IN THE NAME OF ALLAH, THE MOST BENEFICENT, THE MOST MERCIFUL.

“Development, nutritional evaluation and shelf life optimization of Date-fructose biscuit bar”

By

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In

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UNIVERSITY OF AGRICULTURE

FAISALABAD

2016
DEDICATED TO

“Professor Dr. Salim-ur-Rehman
A dynamic supervisor
And
A great legend”
DECLARATION

I hereby declare that the contents of the thesis, title of the thesis are product of my own research and no part has been copied from any published source (except the references, standard mathematical or genetic models/ equations/ formulate/ protocols etc). I further declare that this work has not been submitted for award of any other diploma/ degree. The university may take action if the information provided is found inaccurate at any stage. (In case of any default the scholar will be proceeded against as per HEC plagiarism policy).

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This acknowledgement remains incomplete without paying homage to my late mother, for she laid the very foundation of whatever I achieved in my life. Infact, I owe my life and career to her. May her soul rest in eternal peace. Aameen!
Performa for Sensory Evaluation of date-biscuit bars

Name of the Panelist: ---------------------------------

Evaluation Date : ---------------------------------

Hedonic Scale:

1. Disliked extremely    2. Disliked very much    3. Disliked moderately

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ABSTRACT

The demand of biscuits is increasing day by day, due to change in life style of people and convenient foods with improved formulations are making their way to market shelves. Proximate composition of different materials such as dates, peanuts and vetch protein isolate are found suitable for the preparation of DFBB. Sucrose, the main sweetener for biscuits bars could be replaced with fruit sugars that are more digestible and sweet with fewer calories. Low grade dates were used to prepare date-fructose (DF) by using enzyme assays. DFBB with 15% sucrose replacement with date fructose was found the best in terms of physical and sensory characteristics. For improving the protein quality of date-fructose biscuit bars, protein isolates from cheaper and locally available Indian vetch (*Lathyrus sativus*) were incorporated (IVPI). Sensory parameters especially color, taste, flavor and overall acceptability were affected with the replacement of IVPI and DF in DBB at different levels. 15% IVPI 15% DF replacement into DFBB did not affect the physical and sensory qualities. For 90 days shelf life study, levels of natural antioxidant extracts obtained from peanut peel and *withania coagulans* were optimized by using Response Surface Methodology (RSM). Optimized levels; 0.21% peanut peel and 0.32% *withania coagulans* extracts were found to provide the highest response in DFBB without affecting sensory characteristics during storage. Calorific value, free fatty acids and peroxide value, minerals content were non-significantly affected, while amino acids profile was greatly improved in DFBB. Biochemical serum profile and protein quality of the DFBB were evaluated through Spargue-Dawley rats studies. Biological value of BFBB was improved as a result of added IVPI. Serum electrolytes were affected non-significantly among groups. Lipid profile was affected significantly within diet groups, while study seasons remained non-significant. ALP, Bi.T, ALT, AST and blood glucose were significantly found lower as compared to control. No effect was observed on the *in-vitro* protein (IVPD) and starch digestibilities (IVSD). All the bars were acceptable with good sensory characteristics but bars containing medium level (0.5% of optimized combined levels of extracts) of antioxidant extracts were maximally preferred. DFBB achieved 3.53±0.4Kcal/g calorific value. It was concluded that DFBB containing 15% Indian vetch protein isolate, 15% date fructose and 0.5% peanut peel and *withania coagulans* blended extracts were proved as the best biscuit bars having good sensory qualities and have 90 days shelf stability.
Chapter-1

INTRODUCTION

In bakery products, biscuits are one of the most popular snacks which are available in the market in various varieties at affordable prices and consumed at tea time by almost at all levels of population segments. Major ingredients for soft dough biscuit’s recipe are flour, sugar, fat and water, however, optional ingredients such as nuts, dried grapes and dates can be incorporated to enhance their nutritional value and consumer’s attraction. With the changing life style of a community, the demand of these snacks is boosting up with the passage of time and such convenient foods are making their way to market shelves. In present era, people are more conscious about their health and nutrition. In Pakistan, biscuits are consumed by children and people of all age groups as a popular snack at tea time as well as arranged functions such as marriage ceremonies, workshops, conferences etc. These are most commonly relished by school-going children, who require more quantities of protein per unit body weight than adults. Moreover, the school-going children prefer snacks as compared to regular meals. Development of food bars with different formulations like energy bars, granola bars, nutrition bar etc. is an effective approach because these contains all the main nutritional constituents in balanced amounts. Biscuit bars may be better option as an ideal snack because these are ready-to-eat, offer several advantages like good eating quality, palatability, compactness, convenience, shelf stability and are excellent carrier for fortification with an enhanced nutritional value for target groups of populace. These may be mostly safe from microbiological spoilage, exhibit longer shelf life and high energy density due to low moisture contents than cakes and breads (Wade, 1988; Ajila et al., 2008; Rehman et al., 2013).

School going children and old age people need nutritious and balanced diet for their body development, maintenance of health and immune system and replacement of tissues. These foods should be rich in protein, carbohydrates, fat, vitamins and minerals. The choice available for these groups to buy wholesome and nourishing foods is not sufficient. In the market junk foods of low quality are available such as samosas/pakora, burgers etc. Most of them are less nutritious and contained limited supply of nutrients. So, there is a dire need to conduct research for the development of multiple fortified food products which can fulfill the nutritional requirements of school-going children.
In Pakistan, wheat flour is mainly consumed as a staple diet for energy and protein purposes due to low cost and ample supply. It provides more than 60% protein and calories in the total dietary requirements. Chiefly, it is consumed in the form of chapattis and other flat breads such as naans, khameri rotis etc. It is also utilized for the special baking and production of numerous bakery products such as cakes, biscuits/cookies, doughnuts and crackers, commonly used as snacks. Pakistan has produced 24.231 thousand tons wheat in 2013 (GOP, 2013). Bakery products are an appropriate selection among value added food products, for the supplementation of Protein Isolates (PI) from non-conventional plant sources (Bakke and Vickers, 2007). Biscuit bars prepared from wheat flour alone have not contained good quality protein because there is a deficiency of certain essential amino acids such as lysine. Legumes are important sources of good quality dietary protein carrying good quantity of essential amino acids. A legume, Indian vetch (*Lathyrus sativus* L.) belongs to the family “Fabaceae” and is high in protein (28.70g/100g) and is one of the cheapest legumes rather least investigated source (Hanbury *et al*., 1999; Rehman *et al*., 2014b). Indian vetch protein (IVP) is also high in lysine content. Protein energy malnutrition and micro and macro nutrients deficiencies result in poor health and stunted growth, adults face reduced work efficiency and diseases like Kwashiorkor and Marasmus and learning disabilities in children (Powel, 2007). Economic Survey of Pakistan reported that protein deficiency is increasing at alarming rate in Pakistan like other developing countries. Thus, it is imperative that proper nourishment through balanced diets should be a necessary part of the children growth and healthy immune system for disease prevention (GOP, 2013).

Legume protein isolates (LPI) are recently being used as the ingredients primarily to increase nutritional quality and to provide a variety of functional properties, including desirable structure, texture, flavor, and color characteristics to bakery products. Other functional properties include hydration, dispersibility, solubility and swelling; surface active properties such as emulsification, foaming and adsorption including fat binding; rheological properties such as gelation and texturization, sensory and kinesthetic properties (Nakai and Powrie, 1981; Tang and Ma, 2009; Taherian *et al*., 2011). LPI can serve as a nutritional supplement because protein malnutrition is one of the major problems in the developing countries. Moreover, animal proteins are more expensive as compared to plant proteins and there are constraints to maintain their availability in required demands which has shifted the researchers to route their efforts towards
plant species especially for improving the nutritional status of the masses through good quality proteins (Arulbalachandran and Mullainathan, 2009; Butt and Batool, 2010). Matri (*Lathyrus sativus* L.) is also known as “Mattra” in Pakistan, is an annual legume, having common names like grass pea, chickling pea and Indian vetch (IV) in India, khesari dal in Bangladesh, San Lee Dow in China and Sabberi in Ethiopia. It is commonly grown for its grain, but also used as fodder or forage for animals. In Sindh Province, 60% of the crop is used as forage and out of the harvested portion, 60% is used for animal feed and 40% for human consumption (Khawaja, 1985; Rehman et al., 2014), for growing chicks (Rotter et al., 1991). The grains of IV contain some natural anti-nutritional factors, ODAP (beta-N-oxalyl-L-alpha, beta diaminoproplionic acid) and phytates which have the ability to chelate metals and form insoluble complexes and affect the biological availability of the nutrients. The ODAP ranged from 0.22-7.2g/kg of grass pea which could be depleted to the safer limits by heat treatments that may improve the biological value of its protein isolate (Seena et al., 2006). It can be detoxified to a safer limit and utilized in various bakery products for “nutrient enriched foods” (Rehman et al., 2006a).

In baked goods, sweeteners provide sweetness, texture, humectency (Salminen and Halikainen, 1990) and also enhance shelf stability of food products (Hett and Butterill, 1999). These also reduce/retard starch gelatinization and gluten development. Cane sugar (sucrose) is a main sweetener used in bakery products which provides condensed energy on digestion. It can be replaced with fruit sugars that can easily be digested and provided more sweetness with fewer calories. Dates (*Phoenix dactylifera* L.) are rich in carbohydrates in the form of fructose and glucose, which can easily be absorbed in the human body (Al-Farsi et al., 2005).

In Pakistan, date production was 557.28 thousand tones in the year 2011 (GOP, 2011) and ranked 7th in the top ten date producing countries. More than 150 date varieties are produced in Pakistan such as Dhakki, Aseel, Zahidi, Begum Jangi, Halavi and Khudravi. Their flesh is a good source of sugars (~81-88%, mainly fructose, glucose and sucrose), dietary fiber (~5-8.5%), and small amounts of protein, fat, ash and polyphenols (Al-Farsi et al., 2007; Nadeem et al., 2011).

Globally, prevalence of diabetes is boosting and in the developing countries, the higher level of increase has been recorded (Grol et al., 1997; Kuller, 1997). In 1999, Diabetic Association of Pakistan, had conducted a survey and found that 10% people were suffering
from prevalence of diabetes and same rate of impaired glucose tolerance (Samad-Shera, 1999). In world ranking of the diabetes, Pakistan is 8th and would rank 4th by the year 2015 with almost 14.3 million as per WHO estimates with the rising trend of diabetes. Morbidity and mortality associated with diabetic complications pose a huge burden and threat to the economy of the country (Lorenz et al, 1986; Murray and Lopez, 1996).

Fructose and sugar alcohols such as sorbitol and mannitol are used in special dietary foods as a bulking agent and humectant with sweet taste (Francis, 2000). Now-a-days, people are becoming more quality and health conscious. Their demand for sugar free products without compromising on calories is increasing progressively (Diffy and Anderson, 1998).

Dates are good in the diet of diabetic patients, because these contain invert sugar, which may be better utilized as source of energy by diabetic patients than glucose and sucrose. They provide about 180 Kcal/100 g calories. Date-fructose (DF) can provide more sweetness and energy along with minerals, low glycemic index, easy absorbance by the body and insulin independent make fructose attractive for diabetics. Date’s sugars can be converted into fructose by hydrolysis at low pH. Enzymatic treatments may be applied to extract maximum quantity of fructose from date paste (Al-Farsi et al., 2005; El-Sharnouby et al., 2009).

Quality of biscuits may be affected by oxidation process that oxidizes the fats into free fatty acids causing decrease in flavor, taste and nutritional value of the bakery products along with health concerns (Karren and Rosenthal, 1992). Generally, synthetic antioxidants are incorporated into fatty food formulations to overcome the problem of rancidity during storage. Mostly, BHA (Butylated hydroxyanisole) and BHT (Butylated hydroxytoluene) are added singly or in combined doses at commercial scale in the baking industry, but it has been reported that they might cause health risks for human consumption (Martinez-Tome et al., 2001).

In the last decade, substantial attention has been paid to natural phenolics antioxidants found in plant materials worldwide. Natural antioxidants present in foods have attracted considerable interest because of their presumed safety and therapeutical value that has led to the extraction of antioxidants from many species of fruits and vegetables and their utilization in the form of extracts in food products (Liyana-Pathirana and Shahidi, 2005). Date Pits are by-product of date processing and are a potential source of natural antioxidants like mango peels (Hussein et al., 1998). Antioxidants are the substances that prevent the oxidation of cellular oxidizable
substrates by scavenging action (Halliwell, 1996). These dietary antioxidants play an important role in this defensive mechanism (Besbes et al., 2004) and can be used in bakery products to improve the shelf stability and consumer acceptability (Giovanni, 1983; da Silva Pereira et al., 2014). Therefore, food scientists are more concerned to explore natural sources of antioxidants to replace the synthetic antioxidants in food formulations. Food processing agricultural waste materials like mango peels, date pits, peanut scales, apples pomace, citrus peels and pomegranate skins and other locally available cheaper sources are being used to extract natural antioxidants (Mandadi et al., 2009; Asimi et al., 2013; Sharma and Gujral, 2013; . In particular, such natural constituents are present in a number of plant extracts including peanut skin and Withania coagulans etc.

Earlier, application of natural antioxidants have been seen in the preservation of corn, cottonseed, olive, sunflower, peanut, rapeseed, soybean and fish oils by the addition of extracts from apple pomace, mango peel, pineapple pulpy waste and red beets (Schieber et al., 2001). Moreover, extraction of healthy ingredients from industrial food residues and their supplementation in food products is a possible route of their effective utilization.

To optimize the levels of ingredients and process conditions of food products for effective role, Response Surface Methodology (RSM) has been proved to be an effective tool (Martinez et al., 2004). RSM has been applied to optimize the levels of protein for the preparation of date bars and optimize composite flour for the production and enhanced stability of leavened flat bread (Naan) (Nadeem et al., 2012; Farooq et al., 2012).

Preparation of Indian vetch protein isolate (IVPI) and investigation of its functional and nutritional properties for the preparation in date fructose biscuit bars (DFBB) were the aims of these studied. Therefore, this project was designed to explore the effect of adding IVPI and replacing of sucrose (cane sugar) with date fructose (DF) on the physico-chemical, sensory and nutritional qualities of DFBB. Safety and biological evaluations of the DFBB were conducted by using Sprague Dawley rats. The present project is planned to explicit the following objectives.

- Detoxification of Indian Vetch (Iv) to a safer limit for preparation of protein isolate and to prepare biscuit bars with Iv protein isolates.
- To assess suitability of replacement of cane sugar with date fructose for the development of nutrient dense biscuit bar by applying physical, chemical and sensory tests.
• To evaluate the protein quality of vetch isolates supplemented biscuit bars by protein efficacy studies.

• To screen out natural antioxidants from food processing waste materials and their utilization for their activity and to optimize the antioxidant extract levels by using response surface methodology (RSM) for getting biscuit bars with better sensory acceptability and shelf life stability.
Chapter No 2

REVIEW OF LITERATURE

Biscuits are considered as an ideal vehicle for fortification programs due to less moisture, longer shelf life and worldwide consumer acceptance (Khattak et al., 2003; Sharif et al., 2009). These area cheap nutritional source of calories and protein and are used among the masses in disasters such as worse weather conditions, floods and earth quick areas. For the initiation of nutritional programs in those areas, biscuits may be considered better option, however, consumption for long term basis does not provide all the needs of essential amino acids, minerals and vitamins (Rehman et al., 2001: Akhtar et al., 2009).

2.1. Food bars

Food bars are snack foods of good sensory characteristics due to their constituents, contributing rich contents of proteins, lipids and carbohydrates. The popular varieties of food bars include nutrition, energy, nutraceutical and diet bars. These have huge growth in market potential around the globe; for instance, only in the United States, $1.6 billion cereal bar market is existed. One of the reasons for high demand is the consumer’s awareness of the health benefits of grains (Palazzolo, 2003). Chemical analysis reveals that cereal bars are marginally better than favorite traditional snacks on the basis of their sugar, fat, salt and fiber contents (Boustani and Mitchell, 1990). Food bars can be produced by applying various techniques such as extrusion is a foremost one of such technologies. A flour mixture of peanut and sorghum was used to develop extruded product in the form of wafers. Consumer acceptance for the final product was enhanced by using chocolate and peanut-butter paste as fillers for improving the nutrition of the product (Anderson and Jones, 1999). The development of food bars could be carried out through blending the grains, nuts and other ingredients along with some binding materials including gums, liquid glucose and sucrose etc. The mixture is then shaped into a bar by passing through a roller (Al-Hooti et al., 1997a) or baked in a baking oven at moderate heat i.e. below 150°C (Brisske et al., 2004).

The examination of sensory aspects of food bar snacks presents a picture of consumer attraction to the product. The children mostly like cereal bars having chocolate in their formulations but these bars may not be the better choice because their formulations are not nutritionally healthier than whole grain plain cereal bars (Boustani and Mitchell, 1990). Food
bars containing cereals in their formulations and added peanuts and walnuts have shown to be snack foods of good sensory characteristics and high calorific values. Protein content is higher in the bars having mesquite cotyledons. The thermal processing has no effect on their chemical composition. However, on the basis of sensory characteristics, bars with mesquite cotyledons treated by microwave have shown a higher acceptability (Escobar et al., 2000).

In another investigation, three different cereal bars were prepared with various amounts of oat, wheat germ, and puffed amaranth. During accelerated storage (37°C for 15 days), water activity and moisture content reached to low levels of 0.48 and 5.9%, in the bars, respectively. Peroxide values increased gradually up to 12 and 17 meq/kg at 15 days of storage (Escobar et al., 1994). In a separate study, four different formulations of cereal bars were prepared having microwave treated (6%) mesquite cotyledons and peanuts/walnuts (18%). The bars stored for 90 days at room temperature and evaluated for peroxides values (4.9-13.8 meq/kg of the sample oil) which showed that the product had good shelf stability (Escobar et al., 1998).

In another study, oats and wheat germ containing snack-type food bars were prepared. Natural sweeteners, fats were used in the manufacture by adding two different ratios of walnuts. Out of six bars, two bars contained addition of toasted amaranth, brown sugar cover and wheat germ, while next two had puffed amaranth. Then all the six bars were dried at 120°C for 45min. The bars with walnuts (18%) scored the highest in sensory attributes. Storage studies revealed that all bars showed similar decreasing trends towards moisture and water activity. Peroxide values were remained in the acceptable limits. The bars containing oats, walnuts and wheat germ showed higher protein levels and all the six bars had a good quantity of dietary fiber (Estévez et al., 1995).

In other study, granola bars were developed by using common black and red beans (Phaseolus vulgaris). On appearance basis, the red bean granola bars were more acceptable than the black bean products (Maurer et al., 2005). Five energy bars containing papaya, hazelnuts, almonds, apple and orange chunks showed good microbiological and sensory qualities. The energy value per 100g of the product was 520 Kcal. The shelf life of the product was observed at the temperatures ranging between 20 to 25°C and relative humidity at 55-60%. The results showed that it remained shelf stable for 60 days, whereas, neither rancid odor nor rancid flavor was detected in the product with added preservative and antioxidant. Moreover, at 120 days of storage, no unacceptable changes in flavor were detected (de Penna et al., 1992).
The sportsmen need high energy from protein sources. In a separate study, two candy bars with different formulations were prepared. Soy protein Isolates (SPI), texturized soy flour, cocoa powder, milk solids, oats (toasted), nuts, almonds, artificial flavors, antioxidants and preservatives were used in the preparation of soy-based chocolate coated candy bars especially for sportsmen to meet their higher protein energy needs. The nutritional composition obtained with this formulation included proteins (12.4%), carbohydrates (58.7%) and lipids (9%) energy value (375.2 Kcal per 100g) (de Penna et al., 1993).

Packaging materials play a crucial role in keeping the food bars shelf stable. The double layered polypropylene package film can effectively protect the quality of energy bars having fruit chunks (de Penna et al., 1992). The quality of soy-based candy bars packed in aluminum foil could be maintained for 30 days storage without affecting the quality attributes of the nut candies, and at almost double time, almond candy bars could be stored (de Penna et al., 1993). Similarly, the packaging materials maintained the cereal bars in stable conditions as the moisture content and water activities remained in safer limits during extended periods of shelf life studies. The bars remained acceptable from sensory point of view as well as physic-chemical characteristics (Escobar et al., 1998).

2.2. History and nutritional values of dates

Date palm or Khajoor tree (Phoenix dactylifera L.), a perennial monocot plant belongs to family Palmaceae and is known as an important food crop in many countries the world over (Al-Hooti et al., 1997b). In the Latin name (Phoenix dactylifera L.), where L is the abbreviation of Linnaeus (From the year 1707 to 1778) and the name was given by a Swedish botanist (Amer, 1994). Similarly, the Latin word Dactylifera, is derived from the Dactyllos (Greek word) meaning “Finger” due to its similarity in form. Due to richness in simple sugars and phenolic compounds, dates are considered a valuable source of natural antioxidants and low glycemic index sweetener that may have many health benefits for human beings (Besbes et al., 2004) and can be used in bakery products with enhanced nutritional quality and acceptability of consumers (Al-Hooti et al., 1997a; Ismail et al., 2006). These are also important in human nutrition because of their highly nutritious composition i.e. carbohydrates, dietary fiber, vitamins and minerals, fatty acids, amino acids and protein. It has been mentioned in the Glorious Qur’an (26:148) and Hadith that dates have a digestive effect on normal food and can affect starch digestion at pH 7.4 (Clarke, 1995). Dates are one of the old fruits, a major fruit crop in the Middle East countries. About 75
to 80% of the world production is produced in this region (Yousif et al., 1990). Pakistan is famous worldwide due to its annual production capacity and soil suitability for quality dates. In the year 2006-2007 the production yield was 496.6 thousand tons (GOP, 2007). Fleshy part of the date palm was detected in Egypt, in the earliest samples showed that it was used as beer sweetening in Ancient Egypt (Darby et al., 1977). Dates provide 3000 calories per kg of date fruit. The Hebrew’s drawings depicted the presence of date palm tree in one of their old paintings. They had sensational attachment with the tree and used the name for their girls as ‘Tamara’ derived from ‘Tamar’ stage of date fruit; hoping that their girls may show date palm tree like properties in their physiology (Tall, smart, fertile and pretty) (Qudama, 1985).

The date palm fruit (Phoenix dactylifera L) is an important fruit as it has a large number of applications in the daily life of the masses from very old times (Ahmed et al., 1995). Nutritionally, this fruits in fully ripe form (Tamar stage) has importance as it contains almost all the nutrients needed to survive. On dry weight basis, it contains different moisture levels (10% to 22%), sugars (62% to 75% total sugars including invert sugars and sucrose), some quantities of quality protein (2.2% to 2.7%), fat (0.4 to 0.7%), ample amounts of dietary fiber (5 to 8%), ash (3.5 to 4.2%), vitamin C (30.0 to 50.0 mg) and total acidity (0.06 to 0.20%) (FAO, 1992; El-Sharnouby et al., 2007). Developmental stages of date palm fruit can be divided into three important states on physiological and ripening basis (Khalal, Rutab and Tamar states). The extracted date syrup (debis) is the most accepted of all the derived products from date palm. Normally, people consume ripe dates as such without processing at the Rutab stage (Semi-ripe or Doka stage) and then on the Tamar (fully-ripe or Khajoor stage). But now-a-days the trend has been changed due to mutual cooperation between the food processing industries and the growers with an intention to provide value added products to the consumers (Al-Hooti et al., 1997a). The worldwide trend towards the production, processing, consumption followed by industrialization of dates has been increased at an elevated rate (FAOSTAT, 2004).

The date palm production rate is very high in Kingdom of Saudi Arabia (KSA) due to the reason that Muslims give great importance to the date fruit not only religiously but also nutritionally, especially during the Holy month of Ramadan, the significance of this fruit increases drastically (El-Behissy et al., 1998). Production and processing of dates in KSA has been greatly increasing in few years, and reached at 970,488 tons of annual production, and may
be associated with high consumption and demand of the consumers (Ministry of Agriculture, 2006).

Date fruit is nutritionally rich in vitamins including vitamin A, vitamin C, vitamin B₁ and B₂ and nicotinic acid (Ahmed et al., 1995). Different date varieties were studied by Yousif et al. (1976) and they grouped them into three types i.e., “soft”, “semi dry” and “dry”. Soft dates have high moisture content (>30%). Other types; semi dry and dry contain 20-30% and < 20%, moisture contents, respectively. Moisture changes in dates during three ripening stages were studied: Khalal (Immature stage having hard, yellow-red color appearance, with 30-45°Brix, flavor like astringent but edible), Rutab (fruit with the soft tip, 55-60°Brix, edible), Tamar stage (Fruit is completely ripe, with 60-84°Brix and fully edible). A study was conducted on eighteen varieties of Saudi date cultivars and it was revealed that dates contained 73.8% carbohydrates, 19.6% moisture, 2.31% ash and 2.3% proteins. Minerals present were iron (2.56-10.3 mg), potassium (833-894 mg), copper (2.54-5.68 mg), calcium (184-207 mg) and magnesium (56-60 mg), small amounts of sodium (5-16 mg) and phosphorous (13-16 mg) per 100 mg dry weight (Hussein et al., 1976).

Dried dates are rich in total phenolics and a valuable source of natural antioxidants (Al-Farsi et al., 2007). The quantity of total phenolics may vary among different varieties due to different agro-climatic conditions. Different pigments have been identified in dates like carotenoids, anthocyanins, flavones, flavonoles, lycopene, carotenes, flavoxanthin and leutin. Biglari et al. (2009) studied the phenolic compounds and flavonoid contents during storage of hard and soft dates at 4°C for six months. Then, these were stored at 18°C for seven days. Cluster analysis showed different behavior of flavonoids and phenolics in both types of dates.

Dates contain pits weighing about 13-15% by weight on an average of the whole fruit, and appear as a by-product during date processing. These are mainly used as feed for the ruminants as it contains proteins (5-6%) in crude form, fat (4-10%) as crude fiber (12-27%), ash (1-2%) and NFE (55-73%) (Hussein et al., 1998). These date pits were used in the diet of rats by to evaluate its effect on reproductive hormonal status. Results revealed that these feed formulations did not show any effects on the testosterone levels of rats but in the female rats, the oestradiol level reduced significantly indicating that it may cause reduced fertility in them.

2.3.1. Processing of dates and date products
Dates are utilized in the preparation of many date products. Date flesh minced in the form of date paste has been used as an alternative sweetener against sucrose in baked items and other formulations of foods (Alhamdan and Hassan 1999). Medically, calcium and fiber play a critical role in preventing heart diseases and colon cancer, and elemental fluorine helps to prevent tooth decay (Al-Shahib and Marshal, 2003). Dates contain invert sugar i.e., fructose that can be utilized as an energy source for diabetic patients (Awan and Sohail, 1999).

A blend of date syrup with sesame paste was studied as fat replacer by Razavi et al. (2008). The influence of these fat replacers with starch, guar gum, xanthan gum was observed at different replacement levels using different temperature ranges (at 25, 35, 45 and 55°C). At a temperature of 25-55°C, apparent viscosity was improved and the starch showed better results as compared to others gums (xanthan gum and guar gum).

2.3.1.1. Production of date syrup and its utilization

Sucrose extract in water obtained from date variety (Deglet Nour) was mixed with invertase enzyme to obtain high fructose syrup. It was incubated for 30hr to get concentrated fructose contents in the syrup and then utilized as a low cost sweetener in the food processing industry (Chaira et al, 2011). This syrup can also be crystallized through vacuum evaporation to get crystalline fructose for utilization in the pharmaceutical (as an instant energy source, strong sweetener, highly soluble, prevents crystallization, high osmotic pressure properties as compared to sucrose) as well as food industry applications (Kurup et al., 2005; Tomotani and Vitolo, 2006; Kotwal and Shankar, 2009).

Due to the good sensory properties, the date syrup is found as highly acceptable for use in food products as compared with other sources of syrup extraction which are in use like cane syrup which is also known as black honey(El-Sharnouby et al., 2009). Soft dates of Reziz variety were used to prepare date syrup using different water/date paste concentrations (2:1, 2.5:1, 3:1), pectinase and cellulose enzymes were used to obtain maximum extraction (83.51%)of total sugar content (on dry basis)as compared to control (50-56.30%) without using the enzymes (El-Sharnouby et al., 2009). Moreover, date fruit at tamar stage bears an ample quantity of sugars along with other important constituents like vitamins, pectin, cellulose, ash and water (FAO, 1992).

Date syrup was used in different levels(2%, 4%, 6%, 8% and 10%) with the concentration of sucrose (30g/L and 60 g/L) on the micro propagation. Its application to the
culture media (6% concentration) was used as sucrose replacer that is normally used for culture media of plants (in somatic embryogenesis) and as a replacement of sucrose with date sugars (Al-Khateeb, 2008).

Enzymes, \((\text{Pectinase and cellulose; 1.0\%})\) addition in the date paste was carried for maximum extraction of sugars (up to 80%) and blended with water (in ratio 3:1 paste/water: w/v) and incubated at 40°C for about 24 hrs. The syrup obtained was evaluated for sensory parameters and composition of the extract showed that the extract contained high quantity of sugars and minerals contents. It contained invert sugars (glucose and fructose) in almost equal quantities. So, the potential of syrup obtained enzymatically from Reziz variety of date fruits can be used as sucrose replacement in the food formulations (El-Sharnouby et al., 2009). Enzymatic extraction of a date variety from Iran (Kerman province) was evaluated in a study by Bahramian et al. (2006) and the samples were treated with enzymes and then compared with control samples without enzymes. The results showed that enzymes showed magnificent increase in production as well as clarity of the sugar extract as compared to control. About 46% increases in extraction rate was observed (Bahramian et al., 2011).

Both glucose and fructose are monosaccharide carbohydrates with molecular formula \(\text{C}_6\text{H}_{12}\text{O}_6\). They differ in their molecular structure which makes them isomers. Food and beverage manufacturers often convert glucose to fructose to get ‘Sweeter’ taste. Date syrup was found a good source of macro-elements like Ca, P, K, Mg but significantly low in Na (Al-Hooti et al., 2002).

For the production of low caloric cake for diabetic and health conscious consumers, date syrup containing fructose and sorbitol was used replacing sucrose and its effect on quality of cakes was examined. Date syrup and sucrose was used in five different proportions in preparation of cakes. Evaluation of these cakes on sensory parameters was carried out to find out consumer acceptability. The sensory characteristics i.e. cells, grains, crumb color, texture, flavor and taste etc. of cakes were best when sucrose was replaced with date syrup in the proportion of 50%. There was a declining trend with the passage of time in the sensory characteristics of cakes. The moisture, protein, ash, fiber, fat and NFE contents of cakes ranged from 24.0-26.8%, 7.6-8.1%, 0.8-1.1%, 0.5-0.9%, 31.0-31.1% and 35.8-31.8, respectively (Tufail et al., 2002).

2.3.1.2. Production of date paste and its utilization
Commercially available date products such as date butter, date jam, date jellies and date pickle are being used as appetizer (Sawaya et al., 1983). Dates are used for the preparation of biscuits, date sugar, buns, cakes, date juice and vinegar (Jagirdar, 1998). The physico-chemical properties of two date varieties (Khalas and Barhee) were studied for storage conditions important for industry and commercial needs at different temperature (-3°C and 25°C) for 2 months and 1 year. Analysis of proteins, sugars, minerals contents etc. were carried out. The results obtained at similar conditions were different as both varieties responded differently in similar storage atmosphere. The best results were found at 2 months (-3°C) for Khalas and 1 year (-3°C) for Barhee (Ismail et al., 2008). Date paste can be used to replace refined sucrose in the food formulations. It can also be replaced with different alternative sweeteners like raw cane sugar, honey, plant syrups, molasses, and fruit sugars (fructose) like date sugar replace (Yousif et al., 1987). These sugars are unrefined contrary to sucrose (refined sugar) contain appreciable amounts of antioxidants (Phillips and Carlsen, 2009). Moreover, incorporation of date paste in the preparation of bread and biscuits can replace sucrose and improves their nutritional status (Hoseney et al., 1986). For the preparation of date paste, raw dates were processed through steaming, de-stoning procedures, then, these were minced to form a paste having about 20-23% moisture with about 0.6% or less water activity. Dairy products such as yoghurt can be prepared by adding date paste and depis in the cultured milk. Addition of 10-20% paste with or without 5% depis did not affect the yogurt pH, protein and fat, while pH was decreased and total solids increased significantly during storage. Candy sugar was replaced with date paste in order to determine its nutritional value, processing requirements and sensory properties which showed good acceptability, improved nutrition and storage up to 5 months without affecting the qualities of the candies (Ahmad et al., 2005).

2.4. Role of sugars in bakery products and in human system

Sugars carry significant position in bakery products because of imparting sweet taste, improving texture and crust color with extended shelf-life. Desired properties can be achieved in the product by adding the different kind of sweeteners. Granulated form of sucrose is mainly used in the biscuit formulations. Other natural sources of sugars like honey, molasses, corn syrup, invert sugars, high fructose corn syrup (HFCS) and glucose syrups are also being utilized
but to a less extent. Sugar granule size is very important, as more spread factor of biscuits can be achieved by using coarse sugar grains that imparts better texture and good eating quality. Dense texture is produced in biscuits due to finely granulated sugar because proper aeration of the batter is not achieved during mixing stage of biscuit preparation, and the resultant product shows poor eating quality. Biscuits show moist or soggy appearance and texture when sugars other than sucrose are used that can reduce the sweetness and also impart darker color to the product (James, 2009).

In another study, sucrose was replaced in cookies with fructose, mannitol and sorbitol and studied for shelf life studies for 60 days. Treatment containing 50% sucrose + 50% fructose was accepted at higher score by the judges. Replacement of table sugar at 50% with the fructose improved the cookie width up to 26.4 cm while treatment with 70% fructose + 15% sorbitol + 15% mannitol obtained maximum spread factor (41.5 cm). Moisture changed significantly while non-significant behavior was observed in proteins, fat, fiber, ash and NFE values during storage. Calorific value decreased with dietetic sweetener addition scoring 4.4 Kcal/g in treatment containing 100% sucrose and 3.72 Kcal/g intreatment having fructose 75% + sucrose 25%. Acceptability of cookies was appropriate after 60 days of the storage period (Pasha et al., 2002).

Glycemic index (GI) is a tool to measure the effect of food being swallowed on the glucose levels of the blood stream. Different foods have different properties depending upon type of sugars. If low GI rate food is eaten, it will slow down the digestion and then absorption processes of sugars, resulting gradual infusion of sugars in the blood system. On the other hand, higher rated foods will cause quick rise of blood glucose levels that would in turn stimulate the pancreas to release the insulin more efficiently and quickly to lower down the raised blood glucose level. These rapid fluctuations of blood glucose in drastic manner would not only affect the pancreas but also disturbs the psychological behavior of the people. Insulin production at higher rates affects the production and release of the growth hormones by depressing the immunity. Vitamin C and glucose have similar chemical structure; due to this reason when blood glucose levels rise, glucose and vitamin C compete for chance of entering in the body cells. The entry of vitamin C is suppressed, when high level of glucose in the blood is prevailed and affects the immune system of the body. A clinical study revealed that blood glucose raised up to 120 lowers the body’s phagocytic activity up to 75% which is the strong evidence of weakened immune system (Digirolamo, 1994).
2.4.1. Role of fructose as sucrose replacer in food products

Fructose is a sweetest natural sugar and its sweetness index is 1.3-2.0 times more than that of sucrose. During carbohydrate metabolism, disorder known as HFI-Hereditary fructose intolerance is observed by birth through autosomal form of recessive genes. With this reason, body fails to metabolize fructose and some other sugars. Research carried out on Switzerland people showed greater prevalence of this disorder (1 in about 20,000 births) as compared to other parts of the world. Dietary factors as well as hereditary traits play a role in the development of the disease in humans. Infants showing clear symptoms to the disease are mostly become habitual to unhealthy food stuffs and with that reason, they are able to survive to the adult age and may prove to be fatal due to this disorder. Diet plan excluding fructose should be strictly followed by the affectees to lead a healthy life (Mock et al., 1983).

