CHARACTERIZATION OF MAIZE GERM FROM VARIOUS HYBRIDS AND USE OF ITS COMPONENTS FOR VALUE ADDED BAKED PRODUCTS

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A dissertation submitted in partial fulfillment

of the requirements for the degree of

DOCTOR OF PHILOSOPHY

IN

FOOD TECHNOLOGY

NATIONAL INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY
UNIVERSITY OF AGRICULTURE
FAISALABAD
2009

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The Controller of Examinations,
University of Agriculture,
Faisalabad.

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TO

HAZRAT MUHAMMAD

(Peace Be Upon Him)

ACKNOWLEDGEMENTS

This is all due to mercy and blessings of **ALMIGHTY ALLAH** (The Beneficent); created and blessed me to accomplish the present research project. I submit my modest gratitude from core of my heart to **HOLY PROPHET HAZRAT MUHAMMAD** (**Peace Be upon Him),** The source of knowledge and wisdom.

I expand my sincere admiration to my kind and friendly supervisor, **Dr. Masood Sadiq Butt,** Associate Professor, National Institute of Food Science and Technology, University of Agriculture, Faisalabad for his great support, enlightened guidance and constant encouragement during planning, execution and write-up of this dissertation.

I am extremely thankful to **Prof. Dr. Faqir Muhammad Anjum,** Director General, National Institute of Food Science and Technology, University of Agriculture, Faisalabad for his compassionate and thorough support throughout my career as a student in the Institute.

I am also indebted to my committee members, **Dr. Amer Jamil,** Associate Professor and **Dr. Ijaz Ahmad,** Senior Scientific Officer, PCSIR Laboratories, Lahore, for their caring attitude and kind assistance during the course of study.

I would also like to thank **Higher Education Commission, Pakistan**, for providing funding and support for PhD studies and research both in Pakistan and USA. I pay my heartiest regards to **Dr. Muhammad Siddique, Dr. Kirk D. Dolan, Dr. Janice B. Harte and Dr. Ravi**, Department of Food Science and Human Nutrition, Michigan State University, USA, who greatly helped me to complete part of my research work at MSU, USA.

It has also been pleasurable to work with all my friends and colleagues; always helping, friendly, empathetic and encouraging. It is hard to mention all the names here yet; I would like to mention few out of bunch: **Kamran, Saima and Tauseef**.

I am also privileged to be the son of great **DAD** (late) and **MOM** who helped me in every walk of life. My sisters and brothers have also been very helping and encouraging for the accomplishment of this task. I am also obliged to **my wife, my son** and all other family members and relatives for their consistent support.

MUHAMMAD NASIR

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ABSTRACT

Maize germs from six promising locally grown hybrids, namely Poineer-32-F-10 (P-1), Poineer-32-B-33 (P-2), Monsanto-6142 (M-1), Monsanto-6525 (M-2), Rafhan-2331 (R-1) and commercial were characterized for proximate composition, selected minerals, amino acid & fatty acid profiles and tocopherols. Based on the germ recovery and compositional analysis, one best hybrid germ, Pioneer 32-F-10 (P-1), was selected for further analysis and product development. Refined maize germ oil (MGO) from selected hybrid, was analyzed for various physical and chemical characteristics. The MGO was evaluated for cake preparation through blending with normal shortening at different levels. Moreover, defatted maize germ (DMG) meal was subjected to biological and safety evaluation using Sprague Dawley rats. Later, DMG flour was blended with wheat flour in different combinations; evaluated for functional properties, textural analysis (SMS Texture Analyzer) and Farinographic behavior. Flour blends were further used to develop value-added products like bread and cookies. Germ recovery from the experimental hybrids ranged from 6.31-7.68%, while the highest germ yield was observed in P-1 hybrid. Germ samples were found to be nutrient dense; crude protein 16.34-20.96%, crude fat 32.10-38.80%, crude fiber 2.63-4.79%, ash 3.08-4.94%, α-tocopherol 4.63-9.68 mg/100g and γ-tocopherol 29.52-35.51 mg/100g. The germ samples were rich in polyunsaturated fatty acids (46.74-58.00 g/100g) and essential amino acids (33.30-39.04 g/100g). Additionally, in germ samples, minerals like P (1.06-1.79 g/100g), K (1.19-1.64 g/100g), Mg (0.43-0.78 g/100g) and Fe (9.08-14.46 mg/100g) were in substantial amount. MGO from selected germ (P-1) was successfully incorporated in cake recipe with high sensory quality. Defatted maize germ (DMG) meal was found to be considerable source of protein (32.1%), dietary fiber (31.87%) and allied minerals. In the experimental rats, *in-vivo* protein quality of DMG flour was: 87.10±0.78% true digestibility, 76.70±1.25% net protein utilization, 88.06±o.67% biological value, 5.12±0.21 net protein ratio and 2.15±0.03 protein efficiency ratio were significantly higher than that of wheat-based diet and comparable with casein. Favorable impact of DMG flour on serum biochemical profile was observed; cholesterol, triglyceride, LDL and glucose levels decreased up to 6.80, 12.45, 16.19 and 6.50 %, respectively. DMG flour resulted in improvement of functional properties of DMG flour-wheat flour blends with special reference to improved water & oil absorption capacity, gelling and emulsion properties; revealed its worth in food preparations. Protein and fiber enriched cookies and breads were prepared up to 15% wheat flour substitution with DMG flour. Consumer response to purchase defatted maize germ flour fortified cookies was very positive e.g. 64% of the respondents said that they would prefer to purchase

INTRODUCTION

Bridging the gap between increasing food consumption and production is amongst the most challenging tasks round the globe especially in developing countries. The existing problems of food security and malnutrition coupled with escalating population, uncertain crop yield and high cost animal based food supplies have urged to identify and incorporate unconventional food sources in diet formulations to improve the prevailing situation by introducing value added products.

Maize (*Zea Mays* L.) is world's third leading cereal crop with production of 703 million metric tons during the crop year 2006-07 (CRA, 2007). In Pakistan, the annual maize production has increased significantly in last four years from 1.7 million metric tons during crop year 2003-04 to 3.0 million metric ton in 2006-07 (GOP, 2007). The efforts to develop maize hybrids with higher germ yields and distinct quality traits gained momentum in the last decade; still questions have been raised about possible changes in grain quality associated with them (Bullock *et al.,* 1989). Heterosis or hybrid vigour describes the superior performance of heterozygous F1-hybrid plants in terms of its quality attributes (Swanson-Wagner *et al.,* 2006). Benefits associated with cultivation of maize hybrids have witnessed the increased global acreage; 95% in USA and 65% of worldwide is under hybrids plantation (Meyer *et al.,* 2007).

In Pakistan, hybrids cover maximum acreage of cultivated maize, however, these differ in their grain composition with special reference to germ size and its components. Generally, dry and wet milling processes are employed in the maize processing that separate the maize germ from kernel. Maize germ

constitutes 5-14% of the weight of kernel and is a good source of key nutrients especially 18-41% of oil and 17-35% of protein contents (MPOC, 2008; Johnston *et al.,* 2005). Nutritional profile of maize germ supports its use for oil extraction and supplementation of its defatted portion to improve the protein quality of wheat based products. Keeping in view the germ recovery from various maize hybrids in Pakistan, roughly 225 thousand tons of germs can be obtained annually for oil production and value added products from defatted part.

Pakistan is facing serious shortage of edible oils & fats from the last few decades and oil from indigenous sources fulfills about 30% of domestic needs, while rest is imported. The country has spent US$ 1309.1 millions in 2007-08 for the import of palm and soybean oil reckon as being world's 4th largest edible oil importer. Soaring prices of oils and current food crisis in the world require exploration of alternative sources and introducing new maize hybrids with better oil yields.

Acquaintances with chemical and physical properties of edible oils are imperative as they tie up with processing functionality, storage stability and nutritional behavior. These properties depend primarily on varietal differences, compositions and origin of fats and oils. Technological properties of maize germ oil (MGO) proved it as stable vegetable oil that renders its extensive use as cooking medium, in margarines and salad dressings. High oil yielding maize germ from hybrids could play a key role in fulfilling oil requirements with additional nutritional significance.

Maize germ oil (MGO) is bestowed with the components of nutritional significance like tocopherols and polyunsaturated fatty acids (PUFA). MGO has gained wide popularity as a good source of essential fatty acids with 50-60% of poly unsaturated fatty acids (Lemcke-Norojarvi *et al.,* 2001). Furthermore, MGO is amongst the richest sources of vitamin E as it contains appreciable amounts of α and γ-tocopherols. The supply of these components through diet is of

significant importance as they provide several health benefits especially in coronary cure. Presence of PUFA plays pivotal role in betterment of human health through maintaining the body homeostasis and regulating serum lipid profile (Ramaa *et al.,* 2006). Presence of MGO allied antioxidants in diets improves serum tocopherols especially γ-tocopherol status. Improved antioxidant defense system has the ability to reduce the extent of LDL oxidation and subsequently improving cardiovascular health (Lemcke-Norojarvi *et al.,* 2001; Albertini *et al.,* 2002).

Developing world is facing the menace of protein malnutrition especially in those countries where wheat and rice are the dietary staples. Wheat protein is of poor quality due to lower lysine contents (Anjum *et al.,* 2005). In order to control protein malnutrition, petite efforts are being carried out through diet diversification programs. Residues left after oil extraction, can be a potential food source in composite flour technology as a source of protein, dietary fiber and minerals (Rehman *et al.,* 2007).

