11.18
3	1.5	0.75	0.19	3.2	0.953	26.62	14.45	17.74
4	2.5	0.75	0.19	3.2	0.914	23.18	9.62	11.29
5	1.5	0.5	0.29	3.2	0.949	33.96	13.82	16.28
6	2.5	0.5	0.29	3.2	0.959	30.18	13.01	18.17
7	1.5	0.75	0.29	3.2	0.941	33.18	13.89	19.65
8	2.5	0.75	0.29	3.2	0.962	27.18	12.76	16.62
9	1.5	0.5	0.19	4.8	0.935	22.92	9.365	10.23
10	2.5	0.5	0.19	4.8	0.960	32.66	13.56	17.58
11	1.5	0.75	0.19	4.8	0.944	23.12	10.26	11.36
12	2.5	0.75	0.19	4.8	0.957	25.83	13.42	15.89
13	1.5	0.5	0.29	4.8	0.928	26.98	12.23	14.28
14	2.5	0.5	0.29	4.8	0.959	34.18	14.83	17.19
15	1.5	0.75	0.29	4.8	0.928	33.61	13.55	19.96
16	2.5	0.75	0.29	4.8	0.947	22.19	8.69	12.15

17	1.0	0.63	0.24	4.0	0.953	25.95	13.45	16.78
18	3.0	0.63	0.24	4.0	0.961	31.91	16.12	21.03
19	2.0	0.38	0.24	4.0	0.941	24.71	12.28	17.89
20	2.0	0.88	2.4	4.0	0.935	24.6	11.19	13.15
21	2.0	0.63	0.14	4.0	0.942	30.97	15.77	20.47
22	2.0	0.63	0.34	4.0	0.949	23.19	10.58	11.73
23	2.0	0.63	0.24	2.4	0.936	32.91	13.92	20.51
24	2.0	0.63	0.24	5.6	0.916	31.96	14.17	20.16
25	2.0	0.63	0.24	4.0	0.935	33.25	13.03	20.34
26	2.0	0.63	0.24	4.0	0.948	22.96	10.19	12.46
27	2.0	0.63	0.24	4.0	0.952	32.19	15.48	20
28	2.0	0.63	0.24	4.0	0.94	23.81	9.53	11.08
29	2.0	0.63	0.24	4.0	0.947	32.28	12.28	19.42
30	2.0	0.63	0.24	4.0	0.956	24.58	9.12	12.87

Table 5.37: Coefficient of full second order polynomial model for sensory responses of dietetic *doda burfi*

Factor	Colour	Texture	Flavour	Sweetness	Overall acceptability
Intercept	13.03	5.77	14.66	-2.27	13.00
A- Oat fibre	-1.24	-2.81	-3.37*	-0.35	-2.41
B- Diesol	-2.74	1.06	-25.06	10.06*	-9.41
C- Stevia	-36.43	29.60	6.10	29.60	-2.53
D-Sugar	1.36	1.63	13.02	13.30	-5.45
A*B	0.77	2.71	5.94**	2.64*	3.26*
A*C	0.87	2.13	-1.35	-1.28	0.81
A*D	-0.44	0.60	1.19	-2.58	0.07
B*C	4.53	-19.93	12.34	-33.94*	5.26
B^*D	-12.68	1.20	6.37	1.41	7.03
C*D	16.67	-11.10	-22.18	8.53	-15.31
A^2	0.18	0.06	-0.24	0.02	-4.16×10^{-3}
B^2	3.47	-1.43	6.41	-5.14	-0.83
C^2	51.93	-34.59	-3.70	-15.53	8.28
D^2	5.17	-1.48	-15.90	-14.52	6.36
R^2	0.33	0.43	0.57	0.66	0.38
APV	2.14	3.84	3.97	6.79	3.02
PRESS	9.48	8.15	16.94	6.39	8.91
Model 'F'value	0.52^{ns}	0.81^{ns}	1.40^{ns}	2.11^{ns}	0.67^{ns}

ns non significant, * significant at p< 0.05, **significant at p<0.01

Table 5.38: Coded and real levels of functional *doda burfi* in the two factor CCRD experiment

Factor	Lower limit		Upper limit
	Coded levels		
	-1	0	+1
Oat fibre (%)	1	2	3
Calcium (mg/100ml)	50	75	100

Table 5.39: The CCRD design for manufacture of functional *doda burfi* using culture NCDC 167

Standard order	Factor-1	Factor-2
	Oat fibre %	Calcium (mg/100ml)
1	1.0	50
2	3.0	50
3	1.0	100
4	3.0	100
5	0.59	75
6	3.41	75
7	2.0	39.64
8	2.0	110.36
9	2.0	75
10	2.0	75
11	2.0	75
12	2.0	75
13	2.0	75

The coefficient estimates of texture in terms of linear order, second order and interaction of all the three ingredients as presented in Table 5.41 reveal that oat fibre at quadratic level had significant effect on the texture of *doda burfi* ($P<0.01$). Multiple regression equation generated to predict the texture as affected by different factors is as follows:

Texture = 10.832 – 1.890*Oat fibre – 0.032*Calcium + 0.003*Oat fibre*Calcium – 0.247*Oat fibre^2 + 0.0001*Calcium^2

Interaction among variables and their effect on texture of *doda burfi* is depicted in form of three dimensional graphs {(Fig 5.25(b)}. The positive sign of the coefficient in the equation indicates that there is an increase in score for texture response with an increase in level of ingredients used, while negative sign indicates the reciprocal of the same. Therefore, with the increased level of oat fibre in the product, there was a significant decrease ($p<0.01$) in texture score.

(c) Effect on sweetness score: A wide variation in sweetness score of *doda burfi* was observed and ranged from 6.83 to 7.57 (Table 5.40). The regression analysis of the data is presented in Table 5.41. It reveals that the coefficient of determination (R^2) was 0.86 indicating that the model developed was satisfactory. Further the statistical analysis indicated that the model fit well with the data (model F-value: 9.07). The calculated adequate precision value (APV) was found to be 9.67, which is more the minimum desired value (4) and hence the model can be reliably and satisfactorily used to navigate the design.

The coefficient estimates of sweetness in terms of linear order, second order and interaction of all the three ingredients as presented in Table 5.41 reveal that none of the variables had significant effect at any level. Multiple regression equation generated to predict the sweetness as affected by different factors in terms of actual factors is as follows:

Sweetness = 9.515 – 0.721*Oat fibre – 0.035*Calcium + 0.002*Oat fibre*Calcium + 0.056*Oat fibre^2 + 0.0001*Calcium^2

Interaction among variables and their effect on sweetness of *doda burfi* is depicted in form of three dimensional graphs {(Fig 5.25 (c)}. It shows with the increase in oat fibre the sweetness score decreased. The similar is the case with the increase in calcium addition, though the effect was not much significant.

(d) Effect on flavour score: Flavour is an important criterion for deciding the quality of the product, which in turn determines its ultimate acceptability. The sensory scores for the flavour of *doda burfi* ranged from 6.16 to 7.7 (Table 5.40).

The maximum score was given to the product prepared from least amount of fibre added in the final product (0.59%). This clearly indicates that flavour score increased with decrease in oat fibre percentage.

As indicated by model statistics, the R-square for flavour was 0.840, which is more than the minimum value prescribed by Henika (1982) (0.80) for developing a model for sensory data. Fig 5.26 (a) is a response surface graph depicting interactive effect among variables on flavour and has been presented in the form of three dimensional graphs. The oat fibre at minimum concentrations showed maximum score values whereas the calcium had negligible effect on the flavour score. The coefficients estimates of the flavour model (Table 5.41) show that the level of oat fibre at linear level had a significant ($p<0.01$) effect on flavour.

Multiple regression equation generated to predict the flavour score as affected by different factors in terms of actual factors is as follows:

$$\textbf{Flavour} = 8.713 - 0.876 \times \text{Oat fibre} - 0.010 \times \text{Calcium} + 0.002 \times \text{Oat fibre} \times \text{Calcium} + 0.048 \times \text{Oat fibre}^2 + 0.00002 \times \text{Calcium}^2$$

(e) Effect on overall acceptability score: Overall acceptability is the most active and determining parameter of sensory quality of the product in totality and consists of colour and appearance, flavor, body and textural characteristics. The sensory score for overall acceptability of *doda burfi* ranged from 6.56 to 7.54. (Table 5.40). Overall acceptability is an important criterion for deciding the quality of the ultimate product, which in turn determines its final acceptability by the consumer. The maximum score was given to the product prepared from 1.0% Oat fibre and 50 mg/100ml of calcium in the product whereas the minimum score was obtained in the product with 3.0% oat fibre and 50mg/100ml of calcium.

As indicated by model statistics, the R-square for overall acceptability was 0.83. Value of adequate precision (APV) as 8.09, indicating that the model could be used to navigate the design. The model F-value of 7.17 implies the model is statistically significant. The lack of fit test, which measure the fitness of the model obtained, did not result in a significant F value, indicating that the model is sufficiently accurate for predicting the organoleptic characteristics of functional *doda burfi* made with any combination of ingredient levels within the range evaluated.

Fig 5.26 (b) is a response surface graph depicting interactive effect among variables on overall acceptability and has been presented in the form of three dimensional graphs. The coefficients estimates of the overall acceptability model (Table 5.41) reveal both the variables at lower levels had scores comparable to conventional product and oat fibre at linear level had significant effect ($p<0.01$). The positive sign of coefficient of the equation indicates that there is an increase in score for overall acceptability with an increase in level of ingredients used, while negative sign indicates the reciprocal of the same. Therefore, with the increased level of oat fibre in the product the desirability decreased as evident in Table 5.41.

Multiple regression equation generated to predict the overall acceptability as affected by different factors in terms of actual factors is as follows:

$$\textbf{Overall acceptability} = 9.831 - 1.061{*}\text{Oat fibre} - 0.040{*}\text{Calcium} + 0.003{*}\text{Oat fibre}{*}\text{Calcium} + 0.002{*}\text{Oat fibre}^2 + 0.0002{*}\text{Calcium}^2$$

5.4.1.2 Effect of oat fibre and calcium on instrumental textural attributes

(a) Effect on hardness: The average hardness scores of functional *doda burfi* varied from 24.19 to 64.74 N (Table 5.42). The maximum hardness value was observed for the product prepared from 1.0% oat fibre and 100 mg/100ml of calcium in the product whereas the minimum value was obtained in the product with 3.0% oat fibre and 50mg/100ml of calcium.

As indicated by model statistics, the R-square for hardness was 0.42. Value lesser than 0.80 represents values less than desirable, indicating that the model developed was unsatisfactory. Value of adequate precision (APV) w as 3.39, indicates model could not be used to navigate the design. The model F-value of 1.02 implies the model is non-significant.

Table 5.40: Sensory attributes of functional *doda burfi* manufactured using functional ingredients

Standard order	Oat fibre (%)	Calcium (mg/100ml)	Colour	Texture	Sweetness	Flavour	Overall acceptability
1	1.0	50	7.3	8.21	7.7	7.5	7.54
2	3.0	50	7.0	6.66	6.83	6.16	6.56
3	1.0	100	7.0	7.96	7.6	7.42	7.46
4	3.0	100	7.5	6.81	7.0	6.34	6.86
5	0.59	75	7.3	8.26	7.5	7.7	7.4
6	3.41	75	7.25	6.75	6.87	6.62	6.62
7	2.0	39.64	7.3	7.3	7.5	7.2	7.3
8	2.0	110.36	7.6	7.1	7.1	7.0	6.78
9	2.0	75	7.16	6.83	7.16	7.18	6.83
10	2.0	75	7.5	6.9	7.2	6.85	6.9
11	2.0	75	7.5	7.0	7.0	7.0	7.0
12	2.0	75	7.3	7.3	7.1	6.8	6.74
13	2.0	75	7.0	7.16	7.0	6.81	6.67

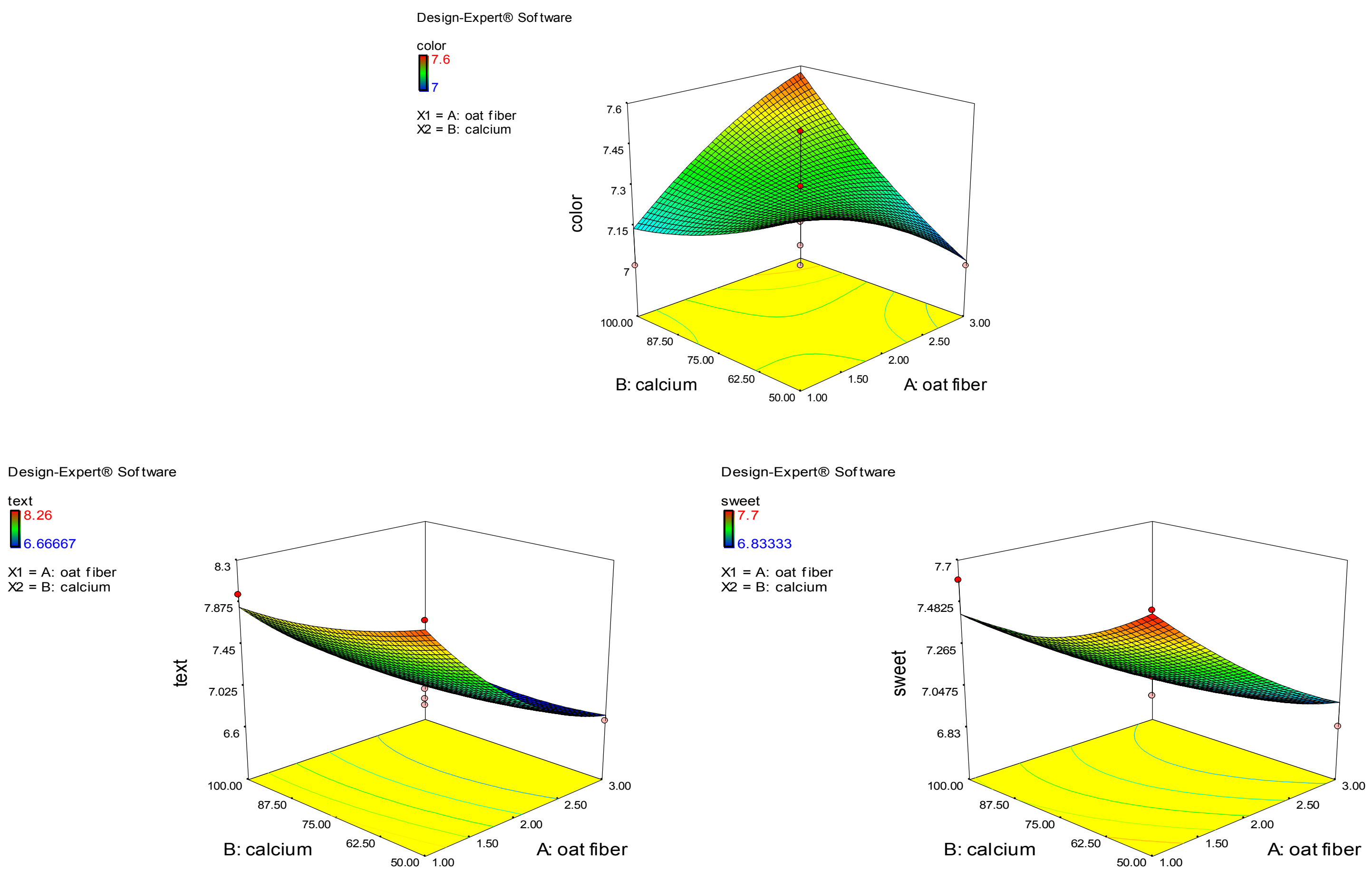
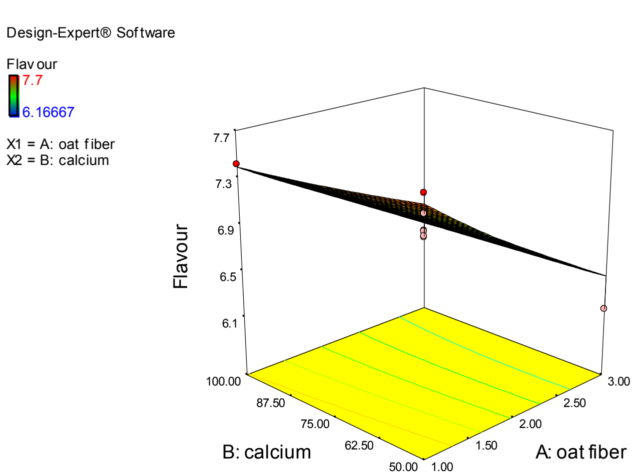


Fig. 5.25 Effect of oat fiber and calcium on sensory attributes of functional *doda burfi*



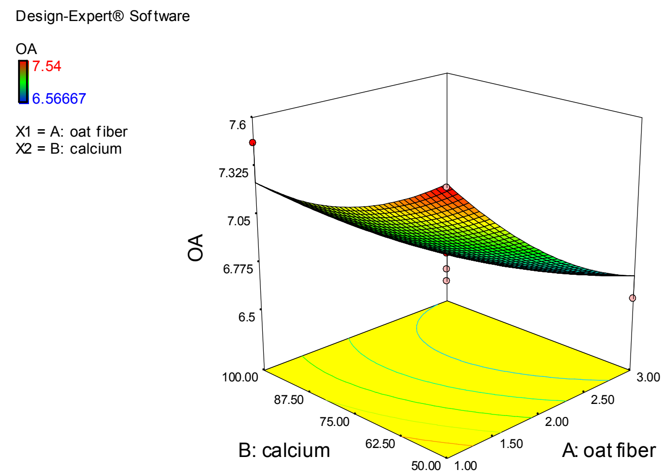


Fig. 5.26 Effect of oat fiber and calcium on sensory attributes of functional *doda burfi*

Table 5.41: Coefficient of full second order polynomial model for sensory responses of functional *doda burfi* using NCDC -167

Factor	Colour	Texture	Flavour	Sweetness	Overall acceptability
Intercept	8.36	10.83	8.71	9.51	9.83
A- oat fibre	-0.38	-1.89	-0.87**	-0.72	-1.06**
B-calcium (mg/100ml)	-0.02	-0.03	-0.01	-0.03	-0.04
A*B	0.008	0.003	0.002	0.002	0.003
A^2	-0.04	0.247**	0.048	0.056	0.11
B^2	0.00006	0.0001	0.00002	0.0001	0.0002
R^2	0.48	0.94	0.84	0.86	0.83
Lack of fit	0.49^{ns}	0.45^{ns}	3.40^{ns}	3.72^{ns}	3.04^{ns}
APV	4.28	15.03	8.86	9.67	8.09
Model 'F' value	1.32	24.53**	7.40*	9.07**	7.17*

ns non significant, * significant at p< 0.05, **significant at p<0.01

Fig 5.30 (a) is a response surface graph depicting interactive effect among variables on hardness and has been presented in the form of three dimensional graphs. The coefficients estimates of the hardness model (Table 5.44) reveal both the variables (oat fibre and calcium) at lower levels of their addition obtained highest scores and were comparable to conventional product. The positive sign indicates that there is an increase in score for hardness with an increase in level of ingredients used, while negative sign indicates the reciprocal of the same.