In a study, fructose was separated from a sugar mixture (fructose, glucose, sucrose) extracted from dates. Vacuum drying of the sample yielded 86.5% total solids. Chromatographic method was used to separate fructose by taking date syrup at the levels of 20%, 30% and 40% on weight basis. Fractions obtained were analyzed after collection (glucose-rich, return fraction, and fructose-rich fraction) fructose separated at 70°C with a flow rate of 0.025 bed volume/min (Al-Eid, 2006).

2.5. Protein-energy malnutrition and its effects on community

Proteins carry vital importance in the normal diet of an individual. In Pakistan, where masses are not getting appropriate nutrients are facing protein-energy malnutrition (PEM), which is a serious and alarming health issue of the country, like other developing countries of the world where PEM rate exceeds 35% of children deaths at early ages (Nahar et al., 2010). Previous literature shows that severely-underweight children under five years of age had more than eight-fold increase in mortality (Black et al., 2008). It is observed frequently in the developing nations but it has also been reported as being increased in Unites States with the symptoms of chronical illness in children and frequent hospitalization (Hendricks, 1995). The effects of fluctuations in environmental conditions also influence the malnutrition because this problem is multi-factorial. When the environmental conditions are poor, they cause infections and enhance the environmental influence in the availability of nutrients. In case of severe and complicated malnutrition, the role of bacterial infection has been reported because they weaken the immune system (Aiken et al., 2011). Burden of population is another reason of poor nutritional status of
people in developing countries that in turn affects the health of the community causing bad impacts on economic and social growth of a country. The result of improper food availability, the nation of Pakistan is estimated to face growth hurdles in growing children as 40 to 50% of growing children of age less than five are stunted and 12% are severely underweight (Pelletier et al., 1994). The malnutrition affects are more adverse as it also causes the fatal diseases to attack through minor infections leading chronic illnesses. Malnourished children undergo negative changes in their behavior, including irritability and unavailability of nutrition at growing age affects the school performance of a child leading to low intellectual abilities and outputs of the school going children (Black et al., 2008).

Cereals have ability to fulfill the overall energy needs of the masses. Among the Pakistani people, wheat fulfills more than 60% of the protein and energy needs of the people as chapatti made from wheat flour, is a staple food of the people, but it is deficient in an important amino acid (Lysine), making it inferior in respect of protein quality. So, cereal based diets are deficient in lysine and tryptophan, and are unable to provide healthier amino acid profile. Thus, some other protein sources should be incorporated in cereal products to improve their amino acid profile (Onwulata et al., 2006).

Malnutrition due to protein deficiency is a dilemma among the masses, whose diet is generally based on cereals or other starchy foods (Barker 2002; Reilly 2002). Feeding on protein-deficient diets can lead towards many disorders such as breast cancer, colon cancer, heart disease and osteoporosis (Alam et al., 2003; Bhan et al., 2003). Therefore, the utilization of protein-enriched diet is important to fight against infections and diseases as it facilitates production of antibodies to activate human immune system (Friedman, 1996; Alexander et al., 1998). Expensive and limited supply of animal protein have geared the present-day research efforts towards the evaluation and utilization of inexpensive locally available protein sources such as protein-rich crops of oilseeds and the legume crops (Enujiugha and Ayodele-Oni., 2003).

Legumes are renowned as important sources of food and feed proteins. In many countries, legumes are used for food as well as for feed purposes; seeds are considered as the distinguish source of protein in the diet (Marcello and Cristina 1997). Moreover, consumption of legumes is also associated with reduction in pro-inflammatory status and improvement in some metabolic features (Hermsdorff et al., 2011). The incorporation of protein-rich legume flour such
as gram flour in bakery products like bread and biscuits can attain the goal of protein enrichment (Patel and Rao, 1996; Singh et al., 1996; Gandhi et al., 2001). Among processed bakery items, biscuits grasp huge acceptance and preference in urban and backward areas amongst both the communities (Agrawal, 1990). The high nutritive protein-enriched cookies were formulated using composite flour of wheat with soy bean and other energy rich plant sources like cottonseed, peanut, pulses, corn germ flour and mustard seed flour (Tsen et al. 2006). The challenge of selecting the best-suited protein source has geared the food processing and baking industry to explore such ingredients that provide the desired nutritional and functional characteristics to the baked products (Tyagi et al., 2006).

2.5.1. Legumes, their protein quality and substitution in bakery products

Protein carry a significant position in our energy sources and are vital for human growth and maintenance due to diversity of functions carried out in the human body by providing building blocks as amino acids. Acquiring of good quality protein is another issue as the animal proteins which have healthier amino acid profile as compared to plant sources are expensive and scarce, thus are becoming unreachable by the people living under the poverty line. This segment of the population is vulnerable to malnutrition (Niiya et al., 2007).

Although, most of the vegetable proteins are of inferior quality than animal proteins but legumes are good sources of proteins, vitamins and minerals (Siddique, 2000; Singh and Misra, 1985). Protein sources of plant origin specially legumes are considered as an important source of dietary protein worldwide and provide healthy nutrition at low cost as compared to animal origin protein sources. Experiments were conducted on Vignaradiata (mung bean) and Vignamungo (mash bean) to determine the proximate composition. The protein isolates from V. radiate and V. mungo were prepared and their functional properties (foaming, nitrogen solubility index and SDS gel electrophoresis) were also analyzed. Results show that they have high protein content and play significant role in human nutrition. This research concluded that V. radiate has high percentage of moisture (9.74 ± 0.19), fat (1.35 ± 0.048) and protein content (22.5 ± 0.24) as compared to V. mungo (7.9 ± 0.06, 1.01 ± 0.01, 21.3 ± 0.24, respectively). Moreover, 54 and 33% of protein isolates were made from V. radiate and V. mungo, respectively. The functional properties analysis enhances their acceptability in food industry (Shaheen et al., 2012). Legumes are considered important sources for supplementations done with cereal based baking formulations which are traditionally being used worldwide. These efforts have provided
innovations and imparted value addition to the products such as biscuits and bread formulations (Patel and Rao, 1995). Supplementation of wheat flour with legume flour has been studied by several research workers to enhance protein quality of cereal with the addition of Lysine. Wheat flour was fortified with other protein-rich materials for improving its nutritional and functional properties. Oilseed meals and gram flours are rich in proteins and can be used in bakery products (Harden, 1974; Rajput et al., 1988) such as gram supplemented biscuits (Yousaf et al., 2012; Passoset al., 2013) and wheat biscuits fortified with defatted Macrotermessub hyalinus (Niaba et al., 2013).

Due to high price of meat and Fish, legumes are preferred as a protein source in developing countries, but the only reason for their restricted use is the presence of anti-nutritional factors including polyphenols, phytic acid, and enzyme inhibitors etc (Lajolo et al., 1991), which can be removed by simple processing methods. Although, most of the vegetable proteins are of inferior quality than animal proteins but legumes are good sources of proteins vitamins and minerals. Cow peas, green grams and black grams contained appreciable amounts of lysine (6.40-7.68g), cystine (0.89-1.31g), methionine (1. 08-1.23g), total S-amino acids (2.02-2.55g/100g) (Siddique, 2000; Singh and Misra, 1985).

Wheat flour substitution for biscuit preparation was done with dehulled pigeon pea (Cajanus cajan L) flour and pigeon pea by-product flour having high protein contents (29.42 g/100g) compared withdehulled pigeon pea flour (24.67g/100g). Wheat flour was replaced at different levels to obtain composite flour. Nutritional value was increased as the protein values rose from 1.3 and 1.4 times, respectively than control. Fiber content increased significantly at a substitution level of 85:15 of dehulled pigeon pea or 90:10 of pigeon pea by-product flour with the acceptable sensory attributes of the prepared biscuits (Tiwari et al., 2011).

In another study on the physicochemical and sensory evaluation of cookies, wheat flour was replaced at different levels and the product was found acceptable at 10% replacement with pumpkin flour. This was done to increase the nutritional quality of the product with improved protein quantity.

2.5.2. Indian vetch as protein source

In Italy, Indian vetch (Lathyrus sativus L.) has been extensively cultivated by organic farmers due to its hardy nature (Mera et al., 2000). In Polish register, it has been reintroduced as an agricultural crop in 1997 (Milczak et al., 2001). It is a popular crop in the famine hit areas of
the world i.e. Ethiopia and Afghanistan (Praveen et al., 1994). Indian vetch (*Lathyrus sativus* L) is commonly known as “Grass pea” or “Chickling pea” and “Matri”. It belongs to the family “Fabaceae”. In Pakistan, it is being cultivated in rain-fed or drought areas, having very low or nil agronomic practices required, as it is adapted to grow under harsh environmental regions of India (Hanbury et al., 1999).

Indian vetch has high contents of good quality proteins (28.70g/100g) and lysine content. Proximate composition of vetch seed per 100g indicates moisture 10g, protein 25g, fat 1g, total carbohydrates 61g, fiber 15g, ash 3g, calcium 110 mg, Iron 5.6, vitamin A 70 I.U, thiamine 0.1000mg, riboflavin 0.40mg (Kay, 1979) but it contains nerve affecting toxic derivative of amino acid (β-N-oxalyl-L-α, β-diamino propionic acid) and limits its uses in food products causing *neurolathyrism* if its consumption is prolonged in raw form in human and animals (Siddique et al., 1996). In other study, it was found that Matri contains moisture 7.5-8.2%, starch 48.0-52.3%, protein 25.6-28.4%, acid detergent fiber 4.3-7.3%, ash 2.9-4.6%, fat 0.58-0.84, calcium 0.07-0.12mg/kg, phosphorus 0.37-0.49mg/kg, lysine 18.4-20.4mg/kg, threonine 10.2-11.5mg/kg, methionine 2.5-2.8mg/kg and cysteine 3.8-4.3mg/kg (Rotter et al., 1991).

The presence of the neurotoxin, β-ODAP (β-N-oxalyl-L-α, β-diaminopropionic acid) which is also named as β-N-oxalyl-α-amino-L-alanine (BOAA), is a major hazard to the utilization of Indian vetch (Kuo et al., 1998). This toxin can cause irreversible paralysis on consumption in large quantities for longer time such as over three months (Spencer et al., 1993). Lab experiments show the symptoms appear in lab. animals (chicks, rodents, horses and other species) due to the neurolathyrysm. However, these do not appear in humans (Roy and Spencer, 1989; Bellido, 1994; Hanbury et al., 2000). The primates feeding on Indian vetch (*Lathyrus sativus* L) for extended periods may develop central motor pathway and hind limb problems which are in resemblance with the symptoms appear in the humans (Spencer et al., 1986). Moreover, heavy consumption of grass pea (Indian vetch) for several weeks or months by animals may cause *lathyrism* resulting muscle cramps, heavy legs, weakness and loss of movement with legs, but the case of severity appears if prolonged consumption without any other dietary additions is done (Haque et al., 1996).

However, *L. sativus* does not affect all species so badly, many species of animals can tolerate this toxin very easily such as sheep, cattle and other species of ruminants can withstand up to 70% *lathyrus* containing 0.09% of the toxin (β-ODAP); perhaps its break down to other
non-toxic compounds in the rumen (Hanbury et al., 2000). Similarly, *L. sativus* level up to 30% when fed to the calves in their daily diet for a long time did not show any symptoms of the disease (Dhiman et al., 1983). However, some pathological changes were noted in liver and kidney cells of the ruminant animal (Chen et al., 1992).

*Neurolathyrism* affected people in the Bangladesh who were consuming green parts of grass pea were investigated for the disease, and found that most of them have the problem of bone pain (12% male patients of age 30-37 years) and showed the symptoms like *osteolathyrism* (skeletal deformity). Two patients were found suffering from failure in fusion of vertebral and iliac epiphyses, when examined through x-rays (Haq et al., 1996). Another investigation regarding the usage of grass pea in food applications was done in the rural Ethiopia. Five-hundred subjects had taken part in this investigation. The target population was served with the gravy (Shiro) prepared from grass pea (91.6% of the total experimental population), boiled grass pea seeds (Nifiro) were fed to 86% population, and roasted (Kollo) to 56.4% of the selected people for about four months. The results showed that a significant interaction between the chances of *neurolathyrism* and consumption of the boiled or roasted grass pea were present, but the population served with the gravy form of grass pea showed no symptoms of the disease (Getahun et al., 2002; Ghanem et al., 2008).

Effective utilization of legumes for improving the nutritional status of the food products requires removal of the unwanted components. For this purpose, the easy, inexpensive and simple techniques could be used such as soaking, fermentation, cooking, and germination to deplete toxic factors. Sometimes, when one technique does not work, then more than one procedure becomes necessary to achieve maximum removal of the toxins. Usually soaking technique is a prior step in cooking of legumes that not only removes the toxin factors but also improves the palatability and aroma of the seeds (Ibrahim et al., 2002; Rehman et al., 2014b). Moreover, β-ODAP is soluble in water, so it could easily be eliminated by leaching from the seed during soaking in plenty of water (Akalu et al., 1998). The process of steeping the seeds of vetch in the cold water (about 3 min) removed less amount of β-ODAP (up to 30%), whereas, with the application of hot water, the results were even higher (Tekele-Haimanot et al., 1993). Toxin removal from husk-free seeds in this manner was 70–80% (Mohan et al., 1966).

Grass pea can safely be utilized up to 50% in whole wheat flour biscuits without posing any deleterious effects on rats (Rehman et al., 1997). In another study, toxin removal techniques
like soaking, steeping, boiling in hot water, germination and other chemical treatments using different chemical were applied and then the toxin was quantified by using thin layer chromatography and spectrophotometric techniques. Chapatti prepared from these samples showed that by using steeping method (soaking in water at 60-70 °C for 8 hrs with seven rinses) was most acceptable. Protein level increased from 124-174g/kg (300g/kg supplementation). Biological evaluation of the product using albino rats showed improved nutritional status of chapatties (Rehman et al., 2006a). In a study, nutritional profile and bioactive properties of C. arietinum and L. sativus pulses were investigated and valorized their traditional consumption and the use in modern diets. The result revealed that process of cooking decreased protein, ash, sugars and organic acids, and increased carbohydrates, fat, tocopherols, bioactive compounds and antioxidant activity while soaking did not significantly influence macronutrients and no differences were obtained in fatty acid composition (Sarmento et al., 2015).

Because of the survival of the crop in adverse environmental conditions and ease in growing with almost no managerial practices, this crop is still known as the survival food for poor populations mainly in Asia and Africa (Tadesse et al., 2003). Another positive aspect is that many varieties of Lathyrus are produced with low levels of the toxin by the crop breeders and genetic engineers (Campbell et al., 1994). Moreover, environment has influence on the β-ODAP content of the crop (Chen et al., 1992; Dixit et al., 1997; Piergiovanni et al., 2011).

In Pakistan, Indian vetch is the cheapest legume and usually its splits are mixed as an adulterant into gram’s splits. Gram (Cicer arietinum) flour adulteration with vetch flour is known which is used to produce certain savory products popular in subcontinent such as pakora. Lathyrus grains are used to prepare daal (curry) in Nepal. The grains are dried, cleaned and split slightly in to pieces by stone grinder (milled) to cook daal that is eaten with the cooked rice. The flour prepared after milling is utilized in pancake-like food formulations (pakora, badi local names of the fried food eaten by local people) (Rehman et al., 2007).

Indian vetch is one of the richest sources of protein among legumes and carries 25-31% protein. Mixing grass pea with cereals is a way of improving the amino acids profile of the final product, as they are complementary in their amino acid compositions (Cheftel et al., 1996). Indian vetch, due to its good quality protein has been recognized as a genuine candidate for protein quality improvement of cereal foods through cereal-legume complementation (Rehman et al., 2014). Its potential usage has been explored in different food products such as biscuits as
described previously (Rehman et al., 1997) and in bread (Lodhi et al., 2003). Indian vetch was used for the preparation of protein rich milk powders (Rehman et al., 2006a), Ice cream (Rehman et al., 2004), addition in chapatti (Rehman et al., 2006b), doughnuts (Rehman et al., 2007a), and milk blends (Rehman et al., 2007b). Bread was prepared by using matri milk powder at 1, 2 and 3% of supplementation and stored for 96 hrs. The bread with 2% level of supplementation was found best regarding sensory characteristics (Rehman et al., 2006c).

2.5.3. Legume protein isolates

Protein isolates are generally extracted by commonly used method called Isoelectric Precipitation (IEP) method and the protein isolates of powdered legume sample are precipitated from the solution on a specific pH. The legume protein supplies ample amount of essential amino acids when incorporated with cereal and other food formulations (Boye et al., 2010a, b). In a study, protein isolate was prepared from defatted flour of *Brassica carinata* through a process carried out at low pH. The resulting protein isolate was analyzed for the chemical and functional attributes. Higher level of protein content (more than 90%) with a good amino acid profile (meeting FAO standards) was found in isolate except lysine. About 90% anti-nutritional components were also reduced by the extraction process (Pedroche et al., 2004).

The interest has been growing in the utilization of legumes in other forms (Flour, concentrate, isolates) rather than the whole seed (Saio, 2000). In order to bridge the ever increasing gap between the human population and protein supply, researchers have been working to explore new protein crops to meet the human needs (Siddhuraju et. al., 1996), as nutritional supplements and a functional food ingredient (Onweluzo et al., 1994). Protein isolates are used in food formulations to obtain some essential amino acids deficient in the diets of inhabitants. Addition of isolates from soy protein and dry skimmed milk of varying proportions, during the production of fortified date bars, resulted in an increase in the level of protein, ash, fiber, fat, Na, K, Ca, P, Zn and essential amino acids(Sawaya et al., 1983).The results showed increased levels of both quality and quantity of protein in date bars, and gluten-soy protein blended cookies(Singh and Mohamed, 2007) and no deleterious effects on overall acceptability were seen. Addition of fruits, cereals and skimmed milk powder along with inexpensive nutrients to the date bars, provided substantial amount of protein, fat, B vitamins and minerals (Kamel and Kramer, 1977; Al-Hooti et al., 1997a). Cereal bars were found stable at room temperature when
studied for 90 days storage period, containing oven-treated mesquite cotyledons (6%) and walnut (18%) (Escobar et al., 1998).

Bioavailability of whey protein isolates increases with enzymatic and thermal treatments and their hydrolysate have shown positive effect in reducing arterial blood pressure (da Costa et al., 2007). Legume proteins can be obtained at 92% extraction rate, having protein content up to 72-75% in their isolates. In a study, chocolate coated candy bars containing soy proteins isolate, soy flour (texturized), milk solids, cocoa powder, oats (toasted), various nuts and antioxidants were prepared for sportsmen to meet their high protein intake needs. These bars contributed 12.4% proteins, 9% fat, 58.7% carbohydrates and 375.2 Kcal/100g energy (de Penna et al., 1993).

In another study, it was found that wheat supplementation with legume protein isolates resulted in energy and nutrition rich food with 73.5% NPU and 3.0 PER values, compared with the control diet having 68.2 NPU and 2.8 PER values (Baskaran and Bhattacharaya, 2004). The potential for mucuna bean protein isolate (MBPI) application as functional ingredient in foods is unknown. In this study, nutritional quality and physicochemical properties of MBPI were investigated. Bean samples were processed for L-dopa extraction in distilled water adjusted to pH 3.2 at 60°C for 48hrs. MBPI was extracted at pH 9.0 and precipitated by isoelectric precipitation at pH 4.5. MBPI from raw and processed seed contained higher protein content (86.7 and 86.9%, respectively) than soybean protein isolate (82.7%). Essential amino acids content of MBPI met FAO/WHO scoring pattern for 2-5 year-old children. SDS-PAGE revealed four main polypeptide protein subunits of apparent MW of 11, 19, 36 and 98 kD in MBPI. A high stability of foam, activity and stability of emulsion, in comparison with soybean protein isolate were exhibited by MBPI. However, poor foam expansion, capacity to absorb water and oil and dark color (Hunter lab “L” value of 36.39) has a negative impact on its prospect as an FFI (functional food ingredient) (Mugendi et al., 2010). In another study, properties of legume seed storage proteins have been determined for their functional properties as purified proteins or as protein isolate (Gueguen and Cerletti, 1994). Soybean protein is widely used in many protein-rich foods as functional and nutritional component, (Gandhi, 2009). In an investigation, functional attributes of commercial as well as membrane processed yellow pea protein isolates (YPPI) were evaluated. Protein isolates were extracted with water and KCl at 25 °C followed by ultra-filtration and dia-filtration at pHs of 7.5 and 7.5/6, respectively from yellow pea flour.
Comparatively superior functional property such as solubility and 28% to 68% reduction in phytic acid in membrane purified proteins was noticed, whereas the lowest foaming stability and apparent viscosity were recorded in commercial YPPI (Taherian, et al. 2011).

2.6. Natural antioxidants, their effects and applications

Chemically, antioxidants have the property to slow down the reaction sequence of a free radical which promotes the oxidation of the lipids. Different chemical compounds, such as tocopherols, phenolic, carotenoids, amino acids, peptides, phytates, protein hydrolysates, phospholipids enzymes and vitamins may be called as natural antioxidants. The most important groups of natural antioxidants include tocopherols, phenolic acids and flavonoids. Antioxidant compounds are easily absorbed by human body in small amounts and pass through metabolic system of a body. Their availability to the biological processes depends on the dietary sources. The antioxidants present in the foods are important for both preserving and preventing deterioration of food product that can be oxidized. The antioxidants found in plants and wheat grain fractions such as bran have a protective effect against diseases caused by Oxidative Stress (OS). (Carlsen et al., 2010; Wang et al., 2010; Khan et al., 2013). The phenolics of plant extracts have been found to be directly linked with antioxidant activity in poultry meat (Ajila et al., 2007; Abdul-Rehman, 2012).

Our liver is an extremely complex organ which has an effect on every physiological process of a body. The liver protects our body from various injurious substances and toxic metabolic by-products, absorbed via intestine like Xenobiotics (Sahu, 2007). Natural antioxidants rich foods are recommended for prevention and cure of liver damage (Morisco et al., 2008) such as pomegranate peel powder (PPP), whey powder (WP) alone or in combination (PPWP) significantly reduced the levels of ALT and AST, compared with control group in liver-damaged rats. Pomegranate peel powder was effective than whey powder, however, the combined treatment was found to be more effective than the single treatment. So, the mixture of pomegranate peel powder and whey powder may be more useful natural antioxidant (Hamad et al., 2011; Hamadi, 2012; da Silva Pereira et al., 2014). Citrus bioactive compounds were also found to improve the bone quality and plasma antioxidant activity of orchidectomized rats (Mandadi et al., 2009).

The polyphenols are important class of phytochemicals that act as antioxidants in food. In a study, turmeric, betel leaves, clove, lemon-grass and G. atriviridis were found to extend the
shef life of the butter cake up to 4 weeks, while, with the addition of BHA, the storage life was extended for only 3 weeks. Antioxidative properties of turmeric and the betel leaves were proved as a very effective tool for food preservation in comparison of synthetic antioxidants BHA/BHT. Anisidin value of the prepared cakes with G. Atriviridis resembled to control cakes but the peroxide values remained low during storage that showed a good potential of G. atriviridisas an antioxidant. Moreover, oxidation of vegetable oils during storage or frying can be lowered by adding natural antioxidants and the effects of antioxidants prove to be effective in inhibition of lipid oxidation in meat and biscuits. (Lean and Mohamed, 1999; Abdul-Rehman, 2012).

The polyphenols can also provide protection against some common health problems. These compounds are effective in protecting the body cells from the damage induced by the free radicals present in the cells. When cholesterol (LDL) is oxidized, it may attach with the arteries causing coronary heart diseases. Moreover, the chemicals and enzymes that are known to promote cancer are also deactivated with the action of polyphenols (Adams et al., 2006). The investigations on the extent of assessment of absorption of the phenolics by consuming the beverages and dried extracts of these natural compounds were carried out by using different procedures. Mainly, DPPH (2,2-diphenyl-1-picrylhydrazyl) method is employed to determine antioxidant activity. Free radical scavenging method (FRSM) and β-carotene bleaching test (BCBT) are also employed depending on the experimental preferences. The DPPH method was proved very convenient, rapid, simple, sample polarity independent, for the quick extraction and screening of the extracts being evaluated for radical scavenging activity as compared to other methods (Koleva et al., 2002). Fruit and vegetable wastes contain ample amounts of antioxidant compounds such as carotenoids, ascorbic acid and flavonoids which could be utilized for extraction and preventing the disease because these natural antioxidants could significantly be absorbed in animals well as humans systems (Lampe, 1999; Knekt et al., 2002; Huxley and Neil, 2003).

2.6.1. Antioxidant extraction from Paneerdodi (Withania coagulans)

The genus Withania belongs to family Solanaceae and has more than 23 species, of which Withania coagulans and Withania somniferaare well known due to their medicinal properties. Withania coagulansis a rigid herb with flowers coloring yellow in the axillary form of cymose clusters, bearing dark brown and ear shaped seeds. The pulp is brown having nauseous and fruity odor (Rahman et al., 1998a). The extract of Withania coagulans is known
for anti-bacterial, and anti-fungal properties (Subramanian et al., 1971). *Withania coagulans* Dunal is a berries bearing plant, locally available throughout Pakistan and is also found in Afghanistan and India (Glotter, 1991). There are different names of the plant depending on different local languages of the *withania* growing areas such as Tukhm-e-kakenaje in Persian, Spiubajja in Afghanistan, Khamjira in Punjab, Punirjafota in Sindh and Indian cheese in India. The fruit of the plant is a berry like that possesses milk coagulating properties; its pulp and husk obtained from the berry is used for this purpose. This property might be due to the presence of enzyme which has milk coagulating activity. The extract of the fruit (one ounce) in a quarter of boiling water makes a decoction that is used for milk coagulation. In some parts of Pakistan and India, berries of paneer dodi are used as blood purifier while the twigs of the plant are used for teeth cleaning and its smoke is inhaled for the relief of toothache (Dymock et al., 1972).

A number of withanolides (steroidal lactones with an ergostane skeleton) have been extracted from plant parts of paneerdodi (Neogiet et al., 1998). The most active forms of withanolides are withaferin A and withanolide D (Kuboyama et al., 2002; Ganzera et al., 2003). *Withanolides* are steroidal lactones commonly present in plants of family *Solanaceae*. Genera *Withania* and *Physalis* have great importance in the indigenous medicinal system of south-east Asia. The *withanolide* skeleton may be defined as 22-hydroxyergostan-26-oic Acid-26,22-olide. The variation in the skeleton forms different types of withanolides. This class of compounds does not occur in all members of the family *Solanaceae*. Members of twelve genera have been reported to contain withanolides, mostly from *Withania* and *Physalis* genera (Cardenas et al., 1994). *Withanolides* are separated from the whole plant and can be used to prevent food spoilage. Anti-bacterial and anti-fungal properties have been demonstrated in the ethanolic extract of the whole plant and leaves, which can be used for extension of shelf life of food products (Khan et al., 1993).

2.6.2. Antioxidants extraction from Peanut skin (*Arachis hypogaea*)

Food processing industries that process peanuts generate huge amounts of peanut skin as a side-product (waste) during the processing operation. More than 750,000 tons of this side-product are being generated annually worldwide, which have only been utilized in animal feed formulations or simply wasted (Sobolev and Cole, 2004). More often, skin removed during processing becomes the industrial waste, and is hardly disposed off for adding in the animal
feeds. This industrial waste is of economic value because it contains abundant quantities of phenolic antioxidants (Lee, 2002).

In a study, three processing techniques were used for removal of peanut skin from the peanut kernel. Then extracts were obtained using three types of solvents and studied composition of extracts through HPLC analysis. Total 90–125 mg/g of the extracts were obtained (Jianmei et al., 2006). The health benefits (weight gain control, prevention against cardiovascular diseases) associated with the consumption of peanuts are evident from literature (Feldman, 1999). The protection from Alzheimer disease is also reported to achieve through peanut consumption (Peanut-Institute, 2002). Moreover, it has role in cancer prevention (Awadet et al., 2000) due to presence of mono and polyunsaturated fatty acids (trans-fatty acids) in the peanut (Sanders, 2001), other nutrients like vitamin E, folic acid, dietary fiber, health promoting phytochemicals, phenolics, minerals like potassium, magnesium, and zinc are present (Sanders et al., 2000). However, a little effort is done to explore the health promoting potential of compounds present in defatted peanut skin (total phenolics about 150 mg/g) (Nepote et al., 2002). Skin peeled from different types of heat treated peanuts (Spanish, Runner, Virginia) was evaluated for total phenolics and antioxidant potential (Yu et al., 2006).

2.7. Effect of storage on the shelf-life of the products

During storage, various factors are involved to affect the quality of food products. Microbial spoilage is the main cause of food wastage followed by oxidation which affects food freshness most commonly causing food spoilage and leads to overt rancidity in fatty foods (Lindley, 1998). Lipid oxidation is also the most momentous of three chemical processes that lead to the undesirable rancidities; the other two processes are lipolysis and flavor reversion. Lipid oxidation (also referred to as ‘oxidative rancidity’ and ‘autoxidation of unsaturated fatty acids’) is irreversible and is initiated by a free radical chain mechanism that results in hydroperoxides formation. A subsequent decomposition reaction produces a variety of secondary oxidative products (Velasco et al., 2004). The initial hydro-peroxides formation is a three step mechanism involving initiation, propagation and termination. The reaction rates for autoxidative processes are influenced by the presence of pro-oxidants, such as trace metals; antioxidants, either indigenous or added; ultraviolet radiation; available oxygen levels; and temperature conditions. The free radicals produced by oxidation of fats and oils present in the foods produce off-flavors. Autoxidation process is increased when the numbers of unsaturated fatty acids are...
increased. The hydro-peroxide compounds produced are unstable, having no color, odor or flavor but on decomposition, the resultant compounds such as alcohols, ketones, aldehydes, esters, acids, lactones, aromatics and hydrocarbons are generated (Gordon, 1990).

2.7.1. Effect of packaging materials on shelf-life of the food products

Shelf life can be enhanced by proper handling, packaging and addition of certain preservatives in food products. In a study, three packaging materials (tortilla-packing paper, low-density polyethylene and high density polyethylene) were used to evaluate tortilla shelf-life at two different temperatures (-10°C and 5°C). At -10°C, the quality of tortillas was not affected significantly during 11 days of storage and the influence of the packaging material was negligible. The tortilla kept at 5°C in a high or a low-density polyethylene, had significantly better quality than the paper-packaged product. Textural changes were best shown by the Instron puncture resistance test than by the cutting resistance test. At 5°C, packaged tortillas had a microbiologically stable level up to seven days that found to significantly improve at -10°C during storage (Nieblas et al., 1991).

2.8. Biological evaluation of food and food products

Food products from safety point of view are commonly evaluated through in-vitro digestibilities to estimate their inherent potential that is expected in metabolic activity after consuming and their effects on the growth or energy needs of the subject.

However, proteins are biologically evaluated on the basis of growth study parameters like Biological Value (BV), Feed Efficiency (FE), Net Protein Ratio (NPR), Protein Efficiency Ratio (PER), Relative Net Protein Ratio (RNPR) and Net Protein Utilization (NPU). Food intake of the subjects (experimental animals) varies individual to individual due to the difference in their metabolic rate requirements and actual protein contents of the experimental diets (Francis et al., 2009; Khan et al., 2011). The diets having PER with score more than 2.0 are known as good source of quality protein. Legume protein isolates have great application in nutraceuticals and health care applications (Seena et al., 2006). Protein quantity present in the experimental feed affects quantity of diet that rats eat, and it also varies depending upon the metabolic and physiological needs of the subjects (White et al., 2000). The protein bioavailability and protein quality are mainly dependent on the processing technology and allied variables prior to the food consumption. The diets formulated with the experimental proteins are fed to the experimental animals for a specific time span and the results are evaluated (Eissen et al., 2010).
Three traditional Nigerian food formulations were evaluated for biological values that showed very good biological scores for BV(86.1+6.78, 84.3+6.7, 72.7+5.3%), while NPR and PER values were found significantly higher as compared to basal diets as described by Essien et al. (2010). In another study, it was found that in-vivo protein digestibility (72.4%) of kidney bean protein was unaffected by thermal heating as compared to broad bean protein value decreased (from 86.5% to 60.5%) on heating (Carbonaro et al., 2000).

Antioxidant potential of pomegranate and whey protein in liver protection was evaluated using whey powder (WP) and pomegranate peel powder (PPP) using equal quantities of both powders. The rats with carbon tetrachloride (CCl4) induced liver injury was used as experimental animals. Antioxidant activity of both treatments (PPP and WP) was observed. The results indicated that whey powder incorporated with the PPP can be utilized as functional food ingredient formulated to cure liver diseases (Ashoush et al., 2013).

2.8.1. Mineral bioavailability of food and food products

Functional food formulations and production using cereals has greatly been boosted in this era. These provide nutrition and health friendly components in our daily meals. Similarly, formulation of biscuits with value addition improves nutritional status of cereal based food products that are obtained by combining the legumes with cereals. They tend to improve nutritional quality by increasing proteins, vitamins, energy, minerals and sensory appeal of the innovated product. However, the absorbing ability of the minerals present in the food depends on the type or chemical composition of the dietary fiber present in food either soluble or insoluble (Figuerola et al., 2005).

Chemical composition of the dietary fiber affects the ability of the minerals to bind with it during digestion of the food in the intestine. Sometimes, there is poor utilization of minerals from rich formulations of foods (Olivares et al., 2001). The reason is that the polysaccharides present in cereals combined naturally with proteins, phytates, polyphenols etc may also affect the mineral binding ability with the dietary fiber (Idouraine et al., 1996). Wheat bran has the ability to significantly bind more calcium, zinc, and magnesium as compared with the rice bran and oat fiber. However, some polysaccharides (indigestible) like inulin and oligo-fructose, potentially enhance the mineral bioavailability of foods prepared from plant origin. Similarly, minerals absorption was found to improve when rats fed on inulin andoligo-fructose (Ohta et al., 1994).
In another study, calcium absorption was found to improve with the presence of inulin and oligo-fructose in the diets (de Muys et al., 1999).

2.8.2. In-vitro protein and starch digestibilities of food and food products

The effect of different processing treatments on a particular diet could be predicted well through in-vitro digestibilities. The results suggested that the legumes containing minimum amount of anti-nutritional factors had the highest protein digestibility (Preet and Punia, 2000). Anti-nutritional factors may be reduced through fermentation technique in legume, and the nutritional status may also be enhanced with an increase in the nutrients essentially needed for physiological functions. As a result, in-vitro protein and starch digestibilities of legumes were improved (Granito et al., 2005). Moreover, different processing treatments applied to horse grams and the cowpeas have improved protein digestibility of the flour. In-vitro protein digestibility of doughnuts was found to enhance from 71.8g to 76.3 g per 100 g by increasing replacement level of vetch flour (0 to 15 g/100 g) in the doughnuts (Rehman et al., 2007a). In another study, 50 Spanish wild taxa of *Lathyrus, Lens, Pisum* and *Vicia* have been compared for their nutritional properties of seed proteins. The genus *Vicia* and *Lens* showed the highest protein richness and the in-vitro protein digestibility, respectively, whereas the genus *Pisum* exhibited the lowest protein-digestibility corrected amino acid score (Pastor-Cavada et al., 2014).

2.9. Sensory evaluation of food products

Sensory evaluation is one of the quality parameters necessary for the development of the food product associated positively with the consumer acceptance. It is an important tool to judge the food product using senses of sight, smell, touch etc to decide about the quality of the product including taste, color, flavor, texture, and overall acceptability on different scales. Hedonic scale is the systematic documentation created to facilitate the judges or panelists in the evaluation of the product. Sensory evaluation analyzed the food product quality efficiently through hedonic scale (Hussain et al., 2006a,b; Mohsen et al., 2009).

The sensory parameters are also used to determine the palatability of foods that has strong influence on food consumption. The color serves as a sign off refreshness of food associated with aroma, taste and flavor, crispiness, texture changes of the product (Sharif et al., 2009). Similarly, supplementation of purified proteins like protein isolates in biscuits was found more acceptable as compared to un-processed raw materials (Pasha et al., 2011). Addition of soy protein isolates up to 20% level in biscuits has its influence on the aroma and quality parameters.
of the biscuits. With an increase in protein contents, positive effect on taste and overall acceptability of the biscuits containing 10% soy protein isolate were observed (Mohsen et al., 2009).