It is need of time to enhance the value of food products in terms of both quality and quantity along with consumer's safety. In developing new food products, it is important to balance ingredients that offer optimum processing functionality, nutritional value and cost effectiveness. In WTO scenario, novel food sources are required to be evaluated for nutritional status along with safety assessment through some animal based trials to ensure and safeguard consumer health. Liver and kidney functioning tests are used to evaluate the safety of food components including new protein sources, plant extracts or other novel food ingredients (He *et al.,* 2008; MacKenzie *et al.,* 2007). Serum proteins and its fractions like albumin and globulin are key parameters to determine dietary protein quality, its digestion and absorption.

Product development is not only restricted to create distinctive food commodities but also includes product relocation, line extension, and

reformulating the existing items. Among different value added food systems, baked products provide an excellent opportunity to incorporate food-grade fractions from grains, legumes, or other non-traditional food sources. Baked foods i.e. bread, cookies and cakes etc are consumed worldwide relatively on larger scale (Bakke and Vickers, 2007). A variety of wheat flour substitutions have been tried in the form of composite flours in different bakery formulations with varying success; for example, soy or defatted soy flour, raw or defatted wheat germ, flaxseed and sunflower meal (Skrbic and Filipcev, 2008; Junqueira *et al.*, 2008).

The cereal based products made from composite flours are widely accepted and have been commercialized in many parts of the world to improve the nutritional and other quality parameters of end products. The market for bakery and cereals is rapidly growing at an average annual rate of 11.7% in Pakistan, thus presenting an excellent tool for fortification to reach the masses (Datamonitor, 2008). Defatted maize germ (DMG) flour, owing to its nutrient-dense attributes and excellent functional properties, offer a potential to be incorporated in wheat flour for improvement in baked products like bread and cookies (Bakke and Vickers, 2007). DMG proteins are of superior nutritional quality consisting mostly of albumin and globulin with protein efficiency ratio comparable to that of casein (Lawton and Wilson, 2003; Moreau, 2006). It has been observed that protein in DMG possesses balanced amino acid profile containing lysine (5-6%) that can meet the amino acid requirements of target populations as per FAO/WHO standards (Gupta and Eggum, 1998).

In addition to nutrition, the functional properties of value added food ingredients also play a key role for their successful incorporation in conventional food formulations. Defatted maize germ (DMG) meal is reported to have better functional behavior with excellent water and oil retention along with good gelling and emulsifying properties thus resulting in better quality products. The

DMG flour, being a cheaper source of nutrients, decreases the cost of composite flour blends or finished product besides improving the nutritional profile (Johnston *et al.,* 2005).

Screening of superior germ with special reference to nutritional significance and high yield potential from various hybrids and further use of its defatted portion could open new avenues to meet the local oil requirements and increasing demand for protein enriched value added products. Although the use of defatted maize germ as a food ingredient has been often proposed yet very limited research has been conducted to exploit in food formulations. Considering the adverse consequences of food crisis, blending of DMG flour in wheat flour is not only feasible but also economical and can play a vital role to cope protein malnutrition. It is imperative to evaluate the composition and nutritional profile of different maize hybrid germs and utilization of their components i.e. oil and defatted portion in cereal based food products for value addition. Accordingly, present project was designed to achieve following objectives:

1. Characterization of maize germs from various hybrids for chemical composition and nutritional status
2. Evaluation of defatted maize germ flour for safety and protein quality through efficacy studies in Sprague Dawley rats
3. Development of maize germ oil and defatted maize germ based value added bakery products

Chapter-II

REVIEW OF LITERATURE

Escalating population seems to impart atrocious consequences on the sustainability of food security situation over the globe. Mounting food prices, uncertain crop yields, increasing inflation rates and decreasing purchasing power are posing serious threats to food availability resulting malnutrition related disorders in developing countries. The scenario has urged to enhance major crop yields, exploring un-conventional food sources coupled with value-addition by utilizing agro industrial by-products/wastes. Maize germ is nutrient dense substance nevertheless its defatted part is entirely destined to animal feeding. Although, defatted germ has potential as food ingredient but still demands further research for its utilization in food industry. For the purpose, present research plan was designed to find out best maize hybrid germ with respect to oil yield and nutrient status as well as utilization of defatted germ meal for product value-addition. The literature regarding different aspects of present research has been reviewed under the following headings.

2.1. Food security and un-conventional foods

2.2. Maize germ characterization

2.3. Protein quality

2.4. Efficacy and safety evaluation

2.5. Composite flour

2.6. Functional and rheological properties

2.7. Product development

2.1. Food Security and Un-Conventional Foods

Developing countries are facing a dilemma of malnutrition due to lack of food resources. High prices of food commodities and policy barriers are the factors aggravating the food crisis in developing countries (Weaver, 1994). Food security is defined as "condition where all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (FAO, 1996). Food supplies can be expanded by exploitation of alternative non-conventional or novel food resources. Food scarcity including edible oil shortage magnifies the need to generate new avenues, purposely un-conventional foods are getting attention as far as nutrition and food security is concerned (FAO, 1994, Michaelsen and Henrik, 1998).

Millennium Development Goal emphasized that the population suffering from hunger must be decreased to its half during 1990 to 2015. It is estimated that 815 million people are chronically food-insecure in developing countries and 5-10% of population is also at risk from acute food insecurity, due to natural and man-made calamities. According to FAO, the goals to alleviate poverty and hunger as set by World Food Summit (1996) and the Millennium Summit (2000) seems difficult to be achieved as 2015 approaching without significant progress, especially in Latin America and Asia. The statistical data for the period 2002-04 give an idea that the number of hungry people has not considerably been decreased in last decade (from 884 to 864 million); two thirds of the undernourished people being in Asia (558 million) and one fourth in Africa (222 million) (FAO, 2005; EC, 2007).

Exploration of un-conventional foods especially rich in protein and oils is needed to overcome malnutrition and allied health disparities in developing

countries like Pakistan. Several efforts have been made for exploitation and utilization of oils and proteins from alternative sources (Becker, 2007).

Different sources have been exploited in the past to cover the shortage of vegetable oil. Germs of cereals like rice, wheat and maize are rich in oil contents and research has already been conducted on various aspects of their oils (Ramezanzadeh *et al.,* 1999a, 1999b; Sugano *et al.,* 1999). These oils hold rich phytochemistry involving tocopherols/tocotrienols (Kim and Godber, 2001; Sierra *et al.,* 2005).

Edible vegetable oils play an important role in human diet being source of essential fatty acids and fat soluble vitamins; E and A. Wheat germ is a by product of wheat milling industry, which constitutes 2-3% of weight of kernel. Germ oils has been extracted and used as source of polyunsaturated fatty acids and natural antioxidants (Anjum *et al.*, 2008). Based on the findings, wheat germ oil has been recommended to be extracted and utilized in food formulations (Lloyd *et al.,* 2000).

Likewise, rice bran is important co-product produced in the rice milling industry (Juliano, 1985). It possess good nutritional profile and is rich in tocotrienols, tocopherols and sterols but currently it is under-utilized in spite of its food potential (Danielski *et al.,* 2005). Rice bran has been extracted in many parts of world and utilized as a source of oil to cope the need (Kim and Godber, 2001; Amarasinghe and Gangodavilage, 2004). Recently, black cumin oil has also been explored for its health benefits and recommended for human consumption by many researchers (Ramadan and Mörsel, 2002; Weinreb *et al.*, 2004; Al-Saleh *et al.,* 2006)

Some un-conventional sources with meal/cake or defatted portion can be effectively used as food source (Arshad *et al.,* 2007). It has been observed that quality and quantity of protein consumed in the diet could possibly be improved

by incorporating un-conventional food sources in the dietary staples, wheat and rice (Anjum *et al.,* 2005).

Many wild unconventional legumes have been identified, yet their utilization is limited due to inadequate attention; *Canavalia gladiata, Canavalia ensiformis, Canavalia maritima* and *Canavalia cathartica* are some of the common under-exploited legume species (Sridhar and Seena, 2006). Vadivel and Janardhanan, (2005) analyzed seeds of some wild legumes to assess their potential as alternative sources of protein. Crude protein ranged from 20.3 to 35.0% containing valine, phenylalanine, tyrosine, isoleucine, histidine and lysine. Previously, some other wild legumes have been characterized for protein content by Arinathan *et al.,* (2003) and found that these can be utilized as source of proteins i.e. *Atylosia scarbaeoides* 17.3%; *Erythrina indica* 21.5%; *Neonotonia wightii* 15.1%; *Rhynchosia filipes* 16.9%; *Tamarindus indica* 14%. These protein sources are comparable with that of edible legumes regarding their protein contents (*Cajanus cajan* 19.4%; *Cicer arietinum*, 20.7%; *Vigna trilobata* 20.2%, and *Vigna unguiculata* 15.9%) (Nwokolo, 1987; Arinathan *et al.*, 2003). In another study, four accessions of velvet bean were explored by Vadivel and Janardhanan (2000) and found to be rich source of protein and essential fatty acids. Recently, proximate composition of eight *Mucuna* accessions was also determined by Sridhar and Bhat (2007); protein content ranged between 24 and 31.44%, all varieties were good in mineral and crude fiber contents.