(b) Effect on adhesiveness: The adhesiveness of *doda burfi* has been shown in Table 5.42, which varied from -1.10 to -3.79 N.s. As indicated by model statistics (Table 5.44), the R-square for adhesiveness was 0.59, indicating that the model developed was unsatisfactory. Value of adequate precision (APV) as

4.42, indicates model could be used to navigate the design. The model F-value of 2.04 implies the model is statistically non-significant.

Fig. 5.30 (b) is a response surface graph depicting interactive effect among variables on adhesiveness and has been presented in the form of three dimensional graphs. The graph reveals that both the variables (oat fibre and calcium) at lower levels of their addition obtained highest adhesiveness and comparable value to the conventional product.

(c) Effect on springiness: The springiness values of *doda burfi* have been shown in Table 5.42, which varied from 0.14 to 0.42. As indicated by model statistics (Table 5.44), the R-square for springiness was 0.65, indicating that the model developed was unsatisfactory. However, value of adequate precision (APV) as 5.71, indicates model could be used to navigate the design (Table 5.44). The model F-value of 2.59 implies the model is statistically non-significant.

Fig 5.30 (c) is a response surface graph depicting interactive effect among variables on springiness and has been presented in the form of three dimensional graphs. The graph shows with the increase in oat fibre the score for springiness decreased whereas increasing the calcium content upto 62.5g/100 ml increased the springiness. Further increase in calcium concentration decreased the springiness.

(d) Effect on cohesiveness: The cohesiveness value for functional doda burfi has been shown in Table 5.42. The cohesiveness varied from 0.14 to 0.24. As indicated by model statistics (Table 5.44), the R-square for cohesiveness was 0.56, indicating that the model developed was unsatisfactory. Value of adequate precision (APV) as 4.09 indicates that the model could be used to navigate the design whereas the model F-value of 1.76 implies the model is statistically non-significant.

Fig 5.30 (d) is a response surface graph depicting interactive effect among variables on cohesiveness and has been presented in the form of three dimensional graphs. The graph shows with the increase in oat fibre the score for cohesiveness decreases whereas with calcium the score decreases with increased addition but then again achieves the same score with further addition of the same.

(e) Effect on gumminess: The gumminess values for *doda burfi* have been shown in Table 5.42 and the minimum value of as low as 3.66 N was obtained whereas the maximum attained value was 15.41 N. As indicated by model statistics (Table 5.44), the R-square for gumminess was 0.57. Value lesser than 0.80 represents values less than desirable, indicating that the model developed was unsatisfactory. Value of adequate precision (APV) as 4.18 (Table 5.44), indicates model could be used to navigate the design but the model F-value of 1.83 implies the model is statistically non-significant.

Fig 5.30 (e) is a response surface graph depicting interactive effect among variables on gumminess and has been presented in the form of three dimensional graphs. The graph shows with the increase in oat fibre the score for gumminess decreased and the similar trend was observed for the calcium addition.

(f) Effect on chewiness: The chewiness values of *doda burfi* have been shown in Table 5.42 and the minimum value of 0.53 N was obtained whereas the maximum attained value was 4.73 N. As indicated by model statistics (Table 5.44), the R-square for chewiness was 0.72. Value lesser than 0.80 represents values less than desirable, indicating that the model developed was unsatisfactory. Value of adequate precision (APV) as 6.79, indicates that the model could be used to navigate the design. The model F-value of 3.56 implies the model is statistically non-significant (Table 5.44).

Fig 5.30 (f) is a response surface three dimensional graph depicting interactive effect among variables on chewiness. The graph shows with the increase in oat fibre and calcium the score for chewiness decreased.

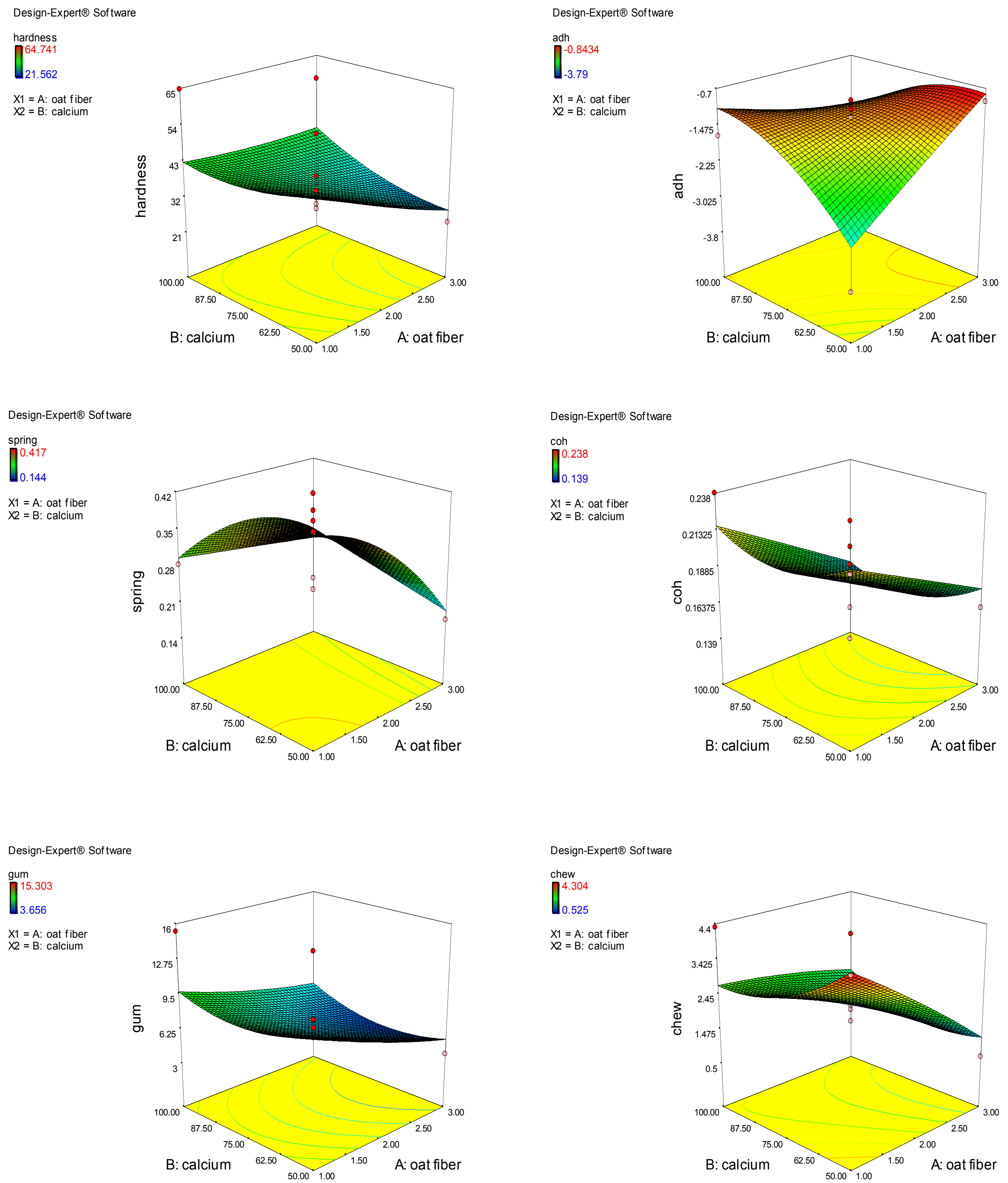


Fig. 5.27 Effect of oat fiber and calcium on instrumental textutral attributes of functional *doda burfi*

Table 5.42: Instrumental textural attributes of functional *doda burfi* manufactured using functional ingredients

Standard order	Oat fibre (%)	Calcium (mg/100 ml)	Hardness (N)	Adhesiveness (N.s)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)
1	1.0	50	62.35	-3.79	0.35	0.22	13.47	4.73
2	3.0	50	24.19	-1.97	0.18	0.16	3.89	0.69
3	1.0	100	64.74	-1.69	0.28	0.24	15.41	4.38
4	3.0	100	37.38	-1.96	0.31	0.17	6.47	2.01
5	0.59	75	32.93	-1.84	0.39	0.20	6.66	2.58
6	3.41	75	25.07	-1.10	0.14	0.15	3.66	0.53
7	2.0	39.64	44.60	-1.69	0.41	0.23	10.09	4.13
8	2.0	110.36	31.56	-1.80	0.30	0.18	5.71	1.74
9	2.0	75	38.56	-1.31	0.34	0.16	6.21	2.12
10	2.0	75	31.94	-1.31	0.42	0.22	7.02	2.93
11	2.0	75	29.96	-1.14	0.37	0.19	5.71	2.09
12	2.0	75	51.52	-1.72	0.24	0.14	7.16	1.69
13	2.0	75	28.46	-1.95	0.35	0.18	5.18	1.79

5.4.1.3 Effect of oat fibre and calcium on instrumental colour values and water activity of *doda burfi*

(a) Effect on lightness (L*): The instrumental lightness values of *doda burfi* have been shown in Table 5.43 and the minimum value obtained was as low as 23.10 whereas the maximum attained was 30.85. As indicated by model statistics (Table 5.44), the R-square for lightness was 0.66. Value lesser than 0.80 represents values less than desirable, indicating that the model developed was unsatisfactory. Value of adequate precision (APV) as 6.06, indicates that the model could be used to navigate the design. The model F-value of 2.73 implies the model is statistically non-significant (Table 5.44).

Fig 5.31 (a) is a response surface graph depicting interactive effect among variables on lightness and has been presented in the form of three dimensional graph. The graph shows that the lightness of the product increased as the levels of both oat fibre and calcium is increased simultaneously.

(b) Effect on redness (a*): The instrumental colour values for redness of *doda burfi* have been shown in Table 5.43 and the minimum value obtained was as low as 8.93 whereas the maximum attained was 13.46.

As indicated by model statistics, the R-square for redness was 0.64. Value lesser than 0.80 represents values less than desirable, indicating that the model developed was unsatisfactory (Table 5.44). Value of adequate precision (APV) as 5.13, indicates that the model could be used to navigate the design. The model F-value of 2.51 implies the model is statistically non-significant.

Fig 5.31 (b) is a response surface three dimensional graph depicting interactive effect among variables on redness. The graph shows with the increase in oat fibre and calcium, the values for redness increased. Though the exact reason behind this could not be ascertained but the similar trend in increase in colour values with the addition of fibre has been observed in *misti dahi* by Raju (2009).

(c) Effect on yellowness (b*): The instrumental colour values for yellowness of *doda burfi* have been shown in Table 5.43. As indicated by model statistics, the R-square for yellowness was 0.53. Value lesser than 0.80 represents values less than desirable, indicating that the model developed was unsatisfactory. Value of adequate precision (APV) as 4.53, indicates that the model could be used to navigate the design. The model F-value of 1.59 implies the model is statistically non-significant (Table 5.44).

Fig 5.31 (c) is a response surface graph depicting interactive effect among variables on yellowness and has been presented in the form of three dimensional graphs. The graph shows with the increase in oat fibre and calcium concentration the instrumental yellowness value decreased.

(d) Effect on water activity: The instrumental water activity values for functional *doda burfi* have been shown in Table 5.43. The water activity of functional *doda burfi* ranged from 0.73 to 0.88. The variation could be attributed to difference in the percentage of added fibre, which being soluble in nature has tendency to retain water thereby influencing the water activity.

As indicated by model statistics, the R-square for gumminess was 0.46. Value lesser than 0.80 represents values less than desirable, indicating that the model developed was unsatisfactory (Table 5.44). Value of adequate precision (APV) as 3.88, indicates model could not be used to navigate the design. The model F-value of 1.20 implies the model is non-statistically significant.

Fig 5.32 is a response surface graph depicting interactive effect among variables on water activity and has been presented in the form of three dimensional graphs. The graph shows with the increase in oat fibre and calcium, the water activity value increased.

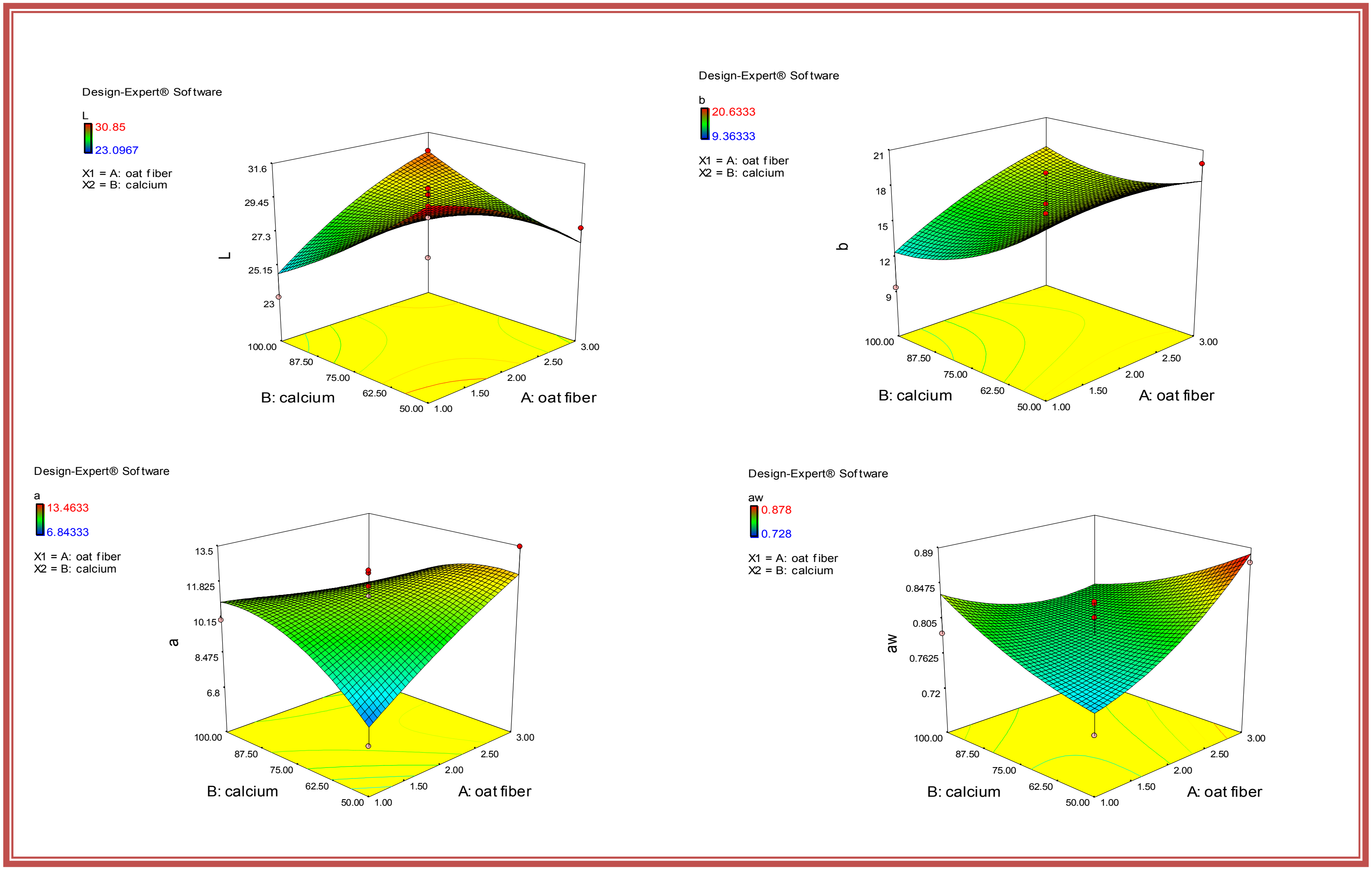


Fig. 5.28 Effect of oat fiber and calcium on instumental colour and water activity values of functional *doda burfi*

Table 5.43: Instrumental colour and water activity values of functional *doda burfi* manufactured using functional ingredients