2.10. Products development and optimization by applying response surface methodology (RSM)

RSM is being widely and successfully used in food industry by the food researchers to optimize the levels of ingredients like sugars, fibers, improvers, thickeners, emulsifiers etc (Collar et al. 1999, 2007). RSM was applied to investigate the influence of milling conditions on the damaged starch and dough stickiness which were utilized to prepare chapatti (Ghodke et al., 2009).

Quantitative form of calculations are required for RSM analysis in the determination and solution of the multivariate equations which optimize the product on behalf of specified set of factors using mathematical modeling, and where interactions among test factors are considered (Giovanni, 1983). Food bars prepared at 15 different combinations were tested by each panelist. Optimum formulation was successfully achieved by applying the model and evaluating the effect of independent variables on dependent physicochemical attributes and sensory properties of the food products (Singh et al., 2004; Alizadeh et al., 2005). RSM was also used for process optimization of blackberry jam (Acosta et al., 2006), preparation of bread (Demirekler et al., 2004), parotha (Indrani and Rao, 2001), paneer (Nanda et al., 2004) and puri (Vatsala et al., 2001). Similarly, RSM has been applied to optimize the levels of protein for the preparation of date bars (Nadeem et al., 2012) and optimization of composite flour for the production and enhanced stability of leavened flat bread (Naan (Farooq et al., 2012).
Chapter-3

MATERIALS AND METHODS

The research trials were conducted in the Postgraduate Laboratories of National Institute of Food Science and Technology, University of Agriculture, Faisalabad. In-vitro biological studies were conducted in the Animal Room of this institute. In the first phase of studies, different extractions were carried out such as extraction of Indian vetch protein isolates (IVPI) from Lathyrus sativus L. Enzymatic conversion of date fruit sugar into fructose and the extraction of antioxidants from peanut peel and Withania coagulan sfruit (paneerdodi or khamjira).

Biscuit bars were prepared using these extracts and then biologically evaluated for efficacy on Spargue-Dawley rats. Albino rats were purchased from NIH, Islamabad. Response Surface Methodology (RSM) was applied to optimize levels of antioxidants.

3.1. PROCUREMENT OF RAW MATERIALS

Wheat flour (maida), Indian vetch, vegetable ghee, eggs, baking powder, dates (Iranian dates), peanut peels, Paneerdodi (Withania coagulans) were purchased from local market, Faisalabad. Analytical grade chemicals, standards, and enzymes (cellulase, hemicellulase, pectinase, glucose isomerase) were purchased from Sigma Aldrich (Seelze, Germany).

3.2. METHODOLOGY

3.2.1: REMOVAL OF β-ODAP FROM MATRI

Indian vetch(IV) seeds were cleaned manually to remove the impurities like dirt particles, stones, metal pieces, straw, insect feaces, damaged seeds, seeds of other crops, weeds etc. Then the seeds were detoxified according to the steeping method as described by Rehman et al (2006a). After cleaning, seeds were immersed in double quantity of water at 60-70°C for 8 hrs, while steeping water was changed every hour, avoiding the temperature change. In this method up to 93.05% neurotoxin (beta-N-oxalyl-L-alpha-beta-diamino propionic acid) is removed. Then, IV seeds were soaked in water at pH 4.0 for 60 min at 100°C to remove the beany odor (Virk, 2000), then sun dried. After drying, IV seeds were milled in the Brabender Quadrumate Senior Mills.

3.2.2. PROXIMATE ANALYSIS OF RAW MATERIALS
Proximate analysis of raw materials and date biscuit bars were analyzed for proximate composition which includes moisture (44-15), crude protein (46-10), crude fat (30-25), crude fiber (32-10) and ash (08-01) according to the standard methods described in AACC (2000).

3.2.2.1. Moisture content

Moisture content of each sample was determined by taking 5g ground sample in pre-weighed heavy-gauge aluminum plates. Duplicate samples were used. Then the plates were covered and weighed at once. These sample plates were placed on the oven shelf uncovered and dried at 130°C±1°C for 60 minutes. Then placed in desiccator, and weighed, according to method No. 44-15 as given in AACC (2000). The moisture was calculated according to the given formula:

\[
\text{Moisture} \, (\%) = \frac{\text{Loss in Wt. (g)}}{\text{Wt. of sample (g)}} \times 100
\]

3.2.2.2. Crude protein

Crude protein was determined by determining Nitrogen present in the sample, using Kjeldahl’s method, with some modifications in the method no. 46-10 given in AACC (2000). Ground sample exactly 2.0g was taken in digestion flask and 25mL of commercial \( \text{H}_2\text{SO}_4 \) in the presence of 5g digestion mixture (\( \text{K}_2\text{SO}_4 \), \( \text{CuSO}_4 \), \( \text{FeSO}_4 \) with 100:10:5 parts respectively) were digested up to 4-6 hrs, till the appearance of light green color. The digested samples were then filtered and volume was made in 250 mL volumetric flask. The 10mL of diluted sample was distilled with 40% \( \text{NaOH} \) into 4% boric acid which was then titrated with \( \text{N/10 H}_2\text{SO}_4 \) to light pink color as end point.

\[
\text{Nitrogen} \, (\%) = \frac{\text{Vol. of 0.1N H}_2\text{SO}_4 \times \text{Vol. of dilution Made} \times 0.0014}{\text{Wt. of sample (g) \times Vol. of dilution Taken (mL)}} \times 100
\]
Where as

\[
\text{Protein (\%)} = \text{Nitrogen \%} \times 6.25
\]

3.2.2.3. Crude fat

Crude fat was determined according to method No. 30-25 given in AACC (2000) in soxhlet apparatus, using hexane as solvent. 2g moisture-free sample was taken in a pre-weighed filter paper; it was wrapped and stapled to avoid sample leakage. This pack was weighed again and placed in the soxhlet jacket which is attached with the condenser having cold water circulation. Hexane (about 250mL) was taken in the flask and attached to the soxhlet apparatus for heating. The fat solvent evaporated by heating and went up via tube in the upper part, where they condensed due to cold water circulation. The condensed solvent drops trickled down in to soxhlet jacket containing sample. This volatilization and condensation was continued till the fat solvent around the sample became colorless and this was achieved with 7-8 siphon-backs, in case of date-biscuit bars. Then the filter paper containing sample was removed from the apparatus. It was placed in an electric oven at 100°C for 10-20 min and then placed in a desiccator for 2-3 minutes. Then it was weighed and loss in weight was noted. Following formula was used to calculate the fat percentage.

\[
\text{Crude Fat (\%)} = \frac{\text{Loss in wt. of sample (g)}}{\text{Wt. of sample (g)}} \times 100
\]

3.2.2.4. Crude fiber

Crude fiber was determined according to method No. 32-10 given in AACC (2000). The sample was ground, defatted with soxhlet apparatus using hexane as solvent and after drying 2g sample was transferred to 600-mL beaker. 200 mL 1.25% H₂SO₄ was added in to the beaker containing the sample to be digested. It was heated at high speed and then at low speed when boiling started, to avoid bumping at simmering. Acid boiling was continued for about 30 min. Then the residue was filtered through muslin cloth giving 6-7 washings and the residue was transferred to the beaker again. Then 1.25% NaOH was added in it and same procedure was revised. After filtration, the residue was transferred to a pre-weighed crucible and dried in hot air oven at 100°C for 24 hrs. Then it was transferred to the muffle furnace at 550-600°C for 4-5 hrs.
and loss in wt. on ignition was noted. The crude fiber content was determined by using the formula.

\[
\text{Crude Fiber (\%)} = \frac{\text{Wt. of dried residue (g)} - \text{Wt. of ash (g)}}{\text{Wt. of sample (g)}} \times 100
\]

3.2.2.5. Total Ash

Ash content was determined according to method No. 08-10 given in AACC (2000). 2g sample was taken in a pre-weighed crucible and charred on a hot plate till the end of smoke. This was followed by incinerating in a muffle furnace at 550°C till a constant weight was obtained. The ash content was determined by the formula:

\[
\text{Total Ash (\%)} = \frac{\text{Wt. of ash (g)}}{\text{Wt. of sample (g)}} \times 100
\]

3.2.2.6. Nitrogen Free Extract (NFE)

NFE was calculated by subtracting the percentages of moisture, crude protein, crude fat, crude fiber, total ash from 100.

\[
\text{NFE (\%)} = 100 - (\text{moisture \%} + \text{Crude Protein \%} + \text{Crude fat\%} + \text{Crude fiber\%} + \text{total ash\%})
\]

3.3. EXTRACTION OF FRUCTOSE FROM DATE PULP

Enzyme assay was applied for the conversion of date pulp into date fructose as described by El-Sharnouby et al. (2009).

Date fruit (ripe) of Iranian variety was collected from local market then pitted and flesh was minced just before chemical analysis, divided into pieces and stored in a refrigerator in sealed polythene bags for further use. Enzymes were purchased from Sigma-Aldrich Company (Dorset, UK). Pectinase (≥9,500 units per mL) and cellulase (≥1000 units per gram) were mixed at ratio 1:1 and added at 1:20, 1:2.5 and 1:30 percent concentration of date pulp and distilled water at 2% enzyme mixture addition. pH was adjusted at 6±0.2 before the addition of enzymes, then placed in incubator at 40°C for 24hs. After incubation, the samples were blended thoroughly and the slurry was filtered to get the clear extract which was then concentrated in
rotary evaporator to get about 72°brix, then packed in sealed glass bottles and stored at room temperature till further analysis. Formula used to get the total solids recovery is as follows

\[
\text{RSS} = \frac{\text{Wt of extract} \times \text{TSS of extract}}{\text{Wt of date pulp taken}}
\]

3.3.1. Conversion and determination of sugars with GC-FID (Gas Chromatograph- Flame Ionizing Detector)

Glucose, fructose and sucrose were determined by using the method described by Medeiros and Simoneit (2007). About 87.3% sugars were collected in the date syrup before glucose isomerase treatment. Then 1 g glucose isomerase enzyme with 5mg magnesium chloride (MgCl₂) was added. Sample was placed on water bath at 60°C with continuous stirring at 70°C till sample contents become highly sweet in taste. Then cooled, freeze dried and stored for analysis.

The extracted sugars were individually analyzed with GC-FID (Gas Chromatograph-Flame Ionizing Detector). Derivatization procedure with slight modifications was followed to determine the monosaccharide sugars as described by Li (1996). Sugar sample (0.6mg) was taken in a test tube and 0.5mg phenyl beta-D-glucopyranoside was added as an internal standard. Then 12.5mg hydroxylamine hydrochloride with 0.5 mL pure pyridine was added, then capped with Tefllon lined cap, mixed by shaking and placed on water bath for 5 min., then mixed and cooled to room temperature. Then 0.5mL hexamethyldissilazane with 0.4mL undiluted Trifluoroacetic acid were added with continuous mixing at 22°C for 10 min., then undiluted isoctane with 4mL De-ionized water was added and thoroughly shaken. The supernatant layer of isoctane was pipette out and stored in vials. Then, injected into the gas chromatography for qualitative and quantitative analysis. Specifications of GC-FID were that Nitrogen gas was used as a carrier, a 30m long DB-5 fused silica capillary column with a film thickness of 1.0micrometer and a 0.25milimeter inside diameter was used. A multi ramp column oven was used to separate monosaccharides with initial temperature 180°C for 5min, with the detector and injection port temperature 300°C and 280°C. The initial temperature of oven was raised to 200°C at a rate of 1.5°C/min for 1min then raised to 200°C at the same rate for the same time, then raised to 260°C
at the rate of 10°C/min which was maintained for 2min, the raised to 290°C at the rate of 6°C/min and maintained for 8min.

3.4. PREPARATION OF BISCUIT BARS (DSB) CONTAINING DATE-SUGAR

3.4.1. Preliminary trials

Preliminary trials carry significance in the development of final recipe and ensure greater chances of moving towards right direction. Following trials were made at the baking plant of National Institute of Food Science and Technology (NIFSAT) for the development of modified biscuit recipe.

In the first trial, conventional recipe sugar was totally replaced by same quantity of fresh paste of soft date variety. Other ingredients remained the same. The biscuits were prepared according to method as described in AACC (2000) with some modifications. The biscuits in the shape of bars were prepared very low in sweetness, and were lacking the crispness. Cardamom powder was used to mask the bland taste, but it was not acceptable due to soggy appearance. The paste was replaced with date sugars enzymatically extracted from soft variety of dates mixing with table sugar at different levels. All the treatments were accepted in sensory evaluation by the panel of judges from teachers and postgraduate students of NIFSAT. It gave good results, acceptable sweetness and texture. Amounts of baking powder, skim milk powder and eggs were also adjusted. On the basis of these trials following recipe for “Date-biscuit bar” was developed (Table 3.1).

3.4.2. Procedure

Biscuit bars containing date-fructose were prepared according to the treatments [$T_0$ (0%), $T_1$ (5%), $T_2$ (10%), $T_3$ (15%), $T_4$ (20%)] for sugar replacement (Table 3.1) with certain modifications in the method as described in AACC (2000) as follows:

The ingredients were weighed accurately. Vegetable ghee and powdered sugar (Sucrose + date fructose) were taken into the bowl of mixer and mixed for 5-6 min till creamy appearance. Then eggs were added one by one, and 2-4 drops of vanilla essence were added. After 2-3 minutes, flour and baking powder were sifted together and added to the sugar-ghee-egg mixture. After getting a homogeneous mass with minimal mixing time, the biscuit batter was taken out of the bowl. It was immediately rolled out with the help of rolling pin. Sheeting of 1cm height was
done and roller cutter having blades of 6cm separation were used to cut the dough sheet into several pieces. Then another roller cutter having 1cm blade separation was rolled over the cut pieces at 90° angle. In this way dough pieces of 6cm length, 1cm width and 1cm height were obtained. These were placed in baking trays and placed in oven at 225°C for 10 min. After getting proper baked color, the trays were removed from the oven. Date-sugar bars were cooled at room temperature for about 10 minutes, packed in the aluminum bags with electric seamer and placed at room temperature for further sensory and texture analysis.

**Table 3.1: Sucrose replacement with date-fructose in Date-sugar bars (DSB)**

<table>
<thead>
<tr>
<th>Ingredients (g)</th>
<th>T0 (0%)</th>
<th>T1 (5%)</th>
<th>T2 (10%)</th>
<th>T3 (15%)</th>
<th>T4 (20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sugars (S:F)</td>
<td>250</td>
<td>250(237.5:12.5)</td>
<td>250(225:25)</td>
<td>250(212.5:37.5)</td>
<td>250(200:50)</td>
</tr>
<tr>
<td>Vegetable Ghee</td>
<td>300</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eggs</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bak. Powder</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 3.1. Systematic Flow sheet for Date-Sugars extraction

Date pulp (100g) + water(300mL)  
↓  
pH(6±0.2)  
↓  
Enzyme addition  
↓  
Incubation (40 °C:24hrs)  
↓  
Blending  
↓  
Filtration  
↓  
Concentration in rotary evaporator  
(70 °C:72 °brix)  
↓  
Glucose isomerase:MgCl₂ addition  
↓  
Heating in water bath(60 °C)  
↓  
Filtration  
↓  
Freeze drying  
↓  
Grinding  
↓  
Storage  
↓  
HPLC analysis
Figure 3.2. Systematic Flow sheet for Date-Dugar Bar Development

Weighing
↓
Mixing
↓
Creaming
↓
Batter formation
↓
Sheeting
↓
Cutting
↓
Baking
↓
Cooling
↓
Packaging (Al foil)
↓
Storage
3.5. EXTRACTION OF PROTEIN ISOLATES

IVPI were prepared by using the method as described by Johnson and Brekke (1983) with certain modifications. 50 g of flour were weighed and 800 mL of alkaline water (pH 9.8) were added to it. This sample was placed on the orbital shaker at room temperature for 40 min and then filtered. Afterward, the sample was subjected to centrifugation at 5000 rpm for 15 min. Due to the presence of protein in the supernatant; precipitation occurred after the adjustment of pH at 4.5 with 1N HCl. Lower layer, which is of high density, serves as the residue. The precipitates formed in supernatant were allowed to settle down. All the proteins were settled at the lower layer, while the upper layer was discarded. The lower layer was collected and centrifuged to get the isolate. The isolate was placed overnight in freeze dryer for drying. Dried isolate was kept in airtight container.

Table 3.2. Addition of IVPI in date-fructose bars.

<table>
<thead>
<tr>
<th>Ingredients (g)</th>
<th>T0 (0%),</th>
<th>T1 (5%),</th>
<th>T2 (10%),</th>
<th>T3 (15%)</th>
<th>T4 (20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sugars(S:F)</td>
<td>250(250:25)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>V. Ghee</td>
<td>300</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eggs</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Baking Powder</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IVPI (g per 500g flour))</td>
<td>0</td>
<td>25</td>
<td>50</td>
<td>75</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 3.3. Systematic Flow sheet for (IVPI) recovery

Sample (50g)
\[ \downarrow \]
Volume (800ml)
\[ \downarrow \]
Stirring (40min:pH9.8)
\[ \downarrow \]
Filtration
\[ \downarrow \]
Centrifugation (20min:3000rpm)
\[ \downarrow \]
Supernatant
\[ \downarrow \]
pH (4.5)
\[ \downarrow \]
Centrifugation
\[ \downarrow \]
IVPI (ppt.)
\[ \downarrow \]
Freeze drying
\[ \downarrow \]
Storage
3.6. EXTRACTION OF ANTIOXIDANTS

Antioxidants were extracted from peanut peel and Paneerdodi. Sample was prepared by the method as described by Khanavi et al. (2010). For the preparation of extracts from the respective samples, various solvents were used i.e. methanol, ethanol and n-hexane. Samples were placed in Soxhlet apparatus for 6-8h at room temperature (AOAC, 1998). The supernatant was filtered with Whatman no.1 filter paper. The solvent from the supernatant was separated at 50°C in a Rotary Evaporator (EYELA, N-N Series, Japan). The extract of each sample was weighted and stored at 4°C. Antioxidant activity of the extracts was determined.

3.6.1. Analysis of antioxidant extracts

The antioxidant extracts obtained from raw materials were analyzed for their antioxidant potential through different tests including total phenolic contents (TPC), antioxidant activity and free radical scavenging activity by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay.

3.6.2. Total phenolic contents

The total phenolic compounds in all the extracts were determined by Folin-Ciocalteu method (Shih et al., 2007). For the preparation of the calibration curve, 1mL aliquots of 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30mg/mL gallic acid solutions in methanol were mixed with 5mL of Folin-Ciocalteu reagent (diluted ten-fold) and 4mL of sodium carbonate solution (75g/L). The absorbance was taken after 30 minutes at 20°C and 760nm using UV/visible light spectrophotometer and the calibration curve was plotted. Each plant extract (1mL) was
Figure 3.4. Systematic Flow sheet for Antioxidant extraction

Dried sample (50g)
↓
Solvent addition (100mL ethanol: water)
↓
Stirring/Shaking (25°C: 3000rpm)
↓
Filtration
↓
Heating (water bath at 60°C till 2mL residue)
↓
Preparation for Analysis
↓
(DPPH method: Absorbance at 528 nm)
↓
Storage
mixed with the same reagents as described above and after 1h the absorbance was measured for the determination of total plant phenolics. Total content of phenolic compounds in each plant extracts in gallic acid equivalents (GAE) was calculated by the following formula:

\[ C = \frac{c \times V}{m} \]

Where as:

\( C = \) Total content of phenolic compounds (mg/g plant extract, in GAE)
\( c = \) Concentration of gallic acid calculated from the calibration curve (mg/mL)
\( V = \) Volume of extract (mL)
\( M = \) Weight of plant methanolic extract (g)

![Standard curve of Gallic Acid](image)

**Figure 3.5. Standard curve of Gallic Acid**

### 3.6.3. Antioxidant activity

Antioxidant activity based on coupled oxidation of β-carotene and linoleic acid was evaluated by using method described by Taga *et al.* (1984) and Yagi *et al.* (2002). β-carotene
(2mg) was dissolved in 20mL of chloroform. A 3mL aliquot of the solution was dissolved with 40mg linoleic acid and 400mg Tween20. Distilled water (100mL) was added into the β-carotene emulsion and mixed well by using a vortex mixer. Aliquots (3mL) of the β-carotene emulsion and phenolic extracts (0.12mL) were placed in capped culture tubes and mixed thoroughly. The tubes were immediately placed in a water bath and incubated at 50°C. Oxidation of β-carotene emulsion was monitored spectrophotometrically by measuring absorbance at 470nm after 30min. The degradation rate of the extracts was calculated according to first order kinetics using following equation (Al-Saikhan et al., 1995).

\[ \text{In}(a/b) \times 1/t = \text{sample degradation rate} \]

Where:

\[ \text{ln} = \text{Natural log} \]

\[ a = \text{Initial absorbance (470nm) at time zero} \]

\[ b = \text{Absorbance (470nm) after 30 min} \]

\[ t = \text{Time (min)} \]

The antioxidant activity (AA) was expressed as % inhibition relative to the control using following equation.

\[ \text{AA} (%) = \frac{\text{Degradation rate of control} - \text{Degradation rate of sample}}{\text{Degradation rate of control}} \times 100 \]

3.6.4. Free radical scavenging activity (DPPH assay)

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of extracts was measured according to the method of Brand-Williams et al. (1995). Solutions were prepared by dissolving 0.025mL of extract in 10mL of ethanol. A fresh solution of DPPH in ethanol (\(6 \times 10^{-5}\)M) was prepared before measurements and 3mL of this solution were mixed with 77μL extract solution in 1cm path length disposable microcuvettes (final mass ratio of extracts to DPPH was approximately 3:1, 1.5:1, 0.75:1). The samples were kept in dark for 15min at room temperature and then decrease in absorbance was measured at 515nm on UV/visible light spectrophotometer. Absorbance of blank sample containing the same
amount of ethanol and DPPH solution was also measured. Radical scavenging activity was calculated by the following formula:

\[
\text{Reduction of absorbance (\%)} = \left[ \frac{(AB - AA)}{AB} \right] \times 100
\]

Where as:

\(AB\) = Absorbance of blank sample \((t = 0 \text{ min})\)

\(AA\) = Absorbance of tested extract solution \((t = 15 \text{ min})\)

Antioxidant activity will be measured by the method as described by Yagi et al. (2002).

3.7 OPTIMIZATION OF LEVELS OF ANTIOXIDANTS USING CENTRAL COMPOSITE DESIGN (CCD)

After the selection of DFBB, the levels of two antioxidant extracts were optimized. Central Composite Design was constructed having two factors by using statistical software package (Minitab Inc. Quality Plaza, 1829 Pine Hall Rd. State College PA. 16801 United States). The two recipe variables optimized were Peanut Peel Extract (PPE) at the levels of 0.1%, 0.2%, 0.3% and Withania coagulans Extract (WCE) at the levels 0.2%, 0.3%, 0.4%. The maximum level of each variable was selected by earlier trials performed to validate the decisions regarding the ingredients. Experimental conditions at the centre point were PPE=0.2% and WCE=0.3%. Color, Flavor, Taste, Texture, Hardness, Fracturability, overall acceptability were the dependent variables (Table 3.3).
Table 3.3. Design for optimizing the antioxidant levels in DFBB

<table>
<thead>
<tr>
<th>Std Order</th>
<th>Run Order</th>
<th>Blocks</th>
<th>Coded Values</th>
<th>Actual Level</th>
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<td>PPE</td>
<td>WCE</td>
</tr>
</tbody>
</table>
3.7.1. PREPARATION OF DATE-FRUCTOSE BISCUIT BARS (DFBB)

Date-fructose biscuit bars were prepared by using different above selected amounts of IVPI and date-fructose with upper and lower limits of different antioxidants by applying Response Surface Methodology (Response Surface Box Behnken Design).

3.8. NUTRITIONAL EVALUATION OF DATE-FRUCTOSE BISCUIT BARS (DFBB)

3.8.1. Proximate analysis

Moisture, total ash, crude fat, crude protein, crude fiber and NFE of Date-fructose biscuit bars were determined by AACC (2000) procedures.

3.8.2. Mineral analysis

Mineral contents of date-fructose biscuit bars were determined by atomic absorption spectrophotometer (AACC, 2000). Sodium and potassium contents were determined by flame photometer according to AOAC (2000). Minerals (Na, K, Ca and Mg) were determined by method No. 40-70 and 40-71 described in AACC (2000). The sample (0.5g) was digested with conc. HNO₃ at low temperature (about 85°C) and then with HClO₄ at high temperature (about 180°C) till 1-2mL of the digested sample remained. This digested sample was then filtered and volume was made to 250 mL. The prepared samples were then run through Atomic Absorption Spectrophotometer according to method No. 40-70 and 40-71 as described in AACC (2000) and then the mineral contents of each sample were calculated.

3.8.3. Amino acid analysis

Date-fructose biscuit bars were evaluated for amino acid profile using amino acid analyzer (Dabbour and Takuri, 2000).

3.8.4. In-vitro starch digestibility (IVSD)

The in-vitro starch digestibility (IVSD) were assayed by employing porcine pancreatic amylase (Singh et al., 1982). The values of starch digestibility were expressed as milligrams of maltose released per gram of sample in dry weight (Chau and Cheung, 1997).

3.8.4.1. Preparation of porcine pancreatic amylase solution

Porcine pancreatic amylase (EC 3.2.1.1, 790 units/ mg of protein; catalog No. A6255, Sigma) was used at a concentration of 0.4mg/mL.
3.8.4.2. Procedure

One unit of amylase liberated 1mg of maltose from starch in 3 min at pH 6.9 at 20°C. In brief, 50mg biscuit bar sample were incubated with 0.5mL of pancreatic amylase solution (0.4mg/ mL) at 20°C for 2 hrs. Then 2mL 3, 5-dinitrosalicylic acid reagent were added, and the mixture was boiled for 5 min. After cooling, the absorbance of the filtered solution was measured at 550 nm with maltose used as the standard. The values of starch digestibility were expressed as milligram of maltose released per gram of sample in dry weight (Chau and Cheng, 1997).

3.8.5. In-vitro protein digestibility (IVPD)

In-vitro protein digestibility was determined according to method described by Saunders et al. (1973).

3.8.5.1. Preparation of pepsin solution

Pepsin enzyme solution (1.5mg/mL) was prepared by using 0.035M solution of HCl with pH near about 2.0 as described in pepsin digestibility method by Mertz et al (1984).

3.8.5.2. Procedure

In a centrifuge tube, 200 mg of the powdered material was suspended in 35mL of pepsin solution and incubated for 2.0 hrs at 37°C with gentle shaking. This solution was centrifuged at 12,000 rpm for 15 min at 40°C and the residue was suspended in 10mL 0035M HCl and the mixture was re-centrifuged. After removal of the supernatant, the residue was collected and dried overnight at 40°C, weighed and then analyzed for Nitrogen by micro-Kjeldahl method. The amount of 0.1N H₂SO₄ used was noted and put in the formula to get the total nitrogen of the dried residue. The blank reading was taken without using the enzyme.

\[
\% \text{ Nitrogen} = \frac{\text{Vol. of } 0.1\text{N H}_2\text{SO}_4 \times \text{Vol. of dilution Made} \times 0.0014}{\text{Wt. of sample (g) \times Vol. of dilution taken (mL)}} \times 100
\]

Where as,

\[
\text{Protein}\% = \text{Nitrogen} \% \times 6.25
\]

3.8.6. Calorific value of the biscuit-bar (gross energy)
Calorific values of the date-biscuit bars were determined by using Oxygen Bomb Calorimeter (IKA-WERKE, C2000 Basic) as described by Krishna and Ranjhan (1981). The amount of heat measured in calories that is released when a substance is completely oxidized in a bomb calorimeter, is called the gross energy of the substance. Finely ground sample of Date-biscuit bar (0.5g) was taken in to the metallic decomposition vial. The vial was unscrewed and fastened a cotton thread onto the middle of the ignition wire with a loop before loading the sample. Then the screw cap was tightened. The decomposition vial was guided into the filler head to the open measuring cell cover until was in place. The start button was pushed and the measuring cell cover was closed. The sample within the vial was burnt through electric spark. Heat produced was noted by the software computer and displayed in the form of a graph denoting the temperature against time. The whole procedure was fully automatic. It gave no. of calories per 1 gram of the sample.

3.9. SENSORY EVALUATION

Date-fructose biscuit bars and normal biscuit bars containing different levels of extracted antioxidants were subjected to sensory evaluation by panel of judges. The attributes of appearance, flavor, texture, and overall acceptability were evaluated by 9-points Hedonic Scale method described by Land and Shepherd (1988).

3.10. SHELF LIFE STUDY OF DATE-FRUCTOSE BISCUIT BARS

The highest ranked bar formulation were selected for shelf life studies. Prepared bars were stored at room temperature for 90 days for evaluation at 15-day interval.

3.10.1. Physical analysis

3.10.1.1. Water activity

Water activity in biscuit-bars during storage was determined by the standard water activity meter method (AOAC, 2000).

Water activity was determined by water activity meter (Rotronic Hygro palm \(A_w\), Series no. 601089738). Hygro palm is a portable humidity temperature indicator, having 9v rechargeable battery. It displays the measurements from the probe connected to the remote unit. The ground sample was filled in the plastic jar and the hygro palm probe was inserted in it. Then the enter key is pressed to access the function of the remote unit. The up and down keys are used
to navigate the function menu the enter key is press to confirm the settings. The display shows the water activity (0-1), along with temperature (°C) and time (s).

3.10.1.2. Texture analysis

Texture analysis of biscuit-bars during storage were determined with the help of texture analyzer (model TA_XT Plus, Stable Microsystems, Surrey, UK) with 5 kg load cell according to the method as described by Rehman and Al-Farsi (2005).

Texture analysis was done with texture analyzer. It is software attached automatic equipment which gives the measurement of the hardness and resistance of the biscuits to bend or snap. It has an accessory attached with it i.e. 3-Point Bending Rig (HDP/3PB) using 5kg load cell Heavy Duty Platform (HDP/90). The two adjustable supports of the rig base plate were placed at about 5cm distance apart so as to support the sample. This distance was kept same for all the samples. The Date-biscuit bar was placed centrally over the supports. The distance at the point of break is the resistance of the sample to bend so relates to the fracturability of the sample i.e a sample that breaks at a very short distance has a high fracturability. The display shows force in grams (g) and distance in millimeters (mm). This data was evaluated by its software for significance of results.

3.10.1.3. Color value (Color test)

Color value was determined with color meter (Neuhaus Color Test – II, Neotec). It was first calibrated with the standards having lower and upper limits (51 and 170 respectively). Then the ground Date-biscuit bar sample was completely filled in the petri plate and the surface was made smooth by removing the extra sample material from the Petri plate, to get the optimum reflection of light, emerged by the photo cells of the color meter, reading was noted from the display. Sample readings were compared with the standard.

3.11. CHEMICAL ANALYSIS OF BISCUIT BARS DURING STORAGE

3.11.1. Peroxide value determination

Biscuit-bar samples were analyzed for peroxide values during storage period using the procedure described in AOAC (2000) with minor modification. Oil was extracted from sample and 5g of the extracted oil was taken in Erlenmeyer flask (250mL) with addition of glacial acetic acid (30mL) and chloroform (3:2 V/V) mixture. The flask was swirled carefully to for about 1
min to mix the contents of the flask. Freshly prepared KI solution (0.5mL) was added in the flask and titrated against Na$_2$S$_2$O$_3$ solution till color disappeared. Then blank reading was taken by continuing titration with addition of 0.5 mL of starch solution as an indicator till blue color just disappeared.

\[
\text{Peroxide value} = \frac{(B-A) \times N}{\text{Wt. of oil used as sample (g)}} \times 100
\]

A= volume Na$_2$S$_2$O$_3$ taken for blank
B= volume of Na$_2$S$_2$O$_3$ used in sample reading
N= Normality of Na$_2$S$_2$O$_3$

### 3.11.2. Free fatty acid determination

Biscuit-bar samples were analyzed for free fatty acid values during storage using the procedure described in AOAC (2000).

Free fatty acids (FFA) were determined according to method No. 58-15 given in AACC (1999). Oil sample was extracted from the product through soxhlet apparatus using hexane. It was then placed in an oven at 60°C for 10 min to evaporate fat solvent from it. Then 5g oil sample was taken into Erlenmeyer flask and 50-100mL hot neutral alcohol was added. This was titrated with 0.1N NaOH, using phenolphthalein as indicator. FFA was calculated with the formula:

\[
\text{FFA} (%) = \frac{\text{NaOH used (mL)} \times \text{Normality of NaOH solution} \times 28.2}{\text{Wt. of sample (g)} \times 1000} \times 100
\]

1 mL of 0.1N NaOH= 0.028g oleic acid

### 3.12. BIOLOGICAL AND EFFICACY STUDIES

#### 3.12.1. Animals, diets and experimental design

Twenty and sixteen weanling albino rats of same age (The Sprague Dawley Strain) for biological study and serum profile for assessing the effect of natural antioxidant extracts were purchased from NIH., Islamabad, respectively. The rats were put on stock diet for seven days prior to the start of experiment. Rats were randomly divided in to four experimental groups each having initial weight 148±1.4g. Each experimental group (Five rats, eight rats for serum profile) was housed in a separate cage. The experimental diets were fed to rats and they had free access
to clean and fresh water ad libitum for a period of 10 days (Biological study) and 30 days (biochemical assessment) (Table 3.4). The temperature of the room was maintained at 25±1°C and 60% humidity, subjected to a 12h dark/light/ period. Composite weight of the each group of rats was recorded daily with the help of a electronic balance. The faecal material from each cage was collected daily, brought to constant weight, dried and stored in aluminum foils for further evaluation. The spilt feed from cages was likewise collected, dried and weighed. Nitrogen intake was calculated from the feed consumed.

After 10 days of experiment, all the rats from G<sub>3</sub>, G<sub>4</sub>, G<sub>5</sub> and G<sub>6</sub> groups were anaesthetized with an over dose of chloroform. Cranial as well as abdominal cavities were opened. Each group of carcass was weighed before and after drying at 105°C to a constant weight. Then, the dried carcasses were ground and the powder form was stored in plastic bags for body nitrogen determination (Miller and Bender, 1955).

For Biochemical study of blood, the rats (G<sub>1</sub> and G<sub>2</sub> group) were deprived of diet for 12h after 30 days, anesthetized with ketamine (90mg/kg b.w) and xylazine (10mg/kg b.w) and sacrificed by extracting blood from the abdominal aorta with a syringe. Serum was obtained by centrifugation at 1500g at 4°C for 5 min and stored at -80°C. .