Some of the studies have been carried out to exploit un-conventional seeds and kernels for human consumption. Detoxified apricot kernels are nutritionally balanced rendering it safe for human consumption (El-Adawy *et al.,* 1994). Defatted tomato seed cake was found to contain 40% protein and 5.1% lysine content (Rao, 1991). Later on, two accessions of mesta (*Hibiscus sabdariffa*) seeds were also analyzed (Rao, 1996) for chemical composition. Protein content of the accessions ranged from 18.8-22.3% whilst dietary fiber 39.50-42.60%. The lysine

and tryptophan contents were high whereas sulphur containing amino acids were limiting. Protein efficiency ratio (PER) and net protein utilization (NPU) after cooking of mesta seed diets were significantly improved than corresponding diets. *Galactia longifolia Benth* (Fabaceae) seeds were characterized for chemical composition by Thangadurai *et al.* (2001). Crude protein, crude fat, ash and NFE observed to be 25.56%, 6.18%, 5.12% and 56.15%,, respectively. The essential amino acids i.e. tryptophan, leucine+isoleucine, arginine and threonine were found to be higher in concentration.

Leaves of some plants hold potential for human consumption with well-balanced amino acid profiles. de Mucciarelli *et al.* (1985) evaluated nutritional quality of leaf proteins from *Atriplex numularia* and reported lysine and methionine contents of 8.50 g/16 g N and 3.00 g/16 g N,, respectively. Problems of their lower net protein utilization (NPU) can be resolved with papain treatment. Later on de Mucciarelli *et al.* (1988) characterized leaves and protein concentrates from *Atriplex lampa* and evaluated its capacity as a complement to wheat flour. The protein concentrate of *Atriplex lampa* contained protein concentration of 59.37% with a chemical score of 85.70. The *Atriplex suberecta* leaves also contain appreciable protein quantity especially lysine thus predicting a good complement for cereals (Cid *et al.,* 1991). Vadivel and Janardhanan (2002) found *Cassia obtusifolia* L. as a good source of protein (18.56-22.93%) containing high levels of essential amino acids with *in-vitro* protein digestibility of 74.66 to 81.44%.

The name, Single Cell Protein (SCP), was given to coin the protein from biomass, produced from different microbial sources such as yeasts, fungi, bacteria and micro-algae. Comprehensive investigations and nutritional evaluation have proved that these proteins are well balanced and comparable to usual proteins from vegetable sources. High production costs and technical difficulties to incorporate the algal material into edible food preparations have

limited their use rendering SCP still in infancy period. Up till now the major part of micro-algal preparations is being sold as health food, cosmetics or as feed (Becker, 2007).

2.2. Maize Germ Characterization

The germ is separated from maize kernel in dry and wet-milling processes; used for the extraction of oil. Hydrocyclones have been employed at pilot-plant scale (Rubens, 1990) but the technique is difficult to apply on laboratory scale maize germ separation. On laboratory scale, maize is usually milled by using 1 kg conventional wet-milling procedure (Eckhoff *et al.,* 1993), though the method has been modified or improved by other researchers (Singh and Eckhoff, 1996; Dowd, 2003).

The laboratory milling process is an acceptable method for preparing germ for evaluation of compositional properties (Johnston *et al.,* 2005). The germ is separated from the first-grind slurry by a flotation or skimming technique, based on difference in density (Eckhoff *et al.,* 1993; Singh *et al.,* 1997; Dowd, 2003). Germ recovery has been difficult to reproduce in laboratory protocols. In industry, the separation of germ is done with germ-clones separating particles by density (Dowd, 2003). A sieving step is often performed to recover germ and fiber together and after the sample is dried, coarse fiber is separated by aspiration (Eckhoff *et al.,* 1996; Singh *et al.,* 1997). Maize germ yield with wet-milling has been reported from 4-8% (Dowd, 2003; Parris *et al.,* 2006) depending upon the variety or hybrid, germ %age, method of separation and process conditions (Singh and Eckhoff, 1996).

Oil and protein contents are the two most valuable components of maize germ (Johnston *et al.,* 2005) as the value of germ is directly related to the amount of these compounds (Ash and Dohlman, 2004; RFN, 2004). Orthoefer *et al.,* (2003) stated that the germ obtained from wet-milling process contains 45% to 50% of

oil. Johnston *et al.* (2005) while comparing the composition of germ derived from different milling processes reported that the protein content ranged from 13.09-22.17% and oil content from 23-40.89%. They further stated that the protein content from quick germ milling process was 21.36%. Montgomery *et al.* (2005) also examined dried full-fat maize germ obtained through corn wet-milling and stated that the germ contained 45% lipid and 12% crude protein on dry weight basis. Kulakova *et al.* (1982) also studied three hybrids of maize and reported that the fat content of wet milled maize ranged from 30.7-33%, while protein was 20-21.1%. It has been reported that the maize germ contains almost 85% of the total kernel oil and clean dried germ contains 45-50% oil (CRA, 2006). Previously, Ruan (2004) also reported similar results for fat (22-25.4%) and protein (14-17%) on wet weight basis.

In a report, Ruan (2004) stated that lightly toasted maize germs composed of 6.5% ash and 46.5% carbohydrates. Johnston *et al. (*2005), while evaluating the maize germs obtained by various milling techniques, mentioned that the ash contents ranged 1.43-3.22% in all the maize germ samples from different milling procedures. Kulakova *et al. (*1982) also reported similar findings for crude fiber content in maize germ. The variation in proximate composition is attributed to the nature of the source of germ or milling process (Johnston *et al.,* 2005).

Inglett and Blessin (1979) determined mineral content in defatted maize germ flour and reported 2.74%, 2.36%, 1.02%, 0.015 % and 0.020 % of phosphorus, potassium, magnesium calcium and iron, respectively. In another study similar results for all these minerals were reported by Garcia *et al. (*1972), while studying the mineral composition of different dry-milled maize and wheat germ samples. The high concentration of potassium in maize germs has the significance as an average human diet is deficient in this mineral (Cuthbertson, 1989).

Edible oils from plant sources are vital, serving as key components of many foods and impart characteristic flavors and textures to finished food products (Rudan-Tasic and Klofutar, 1999). Maize germ oil contains 14% saturated fatty acids, 30% monounsaturated fatty acids and 56% polyunsaturated fatty acids (EFSA, 2005). The information published regarding fatty acid profile of refined corn oil as linoleic acid 54-60%, linolenic 1%, palmitic acid 11-13 % stearic acid 2-3% and oleic acid 25-31% (CRA, 2006). Previously, Rudan-Tasic and Klofutar (1999) found that refined maize germ oil contain 10-15% saturated, 20-35% monounsaturated and 50-60 % of polyunsaturated fatty acids; after that Lemcke-Norojarvi *et al. (*2001) also reported the similar proportions and concentration of fatty acids for corn oil. In another study, Leibovitz and Ruckenstein (1983) evaluated maize germ oil obtained from South Africa and USA for the fatty acid profile. They reported that the triglyceride structure of maize germ oil contained no triglyceride that consisted of completely saturated fatty acids and only small quantity of disaturated triglycerides was found. Triglyceride structure of maize germ oil can be represented as: triglycerides with all saturated fatty acids (S3) = 0%, triglycerides with two-saturated and one-unsaturated (S2U) = 2.2%, triglycerides with one-saturated and two-unsaturated (SU2) = 40.3%, triglycerides with all unsaturated fatty acids (U3) = 57.5%.

Maize germ oil can play a vital role in the diet being a rich source of essential fatty acids (polyunsaturated fatty acids) especially linoleic acid, an essential fatty acid, which helps to regulate blood cholesterol level and elevated blood pressure (Hauman, 1985; Dupont *et al.,* 1990). Later, Hegsted *et al. (*1993) reported that saturated fats raise, polyunsaturated fats lower and monounsaturated fats have no effect on cholesterol blood concentration. Considering the versatile importance of maize germ oil as it is readily available and high in polyunsaturated fatty acids, is used as standard to asses the cholesterol lowering capacities of other oils. Findings of the research conducted

by Jacono and Dougherty (1993) indicated that corn oil diet due to its rich polyunsaturated fatty acids profile reduced the blood pressure about 12% in men and 5% in women, having elevated blood pressure. According to the recommendation of National Research Council and the Food and Agriculture Organization/World Health Organization, about 2-4% of energy should come from essential fatty acids with an additional 3% for pregnant and breast feeding women. Consequently, a tablespoon of maize germ oil can satisfy the requirements for essential fatty acids for healthy child or adult (CRA, 2006).

The knowledge of chemical and physical properties of edible oils is important having role in processing functionality, storage stability and nutritional behavior. Edible oils in general exhibit considerable deviations in their composition, thus it is difficult to define single values for chemical and physical properties of edible oils (Rudan-Tasic and Klofutar, 1999). These properties depend on composition and origin of oil. Iodine value 110.116±0.002 (mmol/kg), acid value 0.1137±0.0001 (g/100 g), saponification value 191.87±0.02 (mg KOH /g), and referactive index 1.4730 has been reported for maize germ oil (Rudan-Tasic and Klofutar, 1999). According to the list of primary specifications for corn oil laid down by Food Chemicals Codex of the National Academy of Sciences/National Research Council (FCC, 2003): color (AOCS-Wesson); not be more than 5.0 red, free fatty acids (as oleic acid); not more than 0.1% , iodine value; 120-130, peroxide value; not more than 10 meq/kg, unsaponifiable matter; not more than 1.5% , and water; not more than 0.1%. Corn Refiners Association has also presented chemical and physical data for the oil (CRA, 2006) which includes iodine value 122-131, saponification value 189-195, refractive index 1.470-1.474, specific gravity 0.922-0.928, weight per gallon 3.49 Kilogram, melting point -11.11 to -8.33 °C, smoke point 229 to 239°C, flash point 332 to 338 °C, fire point 365 to 371 °C and cloud point -13.89 to -11.11 °C. Leibovits and Ruckenstein (1983) also reported that the iodine value of crude maize germ oil processed in

South African factories is in the range of 110-125. They further showed that free fatty acids and POV values of refined maize oil ranged from 0.02-0.03% and 0.0-0.1 meq/Kg, respectively.