Standard order	Oat fibre (%)	Calcium (mg/100 ml)	Water activity	L*	a*	b*
1	1.0	50	0.73	30.85	6.84	19.48
2	3.0	50	0.87	27.58	13.46	19.88
3	1.0	100	0.79	23.10	10.06	9.36
4	3.0	100	0.77	30.32	10.41	15.52
5	0.59	75	0.84	28.38	11.12	13.89
6	3.41	75	0.88	26.73	10.08	17.72
7	2.0	39.64	0.83	30.16	8.93	18.87
8	2.0	110.36	0.85	29.17	9.08	20.63
9	2.0	75	0.82	29.66	11.64	16.56
10	2.0	75	0.77	25.67	11.18	15.58
11	2.0	75	0.73	28.67	12.38	15.19
12	2.0	75	0.81	30.05	10.33	19.12
13	2.0	75	0.83	28.96	10.75	14.89

Table 5.44: Coefficient of quadratic model for instrumental texture of functional *doda burfi*

Factor	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	chewiness	L*	a*	b*	a_w
Intercept	111.58	-9.55	0.62	0.355	29.39	12.68	46.91	-9.84	35.58	0.68
A- oat fibre	-17.12	2.61	-0.03	-0.016	-4.40	-1.99	-4.92	6.310	-0.18	0.07
B-calcium (mg/100ml)	-1.29	0.13	-0.004	-0.003	-0.41	-0.18	-0.33	0.374	-0.49	0.0006
A*B	0.107	-0.02	0.002	-0.0001	0.006	0.016	0.10	-0.06	0.06	-0.001
A^2	-0.14	-0.18	-0.04	-0.0001	0.27	-0.11	-0.69	-0.23	-0.660	0.018
B^2	0.01	-0.0005	$2.65*10^{-7}$	0.00002	0.002	0.0009	0.0005	-0.001	0.002	0.00001
R^2	0.42	0.59	0.65	0.56	0.57	0.72	0.66	0.64	0.53	0.46
APV	3.39	4.42	5.71	4.09	4.18	6.79	6.06	5.13	4.53	3.88
PRESS	6857.87	13.32	0.12	0.02	440.33	36.32	71.48	69.39	317.12	0.070
Model ‘F’ value	1.02^{ns}	2.04^{ns}	2.59^{ns}	1.76^{ns}	1.83^{ns}	3.56^{ns}	2.73^{ns}	2.51^{ns}	1.59^{ns}	1.20^{ns}

5.4.2 Optimized solutions and their validation

The criteria for selecting these responses were based on literature survey as only sensory evaluation affects the acceptability of the finished product to a great extent. Sensory scores were kept in range, as idea of judging product varies from person to person. The goals set for the two factors and five responses are presented in Table 5.45. Thus using the sensory data generated through RSM experiments, eight best solutions were obtained as shown in Table 5.46. For optimization study only sensory responses were taken under consideration. It was noticed that all the first seven solutions had similar desirability value (1.00), whereas only last solution carries a desirability of 0.947. The differences between different solutions were so small that it could be neglected and also on practical basis could not be maintained. So, keeping these all in mind, solution number 1 was tried for the verification purposes.

With sensory attributes and other variables aimed to be ranged, the solution no. 1 (oat fibre level 1.00 %, calcium 50.40 had a fairly high desirability pointing to ideal case (1.00) indicating reliability of predicted values. The predicted yield of eight solutions ranged from 7.37 to 7.38 for colour, 8.10 to 8.12 for texture, 7.65 to 7.68 for sweetness, 7.56 to 7.57 for flavour and 7.52 to 7.55 for overall acceptability.

5.4.2.1 Verification of the optimum formulations

Functional *Doda burfi* was prepared using optimized combinations and was subjected to sensory, textural, colour along with mineral and fibre estimation. The data obtained is depicted in Table 5.46 and 5.47. It was observed that the values were not significantly different from the predicted values with respect to all attributes.

The models that were generated from the response surface analysis of the data were evaluated for testing their fitness. As can be seen from Table 5.41 that the regression models fitted to sensory characteristics of functional *doda burfi* viz. colour & appearance, flavour, texture, sweetness and overall acceptability were significant. It thus appeared that solution produced a formulation which resulted in a product that had comparable sensory attributes, textural attributes and colour values in terms of L*, a* and b*. This combination thus can be considered optimum for the manufacture of functional *doda burfi.*

The optimized product obtained by solution number 1(Photo plate III) was studied for all the response of sensory, physico-chemical, microbiological, texture profile analysis and instrumental color analysis of the optimized functional product using culture NCDC 167 taking lab trials. The average of triplicate values of the same for the optimized functional product using culture NCDC 167 is given in Table 5.48.

5.4.2.2 Selection of level of phytostanol

Based on the preliminary studies, two different levels of phytostanol were tried with the final recipe of functional *doda burfi*. The levels worked upon were 3.0% and 4.5% and the results were compared with control sample with zero or no added phytostanol in it. To select the best optimal level of phytostanol, product was evaluated by a panel of trained judges using a 9-point hedonic scale for colour and appearance, flavor, texture and overall acceptability. The results obtained have been presented in Fig. 5.29. On the basis of results obtained, 3.0% phytostanol was adjudged the best level to be used further in the studies for the development of functional *doda burfi.*

5.4.2.3 Physico-chemical and sensory examination of functional *doda burfi*

The finally optimized functional formulation in respect of sensory, physico-chemical, microbiological, instrumental colour, textural and water activity values were assessed. Sensory scores were fairly acceptable as per the predicted scores received from RSM. Even some of the values surpassed the values predicted and hence indicate a good degree of acceptability of the final product thus made (Table 5.47). The hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness ranged from 42.79 to 51.51, -3.06 to -2.18, 0.27 to 0.48, 0.17 to 0.19, 7.55 to 10.27 and 2.38 to 3.63, respectively. The instrumental color parameters ranged from 33.31 to 33.50, 14.83 to 14.97 and 22.98 to 23.29 for L*, a* and b* respectively. The t-test (Table 5.47) was applied upon the predicted and observed values of solution number 1 for all the responses obtained against colour and appearance of surface, texture, sweetness, flavour and overall acceptability, instrumental colour values in terms of lightness L*, redness a* and yellowness b*. The t-test showed that there were no significant differences for predicted score and observed score for the attributes studied except texture where the scores were statistically significant at $p<0.05$.

Mineral estimation revealed that calcium after fortification @ 50 mg/ 100 ml (of milk) in *doda burfi* came out as 62.32 mg/ 100g of the product obtained. The standard curve of the same has been presented in Fig. 5.30. Fibre after fortification though not changed much and only SDF varied, owing to addition of soluble dietary fibre in the product and results revealed that the final product had IDF and SDF content of 11.47 and 8.25%, respectively (Table 5.48).

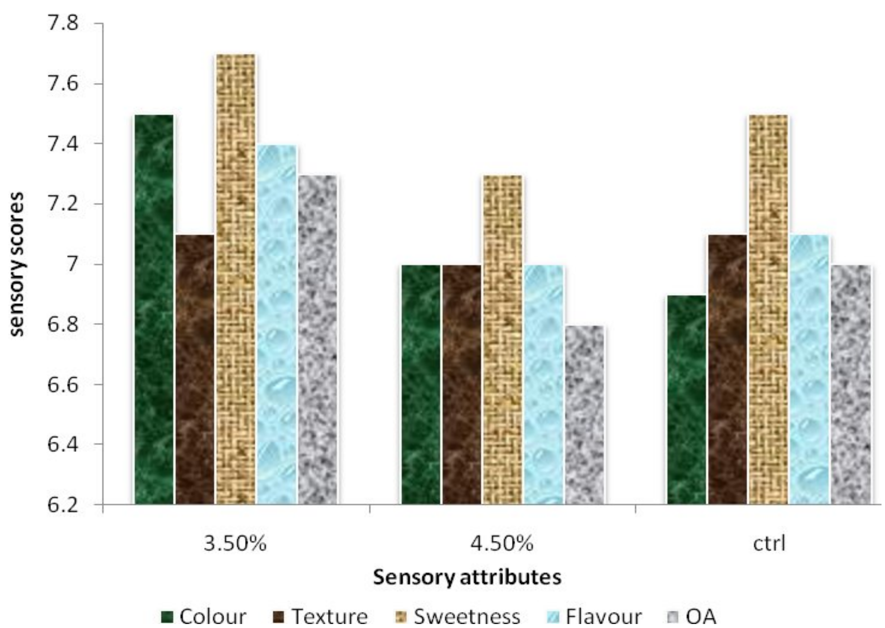


Fig. 5.29 Selection of level of phytostanol using sensory responses

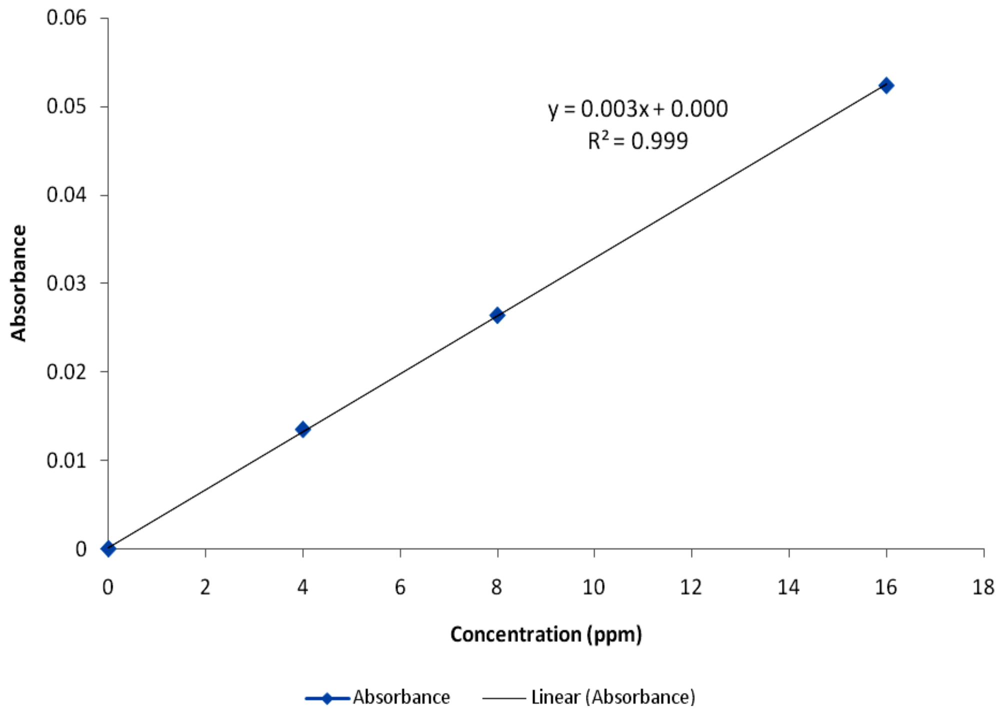


Fig. 5.30 Standard curve obatined for calcium estimation using atomic absoption spectrum

Table 5.45: Goals set for constraints to optimize the technology of functional *doda burfi*

Constraints name	Goal set for optimized product	Lower limit	Upper limit	Lower weight	Upper weight	Importance
Oat fibre	is in range	1	3.0	1	1	3
Calcium	is in range	50	100	1	1	3
Colour	is in range	7.3	7.6	1	1	3
Texture	is in range	8.0	8.26	1	1	3
Sweetness	is in range	7.3	7.70	1	1	3
Flavour	is in range	7.2	7.70	1	1	3
OAA	Maximize	7.2	7.54	1	1	3

Table 5.46: 8 suggested solutions vide RSM design for optimum combinations of oat fibre and calcium for manufacture of functional *doda burfi*

Solutions number	Oat Fibre	Calcium	Colour	Texture	Sweetness	Flavour	OAA	Desirability
1	1.01	50.40	7.38	8.11	7.68	7.56	7.55	1.00
2	1.00	50.67	7.38	8.12	7.67	7.57	7.55	1.00
3	1.01	50.02	7.39	8.13	7.68	7.57	7.55	1.00
4	1.02	50.36	7.38	8.10	7.67	7.56	7.54	1.00
5	1.00	50.24	7.39	8.13	7.68	7.57	7.55	1.00
6	1.02	50.16	7.38	8.11	7.68	7.56	7.54	1.00
7	1.02	50.02	7.38	8.10	7.67	7.56	7.54	1.00
8	1.00	52.31	7.37	8.10	7.65	7.56	7.52	0.947

Table 5.47: Comparison of predicted and observed average values of the optimized product using culture NCDC 167

Parameter	Sensory attributes				
	Colour & appearance of surface	**Texture**	**Flavour**	**Sweetness**	**0verall acceptability**
Predicted values	7.38	8.11	7.67	7.56	7.54
Observed values	7.66	7.58	7.75	7.0	7.25
t-value	-0.32^{ns}	-1.17*	-0.15^{ns}	0.43^{ns}	0.51^{ns}

Figures are the Mean ± SE of three replications

ns not significant (p<0.05), * significant at (p<0.05)

Table 5.48: Characteristics of the developed functional optimized product (n=3)

Property	Attribute	Value
Sensory	Colour and appearance	7.66 ± 0.04
	Texture	7.58 ± 0.24
	Sweetness	7.00 ± 0.91
	Flavour	7.75 ± 0.04
	Overall acceptability	7.25 ± 0.05
Physico-chemical	IDF (%)	11.47 ± 1.47
	SDF (%)	8.25 ± 1.25
	Calcium (mg/100g)	62.32 ± 0.11
	Iron (mg/100g)	3.40 ± 0.005
Textural attributes	Hardness (N)	46.03± 3.41
	Adhesiveness (N.s)	-2.69 ± 0.33
	Springiness	0.338 ± 0.08
	Cohesiveness	0.191± 0.009
	Gumminess (N)	8.81 ± 0.98
	Chewiness (N)	2.94 ± 0.53
Instrumental colour values	Lightness (L*)	33.40 ± 0.05
	Redness (a*)	14.90 ± 0.04
	Yellowness (b*)	23.17 ± 0.09
Microbiological count	Standard plate count (log cfu/g)	$2X10^2$
	Yeast and mould count (log cfu/g)	nil
	Coliform count (log cfu/g)	nil

5.5 SHELF-LIFE STUDIES

Any newly developed product must have a fairly extended shelf life, so that the product could withstand the long journey during marketing and stay for a long time in the seller's shelf or in consumers household. Foods in general are quite susceptible to quality loss due to chemical & microbiological instability depending upon the composition and water activity of the product, processing and packaging techniques used and the environmental conditions to which the product is subjected shelf-life of any food product is governed by the extent of progression of deteriorative changes that occur during storage. Several decomposition and formation processes of chemical and biochemical nature are initiated from the onset of the storage which ultimately renders the product less acceptable. In well packaged foods where protection is afforded against extraneous moisture and microflora, ambient temperature is known to be the principal environmental factor influencing the product shelf-life. The higher the temperature, faster the product loses quality and shorter the shelf-life. The high moisture foods when subjected to thermal processing ensure an extended shelf-life. However, certain physico-chemical and biochemical changes are bound to occur during storage. The extent of deterioration is further influenced by the temperature and period of storage. These changes are of much significance because they not only affect various milk constituents but also affect the overall acceptability. This section of the present investigation deals with the assessment of changes in microbial, physicochemical, and sensory quality of functional *doda burfi* during storage at 30°C. Functional *doda burfi* was manufactured using process standardized and described under section 5.4.

Functional *doda burfi* immediately after manufacture was transferred into shallow trays and allowed to cool at room temperature and then cut in to rectangular pieces of about 150-200g. Each piece was packed in five-ply polyethylene (washed with chlorinated water, 25 ppm) pouch, and sealed thereafter. Similarly prepared functional *doda burfi* was preserved with preservative {(pediocin 0.12%) and microgard 100 (0.5%), potassium sorbate (0.1%) and sodium EDTA (20mM)} and packed in same packing material and stored at 30°C. The samples were withdrawn at predetermined intervals and analyzed for different attributes. The analysis of stored samples was discontinued when product was declared as spoiled by the sensory panel or when got contaminated with visible microbial growth. The results of these studies are presented under different subheads given below. For statistical analysis purposes, the data up to 12 days of storage of samples without preservative (WP) and 27 days of storage with preservative (P) was considered.

5.5.1 Effect of preservative on moisture content during storage

The loss of moisture during storage at high temperature is a common observation for most of the products including sweetmeats. Level of moisture in the product plays a significant role on quality of the product during storage as far as bacterial activity, yeast and mould growth, browning reaction and the acceptability of *doda burfi* is concerned. Moisture content of *khoa* based is very critical factor for spoilage particularly at ambient temperatures as it is directly related to enzymatic and biochemical reactions. Changes in moisture contents of functional *doda burfi* during storages at 30°C are shown in figure 5.31 and Table 5.49. Average moisture content of fresh *burfi* samples was 18.05%. Though not rapid but decrease in the moisture content was observed during storage probably due to high barrier properties of the packaging material. The effect of preservative on moisture content of *doda burfi* was non-significant, however, the moisture content decreased significantly during different periods of storage ($p<0.01$) (Table 5.53). The initial moisture content dropped from 18.05% to 13.95% and 15.1% after 27 and 12 days of storage at 30°C, respectively for product with and without added preservative.