Biological evaluation of protein quality such as digestibility (DIG), biological value (BV), net protein utilization (NPU), net protein ratio (NPR), protein efficiency ratio (PER) and feed efficiency (FE) were determined. The blood sample was allowed to clot and serum was separated at 2500rpm for 15 min and serum biochemical profile including serum enzymes: aspartate aminotransferase (AST, U/L), serum glutamate pyruvate transfaminase (ALT, U/L), serum alkaline phosphatase (ALP, U/L), total bilirubin (BiT, mg/dL), blood glucose (BG, mg/dL), serum calcium (S.CI, mg/dL), were assayed using assay kits. Cholesterol (mg/dL), Triglycerides (mg/dL) and minerals such as Na, K, BC and Cl (mEqual/L) in the blood were also determined as described by previous researchers (Reitman and Frankel, 1957; King and King, 1957; Jendrassik and Grof, 1938; Pellette and Young, 1980; Fararh et al. 2002; Morita et al. 2008). These studies were conducted twice. No significant difference was noticed between two studies. The normal ranges of these parameters are given in Table 3.5.
Table 3.4: Composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>G₁</th>
<th>G₂</th>
<th>* G₃</th>
<th>*G₄</th>
<th>*G₅</th>
<th>G₆</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground Biscuits (g)</td>
<td>100</td>
<td>100</td>
<td>75.5</td>
<td>58.4</td>
<td>64.4</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin mixture (g)</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Salt mixture (g)</td>
<td>-</td>
<td>-</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Corn starch (g)</td>
<td>-</td>
<td>-</td>
<td>17.5</td>
<td>34.6</td>
<td>28.6</td>
<td>72</td>
</tr>
<tr>
<td>Corn oil (g)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Total (g)</td>
<td>100</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*: Diet G₃, G₄, G₅ contained 10% protein
G₁: Control (Without IVPI and DF)
G₂: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts
G₃: Biscuits containing 15% Gluten protein (Control for Biological Studies)
G₄: Biscuits containing 15% Casein protein
G₅: Biscuits containing 15% IVPI
G₆:: Nitrogen free diet
Table 3.5: Normal ranges of serum profile parameters

<table>
<thead>
<tr>
<th>Serum profile parameters</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.F.T (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>Bilirubin Total (mg/dL)</td>
<td>0.1-1.1</td>
</tr>
<tr>
<td>Alkaline Phosphatase (U/L)</td>
<td>80-306 CH:80-640</td>
</tr>
<tr>
<td>SGPT (ALT) (U/L)</td>
<td>M:9-43 F:9-36</td>
</tr>
<tr>
<td>SGOT (AST) (U/L)</td>
<td>8-40</td>
</tr>
<tr>
<td>Blood Glucose Random (mg/dL)</td>
<td>80-140</td>
</tr>
<tr>
<td><strong>Serum Electrolyte</strong></td>
<td></td>
</tr>
<tr>
<td>Serum Calcium (mg/dL)</td>
<td>8.5-10.5</td>
</tr>
<tr>
<td>Serum Sodium (mEq/L)</td>
<td>132-145</td>
</tr>
<tr>
<td>Serum Potassium (mEq/L)</td>
<td>3.5-5.4</td>
</tr>
<tr>
<td>Serum Chloride (mEq/L)</td>
<td>95-105</td>
</tr>
<tr>
<td>Serum Bicarbonate (mEq/L)</td>
<td>22-32</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>180-200</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>&lt;150</td>
</tr>
</tbody>
</table>

**G. Digestibility (DIG):**

Digestibility was calculated by the following formula:

\[
\text{Digestibility} = \frac{I - (F - F_K)}{I}
\]

Where:
I = Nitrogen intake of test diets
F = Faecal nitrogen of test diets
F_K = Faecal nitrogen of non-protein diet.

**H. Net Protein Utilization (NPU):**

Net Protein Utilization was calculated by the following formula:

\[
\text{NPU} = \frac{B - (B_K - I_K)}{B}
\]
Where:

\[ B_K = \text{total body nitrogen of rats on non-protein diet} \]
\[ B = \text{Total body nitrogen of rats on test diets} \]
\[ I_K = \text{Nitrogen intake of rats on non-protein diet} \]
\[ I = \text{Nitrogen intake of rats on test diet} \]

I. Biological Value (BV):

Biological Value of experimental diets was calculated by indirect method using the following formula:

\[
\text{Biological Value} = \frac{\text{Net Protein Utilization}}{\text{Digestibility}}
\]

J. Net Protein Ratio (NPR):

Net Protein Ratio was calculated according to the method by using the following formula:

\[
\text{NPR} = \frac{W + W_n}{g}
\]

Where:

\[ W = \text{weight gain of rats while on test diets} \]
\[ W_n = \text{weight loss of rats while on non-protein diet} \]
\[ g = \text{protein intake of rats while on test diets} \]

K. Protein Efficiency Ratio (PER):

Protein Efficiency Ratio was calculated as:

\[
\text{PER} = \frac{\text{Weight gain}}{\text{Protein consumed}}
\]

L. Feed Efficiency (FE):

Feed Efficiency was calculated as:

\[
\text{Feed Efficiency} = \frac{\text{Feed consumed (g)}}{}$

58
Weight gained (g)

### A. Minerals Mixture (g)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaHPO$_4$</td>
<td>430</td>
</tr>
<tr>
<td>KCl</td>
<td>100</td>
</tr>
<tr>
<td>NaCl</td>
<td>100</td>
</tr>
<tr>
<td>MgO</td>
<td>10.5</td>
</tr>
<tr>
<td>MgSO$_4$</td>
<td>50</td>
</tr>
<tr>
<td>Fe$_2$O$_3$</td>
<td>3</td>
</tr>
<tr>
<td>FeSO$_4$.7H$_2$O</td>
<td>5</td>
</tr>
<tr>
<td>Trace elements</td>
<td>10</td>
</tr>
</tbody>
</table>

Corn starch sufficient for make 1000g

### B. Vitamins Mixture (mg)

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>2000 UI</td>
</tr>
<tr>
<td>Vitamin D$_3$</td>
<td>250 UI</td>
</tr>
<tr>
<td>Vitamin B$_1$</td>
<td>2.0</td>
</tr>
<tr>
<td>Vitamin B$_2$</td>
<td>1.5</td>
</tr>
<tr>
<td>Vitamin B$_3$</td>
<td>7.0</td>
</tr>
<tr>
<td>Vitamin B$_6$</td>
<td>1.0</td>
</tr>
<tr>
<td>Vitamin B$_7$</td>
<td>15.0</td>
</tr>
<tr>
<td>Vitamin B$_{12}$</td>
<td>0.005</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>80.0</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>17.0</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>4.0</td>
</tr>
<tr>
<td>Choline</td>
<td>136.0</td>
</tr>
<tr>
<td>Folic acid</td>
<td>0.5</td>
</tr>
<tr>
<td>Acid PABacid</td>
<td>5.0</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Corn starch sufficient for make 1000g
3.13. STATISTICAL ANALYSIS

Results were statistically analyzed by using RSM analysis of variance technique. Minitab statistical software (Minitab Inc. Quality Plaza, 1829 Pine Hall Rd. State College, PA. 16801, US) was used for optimization studies (Steel et al., 1997).
Chapter-4

RESULTS AND DISCUSSION

The present plan of study consists of three parts. In first part of the study, protein isolate was incorporated into date biscuit bars (DBB). These bars were analyzed for their physical and sensory characteristics to evaluate the suitability and the best level of Indian Vetch (*Lathyrus sativus* L) protein isolate (IVPI) for the preparation of DBB. On the basis of these findings, best suited DBB was selected. In second part, DFBB were developed using date fructose (DF) and best treatment was selected on the basis of best level of combination of protein isolate and date fructose (PIDF). In third part of the study, natural antioxidant extract levels (peanut skin and *withania coagulans* berries locally known as paneerdodi) in the best treatment were optimized by applying Response Surface Methodology (RSM). Date fructose biscuit bars (DFBB), thus, prepared were analyzed for their nutritional, biological and serum profile and shelf stability. The results are discussed under the following plan of work.

4.1. PHYSICOCHEMICAL ANALYSES

4.1.1. OBJECTIVE

Detoxification of Indian Vetch (Iv) to safer limits for preparation of protein isolates and the effect of Indian Vetch Protein Isolates (IVPI) supplementation was determined on physical and sensory properties of DBB.

4.1.2. RESULTS

4.1.2.1. Proximate composition of raw materials

Proximate analyses of raw materials including moisture, crude protein, crude fat, crude fiber and ash content were determined. The mean values of composition of different raw materials are presented in Table 4.1. Proximate analysis of peanuts shows that these contained higher level of crude fat (44.96%) than protein content (35.76%) which was at second in percentage. Moisture, fiber, ash and NFE contents of peanuts were 4.59%, 3.75%, 2.59% and 12.93, respectively. Proximate analysis of vetch showed that protein content of vetch was higher (28.90%) than moisture (8.70%), crude fat (1.27%), crude fiber (2.04%), and ash content (1.52%). Nitrogen free extract of vetch was 66.32%. Analysis of dates revealed that dates
contained 26.02% moisture, 2.61% crude protein, 0.33% crude fat, 3.48% crude fiber, 1.44% ash and 92.14% nitrogen free extract. Indian Vetch protein isolate (IVPI) contained the highest crude protein content (87.54%). Moisture, crude fat, crude fiber, ash and NFE contents were 4.40%, 0.34%, 0.12%, 0.35%, and 11.65%, respectively.

### 4.1.2.2. Physical properties of DBB containing IVPI

Analysis of variance for color values of DBB containing IVPI indicated highly significant difference (P<0.01) among treatments (Table 4.2). Color values for T₀, T₁, T₂, T₃ and T₄ were 176.62±1.23CTn, 174.71±1.89CTn, 171.29±3.36CTn, 162.53± 1.40CTn and 158.44±2.47CTn, respectively. The minimum color value was recorded in T₄ having darkest color, while color value of T₀ was the highest among all treatments indicating light color. Color values of T₂ and T₃ were more acceptable than other treatments due to more appealing moderate color (Table 4.3).

Highly significant (P<0.01) differences were recorded in hardness values of different treatments of biscuit bars (Table 4.2). The highest value of hardness was recorded in treatment T₀ while the lowest value was observed in case of T₄. The treatment T₂ and T₃ showed optimum hardness. Hardness values for T₀, T₁, T₂, T₃ and T₄ were 1871.21±25.06g, 1697.51±12.06g, 1628.81±09.87g, 1513.29±23.15g, and 1476.78±30.69g, respectively (Table 4.3).

Fractureability values also had highly significant (P<0.01) difference among treatments of DBB containing IVPI (Table 4.2). Fractureability values of bars ranged from 73.31±0.78mm to 78.59±0.86mm. Fractureability values of T₀, T₁, T₂, T₃ and T₄ were 73.31±0.78mm, 74.69±0.52mm, 76.05±1.03mm, 76.92±0.44mm, 78.59±0.86mm, respectively. The optimum values were observed in case of treatment T₂ and T₃ (Table 4.3).

Non-significant (P>0.05) differences were found in water activity values of DBB containing IVPI (Table 4.2). Water activity of these bars ranged from 0.215±0.003 to 0.220±0.006. Water activities of T₀, T₁, T₂, T₃ and T₄ were 0.215±0.003, 0.220±0.006, 0.210±0.006, 0.220±0.006 and 0.220±0.006, respectively. The water activity values of all bars were in acceptable range that reflects the shelfstability of DBB (Table 4.3).

### 4.1.2.3. Sensory properties of DBB containing IVPI

Analysis of variance for color score of DBB has been given in Table 4.4. Results showed highly significant (P<0.01) differences in color score of biscuit bars. Color score varied from 7.22±0.14 to 8.41±0.14. The highest color score was achieved by T₂ (8.41±0.14) and T₃
Color scores for \( T_0 \), \( T_1 \), \( T_2 \), \( T_3 \) and \( T_4 \) were 7.50±0.12, 7.22±0.14, 8.41±0.14, 8.21±0.13 and 7.96±0.16, respectively (Table 4.5).

Highly significant (\( P<0.01 \)) differences were recorded in the taste score of DBB containing IVPI (Table 4.4). The results revealed that maximum taste score was obtained by \( T_3 \) (8.09±0.10). It is evident that all treatments were liked by the judges; however, treatment \( T_3 \) was highly acceptable due to its taste. Overall taste score of all treatments varied from 7.31±0.11 to 8.09±0.10. Taste score for treatment \( T_0 \), \( T_1 \), \( T_2 \), \( T_3 \) and \( T_4 \) were 7.45±0.10, 7.31±0.20, 7.50±0.08, 8.09±0.10, 7.33±0.11 respectively (Table 4.5).

Analysis of variance for flavor score of DBB containing IVPI indicated highly significant differences (\( P<0.01 \)). Flavor scores for treatment \( T_0 \), \( T_1 \), \( T_2 \), \( T_3 \) and \( T_4 \) were 7.50±0.10, 7.10±0.12, 8.29±0.07, 7.96±0.13 and 7.27±0.06, respectively (Table 4.5). Maximum flavor score was achieved by treatment \( T_2 \) (8.29±0.07) followed by \( T_3 \) (7.96±0.13).

The statistical analysis of texture score of DBB containing IVPI indicated significant (\( P<0.05 \)) variation among treatments (Table 4.4). Mean values for texture score of bars is given in Table 4.5. Texture values ranged from 8.60±0.11 to 9.30±0.12. Mean values for texture score of bars for treatment \( T_0 \), \( T_1 \), \( T_2 \), \( T_3 \) and \( T_4 \) were 8.90±0.15, 8.60±0.11, 9.10±0.17, 9.30±0.12 and 9.21±0.09, respectively. Texture score of \( T_3 \) was found the highest (9.30±0.12) followed by \( T_4 \) (9.21±0.09).

Highly significant (\( P<0.01 \)) variations exist in mouth feel score of DBB containing IVPI among treatments (Table 4.4). Mouth feel score of DBB containing IVPI varied from 5.35±0.11 to 7.29±0.09 (Table 4.5). The best treatment was \( T_3 \) that achieved the highest mouth feel score (7.29±0.09) followed by \( T_1 \) (7.21±0.12). The mouth feel score of treatments were 5.35±0.11, 7.21±0.12, 6.91±0.08, 7.29±0.09, 7.13±0.12 for \( T_0 \), \( T_1 \), \( T_2 \), \( T_3 \) and \( T_4 \), respectively.

Overall acceptability score of DBB containing IVPI varied from 36.70±0.25 to 40.85±0.72. Overall acceptability score of treatments are 36.70±0.25, 37.44±0.99, 40.21±0.28, 40.85±0.72, 38.90±0.74 for \( T_0 \), \( T_1 \), \( T_2 \), \( T_3 \) and \( T_4 \), respectively. The highest overall acceptability score was achieved by \( T_3 \) (40.85±0.72) followed by \( T_2 \) (40.21±0.28) (Table 4.5).
Table 4.1: Proximate composition of raw materials (%)

<table>
<thead>
<tr>
<th>Raw Material</th>
<th>Moisture</th>
<th>Crude protein</th>
<th>Crude fat</th>
<th>Crude fiber</th>
<th>Ash</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanuts</td>
<td>4.57±0.03</td>
<td>35.78±0.05</td>
<td>44.98±0.05</td>
<td>3.76±0.04</td>
<td>2.55±0.03</td>
<td>12.93±0.04</td>
</tr>
<tr>
<td>Indian Vetch</td>
<td>8.15±0.05</td>
<td>28.45±0.07</td>
<td>1.38±0.02</td>
<td>2.24±0.04</td>
<td>1.61±0.01</td>
<td>66.32±0.08</td>
</tr>
<tr>
<td>Dates</td>
<td>26.02±0.12</td>
<td>2.61±0.05</td>
<td>0.33±0.01</td>
<td>3.48±0.04</td>
<td>1.44±0.02</td>
<td>92.14±0.11</td>
</tr>
<tr>
<td>IVPI</td>
<td>4.40±0.06</td>
<td>87.54±0.14</td>
<td>0.34±0.03</td>
<td>0.12±0.01</td>
<td>0.35±0.05</td>
<td>11.65±0.05</td>
</tr>
</tbody>
</table>

Table 4.2: Analysis of variance (mean squares) for physical properties of DBB containing IVPI

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Color</td>
<td>Hardness</td>
</tr>
<tr>
<td>Treatment</td>
<td>4</td>
<td>186.667**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>14.665</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

Table 4.3: Comparison of mean values for physical properties of DBB containing IVPI

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Color (CTn)</th>
<th>Hardness g (Firmness)</th>
<th>Fracturability (mm)</th>
<th>aw</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>176.62±1.23A</td>
<td>1476.78±30.69D</td>
<td>78.59±0.86A</td>
<td>0.215±0.003</td>
</tr>
<tr>
<td>T1</td>
<td>174.71±1.89A</td>
<td>1513.29±23.15D</td>
<td>76.92±0.44AB</td>
<td>0.220±0.006</td>
</tr>
<tr>
<td>T2</td>
<td>171.29±3.36A</td>
<td>1628.81±09.87C</td>
<td>76.05±1.03B</td>
<td>0.210±0.006</td>
</tr>
<tr>
<td>T3</td>
<td>162.53±1.40B</td>
<td>1697.51±12.06B</td>
<td>74.69±0.52BC</td>
<td>0.220±0.006</td>
</tr>
<tr>
<td>T4</td>
<td>158.44±2.47B</td>
<td>1871.21±25.06A</td>
<td>73.31±0.78C</td>
<td>0.220±0.006</td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05)

CTn: Color Test number
T0= without IVPI; T1= with 5% IVPI; T2= with 10% IVPI, T3= with 15% IVPI; T4= with 20% IVPI
Table 4.4: Analysis of variance (mean squares) for sensory properties of DBB containing IVPI

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Color</th>
<th>Taste</th>
<th>Flavor</th>
<th>Texture</th>
<th>Mouth feel</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>0.73065**</td>
<td>0.30684**</td>
<td>0.72879**</td>
<td>0.23376*</td>
<td>1.97196**</td>
<td>9.34365**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.05628</td>
<td>0.04720</td>
<td>0.03028</td>
<td>0.05088</td>
<td>0.03274</td>
<td>1.31190</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = Significant (P<0.05); ** = Highly significant (P<0.01)

Table 4.5: Comparison of mean values for sensory properties of DBB containing IVPI

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Color</th>
<th>Taste</th>
<th>Flavor</th>
<th>Texture</th>
<th>Mouth feel</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>7.50±0.12C</td>
<td>7.45±0.10B</td>
<td>7.50±0.10C</td>
<td>7.90±0.15AB</td>
<td>5.35±0.11C</td>
<td>35.70±0.25C</td>
</tr>
<tr>
<td>T1</td>
<td>7.22±0.14C</td>
<td>7.31±0.20B</td>
<td>7.10±0.12D</td>
<td>7.60±0.11B</td>
<td>7.21±0.12AB</td>
<td>36.44±0.99BC</td>
</tr>
<tr>
<td>T2</td>
<td>8.41±0.14A</td>
<td>7.50±0.08B</td>
<td>8.29±0.07A</td>
<td>8.10±0.17A</td>
<td>6.91±0.08B</td>
<td>39.21±0.28B</td>
</tr>
<tr>
<td>T3</td>
<td>8.21±0.13AB</td>
<td>8.09±0.10A</td>
<td>7.96±0.13B</td>
<td>8.30±0.12A</td>
<td>8.29±0.09A</td>
<td>40.85±0.72A</td>
</tr>
<tr>
<td>T4</td>
<td>7.96±0.16B</td>
<td>7.33±0.11B</td>
<td>7.27±0.06CD</td>
<td>8.21±0.09A</td>
<td>7.13±0.12AB</td>
<td>37.90±0.74B</td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05)

T0= without IVPI; T1= with 5% IVPI; T2= with 10% IVPI, T3= with 15% IVPI; T4= with 20% IVPI
4.1.3. DISCUSSION
4.1.3.1. Proximate composition of raw materials

The values for proximate composition of peanuts (*Arachis hypogaea* L.) indicated the moisture content, crude protein, crude fat, crude fiber, ash and NFE contained 4.59±0.02%, 35.76±0.07%, 44.96±0.06%, 3.75±0.02%, 2.59±0.02%, and 12.93±0.06%, respectively (Table 4.1). These results are in line with the findings of Atasie *et al.* (2009), who explained that Peanuts contain 47.0% fat, 38.6% protein, 5.8% moisture, 1.8% carbohydrate, 3.7% crude fiber and 3.1% ash. Ng *et al.* (2008) analyzed different peanut varieties for their moisture, protein, sugar, ash and oil contents. Some minor variations exist among varieties that indicated that genetically modified and normal peanuts did not differ significantly for their chemical composition and can be utilized successfully in different food products. Similarly, a study of peanuts composition indicated that peanuts contained 3.53% moisture, 46.35% fat, 29.59% protein, 13.06% carbohydrates, 5.20% total dietary fiber, 1.36% soluble fibers, 3.84% insoluble fibers, 2.27% ash and 587.75 Kcal/100g energy (Sousa *et al.*, 2011).

The vetch grains were detoxified to an extent of 93.05% by steeping in double quantity of water (60-70 °C) for 8 hrs with changing water for 7 times, draining and sun drying (Rehman *et al.*, 2006a; Rehman *et al.*, 2014). Removal of bran portion had further reduced the toxin during milling process. It enhanced the biological value (BV) of vetch flour and had no adverse effect on rats. Net protein utilization, digestibility, biological value and protein efficiency ratio increased and feed gain ratio decreased as a result of removal of toxin and improvement in the quality of protein when added in the chapattis was observed (Rehman *et al.*, 2006a). The values for proximate composition of vetch flour (detoxified) including moisture content, crude protein, crude fat, crude fiber, ash and NFE were found as 8.07±0.02%, 28.90±0.05%, 1.27±0.06%, 2.04±0.01%, 1.52±0.01%, and 67.26±0.07%, respectively. The results are in close agreement with the findings of Rehman *et al.* (1997) who stated that Indian vetch is a rich source of protein carried 28.38% protein and 362.3 Kcal/100g as energy.

The results of proximate composition of dates are in close agreement and comparable to the findings of research work conducted by the previous researchers. Ismail *et al.* (2006) studied five date varieties and reported that moisture, protein and ash contents in date varieties ranged between 20.25-22.14%, 2.3-2.7% and 1.83-2.36%, respectively. In another study, Ismail *et al.* (2008) reported that moisture level in two date varieties ranged from 20.7 to 26.7%, protein ranged from 2.4 to 3.6% and ash from 2.3 to 2.9%. Ramadan (1995) reported that moisture level in different date varieties ranged from 7.58 to 12.56% in dry dates, crude protein in the range of 2.39 to 3.81% and ash in the range of 1.62% to 1.98%. Al-Shahib and Marshal (2003) reported
that moisture content in different date varieties ranged from 9.2% to 32.1%, protein 1.7 to 3.0%, ash 0.3 to 2.4%, fat 0.1 to 0.5% and crude fiber 1.7 to 4.6%.

The values for proximate composition of IVPI i.e. moisture, crude protein, crude fat, crude fiber and ash were 4.40±0.06%, 87.54±0.14%, 0.34±0.03%, 0.12±0.01% and 1.35±0.05%, respectively. An appreciable quantity (87.54%) of IVPI was obtained from Indian Vetch flour. The results are in close agreement with the findings of Onwulata et al. (2001). Despande and Campell (1992) have reported 83.3 to 92.1% yield of grasspea protein isolates depending on solvents used in their preparation. Ant’Anna et al. (1985) used various isolation conditions for protein isolation from pigeonpea and reported the protein yield ranged from 49.7 to 63.6% in different isolates. In another stuy, Taha (1987) used 0.05M NaOH for the extraction of protein isolate from pigeonpea and extracted 92% protein isolate at a pH 4.4 with protein content of 88%. Chavan et al (2001) reported 85.1% protein isolate of beachpea containing a protein content of 86.6%. However, at pH 4.5, the percent recovery from lathyrus clymenum and lathyrus annuus was 60% and the isolates contained 81.07 to 82.4% of protein, respectively (Pastor-Cavada et al., 2011).

4.1.3.2. Physical properties of IVPI incorporated DBB

In this study, the main objective was to evaluate the effect of addition of vetch protein isolate (IVPI) instead of its flour on the physical and sensory properties of DBB. The Indian Vetch was detoxified by following the aforementioned technique (Rehman et al., 2006a). Indian Vetch protein isolate has been proved as an appropriate source of protein supplementation in the development of DBB as it reduced the carbohydrates and enhanced the protein quantity as well as quality and improved nutritional benefits. In the present investigation, it was found that with the addition of IVPI, physical and sensory properties of DBB improved in terms of texture, color, taste and flavor. The color became darker beyond 20% addition of IVPI. Water activity, an indicator for shelf stability of DBB was not significantly affected by replacement of flour with IVPI, while hardness and fractureability were adversely affected. These results are supported by the findings of other researchers (Singh and Mohamed, 2007). It has been reported that protein isolates affected the breaking strength of the cookies. However, a decreasing trend in breaking strength with increasing high protein bean flour was observed (Dreher and Patek, 1984). The main difference between two systems was that they had used bean flour instead of protein isolate. McWatters et al. (2003) reported the harder texture of cookies with an increase in protein content and its interaction with dough development and subsequent baking. Maache-Rezzoug et al. (1998) found that
higher the level of protein isolate, the lower the dough hydration, also the doughs deprived in consistency and were found crumbly. Moreover, composite flours form aggregates with increased number of hydrophilic points available to compete for limited free water in cookies dough (Kissell and Yamzaki, 1975). The results were also in accordance with previous findings in which drier and crumbly dough with 20% and 30% replacement of wheat flour with either soybean or pigeonpea flour was observed (McWatters, 1978; Tiwari et al., 2011).

The results were found contradictory while working on other protein sources for the preparation of bars. Bars containing whey protein isolate and calcium caseinate were evaluated for textural profile analysis. Bars with whey protein isolate showed soft texture throughout storage period due to formation of continuous matrix of protein and sugars, whereas texture become hard in case of bars prepared with calcium caseinate during storage due to migration of water molecule from protein towards glucose and glycerol after 10-18 hrs of preparation of the bars. The results suggested that movement of water molecules and segregation of aggregated protein towards glucose and glycerol caused the hardening of bars during storage (Loveday et al., 2010).

Mango bars with soy protein had higher hardness and springiness. The bars which were fortified with coconut powder had relatively less hardness (Mir and Nath, 1995). Protein bars with added protein, fat, sugars and minimum amount of water (water activity in the range of 6.0-6.5) indicated that fractureability force increased and continued to increase with the passage of time. During this period, rate of chemical reaction decreased and protein particles clustered resulting in precipitation of soluble protein due to migration of moisture. These observations suggested that role of chemical reaction is less as compared to variation in microstructure caused by moisture migration in hardening of protein bars (Loveday et al., 2009).

The effects of ingredients on color, texture, moisture content, water activity and sensory attributes were investigated in pear fruit bars. Pectin is found to be the most considerable independent variable that affects the important properties like sensory characteristics, hardness and chewiness (Huang and Hsieh, 2006). In a similar study, it was found that hardness decreased in date bars with the addition of whey protein concentrate and vetch protein isolates (Nadeem et al., 2012).

Similar results were also observed by some other research workers. Protein addition like whey protein that has considerable viscosity, gel strength and water holding capacity, may contribute in the texture of the bars such as firmness (Uthayakumaran et al., 2000; Ortiz et al., 2008; Shaun, 2008). Firmness in bars, due to protein addition, might be involved moisture
migration between the carbohydrates (such as starches, pectins, sugars and maltodextrin) and the proteins during storage (Shaun, 2008).

The sensory quality parameters are important dimensions of total product quality and are evaluated by the human senses such as taste, sight, smell, touch and hearing (Meilgaard et al., 1991). The sensory characteristics in this study were evaluated on hedonic rating. Among sensory characteristics, color, taste and flavor are important decisive factors. In the present study, all the DBB containing IVPI had achieved good score for color, texture, taste, flavor, mouth feel and overall acceptability. The results suggested that addition of IVPI had improved the sensory characteristics of DBB. These results are agreed with the findings of previous researchers (Grankivist and Biel, 2001; Magnusson et al., 2001; Heinio, 2003). They stated that aroma and flavor had high impact on consumer liking, followed by taste and appearance of cereal bars. Moreover, preference was also associated with sweetness, filling flavor, chew and crunchy texture (Bower and Whitten, 2000). However, food products with darker color are less preferred because of their unappealing color as in case of bars containing black bean obtained less score for color characteristic as compared to bars with red bean (Maurer et al., 2005). In this study, IVPI had not significantly disturbed the color of the DBB and had been utmost liked by the judges.

4.1.4. CONCLUSION

The results regarding the proximate composition suggested that IVPI was found suitable to enhance nutritional value of DBB. Physical properties and sensory characteristics for DBB were evaluated to assess the suitability of replacement of IVPI. The results revealed that replacement of IVPI in DBB contributed hardness in texture but acceptable crispness. It was noted that all sensory parameters such as color, taste, flavor and overall acceptability were significantly affected with more than 15% replacement of IVPI in DBB. On the basis of overall acceptability parameteric results, it was concluded that 15% IVPI could be incorporated into DBB without affecting their physical and sensory qualities.
4.2. PHYSICAL AND SENSORY ANALYSES OF DATE FRUCTOSE BISCUIT BARS (DFBB)

4.2.1. OBJECTIVE

In this study, the main objective was to assess the suitability of replacement of cane sugar with date fructose for the development of nutrient dense biscuit bar by applying physical and sensory tests.

4.2.2. RESULTS

Dates sugars were converted into date fructose by applying enzymes assay. The composition of sugars was determined through GC-FID analysis. 83% total sugars were obtained in which 53% were fructose after conversion with isomerase enzyme. Highest amount of fructose followed by glucose (28%) and sucrose (2%) was obtained. In recipe, sucrose was replaced with date fructose sugar in different levels for the preparation of DFBB.

4.2.2.1. Physical properties of date fructose biscuit bar (DFBB)

Highly significant differences (P<0.01) were observed among treatments of date sugar biscuit bars (Table 4.6). Color values for T0, T1, T2, T3 and T4 were 172.00±2.11 CTn, 168.00±3.49 CTn, 153.00±3.55 CTn, 149.00±2.99 CTn, 142.00±2.75 CTn, respectively (Table 4.7). Minimum color value (142.00±2.75 CTn) was recorded in treatment T4 having darkest color while color value of T0 was the highest (172.00±2.11 CTn) among all treatments indicating light color. Color values of T2 (153.00±3.55 CTn) and T3 (149.00±2.99 CTn) are more acceptable than other treatments due to more appealing color.

Highly significant (P<0.01) differences were recorded in hardness values of different treatments of biscuit bars (Table 4.6). The highest value of hardness was recorded in treatment T0 (2139.21±15.13g) while the lowest value in case of T4 (1369.23±26.25g). Treatment T2 (1897.21±49.91g) showed optimum hardness. Hardness values for T0, T1, T2, T3 and T4 were 2139.21±15.13g, 2087.59±14.46g, 1897.21±49.91g, 1521.79±26.57g, 1369.23±26.25g, respectively (Table 4.7).

Fractureability values also have highly significant (P<0.01) differences among treatments of biscuit bars (Table 4.6). Fractureability values of bars ranged from 62.41±1.21mm to 72.76±0.89mm. Fractureability values of T0, T1, T2, T3 and T4 were 72.76±0.89mm, 71.30±1.48mm, 71.59±1.66mm, 68.39±1.37mm, 62.41±1.21mm, respectively. Optimum values were observed in case of treatment T2 and T3 (Table 4.7).
Highly significant (P>0.01) differences were found in water activity values of biscuit bars (Table 4.6). Water activity of these bars ranged from 0.235±0.003 to 0.335±0.003. Water activities of T₀, T₁, T₂, T₃ and T₄ were 0.235±0.003, 0.240±0.006, 0.330±0.006, 0.330±0.006 and 0.335±0.003, respectively. The water activity values of all biscuit bars were in acceptable range that reflected these bars were shelf stable (Table 4.7).
Table 4.6: Analysis of variance (mean squares) for DFBB

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Color</th>
<th>Hardness</th>
<th>Fracturability</th>
<th>aw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>488.100**</td>
<td>352605.0**</td>
<td>52.1365**</td>
<td>0.00801**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>27.467</td>
<td>2594.0</td>
<td>5.4691</td>
<td>0.00007</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

Table 4.7: Comparison of means of DFBB

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Color</th>
<th>Hardness</th>
<th>Fracturability</th>
<th>aw</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>172.00±2.11A</td>
<td>2139.21±15.13A</td>
<td>72.76±0.89A</td>
<td>0.235±0.003B</td>
</tr>
<tr>
<td>T₁</td>
<td>168.00±3.49A</td>
<td>2087.59±14.46A</td>
<td>71.30±1.48AB</td>
<td>0.240±0.006B</td>
</tr>
<tr>
<td>T₂</td>
<td>153.00±3.55B</td>
<td>1897.21±49.91B</td>
<td>71.59±1.66AB</td>
<td>0.330±0.006A</td>
</tr>
<tr>
<td>T₃</td>
<td>149.00±2.99BC</td>
<td>1521.79±26.57C</td>
<td>68.39±1.37B</td>
<td>0.330±0.006A</td>
</tr>
<tr>
<td>T₄</td>
<td>142.00±2.75C</td>
<td>1369.23±26.25D</td>
<td>62.41±1.21C</td>
<td>0.335±0.003A</td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05)

T₀= without date sugar; T₁= with 5% date sugar; T₂= with 10% date sugar; T₃= with 15% date sugar; T₄= with 20% date sugar
4.2.2. Sensory properties of date fructose biscuit bar (DFBB)

Analysis of variance for color score of DFBB has been given in Table 4.8. Results show non-significant (P>0.05) difference in color score of date fructose incorporated biscuit bars. Color score varied from 7.26±0.12 to 7.54±0.18. The highest color score was achieved by T2 (7.54±0.18) followed by T1 (7.45±0.08). Color scores for T0, T1, T2, T3 and T4 were 7.45±0.08, 7.51±0.20, 7.54±0.18, 7.39±0.11 and 7.26±0.12, respectively (Table 4.9).

Highly significant (P<0.01) differences were recorded in the Taste score of biscuit bars (Table 4.8). The results revealed that maximum taste score was obtained by T2 (6.84±0.06). It is evident that all treatments were liked by the judges but treatments T2 and T3 were maximally liked due to increase in sweetness. Overall taste score of all treatments varied from 5.59±0.07 to 6.84±0.06. Taste score for treatment T0, T1, T2, T3 and T4 were 6.75±0.15, 6.81±0.23, 6.84±0.06, 6.84±0.06 and 5.59±0.07, respectively (Table 4.9).

Analysis of variance for flavor score of date fructose incorporated biscuit bars indicated highly significant differences (P<0.01) among treatments (Table 4.8). Flavor score for treatment T0, T1, T2, T3 and T4 were 6.89±0.14, 7.67±0.21, 7.91±0.21, 7.95±0.11 and 7.21±0.08, respectively (Table 4.9). Maximum flavor score was achieved by treatment T3 (7.95±0.21) followed by T2 (7.91±0.21).

The statistical analysis of texture score of biscuit bars indicated highly significant (P<0.01) variation among treatments (Table 4.8). Mean values for texture score of bars is given in Table 4.9. Texture values ranged from 6.05±0.08 to 8.52±0.10. Mean values for texture score of bars for treatment T0, T1, T2, T3 and T4 were 7.49±0.19, 8.29±0.14, 8.50±0.10, 8.52±0.17 and 6.05±0.08, respectively. Texture score of T3 is the highest (8.52±0.17) followed by T2 (8.50±0.10).

Significant (P<0.05) variations exist in mouth feel score of biscuit bars among treatments (Table 4.8). Mouth feel score of bars varied from 6.60±0.09 to 7.30±0.14 (Table 4.8). The best treatment was T3 that achieved the highest mouth feel score (7.35±0.14) followed by T4 (7.20±0.14). The mouth feel score of treatments were 6.80±0.13, 6.70±0.16, 6.60±0.09, 7.35±0.14, 7.30±0.14 for T0, T1, T2, T3 and T4, respectively (Table 4.9).