Vegetable oils contain several minor compounds including antioxidant or other showing beneficial physiological properties (Deckere and Korver, 1996). Vitamin E is a group of tocopherols and tocotrienols each with the 4 analogs; ∞, β, γ, and δ. Vitamin E, being a lipophilic in nature, is known to be the key chain-breaking antioxidant thus preventing the promulgation of oxidative stress, in biological membranes (Ricciarelli *et al.,* 2001). Maize germ oil is amongst the rich sources of tocopherol, which is preferably retained in the plasma as compared to other family members of vitamin E. Tocopherols and tocotrienols are also helpful in cholesterol lowering or preventing cardiovascular maladies (Lloyd *et. al.,* 2000; Birringer *et al.*, 2002; Sen *et al.*, 2006).

Alpha and gamma tocopherols are the main forms of vitamin E present in the diet; vegetable oils are their richest sources (Eitenmiller, 1997; Parker, 1989). Lemcke-Norojarvi *et al. (*2001) evaluated maize germ oil and its margarine for α- and γ-tocopherol and stated that corn oil is a rich source of these vitamins especially γ-tocopherol. The concentration of α- and γ-tocopherol was 21.3 and 94.1 mg/100 g in oil and 13.2 and 45.4 mg/100 g in margarine, respectively. They also stated that the serum γ-tocopherol concentration in healthy subjects can be improved through intervention of corn oil in diet which in turn might help to prevent the development of atherosclerosis. MGO was compared with MGO high in unsaponifiable matter for chemical composition (Anonymous 2005). Refined MGO contained 0.1 g/100g of total tocopherols against 2.0 g/100g in high unsaponifiable MGO, thus one tablespoon of both oils providing 13 mg and 40 mg, respectively, of total tocopherols (20% α- and 80% γ-tocopherol). Initially Speek *et al. (*1985) analyzed E vitamins in hot and cold pressed seed oils and reported that all the extracted MGO samples contained 1032-1279 μg/g total E

vitamins being rich source of γ-tocopherol (630-890 μg/g) and α-tocopherol (247- 371 μg/g) while low in of β-tocopherol (10-17 μg/g), δ-tocopherol (33-59 μg/g) and α-tocotrienol (13-29 μg/g).

2.3. Protein Quality

The shortage of energy, protein and essential amino acids are amongst major problems of human nutrition in developing and under developed countries including Pakistan. The nutritional quality of food can be improved by augmenting protein content and limiting amino acids especially lysine (Pogna *et al.,* 1994). In food products manufacturing, it is important to balance the quality and quantity of protein keeping in view the nutritional status of populations (Hung and Zayas, 1991). The total protein content and the total essential amino acids are important factors from nutritional view point; essential amino acids should be supplied in adequate amounts in the daily diet (Anjum *et al.*, 2005). Cereal proteins are deficient in few essential amino acids like lysine and tryptophan but these deficiencies are mainly related to endosperm portion of the kernel (Myer *et al.*, 1996). Wheat flour substitution with legume could contribute the increasing demands for protein and energy rich food preparations (Iqbal *et al.*, 2006). However, bioavailability of proteins and energy from raw legumes is poor and require processing prior to consumption (Melcion and Van der Poel, 1993;). Although, they have high protein contents but generally contain low or moderate levels of potentially harmful antinutritional factors (Taiwo, 1998).

The amino acid profile of maize germs is nutritionally valuable being balanced in all essential amino acids, which is in line with FAO/WHO (1973) standards with the exception of isoleucine. Its protein contains 47 % albumin and amino acids particularly lysine and tryptophan are comparable with that of casein (Gupta and Eggum, 1998). It has already been observed by Nielsen *et al. (*1979) that essential amino acids profile of maize germ protein except isoleucine is comparable with the amino acid requirements of preschool children. Inglett

and Blessin (1979) evaluated composition of germ flour proteins from different mills and varieties; reported that there was little difference in amino acid pattern among all maize germs and the lysine accounted for almost 5% of amino acids recovered. Tsen *et al. (*1974) also worked previously on DMG protein quality and reported that the protein in the defatted maize germ contains 6% lysine with good balance of other essential amino acids. Maize germ oil cake has a wide potential of its use in food products as albumin and globulin fractions represent the major portion of the germ proteins (Lawton and Wilson, 2003). In a coherent study Kulakova *et al. (*1982; 1983) compared maize germ protein quality with standard proteins and mentioned that the germ protein was not inferior to egg albumin and casein. Blessin *et al. (*1973) also compared the amino acid profile of defatted maize germ flour with egg and concluded that maize germ flour contained 38.3% of total essential amino acids against 51.3% in egg.

Protein content is the most important factor when evaluating the value of the defatted germ, because the extracted germ is mostly used in animal feed. (Renewable Fuel News, 2004). Maize germ protein has a nutritional quality with balanced amino acid composition (Barbieri and Casiraghi, 1983). Kulakova *et al. (*1982) evaluated maize germ proteins; it contained biologically valuable proteins balanced in all essential amino acids exhibiting conformity with FAO/WHO standards. Kulakova *et al. (*1983) also studied the composition of germ protein and mentioned that the total germ protein and its basic alkali-soluble fraction were comparable to egg albumin and casein.

The albumin and globulin fractions constitute the major part of the germ protein (Lawton and Wilson, 2003). Peri *et al. (*1983) also evaluated proteins in maize germ and reported that it contains mainly albumin and globulin with good nutritional quality having balanced essential amino acid profile (Satterlee *et al.,* 1979). Maize germ also contains better quality protein having 47 % albumin. The amino acid contents like lysine and tryptophan and biological value (BV) are

higher whereas leucine and isoleucine ratio is lower compared to whole maize grain, and are comparable with the requirement of preschool children and casein diet. It has potential to be used in food formulation for human consumption. Processing of defatted maize germ oil cake to produce food grade meal did not affect its amino acid contents (Gupta and Eggum, 1998). Lucisano *et al. (*1984) prepared a maize germ protein meal by drying maize germ at low temperatures and removing oil through solvent extraction. Defatted maize germ protein thus produced could be used as supplement in array of foods. Clark (1986) also tested the maize germ oil cake and reported that it contained high quantity of balanced protein.

Growing rats require balanced dietary protein to meet their protein requirements; growth and maintenance (White *et al.,* 2000). Dietary protein quality can be evaluated by following the protocols described by Miller and Bender, (1955). They defined that protein efficiency ratio (PER), feed efficiency ratio (FER), biological value (BV), total digestibility (TD) and net protein utilization (NPU) as different measure to carry biological assay for proteins. Same methodologies were followed by Escudero *et al. (*1999) to assess the chemical and nutritional composition of the aerial parts of *Amaranthus muricatus;* reported protein content of amaranth flour was 15.74 g/100 g while net protein utilization (NPU), true digestibility (TD) and biological value (BV) measurements presented a nitrogen gain of 74% compared to casein. Milk protein with special reference to casein is well known for its good nutritive functional properties in food formulations and is usually used as reference to evaluate quality of other proteins (Friedman, 1996). Arshad *et al.,* (2007) evaluated the defatted wheat germ supplemented flour for its biological assay compared to casein and reported that diets containing 15% defatted wheat germ meal and wheat blended flour are comparable with that of casein diets.

Protein quality of maize germ is comparable to that of casein. However, its utilization in product development needs to be checked through safety evaluation to meet WTO requirements.

2.4. Efficacy and Safety Evaluation

Efficacy studies are carried out with multiple objectives like assessing the safety of products or specific ingredient, determining its specific quality such as protein and oil quality and in many instances health promoting potential of novel foods. Using animal models to asses the safety of test components is usually focused on treatment-related values representing changes relevant to pharmacological or toxicological effects (Cellini *et al.*, 2004).

Dietary changes may lead to series of reactions which can cause disruption of normal physiological activity bringing changes in biochemical constituents of the body fluid of test animals (Schilter *et al.*, 2003). Blood biochemical screening is a useful indicator for nutritional research. It is supportive for more accurate and reliable diagnosis of various physiological disorders and can be comprehensively interpreted by correlating with other nutritional parameters (Singh *et al.,* 2002). Clinical pathological evaluation is being used as one of the safety assessment tools when some novel food sources are exploited for their appraisal as safe human food ingredient (Malley *et al.,* 2007).

Basal metabolism and body weight of test animals do not always exhibit linear relationship neither between different species of test animals nor within the same specie (Heusner, 1982). For prediction of basal metabolism (BM) and to assess the safety aspects; weight of various organs and tissues together with their specific metabolic activity seems to be promising (Even *et al.,* 2001). Liver and kidney function test along with serum contents were used to evaluate safety of novel foods (Farag *et al.,* 2006). Serum protein and albumin are often used for the

evaluation of the protein (Jung *et al.,* 2003). Physiological responses of growing rats can be useful tool to indicate food safety and quality of test diet (Olivera *et al.,* 2003).