5.5.2 Effect of preservative on pH value during storage

The pH of the fresh functional *doda burfi* samples was 5.77 (Table 5.49). A gradual but statistically significant decrease ($p<0.01$) (Table 5.53) was observed in the pH of *doda burfi* samples in both products with and without added preservatives during storage at 30°C (Fig. 5.32). The decline found was more prominent in case of product prepared without added preservative and may be attributed to the faster microbial growth and production of more amount of metabolites thereof. The final pH recorded was 5.00 in case of *doda burfi* prepared with added preservative whereas the similar *burfi* without added preservative got spoiled after 12 days and the pH recorded was 5.37 in the same. Similar observations had been recorded by Suresh and Jha (1994)[b] in case of *Kalakand*.

5.5.3 Effect of preservative on acidity value during storage

During storage at 30°C a significant ($p<0.01$) increase was noticed in acidity of both the samples (Table 5.53) but none of the samples turned sour upto 12 days and 27 days of storage, respectively prepared using without and with added preservative (Table 5.49 and Fig. 5.33). However, the effect of preservative during storage was not significant statistically. *Doda burfi* prepared using preservative, had a shelf-life of 27 days with good signs of acceptability wherein the acidity increased from the initial value of 1.18% to 1.89% whereas the counter product prepared without added preservative had a shelf life of 12 days at 30°C and the acidity increased up to 1.46% in the course of 12 days. Similar observations were recorded by Londhe (2006), Suresh and Jha (1994)[b] and Prabha (2006), in *Peda, Kalakand* and dietetic *burfi*, respectively.

5.5.4 Effect of preservative on tyrosine value during storage

Proteases indigenous to milk as well as microbial proteases degrade proteins which lead to release of free amino acids like tyrosine, producing bitter taste in dairy products. In addition to milk, added cereals especially GWF, was the prominent source of proteases. The degree of proteolysis in functional *doda burfi* was assessed during storage at 30°C (Table 5.49 and Fig. 5.34) in the terms of tyrosine value. When stored at 30°C, tyrosine value of *burfi* samples prepared without preservative and with preservative increased significantly ($p<0.01$) to 102.12 mg/100g after 12 days of storage and 173.19 mg/100g after 27 days of storage from initial content of 10.35 mg/100g. However, the effect of preservative on tyrosine value was not significant (Table 5.53). The results are in agreement with earlier work on *Khoa* by Patel (1985), Suresh and Jha (1994) and Prabha (2006).

5.5.5 Effect of preservative on HMF value during storage

Millard type browning reaction is accelerated in dairy products during storage of heated milk products especially at elevated temperatures which results in poor palatability diminished physical appeal and losses of nutritive value of the product. Total HMF value was measured in functional *doda burfi* samples to study the extent of browning during storage and the results are shown in Fig. 5.35 and Table 5.49. A highly significant (P<0.01) increase in HMF content of all *burfi* samples was recorded during storage (Table 5.53). The HMF increased from an initial content of 9.34 µmoles/ 100 gm of TS to 23.96 micro moles/100 gm after a storage period of 12 days in samples containing no preservatives whereas the product with added preservative had value of 33.30 µmoles/ 100 gm on 27th day. Increase in HMF values of *burfi* and *peda* during storage has also been reported by earlier workers (Reddy, 1985; Bhatele, 1983; Kumar *et al.* 1997).

5.5.6 Effect of preservative on free fatty acids during storage

Lipid hydrolysis, which causes cleavage of triglycerides thereby releasing short and medium chain fatty acids, is one of the most serious defects which leads to undesirable rancid flavor in the product (Shahin, 1998; Shahin and Fahmy, 1998). Indigenous milk lipase is inactivated by heat treatment during manufacture of functional *doda burfi.* It is mainly microbial and fungal lipase which is responsible for release of FFA in dairy products during storage (Bills *et al*., 1969). The FFA value of freshly prepared *doda burfi* samples was observed to be 4.29 µeq/g at '0' day which gradually shoot to 9.18 µeq/g after a period of 12 days of storage at 30°C whereas the same value increased to 12.83 µeq/g after a period of 27 days for the product prepared with added preservative (Table 5.49 and Fig. 5.36). The values observed were quite comparable with the ones recorded by Prabha (2006) and was also in acceptable limits but at last the product got spoiled before it could be rejected by sensory panelists due to fungal growth.

A gradual but statistically significant (P<0.05) (Table 5.53) increase was observed in FFA content of all functional *doda burfi* prepared using functional ingredients, with and without added preservative, the rise being faster in the later (Table 5.49)

Table 5.49: Changes in physico-chemical attributes of functional *doda burfi* during storage at 30ºC

Storage (Days)	Moisture content (%)		pH		Acidity (% l.a)		Tyrosine (mg/100g)		HMF (µmoles/100g)		FFA (µeq/g)	
	(P)	(WP)	(P)	(WP)	(P)	(WP)	(P)	(WP)	(P)	(WP)	(P)	(WP)
0	18.05	18.05	5.77	5.77	1.18	1.18	10.35	10.35	9.34	9.34	4.29	4.29
3	17.24	17.15	5.675	5.62	1.285	1.3	17.94	29.325	11.89	18.11	4.84	5.27
6	16.35	16.59	5.66	5.59	1.32	1.33	33.12	48.3	14.91	19.67	5.18	6.15
9	16.21	15.28	5.58	5.42	1.37	1.45	45.54	87.63	19.06	22.98	7.24	8.28
12	15.27	15.1	5.47	5.37	1.42	1.46	57.96	102.12	23.49	23.96	8.52	9.18
15	15.21		5.31		1.53		83.49		27.09		8.86	
18	14.85		5.2		1.62		106.26		28.52		10.24	
21	14.73		5.18		1.68		120.06		28.48		10.18	
24	14		5.1		1.71		133.17		31.80		11.29	
27	13.95		5		1.89		173.19		33.30		12.83	

P= With preservative, WP= Without preservative

Nevertheless, none of the stored *doda burfi* samples was criticized for rancid flavour defect by sensory panel until the end of the storage period. Similar trend were evidenced by Palit and Pal, (2005) in FFA of *burfi* stored at 30°C.

5.5.7 Effect of preservative on microbiological count during storage

Milk products serve as a good substrate for the microbial proliferation. Lower moisture content and high sugar content in the sweets pose a hurdle to microbial growth. The product under investigation contained sugar but had high moisture content, so comparatively higher microbial growth was expected. The changes in SPC, yeast and mould and coliform count of stored samples of functional *doda burfi* are illustrated in Table 5.50 and its statistical analysis in Table 5.53, respectively. The SPC was not detected on 0 day in *doda burfi* prepared without and with added preservative. However, the count increased throughout the storage regardless of the preservative used or not, the rate of growth being slower in product with preservative. During storage of packaged sample at 30°C, the SPC increased from initial nil count up to 3.36 log cfu/g after 12 days of storage for the product without preservative and up to 3.63 log cfu/g after 27 days of storage for a product containing preservative. The data revealed that preparation of *doda burfi* prepared with preservative could remarkably retard the rate of microbial proliferation. However, the effect was not statistically significant during storage (Table 5.53). These results are in agreement with the finding of Palit and Pal (2005) and Prabha (2006) and Londhe (2006). Increase in SPC of *burfi* samples during storage had been reported by several workers (Sachdeva, 1980; Bhatele, 1983; Reddy, 1985; Mandokhot and Garg, 1985; Mishra and Kuila, 1988). However, Kumar *et al.* (1997) did not observe any microbial growth in *peda* packaged under MAP and oxygen scavengers.

Yeast and mould growth is one of the main factors of spoilage of the milk sweets thus limiting the shelf-life. In present investigation no mould or yeast counts could be detected in the sample of functional *burfi* during earlier days of storage at 30°C. But during subsequent storage the presence of yeast and moulds were encountered. The spoilage of product can be entirely considered due to growth of yeast and mould count. After 12 and 27 days of storage, in product prepared without and with added preservative the average count was increased from an initial nil count to 2.39 and 2.50 log cfu/g, respectively (Table 5.50). Results are in agreement with the earlier finding (Palit and Pal, 2005). Statistically count had a statistically non-significant effect w.r.t storage and treatments applied.

Coliform that indicate the hygienic conditions maintained during processing and packaging of a product were absent in fresh as well as stored samples irrespective of preservative used.

Table 5.50: Microbiological changes (cfu/g) in without and with added preservative developed functional *doda burfi* during storage at 30°C

Storage (Days)	SPC (P)	SPC (WP)	Yeast and mould (P)	Yeast and mould (WP)
0	Nd	Nd	Nd	Nd
3	Nd	2.72	Nd	Nd
6	2.59	2.96	Nd	1.72
9	2.61	3.08	Nd	2.19
12	2.72	3.36	1.79	2.39
15	2.96		2.04	
18	3.11		2.18	
21	3.40		2.27	
24	3.52		2.32	
27	3.63		2.50	

5.5.8 Effect of preservative on sensory attributes during storage

All deteriorative changes i.e. oxidative, proteolytic, lipolytic, browning, acidity development, microbial and textural changes are collectively reflected in sensory quality and thus lead to unacceptability of the stored product. Changes in the colour score of functional *doda burfi* during storage at 30°C are shown in Figure 5.37 and Table 5.51. The average colour score of freshly prepared *burfi* was 7.67. During storage the sample became dark with advancement of storage period and it resulted in a steady but a very little decrease in their scores. Finally the score reached to 7.25 and 6.60 after 12 days and 27 days of storage of *doda* *burfi* prepared without and with added preservative respectively. The sensory analysis was terminated once the surface growth appeared over the product. The decrease in colour score with the progression of storage period was statistically significant ($p<0.01$). However, the preservative treatment had no significant effect on the colour score of *doda burfi* during storage (Table 5.53).

Changes in body and texture scores in *doda burfi* during storage at 30°C depicted in Figure 5.38 and Table 5.51. With advancing storage, period all samples exhibited a steady decrease but statistically significant decrease ($P<0.01$) in body and texture scores during storage. The stored samples progressively became brittle which lacked cohesiveness. The decrease in body and texture score of samples was observed in both the samples with and without preservative but the decrease was observed to be faster in the product prepared without preservative compared to the samples prepared with added preservative. The difference between scores of both the samples was statistically significant ($p<0.05$) (Table 5.53).

Similarly decrease in flavour score was observed with due course of time and the decline was faster in the product prepared without added preservative compared to product made with added preservative (Table 5.51 and Fig. 5.39). Statistically, the effect of preservative on flavour score during storage was not significant, however the effect of storage period was highly significant (Table 5.53). The decrease in flavour may be attributed to slight loss in freshness, which is inherent with any food product. The changes during storage for sweetness have been depicted in figure 5.40 and Table 5.51. The storage period as well as preservative showed a significant effect on the sweetness attribute ($p<0.05$) during storage of *doda burfi* (Table 5.53).

Table 5.51: Changes in sensory attributes of functional *doda burfi* during storage at 30ºC

Storage	Colour		Texture		Flavour		Sweetness		OA	
(Days)	(P)	(WP)	(P)	(WP)	(P)	(WP)	(P)	(WP)	(P)	(WP)
0	7.67	7.67	7.58	7.58	7.75	7.75	7.80	7.80	7.25	7.25
3	7.60	7.50	7.50	7.40	7.80	7.70	7.60	7.40	7.40	7.40
6	7.50	7.30	7.48	7.40	7.60	7.50	7.50	7.30	7.30	7.40
9	7.30	7.25	7.42	7.30	7.50	7.17	7.48	7.13	7.13	7.25
12	7.25	7.25	7.38	7.15	7.38	7.00	7.38	6.88	6.88	6.59
15	7.15		7.29		7.28		7.21		6.58	
18	7.00		7.20		7.24		7.12		6.57	
21	6.90		7.20		7.00		7.15		6.52	
24	6.78		7.00		6.90		7.00		6.51	
27	6.60		6.90		6.50		7.00		6.40	

The effect of preservative and storage conditions on the overall sensory quality (total rating) of functional *doda burfi* samples have been shown in Figure 5.41. The overall acceptability of stored samples depends upon several factors like degree of proteolysis, lipolysis, flavour changes and microbial activity. The overall acceptability score declined from 7.25 to 6.59 in functional *doda burfi* prepared without added preservative after 12 days of storage whereas the score decreased to 6.40 after 27 days in the product prepared with added preservative (Table 5.51).While the preservative did not have any significant effect on overall acceptability score of *doda burfi*, the storage period significantly affected the overall acceptability ($p<0.01$) (Table 5.53). These results are in agreement with the finding of Prabha (2006) and Londhe (2006).

On the basis of above discussion it is concluded that functional *doda burfi* prepared with added preservative is better approach compared to the product prepared without preservative for maintaining the acceptable sensory quality for longer period and also for restricting the growth of microorganisms, particularly at room temperature storage.

5.5.9 Effect of preservative on changes in instrumental textural attributes during storage

Textural attributes like hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness were determined during the storage period and the same has been shown in Table 5.52 and Fig. 5.42. The changes in hardness of functional *doda burfi* samples prepared without and with preservative and stored at 30°C showed increase in hardness throughout the storage period, owing to evaporation of moisture from the product. It can be observed that initial value of hardness of 47.45 N increased to 57.98 and 62.38N after 12 and 27 days, respectively. Analysis of variance (Table 5.53) revealed that storage period had significant effect on the almost all the textural attributes like hardness, adhesiveness, cohesiveness and gumminess of samples except springiness and chewiness where the effect was found to be non-significant for the samples stored at 30^{o}C. A decreasing trend has been observed in other attributes like adhesiveness, springiness, cohesiveness, gumminess and chewiness (Table 5.50). Adhesiveness showed a decreasing trend for the samples stored, irrespective of the preservative added or not. The initial value of adhesiveness decreased from -2.78 to -1.67 and -2.01 after 27 and 12 days of storage. Similarly, springiness showed the decreasing trend from an initial value of 0.34 to 0.21 and 0.22 after 27 and 12 days of storage.

Cohesiveness like other textural attributes showed a decreasing trend and values decreased from 0.281 to 0.193 and 0.22 after 27 and 12 days respectively. Gumminess, a secondary attribute can be obtained by multiplying hardness with cohesiveness showed the significantly decreasing trend and can be attributed to increase in total solids resulting in increase sugar concentration and gumminess. The value obtained after 12 and 27 days of storage were 12.76N and 12.04N respectively from an initial value of 13.33N. The chewiness of *doda burfi* samples exhibited an decreasing trend throughout the entire storage period in all the samples. The initial value of chewiness 4.56N.s in samples increased to 2.52N.s on 27th day of storage while in product prepared without preservative it increased to 2.81N.s on 12th day at 30^{o}C. These results are in agreement with the finding of Londhe (2006).

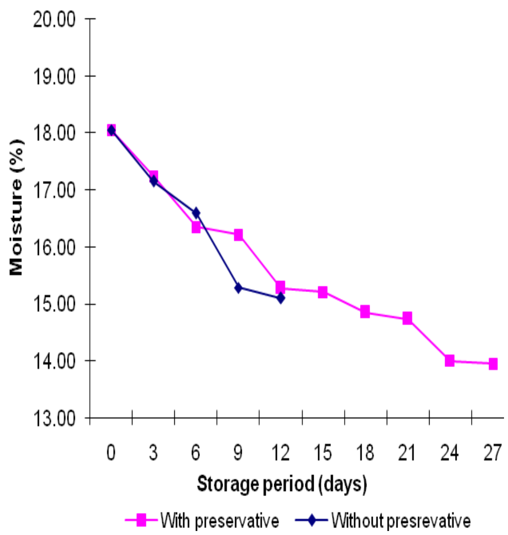


Fig. 5.31 Change in moisture content of packaged *Doda burfi during* storage at 30°C

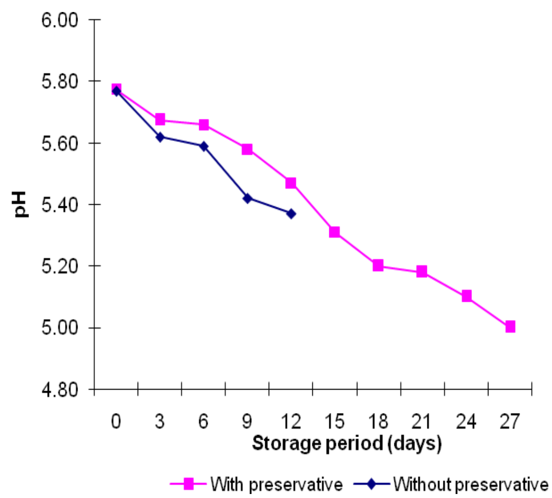


Fig. 5.32 Change in pH of packaged *Doda burfi* during storage at 30°C

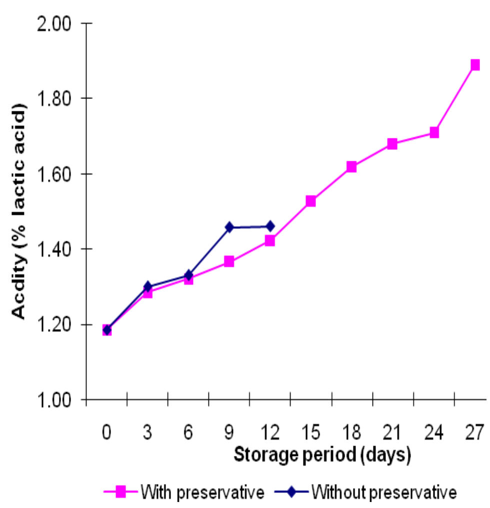


Fig. 5.33 Change in per cent acidity of packaged *Doda burfi during* storage at 30°C