Overall acceptability of bars varied from 33.41±0.85 to 38.05±0.34. Overall acceptability score of treatments are 35.38±0.65, 36.98±0.86, 37.39±0.99, 38.05±0.34, 33.41±0.85 for T0, T1, T2, T3 and T4 respectively. The highest overall acceptability score was achieved by T3 (38.05±0.34) and followed by T2 (37.39±0.99).
Table 4.8: Analysis of variance (mean squares) for sensory evaluation of DFBB

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Color</th>
<th>Taste</th>
<th>Flavor</th>
<th>Texture</th>
<th>Mouth feel</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>0.03705NS</td>
<td>0.86649**</td>
<td>0.47076**</td>
<td>3.00039**</td>
<td>0.29100*</td>
<td>7.32495*</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.06494</td>
<td>0.05082</td>
<td>0.07694</td>
<td>0.06214</td>
<td>0.05290</td>
<td>1.79288</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

Table 4.9: Comparison of means of DFBB

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Color</th>
<th>Taste</th>
<th>Flavor</th>
<th>Texture</th>
<th>Mouth feel</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>7.45±0.08A</td>
<td>6.75±0.15A</td>
<td>6.89±0.14D</td>
<td>7.49±0.19C</td>
<td>6.80±0.13B</td>
<td>35.38±0.65C</td>
</tr>
<tr>
<td>T1</td>
<td>7.51±0.20A</td>
<td>6.81±0.23A</td>
<td>7.67±0.21C</td>
<td>8.29±0.14AB</td>
<td>6.70±0.16B</td>
<td>36.98±0.86AB</td>
</tr>
<tr>
<td>T2</td>
<td>7.54±0.18A</td>
<td>6.84±0.06A</td>
<td>7.91±0.21A</td>
<td>8.50±0.10A</td>
<td>6.60±0.09BC</td>
<td>37.39±0.99A</td>
</tr>
<tr>
<td>T3</td>
<td>7.39±0.11A</td>
<td>6.84±0.06A</td>
<td>7.95±0.11A</td>
<td>8.52±0.17A</td>
<td>7.35±0.14A</td>
<td>38.05±0.34A</td>
</tr>
<tr>
<td>T4</td>
<td>7.26±0.12A</td>
<td>5.59±0.07B</td>
<td>7.21±0.08C</td>
<td>6.05±0.08D</td>
<td>6.30±0.14A</td>
<td>32.41±0.85D</td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05)

T0= without date sugar; T1= with 5% date sugar; T2= with 10% date sugar T3= with 15% date sugar; T4= with 20% date sugar
4.2.3. DISCUSSION

In the present study, the results suggested that color value and hardness of DFBB decreased with the replacement of sucrose with date fructose in DFBB. Decrease in color test value indicated dark color that might be due to the browning reaction (Maillard reaction) between added protein isolate and date fructose (Asikin et al., 2014). Also, it caused serious effects on food properties such as the color, texture, flavor and nutritional values of bakery products (Zhou et al., 2013; Virág et al., 2013). The flavor properties of Maillard reaction products may vary from a pleasant, flowery and fragrant aroma to a burnt, pungent, nutty and caramel-like, depending on the amino acid and types of sugar in the food system and their reaction modes (Wong et al., 2008). Similarly, decrease in hardness in DFBB might be due to more moisture retention capacity of date sugars used in date fructose biscuit bars formulation. Similar results were also observed by researchers working on different types of bars. Sugars, fibers, proteins and polyphenols mainly contribute in chemical reactions in the snack bars (Shaun, 2008).

Generally, health problems are associated with the consumption of refined sugars than raw sugars. During processing vitamins, minerals, enzymes, proteins, fatty acids are removed from the food and the sugar is concentrated. As a result, the body does not metabolize it properly and numerous health problems appear. In a study, immune system suppression was reported by observing live blood under dark field microscope and observed that patients on diets with refined sugars had paralyzed white blood cells. When their meals were changed; the white blood cells became active and started moving again (James, 2009).

Water content changes the texture from soft to hard and brittle texture as the moisture content decreased. However, knowledge of water activity, or the ratio of vapor pressures, is necessary to control shelf stability. Difference in water activity is the dynamic force for moisture movement in candy. Large difference in water activity governs moisture migration. Controlling water activity in confections like candies, toffees with low moisture content render them stable for a very long time (Ergun et al., 2010).

Lowering the water activity below a certain level retards moisture migration in food product that inhibits mold growth and affects its shelf stability for example chocolate and caramel foods can be stored successfully at ambient storage conditions (Bell and Labuza, 2000). For commercial foods like confectionary that usually spoil as a result of surface mold growth,
water activity should below 0.6. Addition of ingredients like proteins, carbohydrates and salts can be a used as a significant tool to lower the water activities by lowering the molecular energy of water i.e. adhesive and cohesive forces and bind water in the food system. Water molecules bind with some specific sites like OH group of polysaccharides and NH$_2$ group of amino acids in protein with hydrogen bonding, dipole bonds and don’t act as a solvent (Loveday et al., 2010).

In the present investigations, water activity of DFBB ranged from 0.235 to 0.335 which were found lower than previous findings. The results revealed that water activity increased within treatments with replacement of sucrose with date fructose. Water holding capacity of DFBB improved with the replacement of sucrose with date fructose resulting in higher water activity than control. Effect of water activity was assessed on the cutting resistance in fruit bars. The cutting force increased from 4.4N to 452N (100 times) with decreasing water activity from 0.86-0.33. The fruit bars become brittle at 0.30 water activities with 68N cutting resistance. Determination of water activity (aw) helps to predict food mechanical properties, stability and shelf life. The water activity of control and fiber-rich bars was 0.483 and 0.460, respectively. The water activity of all the snack bar was 0.44. These all values of water activities were below 0.7,signifying fewer hazards of microbial growth and spoilage (Sun-Waterhouse et al., 2010). Moreover, the lower water activity may be associated with texture, shelf stability, chemical reactivity and enzymatic activity (Labuza, 2000).

In recent years, food industries have articulated a growing concern in the substitution of sucrose as a response to the public curiosity in low-calorie products. For instance, fructose is normally used as replacer of sucrose, because it is the sweetest natural sugar, delivering 1.2-1.8 times the more sweetness than sucrose in the most of the food products. Moreover, it has sweetness synergy with other sweeteners which may permit cost savings (Lai and Lin, 2006; Mariotti and Alamprese, 2012). For the production of low caloric cake for diabetic and health conscious consumers, date syrup containing fructose and sorbitol was used replacing sucrose and its effect on quality of cakes was examined. Date syrup and sucrose was used in five different proportions in the preparation of cakes. Sensory evaluation of cakes at different intervals of storage was carried out to find out consumer acceptability. The sensory characteristics i.e. cells, grains, crumb color, texture, flavor and taste etc. of cakes were maximum when sucrose was replaced with date syrup in the proportion of 50% (Tufail et al, 2002). In the present study, sensory parameters such as taste, flavor, texture, mouth feel and overall acceptability except
color were significantly affected with the replacement of sucrose with date fructose in DFBB. However, in most of the parameters, the effect was non-significant up to the level of 15%.

4.2.4. CONCLUSION

It was concluded from above discussion that all treatments containing different concentrations of date fructose were found acceptable in sensory attributes. However, DFBB with 15% sucrose replacement with date fructose was found the best in terms of physical and sensory characteristics.
4.3. OPTIMIZATION OF ANTIOXIDANT EXTRACT LEVELS BY USING RESPONSE SURFACE METHODOLOGY (RSM)

4.3.1. OBJECTIVES

To optimize the antioxidant extract levels by using response surface methodology (RSM) for getting biscuit bars with better sensory acceptability and shelf life stability

4.3.2. RESULTS

In order to improve the antioxidant level of date fructose biscuit bars, cheap and underutilized sources have been explored. Peanut skin and *withania coagulans* extracts are used in different levels as the antioxidant sources to increase the shelf-life of DFBB. The Phenolic extracts were obtained by treating the dried ground powder of Peanut skin and berries of *withania coagulans* with ethyl alcohol. The Peanut skin (peels) extract and *withania coagulans* contained 64.6% and 57% antioxidant activities and 98.6mg/g and 13.2mg/g total phenolics and 74.2 and 52.5% free radical oxygen scavenging activities, respectively (Omer, 2009). The date fructose biscuit bars (DFBB) were prepared by using the best formulation as selected in the previous studies (15% IVPI+ 15% DF and incorporating peanut peel extract and *withania coagulans* extract at variable levels. Response Surface Methodology (RSM) was applied to estimate the responses of independent variables i.e. peanut peel extract (X) and *withania coagulans* extract (Y) during storage. Fourteen date fructose biscuit bar treatments were generated using a Central Composite Design (CCD) with 2 variables and 3 levels for each variable. Second-order polynomial model was fitted for independent variables i.e. peanut peel extract (X) and *withania coagulans* extract (Y). The regression equations and coefficients were determined by using multiple regression analysis of storage’s data regarding different parameters.

4.3.2.1. Hardness (Firmness) of Date Fructose Biscuit Bars

The responses for hardness from Central Composite Design (CCD) were fitted with second order polynomial equations (Table 4.10). The statistical analysis by applying analysis of variance technique to the full regression of model (Table 4.11) shows significant effect of variables. However, linear terms of variable (X) are observed to positively change the hardness of biscuit bars at all storage intervals, whereas quadratic terms of *withania coagulans* extract (Y) have a negative effect. When interaction of these two terms (XY) was studied, it was found
negative over all storage intervals. The coefficients of determination ($R^2$) were studied as above 98.3%, therefore it could be assured that models are adequately fitted. The data showed that peanut peel extract (X) and *withania coagulans* extract (Y) contributed towards increase in firmness in DFBB at 0 to 90 days storage intervals. The effect of peanut peel extract (X) and *withania coagulans* extract (Y) concentrations in DFBB through three dimensional response surface plots for hardness (firmness) at 0, 30, 60 and 90 days storage intervals are shown in Figure 4.1-4.4, which demonstrates that both variables contribute towards increase in hardness. These graphs indicate a clear effect of two independent variables.

### 4.3.2.2. Fractureability of Date Fructose Biscuit Bars

The models were developed for fractureability of DFBB as affected by independent variables during 90 days storage. The regression coefficients of variables in models are shown in Table 4.12. The data showed that peanut peel extract (X) and *withania coagulans* extract (Y) contributed towards increase in fractureability in date fructose biscuit bars at 0 to 90 days storage intervals. The effect of linear terms of X and Y are statistically significant (P<0.05) for fractureability of date fructose biscuit bars at all days of storage intervals (Table 4.13). The $X^2$ quadratic terms are found significant at 0, 15, 30, 45 and 60 days storage intervals, whereas, the quadratic terms for $Y^2$ are found significant at 0 day. The interaction of two variables (XY) shows non-significant effect on fractureability of date fructose biscuit bars at all storage intervals. The coefficients of determination ($R^2$) were studied as above 96.9%, therefore it could be assured that models are well fitted. The effect of peanut peel extract (X) and *withania coagulans* extract (Y) concentrations in date fructose biscuit bars through three dimensional response surface plots for fractureability at 0, 30, 60 and 90 days storage intervals are shown in Fig. 4.5-4.8, which demonstrates that both variables contribute towards increase in fractureability in date fructose biscuit bars at 0 to 90 days storage intervals. These graphs indicate a clear effect of these independent variables.
### Table 4.10: Response Surface Regression (Analysis of variance) for Hardness.

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Degrees of freedom</th>
<th>Day 0 Mean squares</th>
<th>Day 15 Mean squares</th>
<th>Day 30 Mean squares</th>
<th>Day 45 Mean squares</th>
<th>Day 60 Mean squares</th>
<th>Day 90 Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>5</td>
<td>148.846**</td>
<td>150.090**</td>
<td>153.843**</td>
<td>157.413**</td>
<td>152.583**</td>
<td>160.579**</td>
</tr>
<tr>
<td>Linear</td>
<td>2</td>
<td>327.629**</td>
<td>329.687**</td>
<td>335.455**</td>
<td>341.144**</td>
<td>329.049**</td>
<td>343.923**</td>
</tr>
<tr>
<td>Square</td>
<td>2</td>
<td>44.248*</td>
<td>45.301*</td>
<td>48.221*</td>
<td>51.329*</td>
<td>50.661*</td>
<td>56.137*</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>0.476</td>
<td>0.476</td>
<td>1.863</td>
<td>2.117</td>
<td>3.497</td>
<td>2.772</td>
</tr>
<tr>
<td>Residual Error</td>
<td>3</td>
<td>4.147</td>
<td>4.294</td>
<td>3.839</td>
<td>4.074</td>
<td>4.458</td>
<td>4.238</td>
</tr>
</tbody>
</table>

* = Significant (P<0.1); ** = Highly Significant (P<0.05)

### Table 4.11: Regression coefficients for the models representing as a function of variations in the independent variables (Peanut Peel Extract (X) and Withania Coagulans Extract (Y))

<table>
<thead>
<tr>
<th>Terms of model equations</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>253.46**</td>
<td>253.13**</td>
<td>252.88**</td>
<td>252.56**</td>
<td>251.80**</td>
<td>250.72**</td>
</tr>
<tr>
<td>X</td>
<td>-10.35**</td>
<td>-10.38**</td>
<td>-10.48**</td>
<td>-10.56**</td>
<td>-10.37**</td>
<td>-10.60**</td>
</tr>
<tr>
<td>Y</td>
<td>-1.47</td>
<td>-1.47</td>
<td>-1.45</td>
<td>-1.47</td>
<td>-1.47</td>
<td>-1.54</td>
</tr>
<tr>
<td>X×X</td>
<td>-3.11</td>
<td>-2.99</td>
<td>-3.22</td>
<td>-3.62</td>
<td>-3.70</td>
<td>-4.02</td>
</tr>
<tr>
<td>Y×X</td>
<td>-5.88*</td>
<td>-6.03*</td>
<td>-6.15*</td>
<td>-6.18*</td>
<td>-6.08*</td>
<td>-6.32*</td>
</tr>
<tr>
<td>X×Y</td>
<td>-0.34</td>
<td>-0.35</td>
<td>-0.68</td>
<td>-0.73</td>
<td>-0.93</td>
<td>-0.83</td>
</tr>
</tbody>
</table>

R²: 98.4% 98.3% 98.5% 98.5% 98.3% 98.4%

* = Significant (P<0.1); ** = Highly Significant (P<0.05)
Fig 4.1: Effect of independent variables (X, Y) on hardness in date fructose biscuit bars during storage (at 0 day)

Fig 4.2: Effect of independent variables (X, Y) on hardness in date fructose biscuit bars during storage (at 30 day)
Fig 4.3: Effect of independent variables (X, Y) on hardness in date fructose biscuit bars during storage (at 60 day)

Day=60
Hardness = 201.8089+72.4833*x+368.75*y-370.3333*x*x-93.5*x*y-607.8333*y*y

> 255
< 255
< 250
< 245
< 240
< 235
< 230
< 225

Fig 4.4: Effect of independent variables (X, Y) on hardness in date fructose biscuit bars during storage (at 90 day)

Day=90
Hardness = 198.5661+79.8917*x+380.5*y-402.1667*x*x-83.25*x*y-632.1667*y*y

> 255
< 255
< 250
< 245
< 240
< 235
< 230
< 225

82
Table 4.12: Response Surface Regression (Analysis of variance) for Fracturability

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Degrees of freedom</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>5</td>
<td>1.80115**</td>
<td>1.78953**</td>
<td>1.79416**</td>
<td>1.81864**</td>
<td>1.79597**</td>
<td>1.64730**</td>
</tr>
<tr>
<td>Linear</td>
<td>2</td>
<td>3.82198**</td>
<td>3.85198**</td>
<td>3.85133**</td>
<td>3.89402**</td>
<td>3.81923**</td>
<td>3.60934**</td>
</tr>
<tr>
<td>Square</td>
<td>2</td>
<td>0.68090*</td>
<td>0.62176*</td>
<td>0.63406*</td>
<td>0.65254*</td>
<td>0.67050*</td>
<td>0.50571</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>0.00001</td>
<td>0.00014</td>
<td>0.00002</td>
<td>0.00010</td>
<td>0.00040</td>
<td>0.00640</td>
</tr>
<tr>
<td>Residual Error</td>
<td>3</td>
<td>0.03008</td>
<td>0.03134</td>
<td>0.03650</td>
<td>0.03411</td>
<td>0.03818</td>
<td>0.08846</td>
</tr>
</tbody>
</table>

Table 4.13: Regression coefficients for the models representing as a function of variations in the independent variables (Peanut Peel Extract (X) and Withania Coagulans Extract (Y))

<table>
<thead>
<tr>
<th>Terms of model equations</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>78.4556**</td>
<td>78.3618**</td>
<td>78.3289**</td>
<td>78.2789**</td>
<td>78.2200**</td>
<td>78.0278**</td>
</tr>
<tr>
<td>X</td>
<td>1.1223**</td>
<td>1.1268**</td>
<td>1.1267**</td>
<td>1.1317**</td>
<td>1.1200**</td>
<td>1.0917**</td>
</tr>
<tr>
<td>Y</td>
<td>0.1198</td>
<td>0.1193</td>
<td>0.1200</td>
<td>0.1317</td>
<td>0.1367</td>
<td>0.1067</td>
</tr>
<tr>
<td>X×X</td>
<td>-0.7173*</td>
<td>-0.7002*</td>
<td>-0.7033*</td>
<td>-0.7083*</td>
<td>-0.7200*</td>
<td>-0.6317</td>
</tr>
<tr>
<td>Y×Y</td>
<td>-0.4078*</td>
<td>-0.3627</td>
<td>-0.3733</td>
<td>-0.3883</td>
<td>-0.3900</td>
<td>-0.3267</td>
</tr>
<tr>
<td>X×Y</td>
<td>0.0012</td>
<td>0.0060</td>
<td>-0.0025</td>
<td>0.0050</td>
<td>-0.0100</td>
<td>-0.0400</td>
</tr>
</tbody>
</table>

R²                      99.0%      99.0%      98.8%      98.9%      98.7%      96.9%

* = Significant (P<0.1); ** = Highly Significant (P<0.05)
Fig 4.5: Effect of independent variables (X, Y) on fracturability in date fructose biscuit bars during storage (at 0 day)

Fig 4.6: Effect of independent variables (X, Y) on fracturability in date fructose biscuit bars during storage (at 30 day)
Day=60
Fracturability = 69.12+40.3*x+24.9667*y-72*x*x-1*x*y-39*y*y

Fig 4.7: Effect of independent variables (X, Y) on fracturability in date fructose biscuit bars during storage (at 60 day)

Day=90
Fracturability = 69.8178+37.3833*x+21.4667*y-63.1667*x*x-4*x*y-32.6667*y*y

Fig 4.8: Effect of independent variables (X, Y) on fracturability in date fructose biscuit bars during storage (at 90 day)
4.3.2.3. Color of Date Fructose Biscuit Bars

The regression coefficients are shown in Table 4.14 as well as correlation coefficients obtained for all seven models related to color of date fructose biscuit bars under the influence of independent variables (peanut peel extract (X) and *withania coagulans* extract (Y)) at seven storage intervals (0, 15, 30, 45, 60, 75 and 90 days). The regression coefficients for these models ($R^2 = 94.0\%, 94.0\%, 93.8\%, 94.1\%, 93.9\%, 94.8\%$, respectively) are high enough for a well fitted response surface models.

The effect of linear term of X is statistically non-significant ($P<0.05$) for color of date fructose biscuit bars at all storage intervals, while linear term of Y is statistically significant ($P<0.05$) for color of date fructose biscuit bars at all storage intervals (Table 4.15). The $X^2$ quadratic terms are found significant at all storage intervals whereas, the quadratic terms for $Y^2$ are found non-significant at storage. The interaction of two variables (XY) shows non-significant effect on color at all storage intervals. The effect of peanut peel extract (X) and *withania coagulans* extract (Y) in date fructose biscuit bars for color at 0, 30, 60 and 90 days storage intervals through three dimensional response surface graphs is shown in Fig. 4.9-4.12, which demonstrates that both variables contribute towards color change in date bars at 0 to 90 days storage intervals. These graphs indicate a clear effect of two independent variables.

4.3.2.4. Flavor of Date Fructose Biscuit Bars

Regression coefficients of variables for flavor of date bars during storage have been presented in Table 4.16, whereas analysis of variance for flavor score has been presented in Table 4.17A. The coefficients of determination ($R^2$) showed that >95% variability is covered by the models. The regression coefficients for these models ($R^2 = 95.5\%, 95.8\%, 95.7\%, 96.4\%, 95.8\%, 95.8\%$ and 82.2% respectively) are high enough for a well fitted response surface models. The linear terms for X and Y significantly affect the flavor at 0, 15, 30, 45, 60, 75 and 90 days of storage interval. The interactive term XY also contributes a significant role in flavor over 45 days of storage period. Flavor score increase with an increase in X (peanut peel extract) and Y (*withania coagulans* extract) levels that are indicated by linear terms, but portion of each antioxidant level may vary. Interactive terms of these two variables also have positive effect on flavor perception of judges. The effect of changes in levels of two variables i.e. peanut peel extract and *withania coagulans* extract on the flavor of DFBB over 0, 30, 60 and 90
days storage intervals is depicted in Fig. 4.13-4.16. The surface plots show an increase in flavor score under the influence of *withania coagulans* extract whereas peanut peel extract exhibits little contribution.

4.3.2.5. Taste of Date Fructose Biscuit Bars

Regression coefficients of variables and analysis of variance for taste of date fructose biscuit bars during storage have been presented in Table 4.18 and 4.19, respectively. The coefficients of determination ($R^2$) for these models ($R^2 = 95.0\%, 94.4\%, 94.9\%, 95.2\%, 95.3\%$ and 94.1\% respectively) exhibit the adequacy of models and showed that it covers more variability in data. The data show a good contribution of variables towards achieving good score for taste attribute. The effect of linear terms of $X$ and $Y$ is non-significant for taste of date fructose biscuit bars at 0, 15, 30, 45, 60, 75 and 90 days storage intervals. The $X^2$ quadratic terms are found significant at 0, 15, 30, 45, 60, 75 and 90 days storage intervals, whereas, the quadratic terms for $Y^2$ are also found significant at 0, 15, 30, 45, 60, 75 and 90 days storage intervals. The interaction of two variables ($XY$) shows non-significant effect on taste at all days of storage intervals.

It is evident from data that at low level, independent variables (peanut peel extract and *withania coagulans* extract) have well contributed towards achieving good score for taste of date fructose biscuit bars. However, during the entire storage period, the taste of date fructose biscuit bars is found acceptable. The surface plots for taste of date fructose biscuit bars at 0, 30, 60 and 90 days of storage intervals are depicted in the Fig 4.17-4.20. During the storage, the scores for taste declined during 90 days storage intervals.
Table 4.14: Response Surface Regression (Analysis of variance) for Color

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Degrees of freedom</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>5</td>
<td>0.91968*</td>
<td>0.92711*</td>
<td>0.91655*</td>
<td>0.89566*</td>
<td>0.89774*</td>
<td>0.90785*</td>
</tr>
<tr>
<td>Linear</td>
<td>2</td>
<td>0.60043</td>
<td>0.56754</td>
<td>0.59542</td>
<td>0.54708</td>
<td>0.54591</td>
<td>0.55954</td>
</tr>
<tr>
<td>Square</td>
<td>2</td>
<td>1.58832*</td>
<td>1.63742*</td>
<td>1.59245*</td>
<td>1.59527*</td>
<td>1.59943*</td>
<td>1.61108*</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>0.22090</td>
<td>0.22563</td>
<td>0.20703</td>
<td>0.19360</td>
<td>0.19802</td>
<td>0.19802</td>
</tr>
<tr>
<td>Residual Error</td>
<td>3</td>
<td>0.09736</td>
<td>0.09888</td>
<td>0.10088</td>
<td>0.09570</td>
<td>0.09684</td>
<td>0.08312</td>
</tr>
</tbody>
</table>

* = Significant (P<0.10); ** = Highly Significant (P<0.05)

Table 4.15: Regression coefficients for the models representing as a function of variations in the independent variables (Peanut Peel Extract (X) and Withania Coagulans Extract (Y))

<table>
<thead>
<tr>
<th>Terms of model equations</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>8.044**</td>
<td>8.030**</td>
<td>7.967**</td>
<td>7.901**</td>
<td>7.837**</td>
<td>7.658**</td>
</tr>
<tr>
<td>X</td>
<td>-0.060</td>
<td>-0.053</td>
<td>-0.058</td>
<td>-0.042</td>
<td>-0.037</td>
<td>-0.013</td>
</tr>
<tr>
<td>Y</td>
<td>0.443*</td>
<td>0.432*</td>
<td>0.442*</td>
<td>0.425*</td>
<td>0.425*</td>
<td>0.432*</td>
</tr>
<tr>
<td>X×X</td>
<td>-1.167*</td>
<td>-1.180*</td>
<td>-1.165*</td>
<td>-1.172*</td>
<td>-1.180*</td>
<td>-1.217**</td>
</tr>
<tr>
<td>Y×Y</td>
<td>-0.477</td>
<td>-0.495</td>
<td>-0.485</td>
<td>-0.472</td>
<td>-0.455</td>
<td>-0.362</td>
</tr>
<tr>
<td>X×Y</td>
<td>-0.235</td>
<td>-0.237</td>
<td>-0.228</td>
<td>-0.220</td>
<td>-0.222</td>
<td>-0.222</td>
</tr>
</tbody>
</table>

R² 94.0% 94.0% 93.8% 94.1% 93.9% 94.8%

* = Significant (P<0.10); ** = Significant (P<0.05)
Day=0
Color = -3.5322+53.1167*x+37.7333*y-116.6667*x*x-23.5*x*y-47.6667*y*y

> 8
< 8
< 7.5
< 7
< 6.5
< 6
< 5.5
< 5

Fig 4.9: Effect of independent variables (X, Y) on color in date fructose biscuit bars during storage (at 0 day)

Day=30
Color = -3.6317+52.8417*x+38.0667*y-116.5*x*x-22.75*x*y-48.5*y*y

> 8
< 8
< 7.5
< 7
< 6.5
< 6
< 5.5
< 5

Fig 4.10: Effect of independent variables (X, Y) on color in date fructose biscuit bars during storage (at 30 days)
Day = 60
Color = -3.515 + 53.5083 * x + 36 * y - 118 * x * x - 22.25 * x * y - 45.5 * y * y

Day = 90
Color = -3.0672 + 55.2083 * x + 30.4667 * y - 121.6667 * x * x - 22.25 * x * y - 36.1667 * y * y

Fig 4.11: Effect of independent variables (X, Y) on color in date fructose biscuit bars during storage (at 60 days)

Fig 4.12: Effect of independent variables (X, Y) on color in date fructose biscuit bars during storage (at 90 days)
Table 4.16: Response Surface Regression (Analysis of variance) for Flavor

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Degrees of freedom</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 15</td>
</tr>
<tr>
<td>Regression</td>
<td>5</td>
<td>0.184032*</td>
</tr>
<tr>
<td>Linear</td>
<td>2</td>
<td>0.234042*</td>
</tr>
<tr>
<td>Square</td>
<td>2</td>
<td>0.173225*</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>0.105625</td>
</tr>
<tr>
<td>Residual Error</td>
<td>3</td>
<td>0.014414</td>
</tr>
</tbody>
</table>

* = Significant (P<0.1)

Table 4.17: Regression coefficients for the models representing as a function of variations in the independent variables (Peanut Peel Extract (X) and *Withania Coagulans* Extract (Y))

<table>
<thead>
<tr>
<th>Terms of model equations</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>7.7767**</td>
<td>7.7622**</td>
<td>7.7067**</td>
<td>7.6700**</td>
<td>7.6489**</td>
<td>7.5967**</td>
</tr>
<tr>
<td>X</td>
<td>-0.2367*</td>
<td>-0.2450*</td>
<td>-0.2417*</td>
<td>-0.2300*</td>
<td>-0.2267*</td>
<td>-0.2267*</td>
</tr>
<tr>
<td>Y</td>
<td>-0.1483</td>
<td>-0.1633*</td>
<td>-0.1633*</td>
<td>-0.1683*</td>
<td>-0.1733*</td>
<td>-0.1700*</td>
</tr>
<tr>
<td>X×X</td>
<td>-0.2000</td>
<td>-0.2183</td>
<td>-0.2250</td>
<td>-0.2200</td>
<td>-0.2333</td>
<td>-0.2300</td>
</tr>
<tr>
<td>Y×Y</td>
<td>-0.3650*</td>
<td>-0.3933*</td>
<td>-0.3800*</td>
<td>-0.3950*</td>
<td>-0.3933*</td>
<td>-0.4000*</td>
</tr>
<tr>
<td>X×Y</td>
<td>-0.1625</td>
<td>-0.1800</td>
<td>-0.1900</td>
<td>-0.2000*</td>
<td>-0.1950</td>
<td>-0.2050*</td>
</tr>
</tbody>
</table>

R² 95.5% 95.8% 95.7% 96.4% 95.8% 95.8%

* = Significant (P<0.10); ** = Highly Significant (P<0.05)
Fig 4.13: Effect of independent variables (X, Y) on flavor in date fructose biscuit bars during storage (at 0 day)

Fig 4.14: Effect of independent variables (X, Y) on flavor in date fructose biscuit bars during storage (at 30 days)
Day=60
Flavor = 2.9789+12.9167*x+25.7667*y-23.3333*x*x-19.5*x*y-39.3333*y*y

Fig 4.15: Effect of independent variables (X, Y) on flavor in date fructose biscuit bars during storage (at 60 day)

Day=90
Flavor = 2.81+13.0833*x+26.4*y-23*x*x-20.5*x*y-40*y*y

Fig 4.16: Effect of independent variables (X, Y) on flavor in date fructose biscuit bars during storage (at 90 days)
### Table 4.18: Response Surface Regression (Analysis of variance) for Taste

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Degrees of freedom</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>5</td>
<td>1.08730*</td>
<td>1.08116*</td>
<td>1.07403*</td>
<td>1.07767*</td>
<td>1.13105*</td>
<td>1.13062*</td>
</tr>
<tr>
<td>Linear</td>
<td>2</td>
<td>0.01171</td>
<td>0.00928</td>
<td>0.00894</td>
<td>0.01051</td>
<td>0.00708</td>
<td>0.00341</td>
</tr>
<tr>
<td>Square</td>
<td>2</td>
<td>2.70293*</td>
<td>2.69001*</td>
<td>2.67403*</td>
<td>2.67963*</td>
<td>2.81734**</td>
<td>2.81995*</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>0.00723</td>
<td>0.00723</td>
<td>0.00423</td>
<td>0.00810</td>
<td>0.00640</td>
<td>0.00640</td>
</tr>
<tr>
<td>Residual Error</td>
<td>3</td>
<td>0.09604</td>
<td>0.10623</td>
<td>0.09561</td>
<td>0.09014</td>
<td>0.09295</td>
<td>0.11850</td>
</tr>
</tbody>
</table>

* = Significant (P<0.10); ** = Highly Significant (P<0.05)

### Table 4.19: Regression coefficients for the models representing as a function of variations in the independent variables (Peanut Peel Extract (X) and *Withania Coagulans* Extract (Y))

<table>
<thead>
<tr>
<th>Terms of model equations</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>8.103*</td>
<td>8.061**</td>
<td>8.010**</td>
<td>7.947**</td>
<td>7.912**</td>
<td>7.839**</td>
</tr>
<tr>
<td>X</td>
<td>0.062</td>
<td>0.055</td>
<td>0.053</td>
<td>0.058</td>
<td>0.048</td>
<td>0.033</td>
</tr>
<tr>
<td>Y</td>
<td>0.010</td>
<td>0.008</td>
<td>0.012</td>
<td>0.010</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>X×X</td>
<td>-1.395**</td>
<td>-1.402**</td>
<td>-1.400**</td>
<td>-1.405**</td>
<td>-1.448**</td>
<td>-1.443*</td>
</tr>
<tr>
<td>Y×Y</td>
<td>-0.870*</td>
<td>-0.852*</td>
<td>-0.845*</td>
<td>-0.840*</td>
<td>-0.848*</td>
<td>-0.858*</td>
</tr>
<tr>
<td>X×Y</td>
<td>0.043</td>
<td>0.043</td>
<td>0.033</td>
<td>0.045</td>
<td>0.040</td>
<td>0.040</td>
</tr>
<tr>
<td>R²</td>
<td>95.0%</td>
<td>94.4%</td>
<td>94.9%</td>
<td>95.2%</td>
<td>95.3%</td>
<td>94.1%</td>
</tr>
</tbody>
</table>

* = Significant (P<0.10); ** = Highly Significant (P<0.05)
Day=0
Taste = -5.205+55.1417*x+51.45*y-139.5*x*x+4.25*x*y-87*y*y

Day=30
Taste = -5.1417+55.5583*x+50.1667*y-140*x*x+3.25*x*y-84.5*y*y

Fig 4.17: Effect of independent variables (X, Y) on taste in date fructose biscuit bars during storage (at 0 day)

Fig 4.18: Effect of independent variables (X, Y) on taste in date fructose biscuit bars during storage (at 30 days)
Day=60
Taste = -5.3878+57.2167*x+50.15*y-144.8333*x*x+4*x*y-84.8333*y*y

Fig 4.19: Effect of independent variables (X, Y) on taste in date fructose biscuit bars during storage (at 60 days)

Day=90
Taste = -5.5011+56.8667*x+50.75*y-144.3333*x*x+4*x*y-85.8333*y*y

Fig 4.20: Effect of independent variables (X, Y) on taste in date fructose biscuit bars during storage (at 90 days)
4.3.2.6. Mouth Feel of Date Fructose Biscuit Bars

The regression and correlation coefficients for mouth feel score of date fructose biscuit bars during storage intervals (0, 15, 30, 45, 60, 75 and 90 days) under the influence of independent variables (peanut peel extract and withania coagulans extract) are presented in Table 4.21. The analysis of variance for full regression of models is presented in Table 4.20. The analysis of variance shows significant effects of variables on mouth feel score of date fructose bars during storage intervals at 60 to 90 days. The regression coefficients for these models ($R^2 = 90.9\%, 92.5\%, 92.3\%, 93.2\%, 94.8\%$ and $95.2\%$ respectively) are enough for the well fitted response surface models. The significant results are obtained for linear terms of variables (peanut peel extract and withania coagulans extract). The effect of linear terms of X for mouth feel of date fructose biscuit bars is significant at 15, 30, 45, 60, 75 and 90 days storage intervals. The effect of linear terms of Y is significant for mouth feel of date fructose biscuit bars at 90 days storage intervals. The $X^2$ quadratic terms are found significant at 0, 15, 30 and 45 days storage intervals, whereas, the quadratic terms for $Y^2$ are found significant at all days of storage intervals. The variation effect in the levels of variables i.e. peanut peel extract and withania coagulans extract on the mouth feel score of date fructose biscuit bars at 0, 30, 60 and 90 days storage intervals is given away in Fig. 4.21-4.24.