Chengelis *et al.* (2008) and Morita *et al.* (2008) conducted safety evaluation of novel foods and reported that serum protein, lipids profile, serum enzymes like alanine aminotransferase (ALT) & aspartate aminotransferase (AST), blood hematology and electrolytes balance were found to with in safe limits. They reported total protein, albumin, and globulin were in the range of 6.3-7.0, 4.1-4.3, and 2.1-2.8 g/dL, respectively, whereas, urea and creatinine were in the range of 13.2-16.8 and 0.3-0.4 mg/dL. Enzymes like alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) play important role in normal physiology of the body and usually lie in the range of 109-212, 33-41, and 88-112 U/L, respectively. Petterino and Argentino-Storino (2006) worked out the reference data for Sprague-Dawley rats for both male and female rats. They presented data related to serum biochemical profile with means and their standard deviations at four and thirteen week interval. Data of male rats revealed significant differences between four and thirteen week values of blood cell parameters including red blood cells (RBC), white blood cells (WBC), ALT, ALP, serum proteins, creatinine and triglycerides and in addition, female rats group represented a variant data regarding RBC number and mean corpuscular haemoglobin (MCH), mean red blood cell volume (MCV) and sodium.

Serum electrolytes like calcium, chloride, phosphorus, potassium and sodium are safe in limits of 100-1500 mg/dL, 90-140mg/dL, 7.5-12.0 mg/dL, 5.0-7.50 meq/L and 90-160 meq/L (Petterino and Argentino-Storino, 2006; Morita *et al.,* 2008). Balanced lipid profile is essential for determining proper health of individual and irregularities embark consequences leading to cardiovascular disparities. Nakagawa *et al.* (2008) evaluated novel food components through rats and reported the baseline values for total cholesterol, triglycerides, glucose

and plasma lipids were 71.2 mg/dL, 46.3 mg/dL, 168 mg/dL and 127 mg/dL in male rats. Whilst in female rats, values were 89.0 mg/dL, 105.6 mg/dL, 146 mg/dL and 164 mg/dL for total cholesterol, triglycerides, glucose and plasma lipids, respectively. The data presented is furnished by a claim that it can be useful for the researchers in future, as reference, conducting experimental trials on Sprague-Dawley rats as test animals and for formulations of rules for interpretation by international regulating authorities (Petterino and Argentino-Storino, 2006).

Malley *et al.* (2007) evaluated maize grains including 59122 maize grain, non-transgenic near-isogenic maize grain (091) and a commercially available non-transgenic reference maize grain (33R77) and observed that diets formulated with 35% of either of maize grains fed to Sprague-Dawley rats had no adverse effects with respect to body weight/gain, food consumption/efficiency, clinical signs of toxicity and clinical pathology. He *et al.* (2008) evaluated two types of processed flours from 59122 maize grain or it's near isogenic control line (091) at two concentrations through Sprague-Dawley in a 90 days trial in accordance with Chinese toxicology guidelines. They observed non-significant differences in body weight and feed utilization. Process of safety assessment of transgenic foods includes both in vitro and in vivo studies (Harrison *et al.,* 1996) plus evaluation of protein stability for allergenicity assessment (Astwood *et al.,* 1996) and acute oral toxicity studies (Sjoblad *et al.,* 1992). Recently, Subchoronic rodent feeding study was used as a tool to evaluate dietetic potential of transgenic grain varieties (MacKenzie *et al.,* 2007). Morita *et al.* (1997) explored capability of rice, potato and soybean protein, having low methionine content, to reduce cholesterol. They found that diets with rice, potato and soybean protein reduced serum total cholesterol as compared to casein.

2.5. Composite Flour

Cereal grains like wheat, corn, rice, barley, sorghum, etc. provide 68% of the total world food supplies. Wheat is mainly used as a dietary staple, averaging two-thirds of total consumption (Anjum *et al.,* 2005). Owing to shortage of wheat, several developing countries have devised programs to assess the feasibility of alternate sources for substituting or blending with wheat flour (Abdel-Kader, 2000).

Composite flour technology refers to the process of mixing various flours to make use of local raw material to produce high quality food products in an economical way. Formulation of composite flour is vital for development of value-added products with optimal functionality (Rehman *et al.,* 2007). A variety of wheat flour substitutes have been tried in bakery formulations with varying success; for example, soy or defatted soy flour (Junqueira *et al.,* 2008), defatted wheat germ (Arshad *et al.,* 2007), flaxseed (Koca and Anil, 2007), sunflower seed (Skrbic and Filipcev, 2008), and lupin flour (Hall and Johnson, 2004).

Composite flours prepared by blending wheat and legumes can improve the status of protein and limiting amino acids. In a research trial, layer cakes were successfully prepared from chickpea-wheat (white and whole) composite flour blend (Gomez *et al.,* 2008). In another research trial, sorghum and wheat flour composite blends upto 10% and 20% sorghum resulted in acceptable breads and biscuits (Elkhalifa and El-Tinay, 2002). Composite flours of small red, black, pinto and navy bean flours with wheat flour were successfully used by Anton *et al. (*2008) for tortilla preparation up to 25% of substitution levels. Salem *et al. (*1999) studied the effect of partial replacement of corn tortilla with soybean, chickpea and lupine flours. They reported improvement in color and taste of tortilla with chickpea augmentation. They also found that fortification of tortilla flour with 5% lupine, 15% soybean and 20% chickpea flours improved the sensory and physical properties of the baked tortilla. Blends of soybean flour and cassava flour can be used to prepare biscuits.

The law of complementarity's can be employed to improve the nutritional status of the bakery products by partial replacement of wheat with lysine rich flours e.g. defatted maize germ meal. The wheat-DMG composite flour blends in food formulations could potentially supply most of the nutrients needed in human diets especially essential amino acids, minerals and dietary fiber (Akubor and Ukwuru, 2003).

2.6. Functional and Rheological Properties

Knowledge of functional and rheological properties of un-conventional and/or novel food ingredients is imperative to incorporate successfully in existing food formulations. Blending non wheat flours with wheat might result in technological difficulties and impairment of baking quality. For the purpose, determining potential use of composite flour blends in food formulations, informations regarding functional and rheological properties of blends are essential (Akubor and Ukwuru, 2003).

The excellent functional properties of corn germ flour proteins proved its utilization in the food industry. Lin and Zayas (1987a; 1987b) studied functional properties of two maize germ protein defatted with supercritical fluid extraction or hexane. Incubation temperature affected the functionality while protein solubility was influenced by temperature and pH. Emulsifying capacity increased with pH and stable emulsion was obtained by combining 7% supercritical fluid extraction corn germ proteins with 40% maize oil. Fat binding decreased when heat treatment was applied whereas water retention increased with the increase in temperature up to 70°C. Later, Vani and Zayas (1995) also examined the impact of pH and temperature on water retention, protein solubility, and total solubility of defatted wheat germ protein flour (WGPF) and compared the protein solubility of WGPF with those of maize germ protein flour (MGPF), soy flour (SF), nonfat dried milk (NFDM), and egg white powder (EWP). Water retention, protein solubility and total solubility improved with pH

and maximum value for water retention was obtained at 70°C. Protein solubility of MGPF though equaled WGPF but lesser than that of NFDM, EWP and SF.

The functional properties of solubilized defatted wheat germ protein (DWGP) using alkalase were determined by Claver and Zhou (2004). The nitrogen solubility was 74% at pH 6 while emulsifying activity, capacity and stability noted to be 64, 62 and 57%, respectively. Water retention of DWGP using alkalase was 232% at pH 7 and 70 oC.

Wang and Zayas (1991) applied response surface methodology to study water retention and protein solubility of soy flour, soy concentrate, soy isolate and maize germ protein flour (MGPF). Water retention and Protein solubility improved with pH and incubation temperature but was not influenced with incubation time. Water retention of MGPF was higher than that of soy flour and soy isolate.

Functional properties are also affected with varying solvents. Hexane is commonly used for the extraction of oil from maize germ. However, L'hocine *et al. (*2006) indicated possible potential of ethanol and aqueous extraction as an alternative defatting technique resulting decreased emulsifying activity, whilst improved emulsion stability and foaming properties of the aqueous extracted isolates. Methanol defatting also significantly decreased the fat-holding capacity; therefore, the processing conditions should be modified to produce protein isolates with desired functional properties.

Defatted maize germ protein addition resulted in increased protein decreased batter viscosity, fat, moisture, firmness & shear force, lighter color and meaty aroma in comminuted meat products (Zayas and Lin, 1988). In an experimental study, beef patties were fortified at levels of 10, 20, and 30 % of the weight with slurry of defatted maize germ protein and heated by microwave to study their impact on finished product (Brown and Zayas, 1990). Extended

patties resulted in lower heating loss and higher yield. Incorporation of DMGP in beef patties resulted in decreased protein, fat, and cohesiveness, water and fat retention, decreased with no affect on hardness. Maize germ protein was thus recommended as an extender in coarsely ground meats. In another investigation, dry- and wet-milled maize germ flour at levels of 2.5 and 5% were added to ground pork for patties development (Reitmeier and Prusa, 1991). Addition of maize flour improved the yield by decreasing cooking losses up to 5% and 7.5%,, respectively as compared to control patties. Instron compression values decreased with the amount of dry-milled germ flour while the yellow color ("b") increased and lightness ("L") decreased with augmentation of wet-milled maize germ flour (Zayas and Lin, 1989). The rheological properties were altered with type of flours and their combination ratios. The maize flour replacement significantly effect viscosity and viscoelastic properties of wheat and rice based batter systems (Xue and Ngadi, 2006).