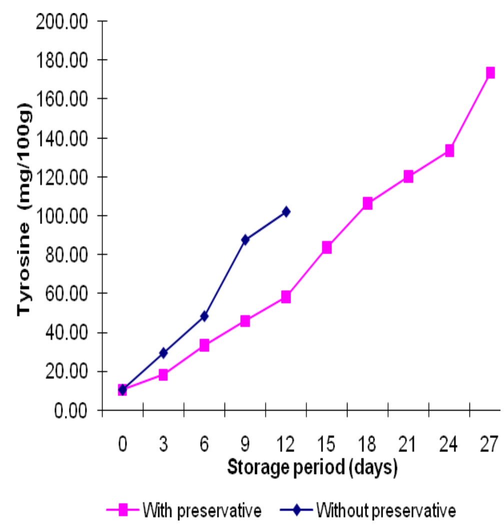
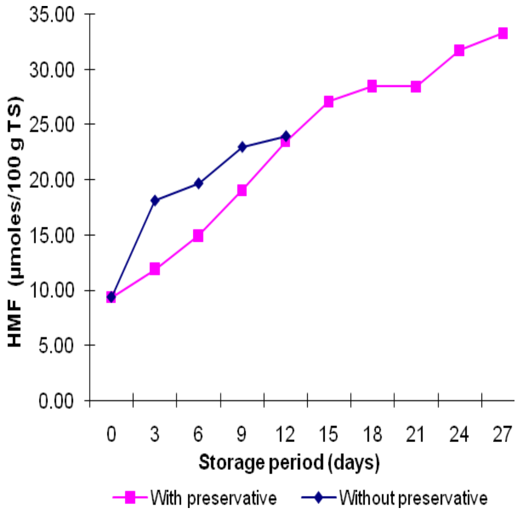


Fig. 5.34 Change in tyrosine value mg/100g) of packaged *Doda burfi* during storage at 30°C



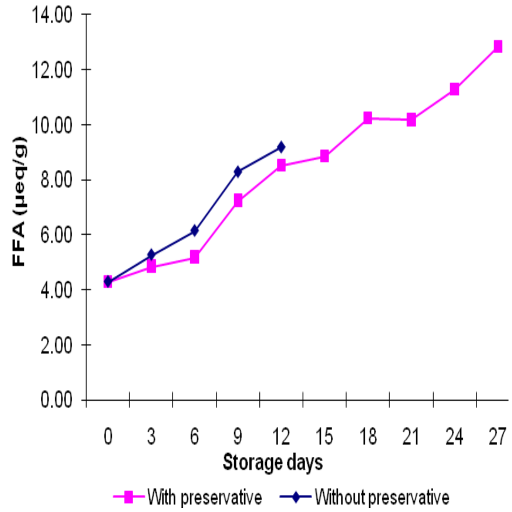
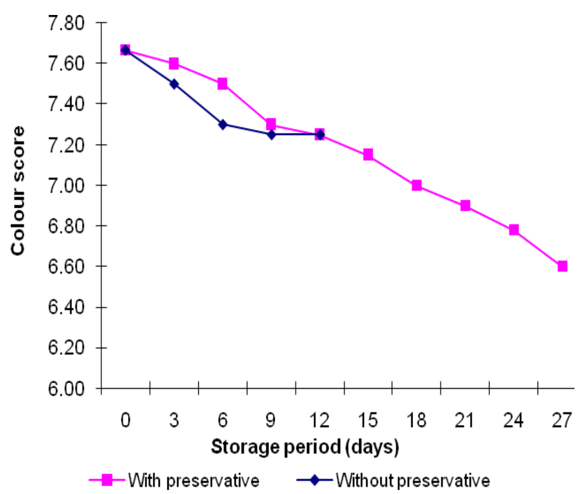


Fig. 5.35 Change in HMF content of packaged *Doda burfi during* storage at 30ºC

Fig. 5.36 Change in free fatty acids (µeq/g) of packaged *Doda burfi* during storage at 30ºC



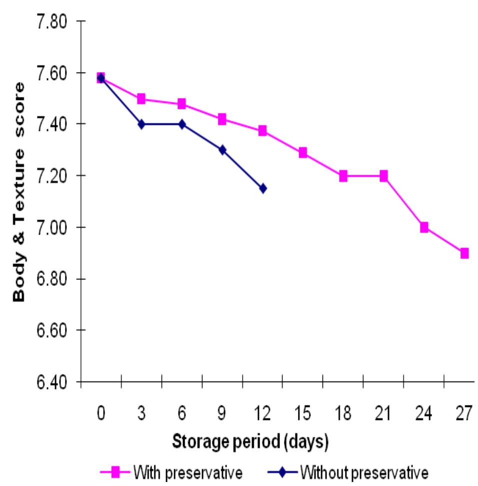
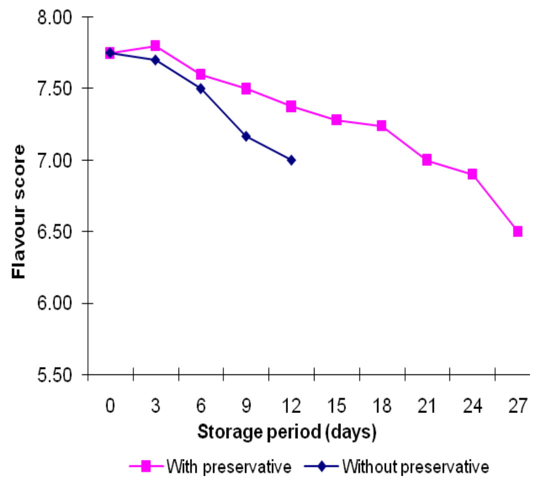


Fig. 5.37 Change in colour score of packaged *Doda burfi during* storage at 30ºC

Fig. 5.38 Change in body and texture score of packaged *Doda burfi* during storage at 30ºC



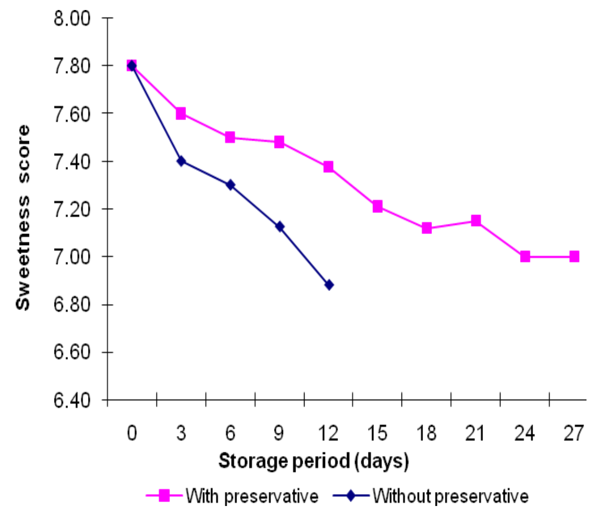


Fig. 5.39 Change in flavour score of packaged *Doda burfi during* storage at 30ºC

Fig. 5.40 Change in sweetness score of packaged *Doda burfi* during storage at 30ºC

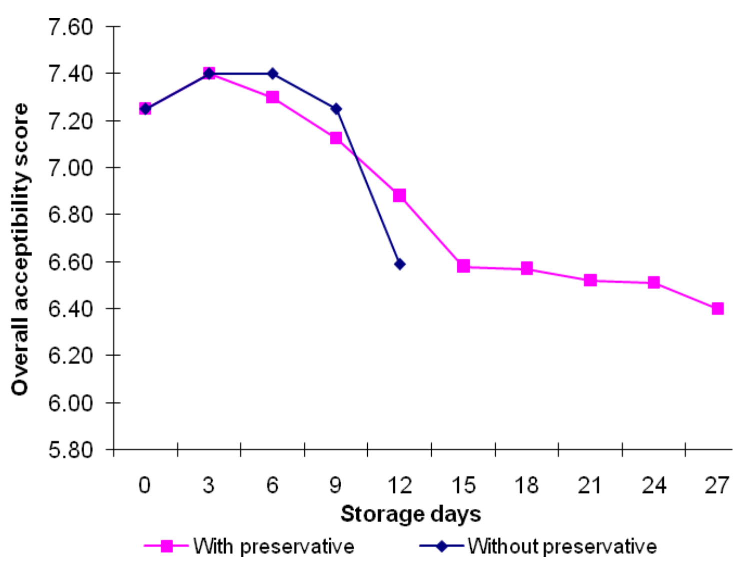


Fig. 5.41 Change in overall acceptability of packaged *Doda burfi during* storage at 30ºC

Table 5.52: Changes in instrumental texture parameters of functional *doda burfi* during storage at 30ºC

Storage	Hardness (N)		Adhesiveness (N.s)		Springiness		Cohesiveness		Gumminess (N)		Chewiness (N)	
(Days)	(P)	(WP)	(P)	(WP)	(P)	(WP)	(P)	(WP)	(P)	(WP)	(P)	(WP)
0	47.45	47.45	-2.78	-2.78	0.34	0.34	0.28	0.28	13.33	13.33	4.56	4.56
3	50.28	49.62	-2.70	-2.68	0.33	0.30	0.28	0.27	13.88	13.60	4.61	4.09
6	53.18	53.29	-2.69	-2.39	0.32	0.25	0.27	0.25	14.41	13.38	4.61	3.34
9	54.28	55.29	-2.51	-2.19	0.31	0.24	0.25	0.23	13.79	12.61	4.27	3.03
12	56.38	57.98	-2.39	-2.01	0.31	0.22	0.24	0.22	13.53	12.76	4.19	2.81
5	59.54		-2.24		0.31		0.24		14.17		4.32	
18	59.82		-2.01		0.29		0.23		13.64		3.89	
21	60.12		-1.99		0.25		0.20		12.08		3.00	
24	61.59		-1.66		0.24		0.20		12.26		2.88	
27	62.38		-1.67		0.21		0.19		12.04		2.52	

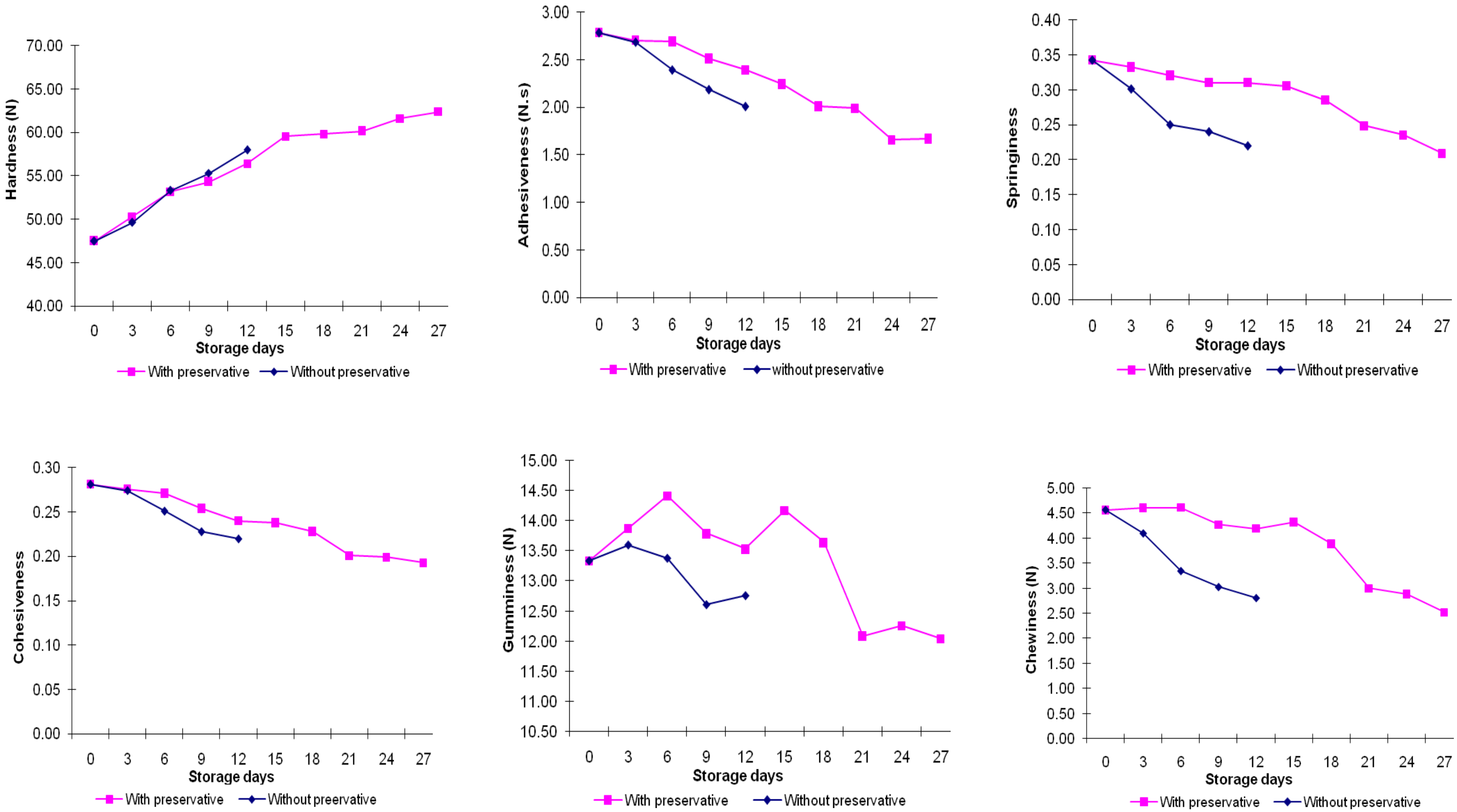


Fig. 5.42 Behaviour of with and without added preservative packaged functional *doda burfi* in terms of instrumental textural response under storage at 30°C

Table 5.53: ANOVA for physicochemical changes in without and with added preservative developed functional *doda burfi* during storage at 30°C

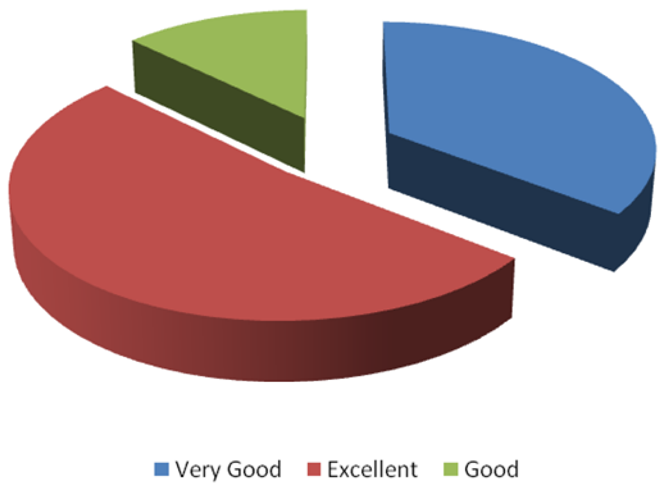
Chemical analysis	Treatments	MSS	F
Moisture	Storage period	2.49	25.58**
	Preservative	0.09	0.92^{ns}
pH	Storage period	0.085	51.78**
	Preservative	0.015	9.26*
Acidity	Storage period	0.05	83.36**
	Preservative	0.002	3.53^{ns}
Tyrosine	Storage period	3507.65	18.26**
	Preservative	1272.72	6.62^{ns}
HMF	Storage period	85.69	23.08**
	Preservative	23.63	6.36^{ns}
FFA	Storage period	10.26	114.51**
	Preservative	0.95	10.59*
	Microbiological analysis		
SPC	Storage period	2.38	4.09^{ns}
	Preservative	1.75	3.00^{ns}
Yeast and mould	Storage period	1.69	3.34^{ns}
	Preservative	2.02	3.99^{ns}
Coliform	Storage period	nd	
	Preservative	nd	
	Sensory analysis		
Colour	Storage period	0.14	40.14**
	Preservative	0.01	3.50^{ns}
Texture	Storage period	0.05	17.79**
	Preservative	0.02	8.38*
Flavour	Storage period	0.20	15.51**
	Preservative	0.08	6.18^{ns}
Sweetness	Storage period	0.11	6.76*
	Preservative	0.15	9.02*
Overall acceptability	Storage period	0.19	14.04**

	Preservative	0.00	0.03^{ns}
	Instrumental textural analysis		
Hardness	Storage period	33.42	84.12**
	Preservative	0.42	1.06^{ns}
Adhesiveness	Storage period	0.06	3.28**
	Preservative	0.16	2.38*
Springiness	Storage period	0.003	4.26^{ns}
	Preservative	0.007	10.40*
Cohesiveness	Storage period	0.001	19.69**
	Preservative	0.000	6.66^{ns}
Gumminess	Storage period	0.79	6.29*
	Preservative	1.07	8.53*
Chewiness	Storage period	2.38	4.09^{ns}
	Preservative	1.74	3.00^{ns}

* Significant at ($p<0.05$), ** Significant at ($p<0.01$), ns non-significant
Nd not detected

5.6 CONSUMER RESPONSE STUDY

Consumer acceptance studies are of utmost importance for estimating the level of acceptability and the scope of a newly developed product in the potential marketplace. With a view to conduct consumer studies, functional *doda burfi* was prepared using representative process developed in the present study. The samples were presented to a total number of 110 respondents representing all age and income group of potential consumers (Appendix IX). This group consisted mainly of employees and students of National Dairy Research Institute, from different regions and different taste preferences. Fifty two percent of total respondents rated the product as excellent, 35 percent as very good and 13 percent as good (Fig. 5.43). No adverse remarks or criticism was received from a single respondent. So it can be inferred that functional *doda burfi* developed through the present study is acceptable enough to be launched at commercial scale.