4.3.2.7. Texture of Date Fructose Biscuit Bars

The regression and correlation coefficients for texture score of date fructose biscuit bars during storage intervals (0, 15, 30, 45, 60, 75 and 90 days) under the influence of independent variables (peanut peel extract and withania coagulans extract) are presented in Table 4.23. The analysis of variance for full regression of models is presented in Table 4.22. The analysis of variance shows significant effect of variables on texture score of date fructose biscuit bars during storage intervals. The regression coefficients for these models ($R^2 = 93.9\%, 94.3\%, 94.0\%, 94.7\%, 93.8\%$ and $93.2\%$ respectively) are enough for the well fitted response surface models. The non-significant results are obtained for linear terms of variables (peanut peel extract and withania coagulans extract). The $X^2$ quadratic terms are found significant at all days of storage intervals, whereas, the quadratic terms for $Y^2$ are found significant at 0, 15, 30 and 45 days of storage intervals. The variation effect in the levels of variables i.e. peanut peel extract and
Table 4.20: Response Surface Regression (Analysis of variance) for Mouth feel

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Degrees of freedom</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>5</td>
<td>0.05957</td>
<td>0.07256</td>
<td>0.07258</td>
<td>0.07259</td>
<td>0.07153*</td>
<td>0.09232*</td>
</tr>
<tr>
<td>Linear</td>
<td>2</td>
<td>0.03904</td>
<td>0.05284</td>
<td>0.05386</td>
<td>0.05920</td>
<td>0.05214</td>
<td>0.08333*</td>
</tr>
<tr>
<td>Square</td>
<td>2</td>
<td>0.10978*</td>
<td>0.12852*</td>
<td>0.12680*</td>
<td>0.12148*</td>
<td>0.12518*</td>
<td>0.14645*</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>0.00022</td>
<td>0.00010</td>
<td>0.00160</td>
<td>0.00160</td>
<td>0.00302</td>
<td>0.00202</td>
</tr>
<tr>
<td>Residual Error</td>
<td>3</td>
<td>0.00998</td>
<td>0.00985</td>
<td>0.01008</td>
<td>0.00885</td>
<td>0.00649</td>
<td>0.00771</td>
</tr>
</tbody>
</table>

Table 4.21: Regression coefficients for the models representing as a function of variations in the independent variables (Peanut Peel Extract (X) and Withania Coagulant Extract (Y))

<table>
<thead>
<tr>
<th>Terms of model equations</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>0.1117</td>
<td>0.1317*</td>
<td>0.1333*</td>
<td>0.1400*</td>
<td>0.1317*</td>
<td>0.1667*</td>
</tr>
<tr>
<td>Y</td>
<td>-0.0233</td>
<td>-0.0167</td>
<td>-0.0133</td>
<td>-0.0117</td>
<td>-0.0067</td>
<td>-0.0000**</td>
</tr>
<tr>
<td>X×X</td>
<td>-0.2417*</td>
<td>-0.2750*</td>
<td>-0.2800*</td>
<td>-0.2633*</td>
<td>-0.2717*</td>
<td>-0.2333*</td>
</tr>
<tr>
<td>Y×Y</td>
<td>-0.2267*</td>
<td>-0.2300*</td>
<td>-0.2200</td>
<td>-0.2283*</td>
<td>-0.2267*</td>
<td>-0.3033*</td>
</tr>
<tr>
<td>X×Y</td>
<td>0.0075</td>
<td>-0.0050</td>
<td>-0.0200</td>
<td>-0.0200</td>
<td>-0.0275</td>
<td>-0.0225</td>
</tr>
</tbody>
</table>

| R²                       | 90.9% | 92.5% | 92.3% | 93.2% | 94.8% | 95.2% |

* = Significant (P<0.10); ** = Significant (P<0.05); *** = Significant (P<0.01)
Day=0
Mouth feel = 3.8428+10.5583*x+13.2167*y-24.1667*x*x+0.75*x*y-22.6667*y*y

> 6.9
< 6.9
< 6.8
< 6.7
< 6.6
< 6.5
< 6.4
< 6.3
< 6.2

Fig 4.21: Effect of independent variables (X, Y) on mouth feel in date fructose biscuit bars during storage (at 0 day)

Day=30
Mouth feel = 3.4433+13.1333*x+13.4667*y-28*x*x-2*x*y-22*y*y

> 6.8
< 6.8
< 6.6
< 6.4
< 6.2
< 6

Fig 4.22: Effect of independent variables (X, Y) on mouth feel in date fructose biscuit bars during storage (at 30 days)
Day=60
Mouth feel = 3.2694 + 13.0083x + 14.0833y - 27.1667x² - 2.75xy - 22.6667y²

> 6.8
< 6.8
< 6.6
< 6.4
< 6.2
< 6

Fig 4.23: Effect of independent variables (X, Y) on mouth feel in date fructose biscuit bars during storage (at 60 days)

Day=90
Mouth feel = 2.6739 + 11.675x + 18.65y - 23.3333x² - 2.25xy - 30.3333y²

> 6.8
< 6.8
< 6.6
< 6.4
< 6.2
< 6

Fig 4.24: Effect of independent variables (X, Y) on mouth feel in date fructose biscuit bars during storage (at 90 days)
### Table 4.22: Response Surface Regression (Analysis of variance) for Texture

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Degrees of freedom</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>5</td>
<td>0.49204*</td>
<td>0.51209*</td>
<td>0.51447*</td>
<td>0.53207*</td>
<td>0.53111*</td>
<td>0.53953</td>
</tr>
<tr>
<td>Linear</td>
<td>2</td>
<td>0.16361</td>
<td>0.16376</td>
<td>0.15527</td>
<td>0.17581</td>
<td>0.18954</td>
<td>0.22453</td>
</tr>
<tr>
<td>Square</td>
<td>2</td>
<td>0.95605*</td>
<td>0.99885*</td>
<td>1.00340*</td>
<td>1.01655*</td>
<td>1.00563*</td>
<td>0.98650</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>0.22090</td>
<td>0.23522</td>
<td>0.25502</td>
<td>0.27562</td>
<td>0.26522</td>
<td>0.27562*</td>
</tr>
<tr>
<td>Residual Error</td>
<td>3</td>
<td>0.05372</td>
<td>0.05114</td>
<td>0.05481</td>
<td>0.05010</td>
<td>0.05821</td>
<td>0.06583</td>
</tr>
</tbody>
</table>

* = Significant (P<0.1)

### Table 4.23: Regression coefficients for the models representing as a function of variations in the independent variables (Peanut Peel Extract (X) and Withania Coagulans Extract (Y))

<table>
<thead>
<tr>
<th>Terms of model equations</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>8.1333**</td>
<td>8.1011**</td>
<td>8.0567**</td>
<td>8.0211**</td>
<td>7.9700**</td>
<td>7.8900**</td>
</tr>
<tr>
<td>X</td>
<td>-0.1583</td>
<td>-0.1567</td>
<td>-0.1433</td>
<td>-0.1600</td>
<td>-0.1683</td>
<td>-0.1867</td>
</tr>
<tr>
<td>Y</td>
<td>0.1717</td>
<td>0.1733</td>
<td>0.1767</td>
<td>0.1817</td>
<td>0.1867</td>
<td>0.2000</td>
</tr>
<tr>
<td>X×X</td>
<td>-0.8050*</td>
<td>-0.8367*</td>
<td>-0.8500*</td>
<td>-0.8567*</td>
<td>-0.8450*</td>
<td>-0.8400*</td>
</tr>
<tr>
<td>Y×Y</td>
<td>-0.5550*</td>
<td>-0.5467*</td>
<td>-0.5300*</td>
<td>-0.5317*</td>
<td>-0.5400</td>
<td>-0.5300</td>
</tr>
<tr>
<td>X×Y</td>
<td>-0.2350</td>
<td>-0.2425</td>
<td>-0.2525</td>
<td>-0.2625</td>
<td>-0.2575</td>
<td>-0.2625</td>
</tr>
</tbody>
</table>

R²: 93.9% 94.3% 94.0% 94.7% 93.8% 93.2%

* = Significant (P<0.10); ** = Highly Significant (P<0.05)
Fig 4.25: Effect of independent variables (X, Y) on texture in date fructose biscuit bars during storage (at 0 day)

Day=0
Texture = -1.69+37.6667*x+39.7167*y-80.5*x*x-23.5*x*y-55.5*y*y

Fig 4.26: Effect of independent variables (X, Y) on texture in date fructose biscuit bars during storage (at 30 days)

Day=30
Texture = -1.8717+40.1417*x+38.6167*y-85*x*x-25.25*x*y-53*y*y
Day=60
Texture = -2.0383+39.8417*x+39.4167*y-84.5*x*x-25.75*x*y-54*y*y

Day=90
Texture = -2.0417+39.6083*x+39.05*y-84*x*x-26.25*x*y-53*y*y

Fig 4.27: Effect of independent variables (X, Y) on texture in date fructose biscuit bars during storage (at 60 days)

Fig 4.28: Effect of independent variables (X, Y) on texture in date fructose biscuit bars during storage (at 90 days)
withania coagulans extract on the texture score of date fructose biscuit bars at 0, 30, 60 and 90 days storage intervals is given away in Fig. 4.25-4.28.

4.3.2.8. Overall Acceptability of Date Fructose Biscuit Bars

Regression coefficients of variables for overall acceptability of date fructose biscuit bars during storage are presented in Table 4.25. The analysis of variance for overall acceptability score has been presented in Table 4.24. The coefficients of determination (R^2) for these models (R^2 = 94.4%, 94.7%, 94.2%, 94.5%, 94.4% and 94.3% respectively) exhibit the adequacy of models and showed that it covers more variability in data. The data showed that peanut peel extract (X) and withania coagulans extract (Y) contribute towards achieving good score for overall acceptability. The effect of linear terms of X and Y is non-significant at 0, 15, 30, 45, 60, 75 and 90 days of storage intervals. The X^2 quadratic terms are found significant at 0, 15, 30, 45 and 60 days of storage intervals for overall acceptability of date fructose biscuit bars, and the quadratic terms for Y^2 are found significant at 0, 15, 30, 45 and 60 days of storage intervals. The interaction of two variables (XY) shows non-significant effect at all days of storage intervals.

The surface plots for overall acceptability of date fructose biscuit bars at 0, 30, 60 and 90 days of storage intervals are shown in the Fig 4.29-4.32 that reflects the effect of changes in levels of two variables i.e. peanut peel extract and withania coagulans extract on the overall acceptability during the storage.
### Table 4.24: Response Surface Regression (Analysis of variance) for Overall acceptability

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Degrees of freedom</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>5</td>
<td>0.67477*</td>
<td>0.69690*</td>
<td>0.69919*</td>
<td>0.69224*</td>
<td>0.69657*</td>
<td>0.71500*</td>
</tr>
<tr>
<td>Linear</td>
<td>2</td>
<td>0.02208</td>
<td>0.02483</td>
<td>0.02908</td>
<td>0.03421</td>
<td>0.03643</td>
<td>0.04388</td>
</tr>
<tr>
<td>Square</td>
<td>2</td>
<td>1.66465*</td>
<td>1.71730*</td>
<td>1.71845*</td>
<td>1.69635*</td>
<td>1.70499*</td>
<td>1.74363*</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>0.00040</td>
<td>0.00022</td>
<td>0.00090</td>
<td>0.00010</td>
<td>0.00003</td>
<td>0.00003</td>
</tr>
<tr>
<td>Residual Error</td>
<td>3</td>
<td>0.06631</td>
<td>0.06437</td>
<td>0.07181</td>
<td>0.06678</td>
<td>0.06898</td>
<td>0.07226</td>
</tr>
</tbody>
</table>

* = Significant (P<0.10)

### Table 4.25: Regression coefficients for the models representing as a function of variations in the independent variables (Peanut Peel Extract (X) and Withania Coagulans Extract (Y))

<table>
<thead>
<tr>
<th>Terms of model equations</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>7.6767**</td>
<td>7.6533**</td>
<td>7.5867**</td>
<td>7.5078**</td>
<td>7.4444**</td>
<td>7.3667</td>
</tr>
<tr>
<td>X</td>
<td>-0.0850</td>
<td>-0.0900</td>
<td>-0.0983</td>
<td>-0.1067</td>
<td>-0.1100</td>
<td>-0.1200</td>
</tr>
<tr>
<td>Y</td>
<td>-0.0117</td>
<td>-0.0133</td>
<td>-0.0050</td>
<td>0.0050</td>
<td>-0.0067</td>
<td>-0.0150</td>
</tr>
<tr>
<td>X×X</td>
<td>-0.9750*</td>
<td>-0.9800*</td>
<td>-0.9850*</td>
<td>-0.9767*</td>
<td>-0.9767*</td>
<td>-0.9800</td>
</tr>
<tr>
<td>Y×Y</td>
<td>-0.8450*</td>
<td>-0.8700*</td>
<td>-0.8650*</td>
<td>-0.8617*</td>
<td>-0.8667*</td>
<td>-0.8850</td>
</tr>
<tr>
<td>X×Y</td>
<td>0.0100</td>
<td>0.0075</td>
<td>0.0150</td>
<td>0.0050</td>
<td>0.0025</td>
<td>-0.0025</td>
</tr>
</tbody>
</table>

R²  | 94.4%     | 94.7%     | 94.2%     | 94.5%     | 94.4%     | 94.3%     |

* = Significant (P<0.10); ** = Highly Significant (P<0.05)
Day=0
Overall acceptability = -3.5633+37.85*x+50.3833*y-97.5*x*x+1*x*y-84.5*y*y

Fig 4.29: Effect of independent variables (X, Y) on overall acceptability in date fructose biscuit bars during storage (at 0 day)

Day=30
Overall acceptability = -3.8367+37.9667*x+51.55*y-98.5*x*x+1.5*x*y-86.5*y*y

Fig 4.30: Effect of independent variables (X, Y) on overall acceptability in date fructose biscuit bars during storage (at 30 days)
Overall acceptability = -4.0072 + 37.8917 * x + 51.8833 * y - 97.6667 * x^2 + 0.25 * x * y - 86.6667 * y^2

Day = 60

Fig 4.31: Effect of independent variables (X, Y) on overall acceptability in date fructose biscuit bars during storage (at 60 days)

Overall acceptability = -4.2483 + 38.075 * x + 53 * y - 98 * x^2 + 0.25 * x * y - 88.5 * y^2

Day = 90

Fig 4.32: Effect of independent variables (X, Y) on overall acceptability in date fructose biscuit bars during storage (at 90 days)
4.3.3. DISCUSSION

Response surface methodology, a statistical technique was applied to determine the true relationship between dependent and independent variables to find out regions of best response values (Cornell, 1990). It was applied to create a design and optimize the response when one or more factors such as process variables or ingredients affect that response (Dean and Voss, 1999). Box and Drapper (1986) described the principles and characteristics of RSM including its efficiency and application to optimize the variables. Minimum time and cost are required to find out the optimum response make it more efficient than the traditional methods. This technique also reduces the size of experiment. Later on many scientists modified its efficiency for creating design and optimizing response. It is also different from traditional technique in that several variables are processed at one time to reduce cost and time. Further, in conventional method of optimization, one variable is changed at a time and keeping the other variables at fixed levels. Due to single dimensional, this arduous and time intensified method usually does not warranty resolving of optimal conditions, neither, considers possible interactions among various factors. Optimization and experimental designs are the tools that facilitate generating models and determining the importance of various factors and their interactions. Moreover, by applying these models, a small number of experimental trials can be run to determin the optimal factor levels to a desired response (Montgomery, 1997; Adnani et al., 2010; Neta et al., 2011).

In this study, effect of two independent variables (peanut peel and withania coagulans extracts) related to natural extracts of antioxidants was assessed on the physical and sensory properties of DFBB during 90 days of storage. The texture in terms of hardness and fractureability was a feature of prime importance in DFBB quality parameters. As far as sensory attributes of DFBB are concerned, flavor, color, taste, mouth feel and overall acceptability were the sensory parameters which showed a declining trend during storage. However, the predited values were in good agreement with the experimental values showing that the model could be used to predict and optimize the levels of both the extracts containing phenolic antioxidants. The regression coefficients for these models ($R^2$) were enough for the well fitted response surface models (Ashoush et al., 2013). The surface plots for flavor, color, taste, mouth feel and overall acceptability at 0 to 90 days showed that the optimum levels of independent variables; peanut peel and withania coagulans extracts were used for achieving acceptable quality attributes regarding shelf stability of DFBB. The color values decreased during storage and color of bar
became darker, which might be happened due to Mailard reaction. It is obvious from the results that each aspect of independent variables suggested different optimized levels which might be occurred due to different composition of phenolic compounds and their antioxidant activities (Ashoush et al., 2013). The phenolic extract was obtained by treating the dried ground powder of peanut peel and *withania coagulans* with ethyl alcohol. The peanut peel extract and *withania coagulans* contained 64.6% and 57% antioxidant activities and 98.6mg/g and 13.2mg/g total phenolics and 74.2 and 52.5% free radical oxygen scavenging activities, respectively. These results are in agreement with previous findings (Omer, 2009). For the target values of texture, color, flavor, taste, mouth feel and overall acceptability, the optimized variable levels were determined as follows:

\[
\text{Peanut Peel Extract (X) = 0.21%}
\]

\[
\text{Withania Coagulans Extract (Y) = 0.32%}
\]

There are many studies conducted previously are supporting the fitness of models and acceptability of coefficients of regression ($R^2$) calculated by applying RSM during the present study (Alizadeh et al., 2005). In previous studies, RSM was successfully applied for optimization of formulations and process conditions of food products (Singh et al., 2004). In a research plan, gluten free bread including potato starch, skim milk and rice flour was optimized with using response surface methodology. HPMC (hydroxypropylmethylcellulose) and water were the independent variables. Optimized level of water was 79% based on the weight of flour/starch and HPMC was 2.2%. For optimized formulation, seven days analysis for shelf-life determination was conducted. During storage, moisture of crumb and crust decreased and firmness of crust increased. RSM can also be applied for optimization of more than two variables for product development. Neta et al. (2011) conducted a study for the optimization of fructose esters synthesis by applying RSM with a central composite rotatable design based on five levels and three experimental operation conditions including temperature, agitation and reaction time and the esterification percentage was considered as dependable variables. In another study, levels of coconut milk (15-35%) and sugar (10-30%) were optimized by taking into account sensory quality attributes (firmness, color and flavor and acceptability) and textural characteristics (chewiness and hardness) as dependant variables of cakes.
In a study, response surface methodology was used to optimize the protein levels in date bars. Vetch protein isolate and whey protein concentrate were used in date bar as independent variables. With the use of central composite design, fourteen treatments of date bars were produced with three levels and two variables and nutritional values and proximate composition of date bar were considered as dependent variables (Nadeem et al., 2012).

4.3.4. CONCLUSION

The results of this study suggested that maximum responses of dependent variables including sensory and physical characteristics of DFBB were achieved by incorporating 0.21% peanut peel extract and 0.32% *withania coagulans* extract during 90 days of storage.
4.4. PHYSICO-CHEMICAL ANALYSES OF DFBB CONTAINING OPTIMIZED ANTIOXIDANTS LEVEL (COMBINED SELECTED LEVELS IN SECTION 4.3.)

4.4.1. OBJECTIVE

To determine the combined effect of selected natural antioxidant extracts (peanut peel 0.21% + withania coagulanse 0.32%) on physicochemical, sensory and nutritional properties of DFBB with different levels during storage.

4.4.2. RESULTS

4.4.2.1. Physical properties of natural antioxidant extracts incorporated DFBB

Non-significant differences (P>0.05) in color values were observed among treatments of DFBB with the addition of antioxidant extracts (Table 4.26). Color values for T1, T2, and T3 were 152.50±0.04CTn, 147.10±0.16 CTn and 144.20±0.12 CTn, respectively (Table 4.27).

Highly significant (P<0.01) differences were recorded in hardness values of different treatments of DFBB (Table 4.26). The highest value of hardness was recorded in treatment T1 (257.93±6.45g) while the lowest value in case of T3 (221.67±3.20g). The treatment T2 (247.32±2.24g) showed optimum hardness. Hardness values for T1, T2, and T3 are 257.93±6.45g, 247.32±2.24g, and 221.67±3.20g, respectively (Table 4.27).

Fractureability values have non-significant (P>0.05) differences among treatments of date fructose biscuit bars containing antioxidant extract (Table 4.26). Fractureability values of T1, T2, and T3 were 76.04±1.97mm, 76.89±1.37mm, and 78.26±0.82mm, respectively (Table 4.27).

Significant (P<0.05) differences were found in water activity values of DFBB containing different levels of antioxidant extracts (Table 4.26). Water activity of these bars range from 0.240±0.006 to 0.261±0.006 that reflects all treatments are shelf stable (Table 4.27). Water activity of T1, T2, and T3 is 0.240±0.006, 0.261±0.006, and 0.260±0.000, respectively.
Table 4.26: Analysis of variance (mean squares) for DFBB containing different levels of extracts

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Color</th>
<th>Hardness</th>
<th>Fracturability</th>
<th>aw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>0.13234&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1042.93**</td>
<td>3.77176&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.0004148*</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>0.04088</td>
<td>56.80</td>
<td>6.43975</td>
<td>0.0000678</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant (P>0.05); * = Significant (P<0.10); ** = Highly Significant (P<0.05)

Table 4.27: Comparison of means

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Color</th>
<th>Hardness</th>
<th>Fracturability</th>
<th>aw</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>152.50±0.04A</td>
<td>257.93±6.45A</td>
<td>76.04±1.97A</td>
<td>0.240±0.006B</td>
</tr>
<tr>
<td>T2</td>
<td>147.10±0.16A</td>
<td>247.32±2.24A</td>
<td>76.89±1.37A</td>
<td>0.261±0.006A</td>
</tr>
<tr>
<td>T3</td>
<td>144.20±0.12A</td>
<td>221.67±3.20B</td>
<td>78.26±0.82A</td>
<td>0.260±0.000A</td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05)

T1: Low level of antioxidant combination (1.75g/500g flour)
T2: Medium level of antioxidant combination (2.50g/500g flour)
T3: High level of antioxidant combination (3.25g/500g flour)
4.4.2.2. Proximate composition of DFBB stored for 90 days

Proximate analysis includes determination of moisture, crude protein, crude fat, crude fiber, ash, and NFE of date sugar biscuit bar samples. The analysis of variance for proximate composition of biscuit bar samples are presented in Table 4.28. The moisture in biscuit bar sample varied from 3.38±0.03% to 3.53±0.03% and the differences among means for moisture were highly significant (p<0.01) (Table 4.29). The highest mean value for moisture (3.53±0.03%) was observed in treatment T3, while the lowest mean value (3.38±0.03%) was recorded in T2. The moisture level in biscuit bars varied non-significantly (P>0.05) among treatments with storage period of 90 days (Table 4.29). The crude protein content of biscuit bar sample varied non-significantly (P>0.05) among treatments and with storage period of 90 days. Similarly, crude fat, crude fiber, ash and NFE contents in biscuit bar samples varied non-significantly (P>0.05) among treatments and with storage period of 90 days (Table 4.30-34)
### Table 4.28: Analysis of variance (mean squares) for proximate analysis of DFBB stored for 90 days

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Fibre</th>
<th>Ash</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (T)</td>
<td>2</td>
<td>0.09081**</td>
<td>0.01609NS</td>
<td>0.00215NS</td>
<td>0.00049NS</td>
<td>0.00047NS</td>
<td>0.02425NS</td>
</tr>
<tr>
<td>Day (D)</td>
<td>5</td>
<td>0.01813NS</td>
<td>0.12167NS</td>
<td>1.07471NS</td>
<td>0.00564NS</td>
<td>0.00109NS</td>
<td>1.77041NS</td>
</tr>
<tr>
<td>T x D</td>
<td>10</td>
<td>0.00596NS</td>
<td>0.00387NS</td>
<td>0.00613NS</td>
<td>0.00001NS</td>
<td>0.00073NS</td>
<td>0.00911NS</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>0.01158</td>
<td>0.60330</td>
<td>0.48622</td>
<td>0.00041</td>
<td>0.00129</td>
<td>4.07652</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant (P>0.05); ** = Highly Significant (P<0.05)

T1: Low level of antioxidant combination (1.75g/500g flour)

T2: Medium level of antioxidant combination (2.50g/500g flour)

T3: High level of antioxidant combination (3.25g/500g flour)
Table 4.29: Comparison of means for moisture content in DFBB during storage (Treatment x Day)

<table>
<thead>
<tr>
<th>Day</th>
<th>Antioxidant levels</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>0</td>
<td>3.40 ± 0.03</td>
<td>3.60 ± 0.08</td>
</tr>
<tr>
<td>15</td>
<td>3.40 ± 0.07</td>
<td>3.45 ± 0.05</td>
</tr>
<tr>
<td>30</td>
<td>3.47 ± 0.07</td>
<td>3.50 ± 0.06</td>
</tr>
<tr>
<td>45</td>
<td>3.44 ± 0.03</td>
<td>3.50 ± 0.08</td>
</tr>
<tr>
<td>60</td>
<td>3.50 ± 0.05</td>
<td>3.60 ± 0.03</td>
</tr>
<tr>
<td>90</td>
<td>3.46 ± 0.05</td>
<td>3.50 ± 0.09</td>
</tr>
<tr>
<td>Mean</td>
<td>3.45 ± 0.02B</td>
<td>3.53 ± 0.03A</td>
</tr>
</tbody>
</table>

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

T1: Low level of antioxidant combination (1.75g/500g flour)
T2: Medium level of antioxidant combination (2.50g/500g flour)
T3: High level of antioxidant combination (3.25g/500g flour)
Table 4.30: Comparison of means for crude protein content in DFBB during storage (Treatment x Day)

<table>
<thead>
<tr>
<th>Day</th>
<th>Antioxidant levels</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>0</td>
<td>15.54 ± 0.24</td>
<td>15.50 ± 0.32</td>
</tr>
<tr>
<td>15</td>
<td>15.40 ± 0.46</td>
<td>15.45 ± 0.47</td>
</tr>
<tr>
<td>30</td>
<td>15.20 ± 0.72</td>
<td>15.22 ± 0.54</td>
</tr>
<tr>
<td>45</td>
<td>15.28 ± 0.11</td>
<td>15.33 ± 0.17</td>
</tr>
<tr>
<td>60</td>
<td>15.39 ± 0.49</td>
<td>15.30 ± 0.70</td>
</tr>
<tr>
<td>90</td>
<td>15.26 ± 0.65</td>
<td>15.20 ± 0.19</td>
</tr>
<tr>
<td>Mean</td>
<td>15.35 ± 0.17A</td>
<td>15.33 ± 0.15A</td>
</tr>
</tbody>
</table>

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

T1: Low level of antioxidant combination (1.75g/500g flour)
T2: Medium level of antioxidant combination (2.50g/500g flour)
T3: High level of antioxidant combination (3.25g/500g flour)
<table>
<thead>
<tr>
<th>Day</th>
<th>Low</th>
<th>High</th>
<th>Medium</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>22.31 ± 0.21</td>
<td>22.25 ± 0.49</td>
<td>22.25 ± 0.36</td>
<td>22.27 ± 0.19A</td>
</tr>
<tr>
<td>15</td>
<td>22.61 ± 0.47</td>
<td>22.55 ± 0.34</td>
<td>22.52 ± 0.43</td>
<td>22.56 ± 0.21A</td>
</tr>
<tr>
<td>30</td>
<td>21.96 ± 0.41</td>
<td>21.90 ± 0.40</td>
<td>22.00 ± 0.14</td>
<td>21.95 ± 0.17A</td>
</tr>
<tr>
<td>45</td>
<td>21.84 ± 0.19</td>
<td>21.80 ± 0.51</td>
<td>21.90 ± 0.52</td>
<td>21.85 ± 0.22A</td>
</tr>
<tr>
<td>60</td>
<td>22.26 ± 0.30</td>
<td>22.30 ± 0.21</td>
<td>22.30 ± 0.30</td>
<td>22.29 ± 0.14A</td>
</tr>
<tr>
<td>90</td>
<td>22.75 ± 0.31</td>
<td>22.80 ± 0.58</td>
<td>22.71 ± 0.65</td>
<td>22.75 ± 0.27A</td>
</tr>
<tr>
<td>Mean</td>
<td>22.29 ± 0.14A</td>
<td>22.27 ± 0.17A</td>
<td>22.28 ± 0.16A</td>
<td></td>
</tr>
</tbody>
</table>

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

T1: Low level of antioxidant combination (1.75g/500g flour)
T2: Medium level of antioxidant combination (2.50g/500g flour)
T3: High level of antioxidant combination (3.25g/500g flour)
Table 4.32: Comparison of means for crude fibre content in DFBB during storage (Treatment x Day)

<table>
<thead>
<tr>
<th>Day</th>
<th>Antioxidant levels</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>0</td>
<td>0.670 ± 0.018</td>
<td>0.666 ± 0.009</td>
</tr>
<tr>
<td>15</td>
<td>0.660 ± 0.007</td>
<td>0.663 ± 0.012</td>
</tr>
<tr>
<td>30</td>
<td>0.651 ± 0.017</td>
<td>0.652 ± 0.009</td>
</tr>
<tr>
<td>45</td>
<td>0.655 ± 0.005</td>
<td>0.651 ± 0.003</td>
</tr>
<tr>
<td>60</td>
<td>0.653 ± 0.012</td>
<td>0.655 ± 0.008</td>
</tr>
<tr>
<td>90</td>
<td>0.648 ± 0.008</td>
<td>0.643 ± 0.004</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean</th>
<th>Low</th>
<th>High</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.654 ± 0.007A</td>
<td>0.656 ± 0.006A</td>
<td>0.656 ± 0.007A</td>
</tr>
</tbody>
</table>

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

T1: Low level of antioxidant combination (1.75g/500g flour)
T2: Medium level of antioxidant combination (2.50g/500g flour)
T3: High level of antioxidant combination (3.25g/500g flour)
### Table 4.33: Comparison of means for ash content in DFBB during storage (Treatment x Day)

<table>
<thead>
<tr>
<th>Day</th>
<th>Antioxidant levels</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>0</td>
<td>0.960 ± 0.013</td>
<td>0.920 ± 0.039</td>
</tr>
<tr>
<td>15</td>
<td>0.967 ± 0.014</td>
<td>0.950 ± 0.018</td>
</tr>
<tr>
<td>30</td>
<td>0.950 ± 0.029</td>
<td>0.940 ± 0.010</td>
</tr>
<tr>
<td>45</td>
<td>0.994 ± 0.006</td>
<td>0.990 ± 0.007</td>
</tr>
<tr>
<td>60</td>
<td>0.980 ± 0.024</td>
<td>0.985 ± 0.021</td>
</tr>
<tr>
<td>90</td>
<td>0.940 ± 0.010</td>
<td>0.950 ± 0.018</td>
</tr>
</tbody>
</table>

**Mean** 0.963 ± 0.029A 0.966 ± 0.026A 0.964 ± 0.031A

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

T1: Low level of antioxidant combination (1.75g/500g flour)

T2: Medium level of antioxidant combination (2.50g/500g flour)

T3: High level of antioxidant combination (3.25g/500g flour)
Table 4.34: Comparison of means for NFE content in DFBB during storage (Treatment x Day)

<table>
<thead>
<tr>
<th>Day</th>
<th>Antioxidant levels</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>0</td>
<td>60.52 ± 0.05</td>
<td>60.60 ± 0.56</td>
</tr>
<tr>
<td>15</td>
<td>60.36 ± 0.52</td>
<td>60.39 ± 0.96</td>
</tr>
<tr>
<td>30</td>
<td>60.24 ± 1.36</td>
<td>61.29 ± 0.54</td>
</tr>
<tr>
<td>45</td>
<td>61.23 ± 0.67</td>
<td>61.23 ± 2.14</td>
</tr>
<tr>
<td>60</td>
<td>60.72 ± 1.21</td>
<td>60.76 ± 0.41</td>
</tr>
<tr>
<td>90</td>
<td>60.86 ± 1.88</td>
<td>60.41 ± 2.36</td>
</tr>
</tbody>
</table>

Mean 60.66 ± 0.40A 60.78 ± 0.49A 60.72 ± 0.33A

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

T1: Low level of antioxidant combination (1.75g/500g flour)

T2: Medium level of antioxidant combination (2.50g/500g flour)

T3: High level of antioxidant combination (3.25g/500g flour)
4.4.2.3. In-vitro starch digestibility (IVSD) of antioxidant incorporated biscuit bars

The analysis of variance (Table 4.35) showed that in-vitro starch digestibility vary non-significantly (P>0.05) among treatments. The mean values for in-vitro starch digestibility of date sugar incorporated biscuit bars have been presented in Table 4.36. The results indicated that in-vitro starch digestibility varied from 381.20±10.13 to 384.20±08.01 (mg maltose/g).

4.4.2.4. In-vitro protein digestibility (IVPD) of antioxidant incorporated biscuit bars

Analysis of variance and mean values for in-vitro protein digestibility have been presented in Table 4.35 and 4.36. In-vitro protein digestibility values showed non-significant variation (P>0.05) among treatments. The in-vitro protein digestibility values are 87.91±1.14, 88.29±1.69 and 87.54±1.49% in treatment T1, T2 and T3, respectively.

4.4.2.5. Gross energy of antioxidant incorporated biscuit bars

Gross energy values differ non-significantly among treatments (Table 4.35). Mean gross energy values are presented in Table 4.36. Mean values ranged from 3.49±0.1 to 3.53±0.48 Kcal/g.

4.3.2.6. Mineral contents of anti-oxidant incorporated biscuit bars

Mineral analysis includes determination of sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc and manganese of date sugar biscuit bar samples. The analysis of variance for mineral composition of biscuit bar samples are presented in Table 4.37. The mineral contents in biscuit bar sample varied non-significant (p>0.05) (Table 4.38). The mean values of sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc and manganese for treatments range from 2.00 to 2.53 mg/100g, 430.61 to 483.89 mg/100g, 41.01 to 45.43 mg/100g, 53.75 to 58.10 mg/100g, 1.30 to 1.84 mg/100g, 0.25 to 0.28 mg/100g, 0.41 to 0.42 mg/100g and 0.32 to 0.38 mg/100g, respectively (Figure 4.33 and 4.34).
Table 4.35: Analysis of variance (mean squares) for in-vitro protein and starch digestibility and calorific value of DFBB

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>IVSD</th>
<th>IVPD</th>
<th>Calorific value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>8.190 NS</td>
<td>8.190 NS</td>
<td>0.42190 NS</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>201.313</td>
<td>201.313</td>
<td>6.35457</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant (P>0.05)

Table 4.36: Comparison of mean values for in-vitro protein (%) and starch digestibility (mg maltose/g) and calorific value (Kcal/g) of date sugar biscuit bars

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IVSD</th>
<th>IVPD</th>
<th>Calorific value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>384.20±08.01</td>
<td>87.91±1.14</td>
<td>3.49±0.1</td>
</tr>
<tr>
<td>T2</td>
<td>383.90±05.88</td>
<td>88.29±1.69</td>
<td>3.53±0.48</td>
</tr>
<tr>
<td>T3</td>
<td>381.20±10.13</td>
<td>87.54±1.49</td>
<td>3.5±0.25</td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05)

T1: Low level of antioxidant combination (1.75g/500g flour)
T2: Medium level of antioxidant combination (2.50g/500g flour)
T3: High level of antioxidant combination (3.25g/500g flour)
Table 4.37: Analysis of variance (mean squares) for mineral analysis of biscuit bars

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>P</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>1</td>
<td>0.20914</td>
<td>2129.01</td>
<td>30.8178</td>
<td>18.966</td>
<td>18.306</td>
<td>0.21874</td>
<td>0.00081</td>
<td>0.00063</td>
<td>0.00253</td>
</tr>
<tr>
<td>Error</td>
<td>4</td>
<td>0.09158</td>
<td>3261.13</td>
<td>16.3685</td>
<td>5.643</td>
<td>22.878</td>
<td>0.15146</td>
<td>0.00291</td>
<td>0.00496</td>
<td>0.00209</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant (P>0.05)
Fig. 4.33: Mean values for mineral contents in date fructose biscuit bars

Fig. 4.34: Mean values for mineral contents in date fructose biscuit bars
4.4.2.7. Amino acids of antioxidant incorporated biscuit bars

Statistical analysis of data regarding amino acid contents in treatments indicated that amino acid varied highly significantly among treatments except few like serine, valine, leucine and histidine. The amino acid also varied among samples except arginine (Table 4.38). Tryptophane was not found in the treatments and in both samples i.e. in protein and in whole sample. Mean values for amino acid contents in treatments are presented in Figure 4.35. The amino acids in date fructose biscuit bars i.e. aspartic acid, alanine, asparagine, glutamic acid, glycine, histidine, leucine, isoleucine, lysine, threonine and tyrosine were increased with the incorporation of vetch protein isolate. The highest quantities of aspartic acid, alanine, asparagine, glutamic acid, glycine, histidine, leucine, isoleucine, lysine, threonine and tyrosine contents were recorded in the date fructose biscuit bars including 3.46 g, 2.41 g, 0.68 g, 10.90 g, 2.20 g, 0.07 g, 1.67 g, 0.73 g, 0.46 g, 0.20 g and 0.82 g/100g, respectively.
Fig. 4.35: Amino acid content (g/100g) of DFBB
### Table 4.38: Mean sum of squares for amino acid contents

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Aspartic acid</th>
<th>Serine</th>
<th>Threonine</th>
<th>Glutamic acid</th>
<th>Proline</th>
<th>Glycine</th>
<th>Valine</th>
<th>Alanine</th>
<th>Cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>19.49**</td>
<td>477\textsuperscript{NS}</td>
<td>0.1472**</td>
<td>7.6202**</td>
<td>10.381**</td>
<td>43.50**</td>
<td>0.873\textsuperscript{NS}</td>
<td>4.582**</td>
<td>2.788*</td>
</tr>
<tr>
<td>Sample</td>
<td>1</td>
<td>165.46**</td>
<td>229**</td>
<td>0.2902**</td>
<td>71.9173**</td>
<td>16.969**</td>
<td>2703.90**</td>
<td>11.896**</td>
<td>108.54**</td>
<td>2.125*</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.536</td>
<td>1445</td>
<td>0.0071</td>
<td>0.1835</td>
<td>1.1280</td>
<td>3.76</td>
<td>0.283</td>
<td>0.023</td>
<td>0.364</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\*\* = Highly significant (P<0.01)

---

**Continue….

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Methionine</th>
<th>Isoleucine</th>
<th>Leucine</th>
<th>Tyrosine</th>
<th>Asparagine</th>
<th>Histidine</th>
<th>Tryptophan</th>
<th>Lysine</th>
<th>Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>3.883 \textsuperscript{NS}</td>
<td>3.750**</td>
<td>16.576 \textsuperscript{NS}</td>
<td>7.636*</td>
<td>3.649*</td>
<td>0.0249 \textsuperscript{NS}</td>
<td>0.000</td>
<td>1.551**</td>
<td>3.769*</td>
</tr>
<tr>
<td>Sample</td>
<td>1</td>
<td>8.106*</td>
<td>6.200**</td>
<td>224.718**</td>
<td>11.704*</td>
<td>36.739**</td>
<td>0.0561*</td>
<td>0.000</td>
<td>4.602**</td>
<td>3.575 \textsuperscript{NS}</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>1.030</td>
<td>0.400</td>
<td>5.468</td>
<td>1.541</td>
<td>0.647</td>
<td>0.0057</td>
<td>0.000</td>
<td>0.176</td>
<td>0.723</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T1: Low level of antioxidant combination (1.75g/500g flour)

T2: Medium level of antioxidant combination (2.50g/500g flour)

T3: High level of antioxidant combination (3.25g/500g flour)
4.4.2.6. Free fatty acid in date sugar biscuit bars

The mean sums of squares and mean values for free fatty acids in biscuit bars during storage period of 90 days have been presented in the Table 4.39 and 4.40, respectively. The mean values of free fatty acids in biscuit bars differ highly significantly (P<0.01) among treatments. Maximum mean value (0.1755±0.004%) for free fatty acids was recorded in T₁ followed by T₂ (0.1663±0.005%), whereas minimum mean value (0.1567±0.005%) for free fatty acids was recorded in biscuit bars with maximum antioxidant level (T₃). The mean values of free fatty acids did not change significantly during storage.