Bombara *et al.* (1997) while, evaluating wheat flour for functional properties reported that wheat flour has optimum functionality for bakery products. The soluble protein content was found to be 65 g/Kg whereas the water absorption capacity and oil absorption capacity were noted as 1.04 mL/g and 0.98 mL/g, respectively. The wheat flour also possessed good emulsifying capacity with the value of 55 mL/g. As for the rheology is concerned the viscosity of wheat flour averaged to 136 kPa.s.

Texture is one of the key factors among various food quality attributes, which consumers use to judge the quality and freshness of foods. Texture is important in determining the eating quality of foods and has a strong influence on food intake and nutrition. Perceived texture is closely related to the structure and composition of the food, and both microscopic and macroscopic levels of structure can influence this parameter (Alvarez *et al.,* 2005). Instrumental methods to measure mouthfeel are based on the science of rheology, which

measures the deformation and flow of materials. Texture and rheology are key factors for foods to be palatable and for the movement of foods through the digestive tract (Bourne, 2002). The flow and deformation behavior of doughs is recognized to be central to the successful manufacturing of bakery products. Thus, rheological measurements are used at numerous points in the development of new products and processes during the optimization stage or manufacturing to ensure consistent quality (Menjivar, 1990).

Universal Testing Machine was employed by Gujral and Pathak (2002) to determine the tensile properties i.e. extensibility, peak force to rupture, modulus of deformation and energy to rupture the chapaties prepared from composite flours to describe texture. The whole wheat flour was partially replaced with flours from rice, corn, barley, millets and black gram. Upon storage of chapaties up to 24 hrs, the extensibility and energy to rupture lowered whereas modulus of deformation and peak load to rupture increased. It was also observed that chapaties prepared from composite flours especially barley resulted in higher extensibility.

Dough rheological properties mainly depend on its protein quantity and quality of flour. In turn, properties of dough play a key role in quality of baked products. The rheological characteristics of flours are also a source for understanding the dough handling behavior in bakery product preparation. Therefore, many types of equipments have been invented to get objective data about rheological behavior of the dough during processing (Bloksma and Bushuk, 1988; Spies, 1990; Lindahal, 1990).

Defatted soya flour was blended with wheat flour in various proportions to study dough characteristics by Senthil *et al. (*2002). Soya flour resulted increase in water absorption and decrease in dough stability evaluated from Farinograms. Matz (1972) reported increased water absorption with the increase in the level of protein content. There was an increase in water

absorption with increase in damaged starch. Nurul Islam and Johansen (1987) determined the water absorption of wheat flour from Indo-Pakistani varieties from 60-76%. Simon (1987) stated that the flour with increased water absorption would result in favorable characteristics of final products, because the products might remain soft for a longer period with improvement in texture and reduced cost.

Protein contents are important in development of continuous phase of protein and decreased protein content <12%, hinder the process thus resulting in increased dough development time during mixing (Finney *et al.*, 1987). In a research study, Corbellini *et al.* (1999) reported dough development time for hexaploid wheat ranged from <90 to 240 s. Reduced farinographic stability and higher degree of softening may contribute towards the shorter dough development time (Borghi *et al.*, 1996). However, whole wheat flour had higher dough development time due to the presence of higher moisture content and bran particles thus interfering with the quicker development of gluten (Haridas Rao *et al.*, 1983).

The rheological behavior of the wheat-guar flour blend was evaluated by Venkateswara *et al.* (1985). Gradual increase in water absorption (49 to 52%) was observed with increasing level of guar gum in flour blends. Tremendous increase in viscosity (cps) was observed from 300 to 2000, 25000 and 90000 units, when the guar gum level was increased from 0.5 to 1, 2 and 3 %, respectively. The viscosity changes have a considerable impact on the dough consistency and handling properties.

2.7. Product Development

To cope protein deficiency malnutrition, cereal based products play a pivotal role as a vehicle for value-addition being consumed by masses. Various food products have been prepared with varying degree of success from defatted

maize germ or from its components. Defatted maize germ protein (3%) was successfully incorporated in comminuted meat products with the improvement of quality characteristics and increased yield (Zayas and Lin, 1988). Defatted maize germ protein was also evaluated as functional or substitute ingredient in frankfurters by Zayas and Lin (1989a; 1989b). Maize germ protein addition in frankfurters as a powder, slurry or emulsion stabilizer resulted in an increase in batter viscosity and shear force values of frankfurters. Sensorial attributes were not affected significantly at all levels. In another study, Zayas and Lin (1989c) observed the impact of pretreatment of maize germ protein on the quality parameters of frankfurters during storage. The results showed that the protein and the methods of pretreatment had no significant effect on meaty aroma, flavor and amino acid pattern of frankfurters. It was concluded that defatted maize germ having high quantity of protein content combined with amino acid profile and low cost showed a potential to be used as an extender, fortificant and binder in frankfurters and other comminuted meat products.

Macaroni, produced from commercial durum semolina, was blended with 10, 20, and 30% DMG flour. Addition of DMG flour resulted in longer dough mixing time, higher water absorption, improved protein content and amino acids profile. % increase in weight of macaroni decreased with the levels of wheat substitution with that of DMG flour. Fortification had no effect on flavor and texture of supplemented product as compared to conventional macaroni evaluated by taste panels but it significantly altered physical parameters of finished product texture resulting in increased firmness (Lucisano *et al.*, 1984)

Maize germ protein flour (MGPF), nonfat dry milk (NFDM), whey protein concentrate (WPC), and sodium caseinate (SC) were used in comminuted meat products by Hung and Zayas (1992) for quality improvement. Fortification resulted in rather improved water holding capacity and decreased the cooking losses. Shear force and firmness of the products was greater than control except

for WPC fortification products. MGPF resulted in highest hue angle while no differences were found between control and milk proteins fortified samples, excluding samples with WPC. In another study conducted by Gnanasambandam and Zayas (1996) acceptibilty of frankfurters extended with wheat germ protein flour (WGPF) by 103 consumer panelists were evaluated through sensory evaluation. The consumers did not distinguish any differences in appearance, flavor, texture, and overall acceptance but the perception of intensities of parameters differed regarding age, frequency of consumption, and sex. Incorporation of defatted maize germ flour has also been studied in different bakery products to evaluate the possibility of this nutritious product for value-addition of the finished products. Blessin *et al. (*1973) reported that addition of DMG flour in a standard cookie formula favorably improved the amino acid and mineral composition of the final product. Similar trend was observed in corn muffins.

Anton *et al. (*2008) investigated composite flours containing 15, 25, and 35% of small red, black, pinto, and navy bean flours with wheat flour for tortilla development. Their findings revealed that tortillas were nutritionally superior with respect to crude protein, total phenols and antioxidant activity and were of with acceptable texture up to 25% of substitution.

For preparation of high quality products breads were prepared from wheat flour supplemented with 5, 10 and 15 % chickpea flour. The legume, augmentation improved crude fiber protein and lysine content at all blending levels. Biological quality of proteins (PER) increased from 0.90 to 1.34 with little deviation in bread protein digestibility (Estevez *et al.,* 1987). Figuerola *et al.* (1987) also studied the practicability of adding chickpea flour with wheat flour for the preparation of leavened bread to improve protein in terms of quantity and quality. Addition of chickpea flour augmented protein, fiber and ash contents in the blends, without sacrificing the quality, up to 15% level of substitution. The

breads prepared from the blends were of good quality without the use of maturing agents.

Breads and biscuits development using sorghum and wheat flour blends and their findings supported 30% substitution for sorghum (Elkhalifa and El-Tinay, 2002). Sakyi-Dawson *et al.* (2006) used composite flours having cassava (45%-70%) and cowpea flour (30%-55%) to bake biscuits which were evaluated for spread factor, hardness, fracturability and color. Addition of cowpea increased degree of browning whereas hardness reduced (30.00N for 30% cowpea-70% cassava to 16.39N for 55% cowpea-45% cassava). The composite flour biscuits were preferred due to decreased hardness as against 100% cassava flour (42.63N). The biscuits prepared from 57.5-42.5% and 48.7-51.3% cassava-cowpea composite flours were most preferred ones regarding hardness, fracturability, and color values. Overall, the addition of cowpea flour in biscuit formulation enhanced the nutritional and sensory quality.

Rehman *et al.* (2007) partially substituted wheat flour with vetch flour to prepare composite flour doughnuts. They conducted sensorial and physical evaluations; doughnuts prepared from composite blends of 15 g/100 g were found to be acceptable. Studies were carried out by Festus *et al. (*1995) to improve the nutritional value of fermented cassava flour, by blending full-fat soya bean flour; resulting in augmentation of protein, total lipids, phosphorus, iron, ash content, and gross energy. Organoleptic qualities of the composite product did not differ from pure cassava flour alone at 10% level of substitution of cassava.

Practical implications of wheat bran blending to prepare value-added high fiber bakery product was studied by Anjum *et al. (*2006). For the purpose wheat bran was blended with wheat flour at 5, 10, 15 and 20% to prepare composite flour for the production of fiber enriched cakes. Crude protein, fiber and total ash of the flour blends increased while calorific value decreased with increasing the level of replacement. Wheat bran blending up to 20% replacement resulted in

acceptable cakes. Nutritional value of wheat based foods especially bakery products can be improved by adding legume flours, owing to their protein quality and fiber content. Physical parameters i.e. volume, symmetry, chroma, and crust and crumb "L" value reduced with increased chickpea flour level in cakes (Gomez *et al.,* 2008). Chickpea flour resulted in enhanced values for initial firmness while cohesiveness and resilience decreased showing hardening tendency. White flours resulted in sponge cakes with increased volume and symmetry.