5.43 Consumer's response on functional *doda burfi*

5.7 COST ANALYSIS FOR THE MANUFACTURE OF FUNCTIONAL *DODA BURFI*

Any newly developed functional product needs to be commercially viable to become attractive for entrepreneurs and industrialist so that its mass production can be taken up for the welfare of mankind. A newly product brought out into the market has to be attractive in terms of palate and price for masses in order to sustain in commercially competitive market. The functional *doda burfi* developed in the present project has the target group consisting of persons suffering from constipation, increased cholesterol and those having heart problems. Apart from this, the product being fortified with mineral, proven a good source of calcium too. Owing to rise at alarming rate in population suffering from above mentioned aliments and growing concern for wellness among masses, there exists a huge market potential for the product. The manufacturing cost is one of the important consideration for establishing a remunerative project. Cost of the functional *doda burfi* was calculated if it is to be manufactured in the experimental dairy of the institute taking in to account daily production of 400 kg of the raw milk taken along with other ingredients. Data provided by Narnaware (2000) for various operational costs was used.

5.7.1 Basic assumptions for cost estimation: The basic assumptions with which the present cost projections have been made are being stated hereunder:

- ✓ It was assumed that the plant for manufacturing of functional *doda burfi* would work in 1 shift per day for 300 days a year.
- ✓ The handling capacity of the plant would be minimum 400 Kg per day.
- ✓ The cold storage capacity would be 1 tonne based on the assumption that product would be dispatched in refrigerated van every alternate day for sale.
- ✓ Various direct (labour and supervision) and indirect (administrative expense) costs have been derived from the recent figures available with the dairy plant having been suitably apportioned.
- ✓ The cost of various ingredients used in the formulation has been worked out on the prevailing market prices.

For convenience in elaborating the data to find out cost per kg steam and refrigeration used, 100 kg of raw milk has taken for cost analysis.

5.7.2 Capital requirements: Total capital required for the manufacture of 180 tonnes of functional *doda burfi* annually has been estimated in this section considering all direct and indirect costs involved.

5.7.2.1 Land: An area of 2000 m^2 (10 times the plant area) was estimated to cost Rs. 6,00,000 at the rate of Rs.300 /m^2.

5.7.2.2 Building: The construction cost of the plant (200 m^2) was computed to be Rs. 5.00,000 at the rate of Rs. 2,500 /m^2.

5.7.2.3 Equipment: The list of various equipments required for manufacture of 400 kg of functional *doda burfi* per day along with their cost and depreciations is given in Table 5.54. The cost of equipment works out to be Rs. 570,000. The cost of installation calculated @ 7.5% of the equipment cost would be Rs. 54,000. The total annual depreciation rate works out to be Rs. 42,322. Thus, the total cost of equipment with total installation charges was calculated to be Rs. 8,16,322.

5.7.2.4 Manpower cost: Requirements for personnel involved for the manufacture of 180 tonnes/annum of functional *doda burfi* has been calculated on the basis of material handling and operating shifts. The annual labour and supervision charges for the personals directly involved have been worked out to be Rs. 10,08,000 (Table 5.55). The costs of personals indirectly involved viz. operational supervisors and administrative expenses in the manufacture schedule have been estimated to be Rs. 1,44000 and Rs. 6,18,000, respectively (Table 5.55). Hence the total annual manpower cost has been estimated to be Rs. 16,26,000.

5.7.2.5 Fixed cost and interest: The total fixed cost that includes land cost, construction cost of building and cost of equipment along with installation charges has been estimated to be Rs. 18,74,000.00 where as the working capital cost (3 month pay of personals) as Rs. 4,06,500. The interest incurred there upon amounts of fixed capital and working capital @ 12 percent were Rs. 2,24,880 and Rs. 48,780, respectively. There for the total interest on total fixed capital and working capital amounts to Rs. 27,3660.00

The total fixed cost is as follows:

Land cost = 6,00,000

Construction cost = 5,00,000

Equipment + installation cost = 7,74,000

Total fixed cost = 18,74,000

Table 5.54: Initial cost and depreciation on plant equipment and machinery

Sr. No.	Item	Nos	Rate (Rs)	Initial Value (Rs)	Scrap Value (Rs)	Life (Yrs)	Annual Depreciation	Capacity
1	Storage tanks	1	50000	50000	5000	17	2647	1000 L
2	Cream separator	1	25000	25000	2500	15	1500	500 L/hr
3	Multipurpose holding vat with agitator	1	50000	50000	5000	15	3000	800 L
4	Boiler and steam system	1	150000	150000	15000	30	4500	50 Kg/hr
5	Cold store & refrigeration system	1	150000	150000	15000	17	7941	1 Tonne
6	Sanitary pump	3	15000	45000	4500	12	3375	500 L/hr
7	Weighing balance	1	20000	20000	2000	17	1059	100 Kg
8	Inoculation hood	1	50000	50000	5000	15	3000	
9	Miscellaneous	**-**	30000	30000	3000	15	1800	
			Total	**570,000**			**28,822**	

Table 5.55: Cost of manpower involved in functional *doda burfi* production

S.No	Staff	Nos.	Monthly Salary	Monthly Cost (total Rs.)	Annual Cost (Rs)
		Operating Labourers			
1	Labourers	4	4500	18000	216000
2	Skilled worker	2	7500	15000	180000
3	Mechanics/electrician	2	9000	18000	216000
4	Boiler attendants	1	9000	9000	108000
5	Lab analysts	1	7500	7500	90000
6	Lab attendants	1	4500	4500	54000
			Sub total (A)	72000	**864000**
		Operational Supervisors			
7	Shift supervisors	1	12000	12000	144000
			Sub total (B)	12000	**144000**
		Administrative Expenses			
1	Plant Manager	1	20000	20000	240000
2	Clerk-cum-Accountant	1	9000	9000	108000
3	Store Keeper	1	9000	9000	108000
4	Attendant	1	4500	4500	54000
5	Security staff	2	4500	9000	108000
			Sub total (C)	51500	**618000**
				Grand Total (A+B+C)	**1626000**

5.7.2.6 Charges on Power and Utilities

Moisture present in 117.2 kg of mixture = 83.5 kg (neglecting moisture content of other ingredients)

Moisture % present in mixture =83.5/117.2*100 = 71.24%

Specific heat of food products

Sp. Heat = $\frac{P}{100} + \frac{0.2\,(100-P)}{100}$ kcal/kg°C

Where, P = % moisture present in food product

So, sp. Heat = $\frac{71.24}{100} + \frac{0.2\,(100-71.24)}{100}$

= 0.769 or 0.77 kcal/kg°C

For first stage heating (5 psi ≈ 34.47 KPa for 1 hour), assuming that during this period, milk heated to boiling point. Heat required to raise the temperature of mixture from 4°C to 100°C

$Q = M\, C_p(T_2\text{-}T_1)$

Where, M = mass ; C_p = specific heat ; T_2= final temp reqd; T_1= initial temp

Q = 8663.4 or 8663 kcal

Steam (at 130°C) at 14.7 psi ≈ 101.35 KPa,

Sensible heat of 1 kg steam, $Q = M\, C_p\,(T_2\text{-}T_1)$

1* 0.60* (130-100) = 18 Kcal

Latent heat of 1 kg steam= 539 kcal

Total heat produced from 1 kg of steam 18+539 = 557 kcal

Heat to be removed from mixture = 8663 kcal

Steam required = 8663/557 = 10.166 kg steam

For second stage heating (15 psi ≈103.42 KPa for 2.5 hours), 8 kg of the sugar is again added to the bulk so the weight of the mixture becomes 117.2+8= 125.2 kg

Mixture is heated till semi-solid consistency conversion or technically to 30% moisture is left after 2nd stage processing operations.

Moisture removed during this duration = 83.5-30= 53.5 kg water

Heat required to remove 1 kg of water at 1 bar or 15 psi =539 kcal/kg

Total heat required to remove 53.5 kg of moisture from the product = 539*53.5 = 28836.5 kcal

Heat produced from 1 kg of steam = 557 kcal

Total heat produced from steam = 28836.5/557 = 51.8 kg

For third stage heating (25 psi ≈172.36 for 0.5 hours), (moisture removed from 30% to 13% at 25 psi)

Water removed during this stage = 30-13 = 17 kg

Heat required to evaporate 17 kg of water present = 17 * 539 = 9163 kcal

Sensible heat of 1 kg steam, $Q = M\ C_p\ (T_2\text{-}T_1)$

1* 0.60* (130-120) = 6 kcal

Latent heat of 1 kg steam=526 kcal

Total heat produced from 1 kg of steam: 6+526 = 532 kcal

Heat to be removed from mixture = 9163 kcal

Steam required = 9163/532 = 17.22 kg steam

Total steam required from all the stages = 15.43+51.8+17.22 = 84.45 kg steam

Assuming that there is 10% loss of steam due to open vat and practical losses taken under consideration

Steam required = 84.45 *1.10 = 92.9 kg

Cost of 1 kg steam = 0.35 Rs.

Total cost = 92.9*0.35 = 32.50 Rs.

Product obtained from 125.2 kg of mixture or 100 kg of milk taken = 50 kg

Cost of steam per kg of product = 32.50/50 = 0.65 Rs. or 65Paisa

Refrigeration cost

Heat to be removed from milk to cool it from 72°C to 4°C

$Q = M\ C_p(T_2\text{-}T_1)$

Q = 100*0.93* (72-4)

= 6324 kcal

1 tonne of refrigeration = 3000 kcal

So, TR = 6324/ 3000 = 2.11

Cost of 1 TR = 8.0 Rs.

Total cost = 2.11*8 = 16.87 Rs.

Product obtained from 100 kg milk = 50 kg

Cost of refrigeration/ kg of product = 16.87/50 = 0.34 Rs. or 34 Paisa

Table 5.56: Charges on power and utilities

		Requirement		Rate	Annual Cost
Sl. No.	**Item**	**Daily**	**Annual**	**(Rs)**	**(Rs)**
1	Steam (kg)	372	111,600	0.35/kg	39,060
2	Refrigeration (TR)	8.44	2532	8.0 Rs. / TR	20,256
3	Electricity (kWh)	10	3000	5 / Unit	15000
4	Water	4000	1200000	2 / 1000 L	2400
				Total	**76,716**

Table 5.57: Requirements and cost of raw materials

Sr. no.	Item	Requirement (kg)		Rate	Annual cost
		Daily	**Annual**	**Rs/Kg**	**(Rs)**
1	Buffalo whole milk	400	120,000	15	450,000
2	Skim milk powder	12	3600	115	414,000
3	GWF	16	4800	60	288,000
4	Diesol	2.0	600	120	72,000
5	Oat fibre	4.0	1200	250	300,000
6	Sugar	64.0	19,200	40	768,000
7	Starter culture	2.0	600	50	30,000
8	Nuts	0.8	240	750	180,000
9	Saturated phytosterol	14.0	4200	$20/kg =1000	4,200,000
		514.8	**154,440**	**Total**	**6,702,000**

Correspondingly for 100 kg: 125.2 would be the daily requirement used for costing of steam and refrigeration neglecting phytosterol content.
Milk assumed with 4% fat, 9% SNF
To makeup MSNF to 12 % on milk basis in mix

Table 5.58: Cost breakup details for 400 kg/day production of functional *doda burfi*

	Sr. No	Cost Component	Rates	Rs. per annum
A		**MANUFACTURING COST**		
	a	**Direct Product Cost**		
	i	Raw materials	Annexure	6,702,000.00
	ii	Packaging material. Annual requirement of boxes (1kg)	Rs. 0.50 per box	38,610.00
	iii	Manpower Cost	Annexure	1626000.00
	iv	Power and utilities	Annexure	76,716.00
	v	Maintenance and repairs	5% of the fixed capital	93700.00
	vii	Laboratory charges	0.2% of cost of raw material	14307.19
	viii	Cleaning and sanitizing material	0.1% of cost of raw material	7153.59
		Sub-total		**8,558,486.78**
	b	**Fixed charges**		
	i	Interest on Total Fixed Capital and Working Capital	12% of Total Fixed Capital and Working Capital	273660.00
	ii	Depreciation on capital investment	Annexure	28,822.00
	iii	Insurance and taxes	2% of the capital investment	37480.00
	iv	Plant overheads	60% of Man Power Cost	975600.00
		Sub-total (b)		**1,315,562.00**
		Manufacturing Cost (A = a + b)		**9,874,048.78**
		MARKETING AND DISTRIBUTION		
B	i	Marketing and distribution	20% of Manufacturing Cost	1,974,809.76
		Total		**1974809.76**
		TOTAL COST (Rs.) per annum (A + B)		**11,848,858.54**
		Cost (Rs.) of Functional *Doda burfi* (per kg) Rs.		**153.44 or 153/-**
		Cost (Rs.) of Standardized *Doda burfi* (per kg) Rs.		**91.34/-**
		Market cost of conventional *doda burfi*		**140-230/kg**

5.7.2.7 Cost of utilities: Utilization of various services is specific for individual plants. For the situation under consideration, the requirements of utility components and their cost have been presented in Table 5.56.

5.7.2.8 Cost of raw materials: The quantity of various ingredients required to manufacture 400 kg of functional *doda burfi*, under the situation have been calculated and presented in Table 5.57. The costing of these ingredients at the current prevailing rates shows that the total cost of raw material is Rs. 6,702,000.

5.7.2.8 Marketing cost: A total marketing cost of Rs. 1,974,809.76 has been calculated on the basis of 20 percent of the manufacturing cost. All direct and indirect costs that have been determined in this section are essentially situation specific. Wherever, necessary, apportioned costs have been considered under similar situation. The costs are expected to remain unaltered but subject to price fluctuation and inflation, the cost needs to be constantly updated from time to time.

5.7.3 Total manufacture cost: This section deals with total cost incurred for the manufacture of 77,220 kg of functional *doda burfi* per annum assuming approximately 50% yield of the total raw materials used. Various direct and indirect costs involved in the production have been presented in Table 5.55. It may be observed that the total cost incurred to manufacture 77,220 kg of functional *doda burfi* per annum is Rs. 11848858.54. Most of the items in costing are situation specific and need to be calculated separately for a given situation of product manufacture. The total fixed charges that includes interest on capital investment and working capital, depreciation on capital investment, insurance and taxes and plant overheads have been estimated to be Rs. 13,15,562 (Table 5.58).

5.7.4 Net manufacturing cost: The net manufacturing cost of functional *doda burfi* is presented in Table 5.58. It may be observed that the net cost of manufacture of functional *doda burfi* including marketing and distribution charges has been worked out to be Rs. 11,848,858.54. Thus the cost of production of one kg of functional *doda burfi* was estimated to be Rs. 153.44 or Rs. 153 only.

Functional *doda burfi*, with proven health benefits, is expected to give same pleasure as that of conventional *doda burfi* to health conscious consumers who are otherwise restricted to relish conventional *doda burfi*. In the current health foods regime, consumers are ready to pay extra for the product that is claimed to possess health benefits. In view of this, the increased cost of production of this premium product would not be a consideration.

chapter-6

Summary and Conclusions

Summary and conclusions

Ethnic dairy based sweet constitute the most promising sector where a lot of market potential exists. To carve a place in the competitive market, dairy industry needs to widen its product baseline and respond to new demands created by health conscious population. *Doda burfi* is a delicious and nutritious delicacy, served on special occasions and also contains cereal as one of the major ingredient, thereby serving as a source of cereal and milk derived nutrients available to the mass along with the appealing taste. But the production at small scale levels and prevailing unhygienic practices urges need to modify the existing process to manufacture *doda burfi* under hygienic conditions of the industry on commercial scale and mechanization.

The study was aimed at optimization of technology for the manufacture of standardized recipe of conventional *doda burfi*. The product so obtained would have superior nutritional quality as well as good storage stability when prepared with additional benefits of bio- preservatives. The study was also intended to fortify the product with fiber, calcium and phytostanol to accrue the health benefits pertaining to the reduced risk of cardiovascular disease (CVD) and osteoporosis. The present investigation was therefore, undertaken with the following objectives:

- **Characterization of the physico- chemical, sensory, rheological and microbiological quality of market samples of *doda burfi.***
- **Standardization of technological parameters for manufacture of acceptable quality *doda burfi***
- **Optimization of the level of functional ingredients in standardized *doda burfi* for enhancing the health attributes**
- **Extension of shelf life of developed product through processing and packaging interventions**

The published literature on *doda burfi* is very scanty. Hence, the literature pertaining to related products i.e. *kalakand, burfi, peda* and milk-cake is given.

There was no documented literature available on the product characteristics and composition. Therefore, it was necessary to first characterize the product through survey and then develop a technology for commercial manufacture of the product of optimum quality.

The present research work was undertaken to characterize *doda burfi* which is a popular variety of *burfi* of Northern India especially in states like Punjab, Eastern U.P and Haryana and to standardize the technology for its manufacture. The first phase of study dealt with analysis of market samples brought from diverse regions of Haryana and Punjab. A process was also standardized for manufacture of *doda burfi* using response surface methodology (RSM) for optimization of processing parameters for different responses using NCDC167. The optimized product obtained through combination of culture NCDC167 and citric acid was studied for storage studies in terms of sensory, physico-chemical, microbiological and instrumental textural changes at 30ºC. Similarly to enhance the health benefits of the product, various nutraceuticals were added and a functional product was developed using a range of fibers and source of calcium with added benefits of phytostanol.