4.4.2.7. Peroxide values in date sugar biscuit bars

The mean sum of squares and mean values for peroxide in biscuit bars during storage period are presented in the Table 4.39 and 4.41, respectively. The mean values of peroxide in biscuit bars differ highly significantly (P<0.01) among treatments. Maximum mean peroxide value (2.943±0.09 meq/Kg) was recorded in T₁ followed by T₂ (2.157±0.05 meq/Kg), whereas minimum peroxide value (1.749±0.07 meq/Kg) was recorded in T₃ with high level of antioxidants. The mean peroxide values did not change significantly during storage period.
Table 4.39: Analysis of variance (mean squares) for free fatty acids and peroxide value.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Mean squares Free fatty acids</th>
<th>Mean squares Peroxide value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AO</td>
<td>2</td>
<td>0.00106**</td>
<td>4.42187**</td>
</tr>
<tr>
<td>Day</td>
<td>5</td>
<td>2.500E-07NS</td>
<td>0.00105NS</td>
</tr>
<tr>
<td>AO x Day</td>
<td>10</td>
<td>6.972E-06NS</td>
<td>0.00014NS</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>4.889E-06</td>
<td>0.00104</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS=Non-significant (P>0.05);  ** = Highly significant (P<0.01)

Table 4.40: Comparison of mean values of free fatty acids in biscuit bars during storage

<table>
<thead>
<tr>
<th>Day</th>
<th>AO.</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>0</td>
<td>0.1770 ± 0.006</td>
<td>0.1557 ± 0.003</td>
</tr>
<tr>
<td>30</td>
<td>0.1763 ± 0.003</td>
<td>0.1560 ± 0.005</td>
</tr>
<tr>
<td>60</td>
<td>0.1757 ± 0.005</td>
<td>0.1570 ± 0.002</td>
</tr>
<tr>
<td>90</td>
<td>0.1730 ± 0.003</td>
<td>0.1580 ± 0.003</td>
</tr>
<tr>
<td>Mean</td>
<td>0.1755 ± 0.004A</td>
<td>0.1663 ± 0.005B</td>
</tr>
</tbody>
</table>

T1: Low level of antioxidant combination (1.75g/500g flour)
T2: Medium level of antioxidant combination (2.50g/500g flour)
T3: High level of antioxidant combination (3.25g/500g flour)
Table 4.41: Comparison of mean values of peroxide value in biscuit bars during storage

<table>
<thead>
<tr>
<th>Day</th>
<th>AO. Low</th>
<th>AO. High</th>
<th>AO. Medium</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.936 ± 0.03b</td>
<td>1.736 ± 0.02h</td>
<td>2.140 ± 0.03g</td>
<td>2.2711 ± 0.15A</td>
</tr>
<tr>
<td>30</td>
<td>2.933 ± 0.15a</td>
<td>1.740 ± 0.07cde</td>
<td>2.163 ± 0.05de</td>
<td>2.2789 ± 0.20A</td>
</tr>
<tr>
<td>60</td>
<td>2.943 ± 0.10a</td>
<td>1.757 ± 0.08def</td>
<td>2.157 ± 0.04fg</td>
<td>2.2856 ± 0.22A</td>
</tr>
<tr>
<td>90</td>
<td>2.960 ± 0.14a</td>
<td>1.763 ± 0.02bc</td>
<td>2.167 ± 0.07cde</td>
<td>2.2967 ± 0.20A</td>
</tr>
<tr>
<td>Mean</td>
<td>2.943 ± 0.09A</td>
<td>1.749 ± 0.07C</td>
<td>2.157 ± 0.05B</td>
<td></td>
</tr>
</tbody>
</table>

T1: Low level of antioxidant combination (1.75g/500g flour)
T2: Medium level of antioxidant combination (2.50g/500g flour)
T3: High level of antioxidant combination (3.25g/500g flour)
4.4.2.8. Sensory properties of antioxidant incorporated DFBB

Analysis of variance for color score of biscuit bar with anti-oxidant has been given in Table 4.42. Results show non-significant (P>0.05) difference in color score of antioxidant incorporated biscuit bars. Color scores for T1, T2 and T3 are 7.80±0.14, 7.50±0.20 and 7.60±0.08, respectively (Table 4.43).

Non-significant (P>0.05) differences were recorded in the taste score of biscuit bars (Table 4.42). The taste score for treatment T1, T2 and T3 are 7.59±0.16, 7.60±0.14 and 7.52±0.06 respectively (Table 4.43).

Analysis of variance for flavor score of date sugar incorporated biscuit bars indicated highly significant differences (P<0.01) among treatments (Table 4.42). Flavor score for treatment T1, T2 and T3 are 6.90±0.06, 7.90±0.21 and 5.60±0.11, respectively (Table 4.43). Maximum flavor score was achieved by treatment T2 (7.90±0.21) followed by T1 (6.90±0.06).

The statistical analysis of texture score of date fructose biscuit bars indicated highly significant (P<0.01) variation among treatments (Table 4.42). Mean values for texture score of bars is given in Table 4.43. Mean values for texture score of bars for treatment T1, T2 and T3 were 7.60±0.05, 8.80±0.17 and 8.10±0.21, respectively. Texture score of T2 is the highest (8.80±0.17) followed by T3 (8.10±0.21).

Highly significant (P<0.01) variations exist in mouth feel score of date fructose biscuit bars among treatments (Table 4.42). The best treatment was T2 that achieved the highest mouth feel score (7.90±0.04) followed by T3 (6.70±0.14). The mouth feel score of treatments were 5.90±0.07, 7.90±0.04 and 6.70±0.14 for T1, T2 and T3, respectively (Table 4.43).

Overall acceptability score of date fructose biscuit bars vary from 35.52±0.22 to 39.70±0.43. Overall acceptability score of treatments were 35.79±0.57, 39.70±0.43 and 35.52±0.22 for T1, T2 and T3, respectively. The highest overall acceptability score was achieved by T2 (39.70±0.43) followed by T1 (35.79±0.57).
## Table 4.2: Analysis of variance (mean squares) for sensory evaluation of DFBB

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Color</th>
<th>Taste</th>
<th>Flavor</th>
<th>Texture</th>
<th>Mouth feel</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>0.07000&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.00570&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.99000**</td>
<td>1.09000**</td>
<td>3.04000**</td>
<td>16.4167**</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>0.06427</td>
<td>0.05100</td>
<td>0.05857</td>
<td>0.07337</td>
<td>0.02563</td>
<td>0.5623</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>3.99000**</td>
<td>0.06427</td>
<td>0.05100</td>
<td>0.05857</td>
<td>0.07337</td>
<td>3.04000**</td>
</tr>
</tbody>
</table>

NS = Non-significant (P>0.05); ** = Highly significant (P<0.01)

## Table 4.43: Comparison of means of sensory characteristics of DFBB

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Color</th>
<th>Taste</th>
<th>Flavor</th>
<th>Texture</th>
<th>Mouth feel</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>7.80±0.14</td>
<td>7.59±0.16</td>
<td>6.90±0.06&lt;sup&gt;B&lt;/sup&gt;</td>
<td>7.60±0.05&lt;sup&gt;B&lt;/sup&gt;</td>
<td>5.90±0.07&lt;sup&gt;C&lt;/sup&gt;</td>
<td>35.79±0.57&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>T2</td>
<td>7.50±0.20</td>
<td>7.60±0.14</td>
<td>7.90±0.21&lt;sup&gt;A&lt;/sup&gt;</td>
<td>8.80±0.17&lt;sup&gt;A&lt;/sup&gt;</td>
<td>7.90±0.04&lt;sup&gt;A&lt;/sup&gt;</td>
<td>39.70±0.43&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>T3</td>
<td>7.60±0.08</td>
<td>7.52±0.06</td>
<td>5.60±0.11&lt;sup&gt;C&lt;/sup&gt;</td>
<td>8.10±0.21&lt;sup&gt;B&lt;/sup&gt;</td>
<td>6.70±0.14&lt;sup&gt;B&lt;/sup&gt;</td>
<td>35.52±0.22&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05)

T1: Low level of antioxidant combination (1.75g/500g flour)
T2: Medium level of antioxidant combination (2.50g/500g flour)
T3: High level of antioxidant combination (3.25g/500g flour)
4.4.3. DISCUSSION

In this study, the main object was to evaluate the combined effect of addition of different levels of optimized levels of antioxidant extracts (0.21% peanut peel extract and 0.32% *withania coagulans* extract) on the physiochemical and sensory properties and shelf stability of developed DFBB during 90 days of storage. The peanut peel (skin) extract and *withania coagulans* berries extract are cheap, locally available and good sources of the antioxidants. The peanut peel extract and *withania coagulans* contained 64.6% and 57% antioxidant activities, 98.6mg/g and 13.2mg/g total phenolics and 74.2 and 52.5% free radical oxygen scavenging activities, respectively. These results are in agreement with the previous findings (Omer, 2009). However, these results are not fully comparable with pomegranate peel and whey protein, some deviation might be occurred due to the difference in sources, methods of extraction and types of solvent (Ashoush *et al.*, 2013). These sources of antioxidant are very economical but under utilized. The changes in free fatty acids and peroxide values are indicators of oxidation. The results revealed that these factors were not significantly affected during storage of DFBB as a function of antioxidant properties of the extracts because radical scavengers could discontinue oxidative reactions through their power to quench the peroxide radical and thus, maintain the quality and shelf stability of the product. Thus, it has been proved that these are appropriate sources for extraction and inclusion of natural antioxidants to control the rancidity and enhance the shelf stability of DFBB. It was found that with the addition of antioxidant, nutritional status, physical and sensory properties of DFBB was found to improve. The protein level of DFBB was found to enhance to the tune of 10.13% as compared to control. These results are also supported by the findings of other researchers (Virág *et al.*, 2013; Sharma and Gujral, 2014).

The balanced level of nutrients in a food formulation is necessary. The proximate composition of DFBB was not found to significantly change with the addition of different levels of antioxidants. The optimization of protein and fat levels in food bars resulted in the product with optimum level of carbohydrates (Bower and Whitten, 2000). It has been observed that the candy bars fortified with soy protein contained 58.7% carbohydrates, 12.4% protein and 9% fat contents (de Penna *et al.*, 1993). In this study, protein level increased from 5.41 to 15.54% in DFBB. Similarly, addition of legume flours increased protein, fat, fiber, ash, minerals and vitamins in chocolate bars (Onwuka and Abasiekong, 2006). In the present investigations, it was found that in-vitro protein and starch digestibilities improved with the addition of vetch
protein isolate. These results were found in line with findings of previous researchers. In-vitro starch digestibility was higher in banana fruit bars that might be due to increased level of rapidly digestible starch in banana flour (Utrilla-Coello et al., 2010). Moreover, date fructose and vetch protein isolate are rich in readily digestible starch and protein that might be involved in increased in-vitro starch and protein digestibility. Starch in the food cannot be digested completely, because some fractions like ungelatinized starch granules, retrograded amylase, physically bind starch, dry heated food starches and chemically modified starches are unable to digest (Lajolo et al., 1991; Kataria et al., 1992; Yadav and Kheterpaul, 1994). However, legumes possess significant amount of incompletely digestible starch (Englyst et al., 1992; Muir and O’Dea, 1992). Protein matrix plays a key role in starch digestibility by encapsulating starch granules resulting in slow rate of starch digestibility and with slow glycemic response in pasta, a carbohydrate based product. However, protein degradation and release from starch granules by sheeting increased the in-vitro starch digestibility (Esther et al., 2008).

In the present study, high nutritional value fortified biscuit bars were developed by improving protein and carbohydrate status. The amino acids profile improved to a large extend by supplementation of IVPI in DFBB as depicted from Fig 4.35. These findings were on the same lines as observed by Siddique, (2000) who stated that quantity of essential amino acids increased by adding 10% gram flour in the chapatties.

Minerals are the elements required by living organisms other than the four elements carbon, hydrogen, nitrogen, and oxygen present in organic molecules. Dietitians may recommend that dietary elements are best supplied by ingesting specific foods rich with the chemical element (s) of interest. These elements may be either naturally present in the food or added to the food products. Thus, intake of mineral elements in correct levels is required to maintain good health. However, the minerals may be present as inorganic or organic salts or may be combined with organic materials, such as the phosphorus is combined with phosphoproteins and metals are combined with enzymes and affect their bioavailability. It is customary to divide the minerals into two groups, the major salt components and the trace elements. The major salt components include potassium, sodium, calcium, magnesium, chloride, sulfate, phosphate, and bicarbonate. Trace elements are usually present in amounts below 50 mg/kg (50ppm). Minerals perform some important functions like act as electrolytes, regulate physiological functions, building component in cellular growth and development of different tissue, co factor of certain
enzymes, activator or inhibitor of enzymatic reaction and contribute in food color, flavor and texture.

In this study, DFBB was prepared with vetch protein isolate, date fructose and antioxidants and subjected to mineral analysis. These biscuit bars were also contain appreciable amount of minerals like sodium, potassium, calcium, magnesium, phosphorus, iron, coper, zinc and manganese. The results of present study are also strengthening by the findings of researchers of previous studies. Yousaf et al. (2012) evaluated wheat flour cookies supplemented with gram flour for nutritional and physical properties. They reported that supplementation of cookies significantly increased the levels of minerals like iron, manganese, magnesium, calcium, copper, zinc, sodium and potassium to 33.45, 7.74, 300.80, 14.68, 2.91, 17.25, 1581.00 and 4074.90mg/kg, respectively (50% replacement of gram flour). The results are also close to the findings of Passos et al. (2013), they analyzed commercial cookies for proximate and mineral contents. Similarly, Niaba et al. (2013) found that mineral contents increased in cookies supplemented with Macrotermes subhyalinus flour. Moreover, nutritional value of cereal based foods may be achieved by mixing the cereal flour with legume flours. They stated that by applying this technique, it is possible to increase protein quantity and quality and some minerals and vitamins in the baked products (Vitali et al., 2008)

These results are in line with the findings of de Penna et al. (1992) who stated that food bars are good source of energy due to their protein, fat and carbohydrate contents along with minerals. These bars can be used for multipurpose such as during sports, emergency situation, as snack food and nutritional serving etc. In a study, energy bars were developed for sportsmen that supplied 520 Kcal/100g with proper supplementation of protein, fat and carbohydrate in the ratio of 8:40:52, respectively. The ingestion of two bars (100g each) had supplied the recommended intake for the sportsmen. Whereas, a soy-based candy bar containing 14% protein, 22% fat and 65% carbohydrates provided 375.2Kcal/100g (de Penna et al., 1993).

The effects of ingredients on color, texture hardness, moisture content, water activity and sensory attributes were investigated. Generally, sugars and proteins play important role in chemical reactions in the snack bars. The sugars imparted sweetness and were involved in modifying water activity, color, hardness and sensory characteristics of the bar, whereas, protein supplementation improved nutritional value and functional properties. The added protein isolate
served to keep the ingredients of bar together, set the structure, increased its strength, and contributed to water holding capacity and Maillard browning. As such, protein addition such as whey protein that has considerable viscosity, gel strength and water holding capacity has found to contribute in bar texture like firmness over shelf life (Uthayakumaran et al., 2000; Ortiz et al., 2008; Shaun, 2008). Moreover, firmness in bars, due to addition of protein, might be involved in moisture migration between the carbohydrates and the proteins. However, high amount of carbohydrates was found to increase the hardening effect during storage (Shaun, 2008).

The sensory characteristics in this study were evaluated on hedonic rating. Among sensory characteristics, taste is of main important decisive factor (Wandel and Bugge, 1997; Schifferstein and Oude Ophuis, 1998; Torjusen et al., 2001). In the present study, all the biscuit bars with added protein isolate and natural antioxidant extracts were ranked the best for taste, texture, flavor, mouth feel and overall acceptability. The importance of taste for product acceptance was in line with priority of sensory characteristics for product preference or purchase on the part of consumers (Grankivist and Biel, 2001; Magnusson et al., 2001).

4.4.4. CONCLUSION

Physico-chemical properties and sensory characteristics of DFBB were evaluated to assess the suitability of addition of antioxidant extracts from two different sources such as peanut peel extract and withania coagulans extract. The results for proximate composition revealed that addition of antioxidant contributed non-significantly effect on proximate composition during 90 days storage. It is also found that addition of peanut peel and withania coagulans extracts at three levels have no effect on the in-vitro protein (IVPD) and in-vitro starch digestibility (IVSD). Sensory parameters such flavor and overall acceptability were affected significantly with the addition of peanut peel and withania coagulans extracts in DFBB. All the bars were acceptable with good sensory characteristics but bars containing medium level (0.5%) of antioxidant were maximally preferred. The results of calorific value indicated that all the biscuit bars provided high calorific value as well. On the basis of results of these parameters, it may be concluded that DFBB containing Indian vetch protein isolate, date fructose and peanut peel and withania coagulans extracts could be considered as the nutrient dense bars with good sensory qualities and 90 days shelf stability.
4.5. BIOLOGICAL EVALUATION OF DATE FRUCTOSE BISCUIT BARS

4.5.1. OBJECTIVES

To evaluate the protein quality of Indian vetch protein isolates supplemented biscuit bars by protein efficacy studies and assessment of effect of optimized dose of antioxidant extracts on serum profile parameters of rats.

4.5.2. RESULTS

Biological evaluation is an appropriate tool for the assessment of nutritional and health aspects of a product. This study was undertaken for biological evaluation of date biscuit bars by using albino rats. Convenient handling, restricted diet, close supervision and feasibility of controlled environmental conditions were the main reasons for conducting trials on rats as these variables are difficult to control in case of human subjects. Moreover, it is hard to find out the volunteers who could restrict themselves on specific diet. Diets prepared from the control and selected treatments were fed to the respective groups of rats. Rats from groups G3 to G6 were sacrificed after 10 days. Body weight, Feed and water intake were measured on daily basis. Moreover, serological characteristics were also determined to resolve the safety concerns of the experimental diets.

4.5.2.1. Feed efficiency

Feed efficiency may be defined as the gain in body weight per unit feed intake. Mean sum of squares and means regarding feed efficiency of rats on different biscuit bars diet groups have shown highly significant differences (p<0.01) among groups as given in Table 4.4 and Table 4.45. Among the test diets normal diet shows low feed efficiency (p≤0.05). Results show that the feed efficiency for casein was 4.07 followed by date fructose biscuit bars (3.73).

4.5.2.2. Net protein utilization

The ratio of feed intake to gain in body weight is called feed utilization. Statistical analysis showed highly significant (p<0.01) variation among rat groups fed on various types of biscuit bar meals (Table 4.44). Mean values (Table 4.45) revealed good utilization of casein biscuit bars (87.37). Net protein utilization of rats group fed on different diets is presented in Table 4.44. The significant statistical difference for protein utilization values in the bar meal diets is observed, where minimum protein utilization was observed for normal biscuit bar meal (71.25) and higher value in date fructose biscuit bars (75.26).
4.5.2.3. Protein efficiency ratio (PER)

Protein efficiency ratio is gain in body weight per unit protein intake. The statistical analysis for Protein efficiency ratio of rats fed on different biscuit bar groups has shown highly significant (p<0.01) variations (Table 4.44). The means pertaining to the protein efficiency ratio are given in Table 4.45. The results revealed that the protein efficiency ratio was higher for casein i.e. 3.060, followed by date fructose biscuit bars (2.74) and normal biscuit bars (2.117).

4.5.2.4. Net protein retention (NPR)

Net protein retention is defined as the ratio of sum of weight gain of test protein group and weight loss of non-protein group to that of protein intake of test protein group. Statistical analysis regarding net protein retention of rats on different biscuit bars diet groups has shown highly significant (p<0.01) differences among groups as given in Table 4.44. Maximum net protein retention value was observed in biscuit bars with casein diet (4.04) followed by date fructose biscuit bars (3.85) and minimum value in case of normal biscuit bars (3.68) (Table 4.45).

4.5.2.5. Protein digestibility (DIG)

Statistical analysis regarding protein digestibility show highly significant (p<0.01) variation among rat groups fed on various bar meals (Table 4.44). Mean values reveal good protein digestibility in biscuit bars with casein (89.25). Protein digestibility of rat groups fed on different diets is presented in Table 4.45. The statistical difference for protein digestibility values in the test protein diets and casein is observed, where minimum protein digestibility was observed for normal biscuit bars (82.55). The digestability of date fructose biscuit bars was 87.91.

4.5.2.6. Biological value (BV)

Biological value of experimental diets was calculated by dividing net protein utilization value with protein digestability. Statistical analysis regarding biological value of rats on different biscuit bars diet groups has shown highly significant (p<0.01) differences among groups as given in Table 4.44. Maximum biological value was observed in biscuit bars with casein diet (96.29) followed by date fructose biscuit bars (92.02) and minimum value in case of normal biscuit bars containing gluten (80.85) (Table 4.45).
Table 4.4: Analysis of variance (mean squares) for biological evaluation of protein

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>BV</th>
<th>PER</th>
<th>NPR</th>
<th>FE</th>
<th>NPU</th>
<th>DIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>2</td>
<td>190.586**</td>
<td>0.69143**</td>
<td>0.09554**</td>
<td>0.0663**</td>
<td>211.455**</td>
<td>37.7478**</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>0.536</td>
<td>0.00049</td>
<td>0.00028</td>
<td>0.0006</td>
<td>0.410</td>
<td>0.3892</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** =Highly significant (P<0.01)

Table 4.45: Biological value (BV), Protein efficiency ratio (PER), Net protein retention (NPR), Feed efficiency (FE), Net protein utilization (NPU) and Digestibility (DIG) values in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>BV</th>
<th>PER</th>
<th>NPR</th>
<th>FE</th>
<th>NPU</th>
<th>DIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>G3</td>
<td>80.85c</td>
<td>2.12c</td>
<td>3.68c</td>
<td>4.07a</td>
<td>71.25c</td>
<td>82.55c</td>
</tr>
<tr>
<td>G4</td>
<td>96.29a</td>
<td>3.060a</td>
<td>4.04a</td>
<td>3.5c</td>
<td>87.37a</td>
<td>89.25a</td>
</tr>
<tr>
<td>G5</td>
<td>92.02b</td>
<td>2.74b</td>
<td>3.85b</td>
<td>3.73b</td>
<td>75.26b</td>
<td>87.91b</td>
</tr>
</tbody>
</table>

G3: Biscuits containing 15% Gluten protein (Control for Biological Studies)

G4: Biscuits containing 15% Casein protein

G5: Biscuits containing 15% IVPI
4.5.2.7. Serum biochemistry

It is observed from the results (Table 4.46, 4.47) that diets (groups) exhibited highly significant while seasons, and their interactions showed non-significant differences in liver functioning tests (alanine aminotransferase, ALT; aspartate aminotransferase, AST; alkaline phosphatase, ALP; total bilirubin). Means regarding blood glucose ranged from 83.67±6.98 to 97.14±4.13mg/dL (Table 4.48). Means regarding diet groups showed that liver and kidney functions tests remained in their normal ranges. Activities of liver enzymes like ALT, ALP and AST ranged from 23.17±3.17 to 31.00±0.92U/L (Table 4.49), 105.00±15.7 to 162.00±2.68U/L (Table 4.50) and 23.83±1.38 to 27.50±0.46U/L (Table 4.51), respectively in the two diet groups. Moreover, bilirubin proteins varied significantly from 0.621±0.02 to 0.695±0.010mg/dL (Table 4.52).

4.5.2.8. Serum electrolytes

Means squares (Table 4.47) depicted that serum electrolytes were affected non-significantly among groups. Means for Na, K and Cl ions (Table 4.52-4.57) indicated non-significant variations due to season.

4.5.2.9. Serum lipid profile

Means squares (Table 4.47) elucidated that lipid profile was affected significantly within diet groups, while study seasons remained non-significant as a function of diets. The mean value of cholesterol was found 74.58±1.87 in G₁ followed by 66.71±1.45 in G₂ (Table 4.58). Means for triglycerides contents (Table 4.59) also indicated significant variations due to diets and non-significant variation due to season.
Table 4.46: Analysis of variance (mean squares) for serum biochemical profile

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BG</td>
</tr>
<tr>
<td>Group</td>
<td>1</td>
<td>544.862(**)</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>4.248(\text{NS})</td>
</tr>
<tr>
<td>Group x Season</td>
<td>1</td>
<td>0.822(\text{NS})</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>8.452</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant (P>0.05); ** = Highly significant (P<0.01)

Table 4.47: Analysis of variance (mean squares) for serum biochemical profile

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Na</td>
</tr>
<tr>
<td>Group</td>
<td>1</td>
<td>27.00(\text{NS})</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>108.00(\text{NS})</td>
</tr>
<tr>
<td>Group x Season</td>
<td>1</td>
<td>300.00(\text{NS})</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>53.51</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant (P>0.05); ** = Highly significant (P<0.01)

Continue…..
Table 4.48: Group x Season means for BG (mg/dL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Season</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Season 1</td>
<td>Season 2</td>
</tr>
<tr>
<td>G1</td>
<td>98.00 ± 1.97</td>
<td>96.00 ± 0.66</td>
</tr>
<tr>
<td>G2</td>
<td>84.00 ± 1.01</td>
<td>83.00 ± 1.50</td>
</tr>
<tr>
<td>Mean</td>
<td>91.00 ± 2.77A</td>
<td>89.81 ± 4.05A</td>
</tr>
</tbody>
</table>

G1: Control (Without IVPI and DF)
G2: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts

Table 4.49: Group x Season means for ALT (U/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Season</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Season 1</td>
<td>Season 2</td>
</tr>
<tr>
<td>G1</td>
<td>31.00 ± 0.45c</td>
<td>31.00 ± 0.21a</td>
</tr>
<tr>
<td>G2</td>
<td>19.00 ± 0.28f</td>
<td>19.33 ± 1.05b</td>
</tr>
<tr>
<td>Mean</td>
<td>25.00 ± 1.63A</td>
<td>25.16 ± 0.91A</td>
</tr>
</tbody>
</table>

G1: Control (Without IVPI and DF)
G2: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts

Table 4.50: Group x Season means for ALP (U/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Season</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Season 1</td>
<td>Season 2</td>
</tr>
<tr>
<td>G1</td>
<td>160.00 ± 4.03</td>
<td>164.00 ± 1.92</td>
</tr>
<tr>
<td>G2</td>
<td>105.33 ± 2.24</td>
<td>105.00 ± 1.70</td>
</tr>
<tr>
<td>Mean</td>
<td>135.00 ± 7.04A</td>
<td>132.50 ± 4.45A</td>
</tr>
</tbody>
</table>

G1: Control (Without IVPI and DF)
G2: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts
Table 4.51: Group x Season means for AST (U/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Season 1</th>
<th>Season 2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>28.00 ± 0.30ef</td>
<td>27.00 ± 0.43f</td>
<td>27.50 ± 0.32A</td>
</tr>
<tr>
<td>G₂</td>
<td>22.00 ± 0.44g</td>
<td>25.67 ± 0.62ef</td>
<td>23.83 ± 1.38B</td>
</tr>
<tr>
<td>Mean</td>
<td>25.00 ± 1.29A</td>
<td>26.33 ± 0.78A</td>
<td></td>
</tr>
</tbody>
</table>

G₁: Control (Without IVPI and DF)
G₂: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts

Table 4.52: Group x Season means for Bi.T (mg/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Season 1</th>
<th>Season 2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>0.690 ± 0.017</td>
<td>0.700 ± 0.012</td>
<td>0.695 ± 0.010A</td>
</tr>
<tr>
<td>G₂</td>
<td>0.610 ± 0.006</td>
<td>0.630 ± 0.017</td>
<td>0.621 ± 0.020B</td>
</tr>
<tr>
<td>Mean</td>
<td>0.667 ± 0.009A</td>
<td>0.650 ± 0.009A</td>
<td></td>
</tr>
</tbody>
</table>

G₁: Control (Without IVPI and DF)
G₂: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts

Table 4.53: Group x Season means for S.Cl (mg/dL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Season 1</th>
<th>Season 2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>8.90 ± 0.21</td>
<td>8.93 ± 0.14</td>
<td>8.91 ± 0.42A</td>
</tr>
<tr>
<td>G₂</td>
<td>9.00 ± 0.25</td>
<td>8.86 ± 0.15</td>
<td>8.93 ± 0.30A</td>
</tr>
<tr>
<td>Mean</td>
<td>8.95 ± 0.15A</td>
<td>8.90 ± 0.14A</td>
<td></td>
</tr>
</tbody>
</table>

G₁: Control (Without IVPI and DF)
G₂: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts
### Table 4.54: Group x Season means for Na (mEq/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Season 1</th>
<th>Season 2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>134.00 ± 3.93</td>
<td>130.00 ± 6.3</td>
<td>132.00 ± 3.44A</td>
</tr>
<tr>
<td>G₂</td>
<td>121.00 ± 3.48</td>
<td>137.00 ± 2.01</td>
<td>129.00 ± 4.00A</td>
</tr>
<tr>
<td>Mean</td>
<td>127.50 ± 1.96A</td>
<td>133.50 ± 1.68A</td>
<td></td>
</tr>
</tbody>
</table>

G₁: Control (Without IVPI and DF)
G₂: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts

### Table 4.55: Group x Season means for K (mEq/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Season 1</th>
<th>Season 2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>3.90 ± 0.03</td>
<td>4.10 ± 0.05</td>
<td>4.00 ± 0.05A</td>
</tr>
<tr>
<td>G₂</td>
<td>3.90 ± 0.09</td>
<td>3.83 ± 0.05</td>
<td>3.86 ± 0.16A</td>
</tr>
<tr>
<td>Mean</td>
<td>3.90 ± 0.09A</td>
<td>3.96 ± 0.10A</td>
<td></td>
</tr>
</tbody>
</table>

G₁: Control (Without IVPI and DF)
G₂: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts

### Table 4.56: Group x Season means for BC (mEq/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Season 1</th>
<th>Season 2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>27.00 ± 0.31</td>
<td>25.00 ± 0.28</td>
<td>26.00 ± 0.48A</td>
</tr>
<tr>
<td>G₂</td>
<td>23.00 ± 0.36</td>
<td>25.00 ± 0.70</td>
<td>24.00 ± 0.57B</td>
</tr>
<tr>
<td>Mean</td>
<td>25.00 ± 0.45A</td>
<td>25.00 ± 0.18A</td>
<td></td>
</tr>
</tbody>
</table>

G₁: Control (Without IVPI and DF)
G₂: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts
### Table 4.57: Group x Season means for Cl (mEq/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Season 1</th>
<th>Season 2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>101.00 ± 1.13</td>
<td>99.00 ± 3.54</td>
<td>100.00 ± 1.72A</td>
</tr>
<tr>
<td>G2</td>
<td>98.00 ± 5.32</td>
<td>103.00 ± 2.78</td>
<td>100.50 ± 2.91A</td>
</tr>
<tr>
<td>Mean</td>
<td>99.50 ± 1.48A</td>
<td>101.00 ± 1.21A</td>
<td></td>
</tr>
</tbody>
</table>

G1: Control (Without IVPI and DF)
G2: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts

### Table 4.58: Group x Season means for Cholesterol (mg/dL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Season 1</th>
<th>Season 2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>74.81 ± 2.86</td>
<td>74.36 ± 0.12</td>
<td>74.58 ± 1.87A</td>
</tr>
<tr>
<td>G2</td>
<td>67.23 ± 0.66</td>
<td>66.20 ± 1.00</td>
<td>66.71 ± 1.45B</td>
</tr>
<tr>
<td>Mean</td>
<td>71.02 ± 0.61A</td>
<td>70.28 ± 0.52A</td>
<td></td>
</tr>
</tbody>
</table>

G1: Control (Without IVPI and DF)
G2: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts

### Table 4.59: Group x Season means for Triglycerides (mg/dL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Season 1</th>
<th>Season 2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>70.00 ± 2.22</td>
<td>70.33 ± 2.93</td>
<td>70.17 ± 2.78A</td>
</tr>
<tr>
<td>G2</td>
<td>61.67 ± 2.04</td>
<td>60.00 ± 4.09</td>
<td>60.83 ± 3.74B</td>
</tr>
<tr>
<td>Mean</td>
<td>65.83 ± 0.84A</td>
<td>65.17 ± 1.19A</td>
<td></td>
</tr>
</tbody>
</table>

G1: Control (Without IVPI and DF)
G2: Biscuits containing 15% IVPI+15DF+ Medium dose of antioxidant extracts
4.5.3. DISCUSSION

Assessment of safety issues and health benefits is an important part of studies of functional and nutraceutical food products which should be addressed before dissemination of findings to the public. Malley et al. (2007) suggested clinical pathological evaluation in animal modeling as one of the safety assessment tools when some novel food sources are exploited for their appraisal as safe food ingredient (Singh et al., 2002; Ashoush et al., 2013). Sprague Dawley strain of rats is commonly used in efficacy studies to achieve multifarious objectives and the present research investigation was conducted following the protocols as described by previous researchers (Pellette and Young, 1980; Fararh et al., 2002; Morita et al., 2008). Rats fed on experimental diets consumed less feed than control that subsequently showed linear correlation with that of body weights; G4 and G5 groups gained more weight as compared to G3 and G6 groups. Body metabolism requires energy; carbohydrate, fat, and proteins act as energy substrates in the body. The intake of quality protein is important due to certain reasons; it acts as building blocks of the body, better body growth, play role in normal functioning of the body and replacement of tissues. Imbalance in essential amino acids leads to low BV as in case of presence of limiting amount of lysine in wheat based bakery products as depicted with diet group G3. In the present investigation, amino acids profile has been augmented by supplementation of IVPI in DFBB. Appreciable increase in the biological parameters such as NPU, NPR and PER was noticed. There was a marked improvement in the biological value of DFBB as a result of the substitution with IVPI. These results are in agreement with the findings of previous researchers who worked on various legumes such as replacement of wheat flour with 25% mothbean flour for the preparation of biscuits. The BV of biscuits enhanced from 46.1 to 56.33% (Awan et al., 1995) and 55.30 to 66.8% with the supplementation of 10% chickpea flour in the chappatties (Siddique et al., 1996). However, Imbalance in protein amino acids levels can generate various health disorders (Wild et al., 2004; Jain et al., 2006; Ugochukwu and Figgers, 2007). Food intake of the experimental animals may vary from individual to individual due to the difference in their metabolic rate requirements and actual protein content of the experimental diets (Francis et al., 2009; Khan et al., 2011). The diets having PER with score more than 2.0 are known as good source of quality protein. In the present investigation, PER was found high (2.74) which might be due to supplementation of IVPI in the diet. Seena et al. (2006) stated that legume protein isolates
have great applications in nutraceuticals and health care supplements. Also, protein quantity present in the experimental feed affects the intake of the diet and it also varies depending up on the metabolic and physiological needs of a subject (White et al., 2000). Moreover, protein bioavailability and protein quality are mainly dependent on the processing technology applied to ingredients prior to consumption by the subjects (Eissen et al., 2010).