A study was conducted by Shittu *et al. (*2007) to investigate the influence of baking temperature and time on physical properties of bread prepared from cassava-wheat composite flour blends at ratio of 10:90 (w/w). The optimum baking temperature and time ranged from 190 to 240 °C and 20 to 40 min,, respectively. There were significant ($p < 0.001$) variations in loaf volume, weight and specific volume ranged from 440-920 cm^3, 162-183 g and 3.31-5.32 cm^3/g,, respectively. The color parameters such as L* (lightness) and brownness index (BI) of the crust varied significantly ($p < 0.01$) from 31-72 and 68-123,, respectively. Fresh crumb moisture, density, porosity, softness and dried crumb hardness also varied with baking temperature and time (34-39%, 0.16-0.20 g/cm^3, 0.69-0.80, 13.00-18.05 mm and 0.90-2.05 kgf,, respectively).

Zhu *et al. (*2006) evaluated defatted wheat germ flour (DWGF) and defatted wheat germ protein isolate (DWGPI) as potential food ingredients. Amino acid profile of protein content of DWGF was comparable to the proposed by Food and Agriculture Organization/World Health Organization reference pattern. DWGPI was easily digested by pepsin in vitro, compared to soybean protein isolate, while DWGF was comparatively less digestible. SDS PAGE analyses showed that interpolypeptide S-S bonds were scanty in wheat germ proteins structure. Arshad *et al. (*2007) prepared cookies by replacing wheat flour with defatted wheat germ (DFWG) at 0–25%. It was observed that 15%

substitution of wheat flour with DFWG produced acceptable cookies similar to 100% white flour cookies.

Various defatted soya-wheat flour blends were utilized for product development through preparation of savoury and sweet snacks by Senthil *et al. (*2002). In general the protein content improved with soya concentration in products and overall variation in acceptability was non-significant among the samples. Color values expressed as "L", "a", "b" also were not markedly changed with soya flour. Hardness of fried snacks, measured as force required for 50% compression, increased with enhanced levels of soya fortification. Recently, Olaoye *et al. (*2006) substituted wheat flour with soy flour and plantain flour from 0-15% for bread production; substitution of wheat flour with 10% plantain flour resulted in bread similar to control in all the sensory aspects. Overall, plantain flour substituted bread was comparable, in sensory and nutritional qualities, with control bread.

Chapter-III

MATERIALS AND METHODS

3.1. Procurement of Raw Material

Maize kernels from five promising locally grown hybrids namely Poineer-32-F-10 (P-1), Poineer-32-B-33 (P-2), Monsanto-6142 (M-1), Monsanto-6525 (M-2) and Rafhan-2331 (R-1) were procured from Sanghera Agriculture Farms, Depalpur whereas commercial maize kernels were obtained from Rafhan Maize Products, Faisalabad.

3.2. Maize Germ Separation

Germs were separated from maize hybrids using quick germ milling process according to the method described by Singh and Eckhoff (1996). One Kg maize sample was soaked in water for 12 hrs followed by coarse grinding (…….) to liberate germs. The germs were then recovered by floatation and skimming process. The germs thus obtained were sun dried in open air for 10-12 hrs. Germ recovery (yield) was calculated as the amount of germ recovered per 100 g of maize kernels and expressed in percentage.

3.3. Analysis of Germ

Germ sample of each hybrid was coarsely ground with lab-scale grinder and packed in polyethylene bags. The samples were stored at 4 °C for further analyses.

3.3.1. Proximate Analysis

The germ samples were analyzed on dry weight basis for crude fat (Method no. 30-25), crude protein (Method no. 46-30), ash content (Method no. 08-01), crude fiber (Method no. 32-10) and nitrogen free extract (NFE) according to their respective procedures (AACC, 2000) as given below

3.3.1.1. Crude Protein

Nitrogen content in each germ sample was determined by using Kjeltech Apparatus (Model: D-40599, Behr Labor Technik GmbH, Germany). The protein percentage was calculated as %N × 6.25.

3.3.1.2. Crude Fat

Crude fat content was determined through Soxtec System (Model: HT2 1045 Extraction Unit, Hoganas, Sweden) with continuous refluxing of sample using n-hexane as solvent.

3.3.1.3. Crude Fiber

Crude fiber content of each germ sample was determined by digesting dry sample with 1.25% H_2SO_4, followed by 1.25% NaOH solutions in Labconco Fibertech (Labconco Corporation, Kansas, USA).

3.3.1.4. Ash Content

Ash content in dry sample was determined by incinerating 3 g sample in a Muffle Furnace (MF-1/02, PCSIR, Pakistan) at 550 °C.

3.3.1.5. Nitrogen Free Extract (NFE)

NFE was calculated according to the following expression:

NFE = 100 – (% Crude protein + % Crude fat + % Crude fiber + % Ash).

3.3.2. Minerals

Maize germs were examined for phosphorus (Method no. 965.17), potassium (Method no. 968.08), magnesium (Method no. 968.08), iron (Method no. 985.01) and calcium (Method no. 968.08) using Atomic Absorption Spectrophotometer (Model: AA240, Varian, Australia) after digesting the samples applying their respective methods given in AOAC (2006).

3.3.3. Amino Acid Profile

Essential and non-essential amino acids of maize germ samples were determined according to the modified method of Adeyeye and Afolabi (2004) by injecting a known volume (20µl) of the supernatant of the prepared samples using high Speed Amino Acid Analyzer (Model: L-8500 A, Hitachi, Japan), while tryptophan was determined according to the method described by Delhaye and Landry (1992). Sample was hydrolyzed in the presence of $Ba(OH)_2$, isolated through gel filtration and analyzed with calorimetry.

3.3.4. Fatty Acid Profile

Oil was extracted from maize germ samples for the analysis of fatty acid composition by following IUPAC protocol (IUPAC, 1987). The fatty acids were

converted to their respective methyl esters and extracted with HPLC grade hexane. The fatty acid methyl esters (FAMEs) were analyzed by Gas Liquid Chromatograph (Model: 14-A, Shimadzu, Japan) using methyl lignose coated (film thickness: 0.25 μm) polar capillary column (SP-2330, 30 m x 0.32 mm) and flame ionization detector (FID). Nitrogen gas was used as a carrier. FAMEs were injected and the fatty acids were quantified by comparing the relative retention time of test samples with standards using a software program (CSW32) and data processor (C-R4A CHROMATOPAC).

3.3.5. Tocopherol Contents

Tocopherol content of the germ samples were analyzed through HPLC (Model: Perkin Elmer Series 200, USA) by using protocol described by (Katsanidis and Addis, 1999). The oil was extracted from samples and dissolved in hexane. A normal phase HPLC column (5 lm particle size, 4.6 9 250 mm) was employed for separation of tocopherol isomers. The HPLC system consisted of a UV detector, which was set at 290 nm excitation and 400 nm emission wavelengths. The column temperature was 35 ^{0}C. An external calibration from both tocopherol standards (α- and γ-tocopherol) was employed to determine the concentration of tocopherols in the samples.

3.4. Maize Germ Selection

Based on the germ yield and other compositional analyses one best germ from various hybrids was selected for further assay and product development.

3.5. Oil Extraction

Extraction of maize germ oil (MGO) was done through solvent extraction (*n*-hexane) technique with Soxtec System through continuous refluxing of solvent until the solvent in the extraction tube was clear. Solvent from oil was removed by distillation process followed by mild heating to remove the traces of solvents. MGO was then kept in desiccator over anhydrous calcium chloride for twenty four hrs to ensure the complete removal of moisture.

3.6. Refining

MGO was refined to remove unsaponifiable matter, pigments and to some extent the partial esters. The refining included the processes of dewaxing, degumming, neutralization, bleaching and deodorization as described below:

3.6.1. Dewaxing

Dewaxing of crude MGO was performed by cooling the oil at 15 °C and allowing the waxes to crystallize and thus removed by centrifugation.

3.6.2. Degumming

MGO was first heated and then citric acid solution was added along with water for hydration. The gums were separated by centrifugation.

3.6.3. Neutralization

Neutralization was achieved by treating MGO with NaOH solution and free fatty acids were removed in the form of soap.

3.6.4. Bleaching

Bleaching of MGO was carried out to remove pigments, oxidized lipids and polar compounds by treating with acid activated bleaching clay.

3.6.5. Deodorization

Deodorization of oil was performed by steam distillation process.

3.7. Analysis of Refined Maize Germ Oil (MGO)

The Refined MGO was analyzed for physical characteristics i.e. color (Cc 13j-97), flavor/odor, specific gravity (Cc 10a-25), refractive index (Cc 7-25) and chemical characteristics i.e. free fatty acids (Ca 5a-40), peroxide value (Cd 8-53), acid value (Cd 3d-63), saponification value (Cd 3-25), iodine value (Cd 1d-92) using their respective methodology (AOCS, 1998; AOAC, 2006).

3.7.1 Physical Characteristics

MGO was subjected to following physical characteristics:

3.7.1.1. Color

The color of oil was compared with standard colored glasses (red & yellow) in a Lovibond Tintometer cell.

3.7.1.2. Odor

MGO sample was taken in a glass beaker and capped followed by heating. Beaker was then swirled and lifted to nose to sniff the odor of the oil.