6.1 MARKET SURVEY

Sixteen different lots of fresh *doda burfi* samples, four each from four market areas viz., Karnal, Delhi, Ludhiana and Jalandhar were brought to Dairy Technology Division, NDRI, Karnal, in insulated containers within 24 hrs of manufacture. The market samples of *doda burfi* brought from four diverse regions of Karnal, Delhi, Ludhiana and Jalandhar, were subjected to descriptive sensory analysis, physico-chemical, microbiological, instrumental colour and textural profile analysis. A standardized descriptive language for colour and appearance, texture and flavour of *doda burfi* was developed and validated. The market samples were evaluated sensorily by a trained panel of minimum of 4 judges. Out of the twenty attributes studied, for sensory attributes like colour and appearance, texture and flavour, only three of them were found significant ($p<0.05$) between market areas. The similar results were analyzed using principal component analysis (PCA) which revealed that major attributes were positively loaded in the Ludhiana city, with least desirable product attributes majorly dominated in Karnal samples.

6.1.1 Application of Principal Component Analysis (PCA)

A highly trained panel (n=4) participated in round table discussion to generate descriptive terms which were later refined and references were identified. *Doda burfi* samples were presented to panel for validation with the identified lexicon. The panel differentiated the sixteen *doda burfi* samples as determined by Univariate analysis of variance. A total of twenty terms were identified to describe *doda burfi* for colour and appearance, texture and flavour. The panelists also found potential to further separate terms. In colour and appearance of surface, the terms like glossiness and colour of the product ranging from light amber to dark chocolate brown were added. For flavour the terms like, nutty, doughy, unclean, acidic, bitter, foreign and oxidized were used whereas for texture the terms included were granularity, crumbliness, springiness, cohesiveness, chewiness and gumminess & finally overall acceptability for the samples was evaluated to reach at some final conclusion. The average sensory score of the four market areas were compared and correlated by principal component analysis (PCA). The results revealed that on the basis of eigenvector loadings, first principal component with an eigen value of 9.814 was able to explain 49.07% of the total variation whereas second PC with an eigen value 6.287 explained 31.34% of the variation and both the PC's (PC1 and PC2) explained 80.50% of the total variation. The characteristics of prime importance were found loaded in Ludhiana city whereas doughy, nutty, acidic, gummy, bitter, oxidized were dominating characters in Jalandhar samples. Granularity the identifying and important attribute was found highest in Karnal samples.

The loading graph, correlation matrix and influence graph depicted and validated the sensory results where the Ludhiana samples were found best in terms of positive loadings and maximum scoring by the panelists.

6.1.2 Physico-chemical, microbiological, instrumental texture and colour variation

The proximate composition of the product in terms of moisture, fat, protein, pH, acidity, ash and dietary fiber (insoluble and soluble) along with instrumental texture analysis was carried out. The analysis of variance revealed significant ($p<0.01$) differences between all the four market areas for physico-chemical attributes studied. Similarly, instrumental texture profile analysis (TPA) revealed statistically significant results (among cities as well as samples). The chemical composition of the samples collected from different shops of a same city were expected to show variation, but it was strange to experience variability even within the samples collected from different shops of same city. The wide variability among different compositional attributes could be attributed to difference in manufacturing practices, varying ingredient quantity and quality and the storage temperature of the final product. Microbiologically, the product quality was checked using SPC, yeast and mould count and coliform count. Being highly loaded with sugar, yeast and mould count was found dominant within the market samples. Statistically the results revealed that there were significant differences between the cities surveyed. However, the samples from same city but different market areas (shops) had non- statistically difference between them. Instrumental colour values revealed highest darker colour (lowest L* value) in samples from Ludhiana samples followed by Delhi Jalandhar and was lowest in Karnal. The analysis for lightness (L*), redness (a*) and yellowness (b*) revealed significant results ($p<0.01$) for samples as well as for cities surveyed.

6.2 OPTIMIZATION OF STANDARDIZED *DODA BURFI* USING RESPONSE SURFACE METHODOLOGY

The critical compositional variables of standardized *doda burfi* i.e. fat level in milk (3-6%), germinated wheat flour (GWF) (2.5-5.5%) and sugar (14-18%) were optimized depending on their effects on sensory profile of the final product. The level of milk solid-not-fat (MSNF) was kept constant at 12% in all the formulations. Besides optimization, combined effect of these variables were also studied on instrumental texture, colour parameters and water activity values, though not taken up during optimization of recipe. A total of 17 experiments were carried out in accordance to Box-behnken design. The obtained data was modeled using quadratic multiple regression technique.

6.2.1 Effect of milk fat, GWF and sugar on sensory attributes

Variation in the compositional variables was expected to affect sensory characteristics of *doda burfi* to a greater extent. Based on the maximum colour score values on 9-point hedonic scale, the colour score of experimental *doda burfi* was found to range from 7.2 to 8.5. It was observed that colour score of *burfi* significantly improved ($p<0.01$) with increasing sugar concentration. Fat showed the increasing trend when increased upto 4.5% for colour which decreased afterwards whereas GWF showed no significant or null effect on colour development score values. Similar trend was observed with the increase in sugar and fat content wherein fat though showed increasing trend but the effect of sugar was quiet significant in the development of colour.

A wide variation in texture score of *doda burfi* was observed and ranged from 6.71 to 8.28. Texture showed a slight increasing trend with increase in GWF addition whereas fat showed steep increase in texture score when increased upto a level of 4.5% but further decreased when increased beyond this level. Sugar showed the same increasing trend for texture score whereas with GWF addition, score first increased and thereafter decreased beyond 4 per cent of its addition.

Sweetness score showed an increase trend with respect to GWF and fat addition wherein score for sweetness increased between 3.0- 4.5% but further decreased when added beyond this level. Sugar and GWF also played a critical role at linear level for improved sweetness. Fat had been found to have a positively significant variable for flavour and overall acceptability of the product for the production of optimized *doda burfi*. Increased amount of GWF showed the same trend but not much increase was observed in overall acceptability of *doda burfi*. A slight increase in overall acceptability score was observed with increase in sugar concentration from 14 to 17%, which decreased thereafter but drastic increase in fat up to a level of 5.25% fat was observed.

The five solutions (vide Table 5.29) were obtained during optimization with culture NCDC 167 with very minute differences found within and impossible to be maintained at practical levels. Thus, solution no. 1 with 4.72% fat, 4.01% GWF and 15.95% sugar with desirability value of 0.931, was opted for the manufacture of optimized standardized recipe and the product thus made was evaluated sensorily and the scores thus obtained were compared with the predicted scores obtained. None of the variable evaluated revealed significant difference indicating that the sensory responses of the optimized product were in close agreement with the predicted values.

The final optimal product had a composition of 15.28% moisture content, fat 18.07%, protein 15.31%, reducing and total sugar, 13.68 and 15.27%, ash 2.43% with soluble and insoluble dietary fiber, contributing factor from GWF as 6.02 and 12.95% having a pH of 5.61 bearing acidity 1.15%, in fresh sample with acceptable and comparable instrumental textural and colour values from market samples along with better control check over the microbiological quality.

6.3 DIETETIC PRODUCT DEVELOPMENT

Various preliminary trials were conducted to select bulking agents and fibers from the wide availability of materials available along with artificial sweetener to develop a dietetic product, suitable for diabetic and obese persons. The levels of best two fibers amongst five different fibers studied namely wheat fiber, oat fiber, Diesol™ (hydrolyzed gum acacia), microcrystalline cellulose and inulin were selected on the basis of sensory studies. The maximum range for various fibers except Diesol was taken as 5% whereas the minimum was 2.5% to enrich and label the product as “fortified with dietary fibers” whereas the minimum and maximum limits for Diesol worked out as 0.5 and 0.75 % respectively. In the screening process, oat fiber and Diesol at levels of 2.5 and 0.75 per cent, respectively were adjudicated as best to be used further in the study. Stevia alone was not able to provide the desirable body and texture to the product thus replacement was then made on minimum sugar basis and few preliminary trials consisting of sugar and stevia in the ratio 25:75, 50:50, 75:25, 100:0 (control) were conducted for standardizing the level of sugar and stevia wherein 25%

Stevia and 75% sugar (of the total sugars) was finally used to develop a dietetic product.

A CCRD deign experiment consisting of 30-experiments with 4-varibles taken for optimization studies was carried out but none of the combinations tried was sensorily acceptable as it lacked typical granularity associated with the traditional *doda burfi*. Hence, it was concluded that the sugar is an essential component of *doda burfi* as it is required for proper body and texture as well as for colour development, and therefore, it is not possible to replace it with artificial sweeteners. Hence, the idea of developing a dietetic product was abandoned and the study on development of functional *doda burfi* using functional ingredients such as dietary fiber, calcium and phytostanol was pursued further.

6.4 DEVELOPMENT OF FUNCTIONAL *DODA BURFI*

Based on the preliminary studies, two different levels of phytostanol were tried with the final recipe of functional *doda burfi*. The levels worked upon were 3.0% and 4.5% and the results were compared with control sample with zero or no added phytostanol in it. To select the best optimal level of phytostanol, product was evaluated by a panel of trained judges and on the basis of results obtained, 3.0% phytostanol was adjudged the best level to be used further in the studies for the development of functional *doda burfi* whereas the levels for the addition of fibers were taken as those finalized previously with the *doda burfi* containing artificial sweetener.

Optimization studies with oat fiber (1-3%) and calcium (50-100mg/100ml) of milk was carried out and the study revealed that both the variables at linear level had a negative effect on the sensory attributes studied. Sensory attributes like colour, texture, flavour, sweetness and overall acceptability decreased with increase in oat fiber and calcium content. The results of instrumental texture and colour analysis revealed that none of the variables studies had significant model 'F' value along with less than the desirable R^2 value. Also, none of the attributes had significant effect on variables at linear, interactive or quadratic level. The maximum score for overall best acceptable product was given to the product prepared from 1.0% Oat fiber and 50 mg/100ml (milk basis) of calcium in the product whereas the minimum score was obtained in the product with 3.0% oat fiber and 50mg/100ml of calcium.

The set goals revealed eight best solutions with desirability approaching to 1.00 wherein the solution no. 1 (oat fiber level 1.00 %, calcium 50.40mg/100ml) had a fairly high desirability pointing to ideal case (1.00) indicated reliability of predicted values. Hence, optimized product thus made from the obtained composition had non-significant values when compared with those obtained from the predicted values with respect to all sensory attributes studied except texture.

The final optimized product had the composition 18.05% moisture with almost similar values of fat and protein content compared to standardized and optimized recipe of *doda burfi.* Mineral estimation of functional product revealed that calcium after fortification @ 50 mg/ 100 ml (of milk) in *doda burfi* came out as 62.32 mg/ 100g of the product. Since the product was fortified with a source of soluble fiber only, SDF content increased and the final product contained 11.47 and 8.25% of IDF and SDF respectively, which provides more than half of daily requirement of dietary fiber on consumption of 25 g product/day.

6.5 SHELF-LIFE STUDIES ON FUNCTIONAL *DODA BURFI*

Functional *doda burfi* immediately after manufacture with and without added preservative was packaged and stored at 30ºC. The samples were withdrawn at predetermined intervals and analyzed for different attributes. The analysis of stored samples was discontinued when product was declared as spoiled by the sensory panel or when got contaminated with visible microbial growth.

6.5.1 Physico-chemical changes

A gradual increase was observed in the acidity, free fatty acids, tyrosine and HMF content of *doda burfi* samples in both products with and without added preservatives during storage at 30°C, whereas decrease in pH and moisture was observed in the stored samples irrespective of preservative used or not. Moisture content decreased after 27 days and 12 days of storage from an initial vale of 18.05% to13.95% and 15.1% respectively, whereas pH declined from a value of

5.77 to 5.00 and 5.37 in product prepared with and without added preservative. Though the decline found was more prominent in case of product prepared without added preservative and may be attributed to the faster microbial growth and production of more amount of metabolites thereof.

A significant shoot was observed in FFA value of freshly prepared *doda burfi* samples from a value 4.29 μeq/g to 9.17 and 12.83 μeq/g after the end of storage period in *doda burfi* prepared without and with added preservative, respectively. When stored at 30°C, tyrosine value of *burfi* samples prepared without preservative and with preservative increased to 102.12 mg/100g after 12 days of storage and 173.19 mg/100g after 27 days of storage from initial content of 10.35 mg/100g. The HMF increased from an initial content of 9.34 μmoles/ 100 gm of TS to 23.96 micro moles/100 gm after a storage period of 12 days whereas the product with added preservative had value of 33.30 μmoles/ 100 gm on 27th day. A highly significant increase in HMF content of all *burfi* samples was recorded during storage.

6.5.2 Microbiological changes

Average initial SPC in functional *doda burfi* was 2.59 and 2.72 at 6th and 3rd day of storage in the product prepared with and without added preservative. In the present investigation, initially no mould or yeast counts could be detected in the sample of functional *burfi* during earlier days of storage. But during subsequent storage the presence of yeast and moulds were encountered at 30°C storage temperature. The spoilage of product can be entirely considered due to growth of yeast and mould count. After 12 and 27 days of storage, in product prepared without and with added preservative the average count increased from an initial content of 1.72 and 1.79 (observed on 6th and 12th day) to 2.38 and 2.50 log cfu/g, respectively whereas coliforms were absent in fresh and stored samples.

6.5.3 Changes in sensory attributes

The sensory quality of functional *doda burfi* samples consistently became less acceptable with the advancement of storage period as it resulted in a steady decrease in colour, body and texture, flavour, sweetness and overall acceptability. The sensory analysis was terminated once the surface mould growth appeared over the product. During storage the sample became stale with advancement of storage period and it resulted in a steady but a very little decrease in their scores. Finally the score reached at 7.25 for the colour of *doda burfi* prepared without added preservative after 12 days of storage whereas the product with added preservative lasted till 27 days and score reached to 6.78 after the storage period at 30°C from an initial score of 7.66. The decrease in colour score with the progression of storage period and was statistically significant. Similarly, a decreasing trend was observed for texture, flavour, sweetness and overall acceptability. The stored samples progressively became brittle which lacked cohesiveness.

The study proved that functional *doda burfi* prepared with added preservative is better approach compared to the product prepared without preservative for maintaining the acceptable sensory quality for longer period and also for restricting the growth of microorganisms, particularly at room temperature storage.

6.6 CONSUMER RESPONSE STUDY

The functional *doda burfi* samples were presented to a total number of 110 respondents to elicit their response to the product acceptability. The assessment revealed that fifty two percent of total respondents rated the product as excellent, 35 percent as very good and 13 percent as good. No adverse remarks or criticism was received from a single respondent. So it can be inferred that functional *doda burfi* developed through the present study is acceptable enough to be launched at commercial scale.

6.7 COST ANALYSIS FOR THE MANUFACTURE OF CONVENTIONAL AND FUNCTIONAL *DODA BURFI*

The total cost of production of conventional as well as functional *doda burfi* was computed in order to assess the economic feasibility of its commercial production. The total cost of production was estimated to be Rs. 91.34/kg for conventional standardized recipe of *doda burfi* and Rs. 153.44/kg functional *doda burfi* with added oat fiber, calcium and phytostanol.

6.8 FUTURE RECOMMENDATIONS

The interaction of different fibers, phytostanol and calcium with basic ingredients of *doda burfi* needs to be studied at molecular level so that exact underlying chemical processes that affect the quality parameters of functional *doda burfi* can be explained. Validation studies using clinical trials would be best approach to carry out to depict the functionality of functional ingredients used and will boost its commercialization with strong evidences. The microstructural studies would also help to elucidate the effect of these interactions and bio-preservatives used.

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Appendix-I

Developed language used for sensory assessment of *doda burfi*

Attribute name	Attribute definition
Glossiness	Having a smooth, shiny, lustrous surface
Colour (towards brown)	The intensity of brown color from light to dark colour
Caramelized	Aromatics associated with caramelized milk products
Cereal like/ doughy	Textural property resembling an unbaked water and flour mixture; pasty, soft and heavy
Nutty	A light, brown, slightly musty aromatic associated with nuts and certain whole grain
Unclean	Covered or stained with or as if with dirt or other impurities
Acidic	Taste associated with dilute aqueous solutions of acids
Rancid	Having the disagreeable odor or taste of decomposing oils or fats
Stale	Not fresh, rapid from age
Off-flavour	abnormal unappetizing flavor.
Bitter	Unpleasant biting taste perceived at back of tongue characterized by aqueous solution of quinine
Oxidized	Painty aroma associated with aged oils.
Granularity	Geometrical textural attribute relating to perception of size and shape of particles
Cohesiveness	Property of unconsolidated fine-grained sediments by which the particles stick together by surface forces
Firmness	Stress/force necessary to attain a given recoverable viscoelastic deformation.
Chewiness	Mechanical textural attribute related to cohesiveness and to length of time or the number of chews required to masticate a solid product into a state ready for swollowing
Stickiness	Having the property of adhering or sticking to a surface
Crumbliness	The consistency of product that breaks under pressure.
Gumminess	Effort required to disintegrate the product to be state ready for swallowing
Overall acceptability	Adequate to satisfy a need, requirement, or standard

Appendix-II

SENSORY PROFILE ANALYSIS OF DODA BURFI

Sample Code ______________
Date: ______________

Kindly evaluate the given samples by making a vertical linear mark and indicating the sample number along the horizontal linear scale for a particular attribute. You may please follow the suggested sequence of steps for product examination.