According to definition, an antioxidant is a molecule that slows down a free radical chain reaction propagating the oxidation of lipids in biological contexts. The previous research findings have shown that the development of atherosclerosis was preceded by the accumulation of LDL particles within sub endothelial macrophages when they are structurally and chemically modified by oxidation of the polyunsaturated liquids within these particles.

Phenolic compounds have shown a huge antioxidant potential in-vitro as well as in-vivo studies and their advantageous effects are extensively reported in the models concerning oxidative stresses caused by atherogenic as well as hypercholesterolemic diets (da Silva Pereira et al., 2014; Ktari et al., 2014). Serum protein profile parameters are considered important in determining the safety of functional ingredients and quality of a final product (Farag et al., 2006; Patel et al., 2008). The results obtained in the present investigation showed a significant change in the levels of blood glucose (BG), triglyceride, total cholesterol when compared G2 (DFBB) group with G1 (control). The activities of the ALT, AST, ALP and Bi.T enzymes were significantly reduced in the serum of G2 group, while serum electrolytes such as calcium, sodium, potassium, chloride except bicarbonates are non-significantly affected as a result of antioxidants. Also, no difference was recorded in feed intake and body weight of rats in both groups. However, all these parameters remained in the normal ranges (Table 3.5) as described in safety studies conducted previously (Chengelis et al., 2008; Morita et al., 2008). They reported that total protein, albumin, and globulin were found in the range of 6.3-8.0, 2.6-4.6, and 2.1-4.8 g/dL, respectively. Moreover, urea and creatinine were found in the range of 23.2-36.8 and 0.3-1.20mg/dL, while values of ALK, ALT and AST were found to lie in the range of 139-260, 33-81 and 88-162 U/L, respectively in the diets having antioxidant extracts (Petterino and Argentino-Storino, 2006). Conclusive approach drawn from this section of efficacy study highlighted that DFBB containing antioxidants hold potential to significantly control the levels of triglycerides and cholesterol.
in the rats (Mandadi, et al., 2009). Thus, consumption of supplements containing crude natural antioxidants may be more beneficial way to control oxidative stress than purified antioxidants (Hajiani et al., 2008). Moreover, antioxidants from combined sources are more effective than single source (Ashoush et al., 2013). The bioactive phenolics are able to quench oxygen derived free radical and substrate free radicals by supplying a hydrogen atom to the free radical, save cell constituents against oxidative damage and thus, elevate the risk of many diseases related to oxidative stress (Aruoma, 1998). The results are also in agreement with the findings of previous researchers who have worked on phenolics derived from various sources such as Indian spices (Asimi et al., 2013), fenugreek seeds extract (Abdul-Rahman, 2012; Akbar et al., 2012), tomato powder phenolics (Alshatwi et al., 2010), effect of fenugreek extract to inhibit fat accumulation and ameliorates dyslipidemia in high fat diet-induced obese rats (Kumar et al., 2014) and on oxidative stress in alloxan diabetic rabbits (Hamadi, 2012).

**COST ANALYSIS OF DATE FRUCTOSE BISCUIT BARS**

The cost of production of bars is given may be commercialized and become a source of foreign exchange. The cost of production/Kg of biscuit bars is given in Table 4.60. The protein level of BB was found to enhance to the tune of 10.10% as compared to control. Maximum biological value was observed in biscuit bars with casein diet (96.29) followed by date fructose biscuit bars (92.02). Mean values ranged from 3.49±0.1 to 4.34±0.2 Kcal/g. The calorific value reduced as a result of substitution of sucrose with date fructose. Cost of one kg biscuit bar has been calculated which increased to the tune of Rs 16.0 as result of substitution of 15% protein isolate and 15% date fructose.

**Table 4.60: Cost of DFBB (Kg).**

<table>
<thead>
<tr>
<th>Ingredients (g)</th>
<th>DFBB (Rs./Kg)</th>
<th>Rate (Rs./Kg)</th>
<th>Ingredient cost (Rs.)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>500</td>
<td>35</td>
<td>17.5</td>
<td>17.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>212.5</td>
<td>60</td>
<td>12.75</td>
<td>15</td>
</tr>
<tr>
<td>Date Fructose</td>
<td>37.5</td>
<td>125</td>
<td>4.7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>170</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>------------------</td>
<td>-----</td>
<td>-----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>V. Ghee</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>100</td>
<td>180</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Baking Powder</td>
<td>12</td>
<td>125</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>IVPI</td>
<td>75</td>
<td>300</td>
<td>22.5</td>
<td>-</td>
</tr>
<tr>
<td>Packaging</td>
<td></td>
<td></td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Overhead charges (10%)</td>
<td></td>
<td></td>
<td>13.0</td>
<td>10.8</td>
</tr>
<tr>
<td>Total Cost</td>
<td>1237</td>
<td>1162</td>
<td>146</td>
<td>119</td>
</tr>
<tr>
<td>Total Cost/Kg</td>
<td></td>
<td></td>
<td>118</td>
<td>102</td>
</tr>
</tbody>
</table>

### 4.5.4. CONCLUSION

In conclusion, the results of present investigations suggested that peanut peel and *withania coagulance* extracts contained an abundant amount of potential phenolics sufficient to enhance the shelf stability of DFBB. The in-vivo results suggested that on consumption, they control the oxygen scavenging capacity in the body which might be associated with enzymes system of the body. These findings strongly suggested that DFBB might have noteworthy health benefits. Moreover, replacement of wheat protein with legumes isolate enhanced the biological parameters of the DFBB. The rats who consumed phenilics diet had better serum lipid profile. Thus, it could be concluded that peanut peel and *withania coagulance* extracts (Agricultural waste) could be utilized in neutraceutical and functional foods as a cost effective therapeutic diet to treat a target populace. Cost of one kg biscuit bar has been calculated which increased to the tune of Rs 16.0 as result of substitution of 15% protein isolate and 15% date fructose.

### LIMITATIONS

- The limiting factors of this study are the high cost of conversion of date sugars into fructose and protein isolate extraction due to higher prices of enzymes and extraction chemicals, respectively.
Chapter No: 5  

SUMMARY

The main attraction of biscuit is its variety, convenience and long shelf life. However, they are specifically high in fat and sugars which make them unhealthy. With the changing in life style of a community, the demand of these snacks is increased. In Pakistan, biscuits are commonly consumed by children and people of all age groups as a popular snack. Keeping in view the popularity and other benefits of biscuits, Date-Fructose Biscuit Bar (DFBB) was prepared, as a healthy and nutritious food for people of all age groups using locally available raw materials.

Proximate analyses of raw materials including moisture, crude protein, crude fat, crude fiber and ash content were determined. Proximate analysis of peanuts shows that these contained higher level of crude fat (44.96%) than protein content (35.76%), moisture, fiber, ash and NFE contents of peanuts were 4.59%, 3.75%, 2.59% and 12.93, respectively. Indian vetch contained proteint (28.90%), moisture (8.70%), crude fat (1.27%), crude fiber (2.04%), and ash content (1.52%). Dates contained 26.02% moisture, 2.61% crude protein, 0.33% crude fat, 3.48% crude fiber, 1.44% ash and 92.14% nitrogen free extract. Indian Vetch protein isolate (IVPI) contained the highest crude protein content (87.54%). Moisture, crude fat, crude fiber, ash and NFE contents were 4.40%, 0.34%, 0.12%, 0.35%, 11.65, respectively.

In first part of study, protein isolate was incorporated in to date biscuit bars (DBB). These bars were analyzed for their physical and sensory characteristics to evaluate suitability and best level of Indian Vetch (Lathyrus sativus L) protein isolates (IVPI) for the preparation of DBB. On the basis of these findings, best suited DBB was selected. DBB containing IVPI indicated highly significant difference (P<0.01) among treatments. Color values for T₀, T₁, T₂, T₃ and T₄ were 176.62±1.23CTn, 174.71±1.89CTn, 171.29±3.36CTn, 162.53± 1.40CTn and 158.44±2.47CTn, respectively. The minimum color value was recorded in T₄ having darkest color, while color value of T₀ was the highest among all treatments indicating light color.
Color values of T₂ and T₃ were more acceptable than other treatments due to more appealing moderate color.

- Highly significant (P<0.01) differences were recorded in hardness values of different treatments of biscuit bars. The highest value of hardness was recorded in treatment T₀ while the lowest value was observed in case of T₄. The treatment T₂ and T₃ showed optimum hardness. Hardness values for T₀, T₁, T₂, T₃ and T₄ were 1871.21±25.06g, 1697.51±12.06g, 1628.81±0.87g, 1513.29±23.15g and 1476.78±30.69g, respectively.

- Fractureability values also had highly significant (P<0.01) difference among treatments of DBB containing IVPI. Fractureability values of bars ranged from 73.31±0.78mm to 78.59±0.86mm. Fractureability values of T₀, T₁, T₂, T₃ and T₄ were 73.31±0.78mm, 74.69±0.52mm, 76.05±1.03mm, 76.92±0.44mm, 78.59±0.86mm, respectively. The optimum values were observed in case of treatment T₂ and T₃. Non-significant (P>0.05) differences were found in water activity values of DBB containing IVPI. Water activity of these bars ranged from 0.215±0.003 to 0.220±0.006. Water activities of T₀, T₁, T₂, T₃ and T₄ were 0.215±0.003, 0.220±0.006, 0.210±0.006, 0.220±0.006 and 0.220±0.006, respectively. The water activity values of all bars were in acceptable range that reflects the shelf-stability of DBB. Results showed highly significant (P<0.01) differences in color score of biscuit bars. Color score varied from 7.22±0.14 to 8.41±0.14. The highest color score was achieved by T₂ (8.41±0.14) and T₃ (8.21±0.13). Color scores for T₀, T₁, T₂, T₃ and T₄ were 7.50±0.12, 7.22±0.14, 8.41±0.14, 8.21±0.13 and 7.96±0.16, respectively.

- Highly significant (P<0.01) differences were recorded in the taste score of DBB containing IVPI. The results revealed that maximum taste score was obtained by T₃ (8.09±0.10). It is evident that all treatments were liked by the judges; however, treatment T₃ was highly acceptable due to its taste. Overall taste score of all treatments varied from 7.31±0.11 to 8.09±0.10. Taste score for treatment T₀, T₁, T₂, T₃ and T₄ were 7.45±0.10, 7.31±0.20, 7.50±0.08, 8.09±0.10, 7.33±0.11 respectively.

- Analysis of variance for flavor score of DBB containing IVPI indicated highly significant differences (P<0.01). Flavor scores for treatment T₀, T₁, T₂, T₃ and T₄
were 7.50±0.10, 7.10±0.12, 8.29±0.07, 7.96±0.13 and 7.27±0.06, respectively. Maximum flavor score was achieved by treatment T₂ (8.29±0.07) followed by T₃ (7.96±0.13). The statistical analysis of texture score of DBB containing IVPI indicated significant (P<0.05) variation among treatments. Texture values ranged from 8.60±0.11 to 9.30±0.12. Mean values for texture score of bars for treatment T₀, T₁, T₂, T₃ and T₄ were 8.90±0.15, 8.60±0.11, 9.10±0.17, 9.30±0.12 and 9.21±0.09, respectively. Texture score of T₃ was found the highest (9.30±0.12) followed by T₄ (9.21±0.09).

- Highly significant (P<0.01) variations existed in mouth feel score of DBB containing IVPl among treatments. Mouth feel score of DBB containing IVPI varied from 5.35±0.11 to 7.29±0.09. The best treatment was T₃ that achieved the highest mouth feel score (7.29±0.09) followed by T₁ (7.21±0.12). The mouth feel score of treatments were 5.35±0.11, 7.21±0.12, 6.91±0.08, 7.29±0.09, 7.13±0.12 for T₀, T₁, T₂, T₃ and T₄, respectively.

- Overall acceptability of DBB containing IVPI varied from 36.70±0.25 to 40.85±0.72. Overall acceptability score of treatments are 36.70±0.25, 37.44±0.99, 40.21±0.28, 40.85±0.72, 38.90±0.74 for T₀, T₁, T₂, T₃ and T₄, respectively. The highest overall acceptability score was achieved by T₃ (40.85±0.72) and followed by T₂ (40.21±0.28).

- In the second phase of study, DFBB were developed using date fructose (DF) and best treatment was selected on the basis of best level of combination of protein isolate and date fructose (PIDF). Dates sugars were converted into date fructose by applying enzymes assay. The composition of sugars was determined through GC-FID analysis. 83% total sugars were obtained in which 53% were fructose after conversion with isomerase enzyme. The highest amount of fructose followed by glucose (28%) and sucrose(2%) was obtained. In recipe, sucrose was replaced with date fructose sugar in different levels for the preparation of DFBB.

- Highly significant differences (P<0.01) were observed among treatments of date sugar biscuit bars .Color values for T₀, T₁, T₂, T₃ and T₄ were 172.00±2.11 CTn, 168.00±3.49 CTn, 153.00±3.55 CTn, 149.00±2.99 CTn, 142.00±2.75 CTn, Minimum color value (142.00±2.75 CTn) was recorded in treatment T₄ having
darkest color while color value of T₀ was the highest (172.00±2.11 CTn) among all treatments indicating light color. Color values of T₂ (153.00±3.55 CTn) and T₃ (149.00±2.99 CTn) are more acceptable than other treatments due to more appealing color. Highly significant (P<0.01) differences were recorded in hardness values of different treatments of biscuit bars. The highest value of hardness was recorded in treatment T₀ (2139.21±15.13g) while the lowest value in case of T₄ (1369.23±26.25g). Treatment T₂ (1897.21±49.91g) showed optimum hardness. Hardness values for T₀, T₁, T₂, T₃ and T₄ were 2139.21±15.13g, 2087.59±14.46g, 1897.21±49.91g, 1521.79±26.57g, 1369.23±26.25g, respectively. Fractureability values also have highly significant (P<0.01) differences among treatments of biscuit bars. Fractureability values of bars ranged from 62.41±1.21mm to 72.76±0.89mm. Fractureability values of T₀, T₁, T₂, T₃ and T₄ were 72.76±0.89mm, 71.30±1.48mm, 71.59±1.66mm, 68.39±1.37mm, 62.41±1.21mm, respectively. Optimum values were observed in case of treatment T₂ and T₃. Highly significant (P>0.01) differences were found in water activity values of biscuit bars. Water activity of these bars ranged from 0.235±0.003 to 0.335±0.003. Water activities of T₀, T₁, T₂, T₃ and T₄ were 0.235±0.003, 0.240±0.006, 0.330±0.006, 0.330±0.006 and 0.335±0.003, respectively. The water activity values of all biscuit bars were in acceptable range that reflected these bars were shelf stable. Results show non-significant (P>0.05) difference in color score of date fructose incorporated biscuit bars. Color score varied from 7.26±0.12 to 7.54±0.18. The highest color score was achieved by T₂ (7.54±0.18) followed by T₁ (7.45±0.08). Color scores for T₀, T₁, T₂, T₃ and T₄ were 7.45±0.08, 7.51±0.20, 7.54±0.18, 7.39±0.11 and 7.26±0.12, respectively.

Highly significant (P<0.01) differences were recorded in the Taste score of biscuit bars. The results revealed that maximum taste score was obtained by T₂ (6.84±0.06). It is evident that all treatments were liked by the judges but treatments T₂ and T₃ were maximally liked due to increase in sweetness. Overall taste score of all treatments varied from 5.59±0.07 to 6.84±0.06. Taste score for treatment T₀, T₁, T₂, T₃ and T₄ were 6.75±0.15, 6.81±0.23, 6.84±0.06, 6.84±0.06 and 5.59±0.07, respectively. Analysis of variance for flavor score of date sugar incorporated biscuit bars indicated highly significant differences (P<0.01) among treatments. Flavor
score for treatment $T_0$, $T_1$, $T_2$, $T_3$ and $T_4$ were 6.89±0.14, 7.67±0.21, 7.91±0.21, 7.95±0.11 and 7.21±0.08, respectively. Maximum flavor score was achieved by treatment $T_3$ (7.95±0.21) followed by $T_2$ (7.91±0.21). The statistical analysis of texture score of biscuit bars indicated highly significant ($P<0.01$) variation among treatments. Texture values ranged from 6.05±0.08 to 8.52±0.10. Mean values for texture score of bars for treatment $T_0$, $T_1$, $T_2$, $T_3$ and $T_4$ were 7.49±0.19, 8.29±0.14, 8.50±0.10, 8.52±0.17 and 6.05±0.08, respectively. Texture score of $T_3$ is the highest (8.52±0.17) followed by $T_2$ (8.50±0.10). Significant ($P<0.05$) variations exist in mouth feel score of biscuit bars among treatments. Mouth feel score of bars varied from 6.60±0.09 to 7.30±0.14. The best treatment was $T_3$ that achieved the highest mouth feel score (7.35±0.14) followed by $T_4$ (7.20±0.14). The mouth feel score of treatments were 6.80±0.13, 6.70±0.16, 6.60±0.09, 7.35±0.14, 7.30±0.14 for $T_0$, $T_1$, $T_2$, $T_3$ and $T_4$, respectively.

- Overall acceptability of bars varied from 33.41±0.85 to 38.05±0.34. Overall acceptability score of treatments are 35.38±0.65, 36.98±0.86, 37.39±0.99, 38.05±0.34, 33.41±0.85 for $T_0$, $T_1$, $T_2$, $T_3$ and $T_4$ respectively. The highest overall acceptability score was achieved by $T_3$ (38.05±0.34) and followed by $T_2$.

- Peanut peel extract and *withania coagulans* extract were used as the antioxidants in different levels to increase the shelf-life of DFBB. The phenolic extracts were obtained by treating the dried ground powder of Peanut peel and *withaniacoagulans* with ethyl alcohol. The Peanut peel extract and *withania coagulans* contained 64.6% and 57% antioxidant activities and 98.6mg/g and 13.2mg/g total phenolics and 74.2 and 52.5% free radical oxygen scavenging activities, respectively. The date fructose biscuit bars (DFBB) were prepared by using the best formulation as selected in the previous studies (15% IVPI+ 15% DF and incorporating peanut peel extract and *withania coagulans* extract at variable levels. Response Surface Methodology (RSM) was applied to estimate the responses of independent variables i.e. peanut peel extract (X) and *withania coagulans* extract (Y) during storage. Fourteen date fructose biscuit bar treatments were generated using a Central Composite Design (CCD) with two variables and three levels for each variable. Second-order polynomial model was fitted for independent variables i.e. peanut peel extract (X) and *withania*
coagulans extract (Y). The regression equations and coefficients were determined by using multiple regression analysis of storage’s data regarding different parameters.

- The responses for hardness from Central Composite Design (CCD) were fitted with second order polynomial equations. The statistical analysis shows significant effect of variables by applying analysis of variance technique to the full regression of model. However, linear terms of variable (X) are observed to positively change the hardness of biscuit bars at all storage intervals, whereas quadratic terms of withania coagulans extract (Y) have a negative effect. When interaction of these two terms (XY) was studied, it was found negative over all storage intervals. The coefficients of determination (R^2) were studied as above 98.3%, therefore it could be assured that models are adequately fitted. The data showed that peanut peel extract (X) and withania coagulans extract (Y) contributed towards increase in firmness in DFBB at 0 to 90 days storage intervals. The effect of peanut peel extract (X) and withania coagulans extract (Y) concentrations in DFBB through three dimensional response surface plots for hardness (firmness) at 0, 30, 60 and 90 days storage intervals.

- The models were developed for fractureability of DFBB as affected by independent variables during 90 days storage. The data showed that peanut peel extract (X) and withania coagulans extract (Y) contributed towards increase in fractureability in date fructose biscuit bars at 0 to 90 days storage intervals. The effect of peanut peel extract (X) and withania coagulans extract (Y) concentrations in date fructose biscuit bars through three dimensional response surface plots for fractureability at 0, 30, 60 and 90 days storage intervals. The regression coefficients as well as correlation coefficients obtained for all seven models related to color of date fructose biscuit bars under the influence of independent variables (peanut peel extract (X) and withania coagulans extract (Y)) at seven storage intervals (0, 15, 30, 45, 60, 75 and 90 days). The regression coefficients for these models (R^2 = 94.0%, 94.0%, 93.8%, 94.1%, 93.9%, 94.8%, respectively) are high enough for a well fitted response surface models.

- The effect of linear term of X is statistically non-significant (P<0.05) for color of date fructose biscuit bars at all storage intervals, while linear term of Y is statistically
significant (P<0.05) for color of date fructose biscuit bars at all storage intervals. The $X^2$ quadratic terms are found significant at all storage intervals whereas, the quadratic terms for $Y^2$ are found non-significant at storage. The interaction of two variables (XY) shows non-significant effect on color at all storage intervals.

- The analysis of variance shows significant effect of variables on mouth feels score of date fructose bars during storage intervals at 60 to 90 days. The regression coefficients for these models ($R^2 = 90.9\%, 92.5\%, 92.3\%, 93.2\%, 94.8\%$ and $95.2\%$ respectively) are enough for the well fitted response surface models. The significant results are obtained for linear terms of variables (peanut peel and withania coagulans extracts). The effect of linear terms of X for mouth feel of date fructose biscuit bars is significant at 15, 30, 45, 60, 75 and 90 days storage intervals. The effect of linear terms of Y is significant for mouth feel of date fructose biscuit bars at 90 days storage intervals. The $X^2$ quadratic terms are found significant at 0, 15, 30 and 45 days storage intervals whereas, the quadratic terms for $Y^2$ are found significant at all days of storage intervals.

- The analysis of variance shows significant effect of variables on texture score of date fructose biscuit bars during storage intervals. The regression coefficients for these models ($R^2 = 93.9\%, 94.3\%, 94.0\%, 94.7\%, 93.8\%$ and $93.2\%$ respectively) are enough for the well fitted response surface models. The non-significant results are obtained for linear terms of variables (peanut peel and withania coagulans extracts). The $X^2$ quadratic terms were found significant at all days of storage intervals whereas, the quadratic terms for $Y^2$ were found significant at 0, 15, 30 and 45 days of storage intervals.

- To determine the combined effect of selected natural antioxidant extracts (peanut peel 0.21% + withaniacoagulanse 0.32%) on physico-chemical, sensory and nutritional properties of DFBB with different levels during storage. Non-significant differences (P>0.05) in color values were observed among treatments of DFBB with the addition of antioxidant extarcts .Color values for $T_1$, $T_2$, and $T_3$ were $152.50\pm0.04CTn$, $147.10\pm0.16 CTn$ and $144.20\pm0.12 CTn$, respectively. Highly significant (P<0.01) differences were recorded in hardness values of different treatments of DFBB. The highest value of hardness was recorded in treatment $T_1$ ($257.93\pm6.45g$) while the
lowest value in case of T3 (221.67±3.20g). The treatment T2 (247.32±2.24g) showed optimum hardness. Hardness values for T1, T2, and T3 are 257.93±6.45g, 247.32±2.24g, and 221.67±3.20g, respectively.

- Fractureability values have non-significant (P>0.05) differences among treatments of date fructose biscuit bars containing antioxidant extract. Fractureability values of T1, T2, and T3 were 76.04±1.97mm, 76.89±1.37mm, and 78.26±0.82mm, respectively.
- Significant (P<0.05) differences were found in water activity values of DFBB containing different levels of antioxidant extracts. Water activity of these bars range from 0.240±0.006 to 0.261±0.006 that reflects all treatments are shelf stable. Water activity of T1, T2, and T3 is 0.240±0.006, 0.261±0.006, and 0.260±0.000, respectively.
- The moisture in biscuit bar sample varied from 3.38±0.03% to 3.53±0.03% and the differences among means for moisture were highly significant (p<0.01). The highest mean value for moisture (3.53±0.03%) was observed in treatment T3, while the lowest mean value (3.38±0.03%) was recorded in T2. The crude protein content of biscuit bar sample varied non-significantly (P>0.05) among treatments and with storage period of 90 days. Similarly, crude fat, crude fiber, ash and NFE contents in biscuit bar samples varied non-significantly (P>0.05) among treatments and with storage period of 90 days.
- The mineral contents in biscuit bar sample varied non-significant (p>0.05). The mean values of sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc and manganese for treatments range from 2.00 to 2.53 mg/100g, 430.61 to 483.89 mg/100g, 41.01 to 45.43 mg/100g, 53.75 to 58.10 mg/100g, 1.30 to 1.84 mg/100g, 0.25 to 0.28 mg/100g, 0.41 to 0.42 mg/100g and 0.32 to 0.38 mg/100g, respectively.
- The amino acids in date fructose biscuit bars i.e. aspartic acid, alanine, asparagine, glutamic acid, glycine, histidine, leucine, isoleucine, lysine, threonine and tyrosine were increased with the incorporation of vetch protein isolate. The highest quantities of aspartic acid, alanine, asparagine, glutamic acid, glycine, histidine, leucine, isoleucine, lysine, threonine and tyrosine contents were recorded in the date fructose
biscuit bars including 3.46 g, 2.41 g, 0.68 g, 10.90 g, 2.20 g, 0.07 g, 1.67 g, 0.73 g, 0.46 g, 0.20 g and 0.82 g/100g, respectively.

- The mean values of free fatty acids in biscuit bars differ highly significantly (P<0.01) among treatments. Maximum mean value (0.1755±0.004%) for free fatty acids was recorded in T₁ followed by T₂ (0.1663±0.005%), whereas minimum mean value (0.1567±0.005%) for free fatty acids was recorded in biscuit bars with maximum antioxidant level (T₃). The mean values of free fatty acids did not change significantly during storage.

- The mean values of peroxide in biscuit bars differ highly significantly (P<0.01) among treatments. Maximum mean peroxide value (2.943±0.09meq/Kg) was recorded in T₁ followed by T₂ (2.157±0.05meq/Kg), whereas minimum peroxide value (1.749±0.07meq/Kg) was recorded in T₃ with high level of anti-oxidants. The mean peroxide values did not change significantly during storage period. Results show non-significant (P>0.05) difference in color score of antioxidant incorporated biscuit bars. Color scores for T₁, T₂ and T₃ are 7.80±0.14, 7.50±0.20 and 7.60±0.08, respectively. Non-significant (P>0.05) differences were recorded in the taste score of biscuit bars. The taste score for treatment T₁, T₂ and T₃ are 7.59±0.16, 7.60±0.14 and 7.52±0.06 respectively.

- Analysis of variance for flavor score of date sugar incorporated biscuit bars indicated highly significant differences (P<0.01) among treatments. Flavor score for treatment T₁, T₂ and T₃ are 6.90±0.06, 7.90±0.21 and 5.60±0.11, respectively. Maximum flavor score was achieved by treatment T₂ (7.90±0.21) followed by T₁ (6.90±0.06).

- The statistical analysis of texture score of date fructose biscuit bars indicated highly significant (P<0.01) variation among treatments. Mean values for texture score of bars for treatment T₁, T₂ and T₃ were 7.60±0.05, 8.80±0.17 and 8.10±0.21, respectively. Texture score of T₂ is the highest (8.80±0.17) followed by T₃ (8.10±0.21). Highly significant (P<0.01) variations exist in mouth feel score of date fructose biscuit bars among treatments. The best treatment was T₂ that achieved the highest mouth feel score (7.90±0.04) followed by T₃ (6.70±0.14). The mouth feel score of treatments were 5.90±0.07, 7.90±0.04 and 6.70±0.14 for T₁, T₂ and T₃.
respectively. Overall acceptability score of date fructose biscuit bars vary from 35.52±0.22 to 39.70±0.43. Overall acceptability score of treatments were 35.79±0.57, 39.70±0.43 and 35.52±0.22 for T₁, T₂ and T₃, respectively. The highest overall acceptability score was achieved by T₂ (39.70±0.43) followed by T₁ (35.79±0.57). Diets prepared from the control and selected treatments were fed to the respective groups of rats. Rats from groups G₃ to G₆ were sacrificed after 10 days. Body weight, Feed and water intake were measured on daily basis. Moreover, serological characteristics were also determined to resolve the safety concerns of the experimental diets. Feed efficiency may be defined as the gain in body weight per unit feed intake. Among the test diets normal dietary shows low feed efficiency (p≤0.05). Results show that the feed efficiency for casein was 4.07 followed by date fructose biscuit bars(3.73).

- The ratio of feed intake to gain in body weight is called feed utilization. Statistical analysis showed highly significant (p<0.01) variation among rat groups fed on various types of biscuit bar meals. Mean values revealed good utilization of casein biscuit bars(87.37). The significant statistical difference for protein utilization values in the bar meal diets is observed, where minimum protein utilization was observed for normal biscuit bar meal(71.25) and higher value in date fructose biscuit bars (75.26).

- Protein efficiency ratio is gain in body weight per unit protein intake. The statistical analysis for Protein efficiency ratio of rats fed on different biscuit bar groups has shown highly significant (p<0.01) variations. The results revealed that the protein efficiency ratio was higher for casein i.e. 3.060, followed by date fructose biscuit bars (2.74) and normal biscuit bars.

- Statistical analysis regarding net protein retention of rats on different biscuit bars diet groups has shown highly significant (p<0.01) differences among groups. Maximum net protein retention value was observed in biscuit bars with casein diet (4.04) followed by date fructose biscuit bars (3.85) and minimum value in case of normal biscuit bars (3.68).

- Statistical analysis regarding protein digestibility show highly significant (p<0.01) variation among rat groups fed on various bar meal. Mean values reveal good protein
digestibility in biscuit bars with casein (89.25). The statistical difference for protein digestibility values in the test protein diets and casein is observed, where minimum protein digestibility was observed for normal biscuit bars (82.55).

- Statistical analysis regarding biological value of rats on different biscuit bars diet groups has shown highly significant (p<0.01) differences among groups. Maximum biological value was observed in biscuit bars with casein diet (96.29) followed by date fructose biscuit bars (92.02) and minimum value in case of normal biscuit bars containing gluten (80.85). Diets (groups) exhibited highly significant while seasons, and their interactions showed non-significant differences in liver functioning tests. Activities of liver enzymes like ALT, ALP and AST ranged from 23.17±3.17 to 31.00±0.92, 105.00±15.7 to 162.00±2.68 and 23.83±1.38 to 27.50±0.46 U/L, respectively in the two diet groups. Moreover, bilirubin proteins varied significantly from 0.621±0.02 to 0.695±0.010 mg/dL. Serum electrolytes were affected non-significantly among groups. Means for Na, K and Cl ions were non-significant. Lipid profile was affected significantly within diet groups, while study seasons remained non-significant as a function of diets. The mean value of cholesterol was found 74.58±1.87 in G1 followed by 66.71±1.45 in G2. Means for triglycerides contents also indicated significant variations due to diets and non-significant variation due to season.
These results suggest that date fructose biscuit bars containing 15% Indian vetch protein isolate, 15% date fructose and 0.5% of peanut peel and *withania coagulans* optimized levels of extracts were proved as the best biscuit bars having good sensory qualities and could be stored for 90 days.
IMPORTANCE OF RESULTS AND MAJOR FINDINGS

The results regarding the proximate composition suggested that IVPI was found suitable to enhance nutritional value of date biscuit bars. Physical properties and sensory characteristics for DBB were evaluated to assess the suitability of replacement of Indian vetch protein isolate (IVPI). The results revealed that replacement of IVPI in DBB contributed hardness in texture. All sensory parameters such as color, taste, flavor and overall acceptability were significantly affected with more than 15% replacement of IVPI in DBB. On the basis of overall acceptability results, it was concluded that 15% IVPI could be incorporated into DBB without affecting their physical and sensory qualities.

Dates sugars were converted into date fructose by applying enzymes assay. 83% total sugars were obtained in which 53% was fructose after conversion with isomerase enzyme. In recipe, sucrose was replaced with date fructose sugar in different levels for the preparation of DFBB. The results suggested that color value and hardness of DFBB decreased with the replacement of sucrose with date fructose in DFBB. All treatments containing different concentrations of date fructose were found acceptable in sensory attributes. However, DFBB with 15% sucrose replacement with date fructose was found the best in terms of physical and sensory characteristics.

Response surface methodology, a statistical technique was applied to determine the true relationship between dependent and independent variables to find out regions of best response values. Maximum responses of dependent variables including sensory and physical characteristics of DFBB were achieved by incorporating 0.21% peanut peel extract and 0.32% withania coagulans extract during 90 days of storage.

Physico-chemical properties and sensory characteristics of DFBB were evaluated to assess the suitability of addition of antioxidant extracts from peanut peel and dried berries of withania coagulans . The results for proximate composition revealed that addition of antioxidant contributed non-significantly effect on proximate composition during 90 days storage. It is also found that addition of peanut peel and withania coagulans extracts at three levels have no effect on the in-vitro protein (IVPD) and in-vitro starch digestibilitis (IVSD). Sensory parameters such flavor and overall acceptability were affected significantly with the addition of peanut peel and withania coagulans extracts in DFBB. All the bars were
acceptable with good sensory characteristics but bars containing medium level (0.5%) of antioxidant were maximally preferred. The results of calorific value indicated that all the biscuit bars provided high calorific value as well. DFBB containing Indian vetch protein isolate, date fructose and peanut peel and *withania coagulans* extracts could be considered as the nutrient dense bars with good sensory qualities and 90 days shelf stability.

Assessment of safety issues and health benefits is an important part of studies of functional and neutraceutical food products which should be addressed before dissemination of findings to the public. The results suggested that peanut peel and *withania coagulans* extracts contained an abundant amount of potential phenolics sufficient to enhance the shelf stability of DFBB. The in-vivo results suggested that on consumption, they control the oxygen scavenging capacity in the body which might be associated with enzymes system of the body. These findings strongly suggested that DFBB might have noteworthy health benefits. Moreover, replacement of wheat protein with legumes isolate enhanced the biological parameters of the DFBB. The rats who consumed phenilics diet had better serum lipid profile. Thus, it could be concluded that peanut peel and *withania coagulans* extracts (Agricultural waste) could be utilized in neutraceutical and functional foods as a cost effective therapeutic diet to treat a target populace.

**CONCLUSIONS**

The results of these studies suggested that DFBB containing 15% Indian vetch protein isolate, 15% date fructose and 0.5% of peanut peel and *withania coagulans* optimized levels of extracts were proved as the best biscuit bars having good sensory qualities and have 90 days shelf stability. By adding protein isolate and date fructose; nutritional values, sensory properties, amino acid profile and biochemical serum profile of rats were found to be improved.

Response surface methodology, a statistical technique was found suitable to find out regions of best response values and relationship between dependent and independent variables.
RECOMMENDATIONS

- Legume protein isolate enriched food products should be encouraged to cope with protein energy malnutrition prevailing amongst the population.
- Awareness should be created amongst the masses by diet planners about the importance of sucrose replacement with fructose in food formulations as it reduces calorie intake giving the same level of sweetness provided by almost double quantity of table sugar.
- Public awareness campaigns should be launched to highlight the importance of eating healthy food and the risks associated with the life of undernourished children and adults at national levels.
FUTURE RESEARCH DIRECTIONS

- *Lathyrus* species of indigenous legumes should be explored in future research efforts for quality protein sources and should be recommended as a valuable cost effective protein source in food formulations.
- Natural antioxidants extracted from agriculture based processing industries waste materials should be explored to enhance the shelf life of bakery products. Thus, to avoid the health risks associated with the use of synthetic antioxidants.
- Food processors and food technologists with collaboration of food scientists should develop healthy, ready to eat food products like food bars, biscuit bars for masses of all age groups to cope with the menace junk foods.
- Multiple fortification in wheat flour with macro and micronutrients may be done to cope with the problem of malnutrition among population segments.
- IVPI may be incorporated in other bakery products such as chapatti, naan, bread, cakes etc.
- Other cheap sources of protein may be incorporated to enhance the protein level of biscuit.
- Whey protein may be tried in date fructose biscuit bars.
- IVPI may be used to prepare meat ball analog by applying extrusion technology.
- IVPI can be incorporated as binder to increase the protein status of sausages.
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