1. Colour and appearance of surface

Glossy	Nil/ dull	Slightly	Moderate	Definite	Extreme
Colour: Amber yellow to chocolate brown	Amber yellow	Dark amber yellow	Moderately brown	Dark brown	Chocolate brown

2. Flavour

Caramelized	Nil	Slight	Moderate	Definite	Extreme
Cereal like/ doughy	Nil	Slight	Moderate	Definite	Extreme
Nutty	Nil	Slight	Moderate	Definite	Extreme
Unclean / foreign	Nil	Slight	Moderate	Definite	Pronounced
Acidic	Nil	Slight	Moderate	Definite	Extreme
Rancid	Nil	Slight	Moderate	Definite	Pronounced
Stale	Nil	Slight	Moderate	Definite	Pronounced

Attribute					
Off flavor	Nil	Slight	Moderate	Definite	Pronounced
Bitter	Nil	Slight	Moderate	Definite	Pronounced
Oxidized	Nil	Slight	Moderate	Definite	Pronounced

3. Texture

Attribute					
Granularity	Nil	Slight	Moderate	Definite	Pronounced
Cohesiveness	Nil	Slight	Moderate	Definite	Extreme
Firmness Very weak to hard	Nil	Slight	Moderate	Definite	Extreme
Chewiness Slight to extremely chewy	Nil	Slight	Moderate	Definite	Pronounced
Stickiness	Nil	Slight	Moderate	Definite	Pronounced
Crumbliness Nil to Extreme	Nil	Slight	Moderate	Definite	Pronounced
Gumminess	Nil	Slight	Moderate	Definite	Pronounced
Overall acceptability	Extremely disliked	Moderately disliked	Neither liked Nor disliked	Moderately liked	Extremely liked

Appendix III

Descriptive Sensory analysis for colour and appearance and flavour of *doda burfi* from different cities

Location	Market	Glossy	Colour	CM	Doughy	Nutty	Unclean	Acidic	Rancid	Stale	Off-flavour	Bitter	Oxidized
1	1	59	54	61	61	67	0	0	0	0	0	0	0
1	1	64	64	80	67	75	10	10	46	4	0	6	34
1	1	73	83	65	49	77	0	13	0	0	0	0	0
1	1	63	74	61	35	30	4	17	7	7	7	11	10
1	2	50	50	50	52	75	0	0	0	0	0	0	0
1	2	13	52	75	54	67	10	5	31	7	0	15	11
1	2	69	90	71	29	82	0	5	0	0	0	0	0
1	2	54	70	72	40	33	3	12	5	5	3	6	5
1	3	83	68	71	64	47	0	0	0	0	0	0	0
1	3	70	56	70	76	67	14	1	21	15	15	20	25
1	3	71	78	60	49	77	6	25	0	0	0	0	0
1	3	22	66	54	30	38	2	39	3	3	2	4	3
1	4	72	64	69	54	65	0	0	9	4	0	2	25
1	4	71	79	70	71	67	0	0	5	4	0	0	37
1	4	44	40	41	56	68	0	0	0	0	0	0	0
1	4	54	30	50	57	27	0	0	0	0	0	0	0
2	1	57	47	49	74	43	7	0	11	4	2	2	25
2	1	35	55	75	61	69	0	0	0	0	0	0	0
2	1	0	12	60	9	13	0	0	0	0	0	0	0
2	1	73	50	63	39	50	0	0	0	0	0	0	0
2	2	74	73	69	58	75	0	0	11	4	0	2	25
2	2	77	75	78	74	75	0	0	0	0	0	0	0
2	2	75	64	25	25	50	50	50	0	0	0	0	0
2	2	78	70	77	50	60	0	25	16	0	0	0	0
2	3	72	64	69	58	62	0	0	11	4	0	2	25
2	3	70	70	62	57	79	0	0	0	0	0	0	0

2	3	50	42	50	34	25	29	13	0	0	0	0	0
2	3	73	62	80	43	56	0	4	0	0	0	0	0
2	4	67	89	69	58	75	0	0	11	4	0	2	25
2	4	87	72	67	66	65	3	0	0	0	0	0	0
2	4	62	50	37	50	75	0	7	0	0	0	0	0
2	4	83	65	86	45	65	0	7	3	0	0	0	0
3	1	61	62	52	35	54	0	0	0	0	0	0	0
3	1	75	78	25	25	50	0	0	0	0	0	0	0
3	1	71	79	70	71	75	0	0	5	4	0	0	37
3	1	50	54	50	50	50	0	0	0	0	0	0	0
3	2	60	70	71	37	55	4	2	0	5	1	4	3
3	2	54	79	80	82	82	0	0	0	0	0	0	0
3	2	55	50	50	29	50	0	0	0	0	0	0	17
3	2	72	69	76	46	34	0	0	0	0	14	0	0
3	3	72	64	69	58	62	0	0	11	4	0	2	25
3	3	69	90	71	29	82	0	5	0	0	0	0	0
3	3	54	54	57	52	50	0	0	0	0	0	0	0
3	3	83	65	86	45	65	0	7	3	0	0	0	0
3	4	67	89	69	58	75	0	0	11	4	0	2	25
3	4	83	65	86	45	65	0	7	3	0	0	0	0
3	4	75	64	25	25	50	50	50	0	0	0	0	0
3	4	43	75	70	38	33	10	14	4	3	3	2	2
4	1	52	78	78	28	21	36	0	0	0	8	0	0
4	1	43	75	70	38	33	10	14	4	3	3	2	2
4	1	65	59	66	0	33	0	0	0	0	0	0	0
4	1	25	75	50	75	25	0	0	25	25	0	0	50
4	2	44	40	41	56	45	0	0	0	0	0	0	0
4	2	77	47	50	62	67	21	0	0	0	5	0	0
4	2	33	50	34	80	13	19	21	7	11	9	5	5
4	2	50	90	88	0	33	0	0	0	0	0	0	0
4	3	60	68	30	32	61	0	0	6	0	0	0	0
4	3	49	30	50	57	27	0	0	0	0	0	0	0

4	3	57	50	53	61	61	0	10	25	8	0	0	8
4	3	50	50	0	25	0	0	0	0	25	0	0	0
4	4	68	76	47	25	69	0	0	0	0	0	0	0
4	4	51	50	50	50	37	0	0	0	0	0	0	0
4	4	67	75	61	41	75	0	10	11	0	0	0	8
4	4	50	75	0	0	75	0	0	0	0	0	0	0

Appendix IV

Descriptive Sensory analysis for texture of *doda burfi* from different cities

Location	Market	Granularity	Cohesiveness	Firmness	Chewiness	Stickiness	Crumbliness	Gumminess	OA
1	1	52	75	54	82	75	26	59	92
1	1	64	70	54	51	44	54	22	68
1	1	64	66	59	60	62	31	34	87
1	1	60	56	46	44	56	21	69	77
1	2	49	60	52	54	52	12	52	100
1	2	17	29	32	16	15	77	9	39
1	2	73	74	69	53	52	38	27	94
1	2	79	67	41	54	63	17	71	83
1	3	75	92	61	89	92	19	92	75
1	3	77	86	32	88	90	81	82	24
1	3	58	56	53	66	71	27	40	78
1	3	21	46	35	31	28	29	36	71
1	4	85	75	52	25	18	32	76	74
1	4	77	74	72	75	32	25	56	85
1	4	50	54	48	52	54	28	67	45
1	4	42	41	45	42	74	24	62	67
2	1	75	75	61	47	18	50	71	55
2	1	52	70	67	75	67	75	61	80
2	1	14	100	75	25	25	50	13	25
2	1	65	75	50	55	43	13	40	82
2	2	100	75	72	64	18	17	74	81
2	2	67	74	67	73	82	83	67	92
2	2	50	50	50	50	50	12	50	50
2	2	78	79	55	65	48	6	48	75
2	3	85	75	52	58	18	17	67	74
2	3	61	79	58	79	64	78	75	88
2	3	50	50	50	50	50	12	66	50

2	3	56	83	39	50	57	3	43	83
2	4	85	75	75	74	18	17	67	65
2	4	47	79	50	75	67	78	57	82
2	4	50	50	50	50	50	12	50	75
2	4	59	82	43	53	45	8	38	78
3	1	50	50	50	50	39	21	42	93
3	1	75	50	50	75	75	19	42	100
3	1	77	74	72	75	32	15	12	89
3	1	52	54	50	56	56	5	50	100
3	2	59	78	66	77	80	75	76	75
3	2	80	80	50	71	50	5	59	83
3	2	75	74	50	50	63	4	25	56
3	2	46	66	55	64	50	4	66	67
3	3	85	75	52	59	18	17	67	74
3	3	73	74	69	53	52	38	27	94
3	3	50	55	56	54	61	12	57	65
3	3	59	82	43	53	45	8	38	78
3	4	85	75	58	29	18	17	67	65
3	4	59	82	43	53	45	8	38	78
3	4	50	50	50	50	50	12	50	50
3	4	39	72	60	68	58	15	70	54
4	1	48	89	17	74	89	19	92	89
4	1	39	72	60	68	58	15	70	54
4	1	75	36	50	50	69	22	50	78
4	1	50	75	65	75	62	11	65	68
4	2	50	54	48	52	54	19	54	28
4	2	52	73	61	47	77	17	71	38
4	2	62	31	40	41	41	53	44	31
4	2	56	75	87	85	84	19	82	39
4	3	86	69	71	75	53	28	21	75
4	3	42	41	45	42	74	24	62	67
4	3	75	61	49	45	28	11	50	60

4	3	85	35	58	59	32	19	49	75
4	4	76	65	60	61	48	32	18	88
4	4	50	50	50	50	56	11	50	75
4	4	75	78	56	55	28	11	40	79
4	4	75	25	50	50	25	19	75	100

Appendix V

Proximate composition of *doda burfi* from different cities

City		Moisture	Fat	Protein	Reducing sugars	Total sugars	Acidity	pH	Ash	SDF	IDF	a_w
Karnal	Market 1	12.31	12.23	8.56	11.34	26.34	1.56	6.04	1.61	2.50	12.07	0.67
	Market 2	14.19	41.05	4.31	8.82	20.67	1.53	5.81	1.74	1.55	7.85	0.69
	Market 3	12.79	9.11	6.39	8.52	16.64	1.58	6.02	2.18	2.65	7.36	0.67
	Market 4	11.08	18.66	10.79	13.49	24.69	1.52	7.88	2.16	2.60	8.73	0.72
Delhi	Market 1	10.83	23.04	7.04	14.89	26.52	1.68	6.16	1.77	3.16	7.46	0.69
	Market 2	7.60	16.11	10.44	9.47	24.82	1.61	6.18	1.98	3.47	11.92	0.65
	Market 3	16.51	23.83	10.14	11.90	18.34	0.82	5.92	2.76	2.55	8.57	0.75
	Market 4	12.49	26.68	8.60	13.30	21.14	0.78	5.96	2.59	5.28	6.73	0.71
Ludhiana	Market 1	10.38	12.16	10.31	16.01	26.59	2.18	5.16	1.78	3.58	3.56	0.71
	Market 2	6.77	12.10	15.09	13.80	19.05	1.85	5.04	2.00	3.86	14.66	0.65
	Market 3	9.00	24.66	10.00	11.51	24.44	2.09	5.19	1.85	2.60	11.42	0.66
	Market 4	15.45	21.86	12.06	20.81	24.72	0.48	5.05	2.17	3.91	14.15	0.77
Jalandhar	Market 1	11.86	21.86	14.01	13.95	23.36	0.09	5.71	1.84	3.50	7.86	0.74
	Market 2	14.56	25.09	14.01	12.25	21.45	1.37	5.62	0.56	3.48	8.83	0.71
	Market 3	17.31	4.70	14.25	11.18	18.65	2.09	5.99	1.60	4.85	8.76	0.73
	Market 4	14.77	12.10	9.23	13.19	21.76	1.85	5.89	1.65	4.91	12.04	0.66
	Average	**12.368**	**19.702**	**10.32**	**12.77**	**22.43**	**1.442**	**5.85**	**1.89**	**3.403**	**9.49**	**0.701**
	S.D	**0.760**	**2.400**	**0.759**	**0.790**	**0.806**	**0.150**	**0.167**	**0.121**	**0.252**	**0.737**	**0.008**
	Range	**6.77-17.31**	**4.70-41.05**	**4.31-15.09**	**8.52-20.81**	**16.34-26.59**	**0.09-2.18**	**5.04-7.88**	**0.56-2.76**	**1.55-5.28**	**3.56-14.66**	**0.65-0.77**

Appendix VI

Mean instrumental colour parameters and microbiological count of *doda burfi* from different cities

City		SPC	Yeast and mould count	Coliform	Lightness L*	Redness a*	Yellowness b*
Karnal	Market 1	3.73	3.87	0.67	40.31	11.84	24.99
	Market 2	3.33	3.56	Nd	30.77	12.29	19.45
	Market 3	1.66	3.83	0.67	36.55	12.09	22.28
	Market 4	3.33	3.59	Nd	34.25	12.34	23.04
Delhi	Market 1	5.49	3.52	1.43	31.75	12.58	24.19
	Market 2	3.42	4.51	1.65	33.18	11.61	20.47
	Market 3	3.65	4.28	1.43	35.45	12.19	22.18
	Market 4	1.66	3.50	2.23	34.68	12.68	22.22
Ludhiana	Market 1	3.43	2.40	2.46	32.18	14.26	23.39
	Market 2	3.43	3.63	0.82	30.45	13.67	20.79
	Market 3	Nd	1.26	2.26	33.37	12.37	19.62
	Market 4	1.67	4.06	2.49	30.59	12.61	20.67
Jalandhar	Market 1	5.42	4.27	0.67	36.89	15.69	24.04
	Market 2	3.43	2.53	0.89	31.32	13.35	20.23
	Market 3	5.59	3.56	0.77	34.16	11.42	23.86
	Market 4	5.89	4.11	1.50	35.24	11.13	23.53
	Average	**3.44**	**3.53**	**1.24**	**33.82**	**12.63**	**22.18**
	S.D	**1.64**	**0.83**	**0.81**	**2.70**	**1.14**	**1.77**
	Range	**0-5.89**	**1.26-4.51**	**0-2.49**	**30.45-40.31**	**11.13-15.69**	**19.45-24.99**

Appendix VII

Mean instrumental textural profile analysis of *doda burfi* from different cities

City		Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness
Karnal	Market 1	107.07	3.43	0.16	0.18	20.09	3.23
	Market 2	53.90	2.70	0.34	0.21	11.66	4.01
	Market 3	79.85	1.62	0.27	0.19	15.73	4.29
	Market 4	86.74	0.80	0.15	0.20	17.56	2.62
Delhi	Market 1	97.68	1.71	0.24	0.21	20.37	4.66
	Market 2	87.30	0.77	0.09	0.16	13.98	1.31
	Market 3	143.72	2.05	0.09	0.12	22.44	2.07
	Market 4	58.20	0.84	0.39	0.18	10.63	4.14
Ludhiana	Market 1	161.02	0.63	0.17	0.19	31.29	5.41
	Market 2	88.47	1.36	0.18	0.18	16.30	2.99
	Market 3	124.02	0.87	0.22	0.19	23.32	4.83
	Market 4	117.15	0.86	0.17	0.19	22.32	3.81
Jalandhar	Market 1	93.91	4.11	0.56	0.19	18.32	9.95
	Market 2	56.11	4.50	0.57	0.28	15.96	10.25
	Market 3	40.47	0.92	0.15	0.16	6.63	0.99
	Market 4	97.53	2.06	0.17	0.19	18.32	3.10
	Average	**93.32**	**1.82**	**0.24**	**0.19**	**17.80**	**4.22**
	S.D	**32.77**	**1.24**	**0.15**	**0.032**	**5.80**	**2.59**
	Range	**40.47-161.02**	**0.63-4.5**	**0.09-0.57**	**0.12-0.28**	**6.63-31.29**	**0.99-10.25**

Appendix-VIII

Dairy Technology Division
NATIONAL DAIRY RESEARCH INSTITUTE
Karnal (Haryana)

Score Card for Sensory Evaluation of *Doda Burfi*

Product particulars: ***Doda burfi*** **Date:** _________

Kindly evaluate the given samples of *Doda Burfi* for appearance, texture, sweetness, flavour and overall acceptability using the following 9-point hedonic scale and enter the score for each sample in the space provided in the below table.

Hedonic ratings	**Score**
Liked Extremely	9
Liked Very Much	8
Liked Moderately	7
Liked Slightly	6
Neither Liked nor Disliked	5
Disliked Slightly	4
Disliked Moderately	3
Disliked Very Much	2
Disliked Extremely	1

Sensory Attributes	***Sample Code***				
Color & Appearance					
Texture					
Sweetness					
Flavour					
Overall Acceptability					

Remarks (if any**):** _________________________ Signature: ______________

Appendix- IX

CONSUMER SURVEY OF DODA BURFI

You are requested to give your honest opinion about the product that will help us to decide its acceptability amongst randomly selected consumers across widely distributed population.

1. Name of the consumer ---------------------------------
2. Occupation/Designation ----------------------------------
3 Organization / Firm working with -------------------------------

4 Did you like this *doda burfi* Yes ___________ No ___________

If yes, degree of liking

Excellent --------------

Very good --------------.

Good --------------

How often you take *doda burfi*

Alternate day ………..

Weekly ………...

Fortnightly ………..

Rarely ………..

Never ………..

Wound you like to eat this *doda burfi* in place of conventional *doda burfi*, if it is available at comparable cost

Yes ……… No ………

Date:

Place: ______________________ Signature.