3.7.1.3. Specific Gravity

Specific gravity of MGO sample was determined at 15.6 °C with specific gravity bottle (Pyrex) based on specific gravity of water (1) at the same temperature.

3.7.1.4. Refractive Index

The refractive index of the oil was determined by means of Abbe's refractometer (Model: 2WAJ).

3.7.2. Chemical Characteristics

The chemical characteristics of MGO are given as under:

3.7.2.1. Free Fatty Acids

Free fatty acid content was determined by titrating MGO in neutralized 95% ethanol against NaOH solution.

3.7.2.2. Peroxide Value

MGO sample, glacial acetic acid–chloroform mixture and iodide solution were taken in 250 mL Erlenmeyer flask. After swirling, water and starch solution were added and the contents were titrated against sodium thiosulphate solution to determine peroxide value.

3.7.2.3. Acid Value

Acid value was obtained by adding neutral alcohol to MGO sample and titrating against KOH solution.

3.7.2.4. Saponification Value

Oil sample was refluxed with alcoholic potash in water bath and titrated against HCl solution to calculate the saponification value

3.7.2.5. Iodine Value

Iodine value was determined by using Wij's method. The oil sample was dissolved in carbon tetrachloride and after addition of Wij's solution, distilled with potassium iodide solution and water. The contents were titrated against sodium thiosulphate solution to calculate iodine value.

3.8. Product Development

Refined MGO from selected germ was blended with normal shortening (NS) at different levels (Table 1) and used in cake formulation.

Table 1. Treatments used for cake production

Treatments	Normal shortening (%)	Maize germ oil (%)
T_0	100	-
T_{20}	80	20
T_{40}	60	40
T_{60}	40	60
T_{80}	20	80
T_{100}	-	100

T_0 act as control

3.8.1. Cake Preparation

Cakes were prepared, from control and all the MGO-NS blends according to AACC (2000) method with some modifications using following recipe: flour 312 g, sugar 250 g, shortening 250 g, baking powder 12.5 g, six eggs and milk 150 mL. Creaming of shortening and sugar was done through Hobart Mixer (Model N-50, Hobart Corp. Troy, Ohio, USA). The rest of the ingredients were added as per sequence and mixed to homogenous mass. The batter was put in greased pans and baked at 180 °C for 45-50 min. The cakes were then cooled and packed for further analysis.

3.8.2. Physical Parameters

Volume of sample cakes was determined by rapeseed displacement method (AACC, 2000; 10-10B). Weight of each cake sample was recorded by using 2-decimal digital weighing scale while cake density was calculated by dividing cake weight by volume.

3.8.3. Hunter Crumb Color

The color of the cake crumb was measured with Hunter Color Meter (Model: D25 L optical sensor, Hunter Associates Lab., Reston, Virginia, USA). For the purpose 150 g of cake crumb was placed in the sample cup and color values

were recorded as "L" (0 black; 100 white), "a" (–a greenness; +a redness), and "b" (–b blueness; +b yellowness). The standard white tile, supplied by the manufacturer, had "L", "a", and "b" values of 94.8, –0.7, and 2.7, respectively. The data thus obtained was used to calculate Chroma and Hue angle according to the method of Little (1975).

3.8.4. Crumb Texture

Textural analysis of cake; as force required to compress 50% of the original height of the cake slices of 25 mm thickness was done using a Stable Micro System (SMS) Texture Analyzer (Model: TA-XT2*i*, Texture Technologies Corp., Scarsdale, New York, USA). The individual cake slices were tested under the compression mode using 35-mm dia. compression probe. A crosshead speed of 100 mm/min was used to record the maximum force expressed as the firmness of the cake crumb in Newton (N).

3.8.5. Sensory Evaluation

Cakes prepared with normal shortening and MGO blended shortening were subjected to sensory evaluation by a trained taste panel of 10 judges as described by (Meilgaard *et al.,* 2007). Evaluation was carried out by the panelists using 15-cm unstructured line for parameters of uniformity of cells, softness of texture, crumb color, aroma, taste and overall quality on a sensory evaluation Performa (Appendix I) All evaluations were conducted at room temperature on the same day in the National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad.

Cake slices were placed in transparent cups, labeled with 3-digit random codes. Panelists were provided with distilled water and unsalted crackers to clean their mouths between the samples. The cake samples were presented in random order and panelists were asked to rate their acceptance by marking a cross on the line for all the parameters. The data thus obtained was converted to numerical scores using metric scale.

3.9. Preparation of Defatted Maize Germ (DMG) Flour

The DMG, left after oil extraction from selected germ was processed according to the method of Tate *et al.* (1990) to produce food-grade DMG flour. DMG was ground in Disk Mill (Model: FFC-15, Shandong-Jimo Agricultural Machinery, China) at speed of 8800 RPM. The flour was sieved using 250-µm sieve (W.S. Tyler Co., Mentor, Ohio, USA). The meal was stored in polyethylene bags at room temperature for further analysis and product development.

3.10. Chemical Analysis

DMG flour was evaluated for proximate composition, mineral assay (P, K Mg, Ca and Fe) and amino acid profile for both essential and non-essential amino acids using their respective procedures as mentioned in preceeding section. Dietary fiber content was determined by using Megazyme TDF Test Kit (AACC Method no. 32-05 and AOAC Method no. 985.29) (AACC, 2000; AOAC, 2006).

3.11. Biological Evaluation

Quality evaluation of DMG flour protein was done through feeding flour diets namely; DMG flour diet, wheat flour diet, casein diet and no-protein diet (Table 2) to four groups of rats.

Table 2. Formulation of diets (moisture free basis)

Diet Constituents	DMG flour diet	Wheat flour diet	Casein diet	No protein diet
DMG flour (g)	36.3	0.0	0.0	0.0
Casein (g)	0.0	0.0	10.0	0.0
Wheat flour (g)	0.0	76.9	0.0	0.0
Corn oil (g)	5.0	5.0	5.0	5.0
Mineral mixture (g)	5.0	5.0	5.0	5.0
Vitamin mixture (g)	1.0	1.0	1.0	1.0
N-free mixture (g)	52.7	12.1	79.0	89.0
Total diet weight (g)	100	100	100	100

3.11.1. Housing of Rats

Forty weanling male Sprague Dawley rats were purchased from National Institute of Health (NIH), Islamabad and were housed in animal room of NIFSAT. The rats were fed on basal diet for a period of one week and randomly divided into four groups, ten in each. The groups were fed separately on DMG flour diet, casein diet, wheat flour diet and no protein diet for a period of 10 days. The diets were made isonitrogenous by adjusting the protein content of the test diets to 10% by using N-free mixture consisting of corn starch, sucrose and cellulose (Eggum, 1973). The composition of mineral mixture, vitamin mixture and N-free mixture is given in Appendix II. The temperature (23±2 ^{o}C) and humidity (50±5%) were maintained throughout the experimental period with 12-hr light-dark cycle. Feed intake, water intake and body weight were recorded for all groups. Spilled diet and feces were collected throughout the study period. At the end of study, overnight fasted rats were decapitated and their bodies were dried in an electric oven.

3.11.2. Protein Quality Evaluation

The spilled diet, feces and dried rats bodies were subjected to nitrogen analysis to determine true digestibility (TD), net protein utilization (NPU), biological value (BV), net protein ratio (NPR) and protein efficiency ratio (PER) following the procedures described by Pellet and Young (1980).

3.12. Safety Evaluation

Safety evaluation of DMG flour was also accomplished through serum bio-chemical profile analysis of Sprague Dawley rats fed on DMG flour based diet up to 45 days of study period.

3.12.1. Rats Modeling

Forty nine male Sprague Dawley rats of eight weeks of age were purchased from NIH, Islamabad for safety assessment of DMG based diet. Rats were housed under the same conditions as mentioned earlier in biological

evaluation study. Seven rats were decapitated at the initiation of study for blood collection to get baseline values as remaining 42 rats were divided in two groups, 21 in each. The groups were fed separately on DMG diet and basal diet (without DMG). Seven rats from each group were decapitated fortnightly up to 45 days of study period. The blood samples were allowed to stand at least for 30 min and then serum was separated by centrifugation as mentioned by Uchida *et al.* (2001). The collected serum samples were frozen at -20 °C for further assay.

3.12.2. Serum Bio-Chemical Profile

Collected serum was subjected to the following analysis to determine the effect of DMG flour on serum bio-chemical profile.

3.12.2.1. Cholesterol

Cholesterol in serum samples was measured by liquid cholesterol CHOD–PAP as described by Stockbridge et al. (1989).

3.12.2.2. High Density Lipoprotein (HDL)

Serum HDL was determined by HDL cholesterol precipitant method as described by Assmann (1979).

3.12.2.3. Low Density Lipoprotein (LDL)

LDL in all the serum samples was estimated by using the procedure outlined by McNamara *et al.* (1990).

3.12.2.4. Triglycerides (TG)

Triglycerides in the serum were determined by liquid TG GPO-PAP (Annoni *et al.,* 1982).

3.12.2.5. Glucose

Glucose concentration in serum samples was analyzed by GOD-PAP method as described by Thomas and Labor (1992).

3.12.2.6. Proteins

Total protein, albumin and globulin in serum samples were determined while albumin/globulin ratio was calculated through their respective procedures as described by Bradford (1976).

3.12.2.7. Electrolytes (K, Ca, Na)

Serum electrolytes i.e. potassium, calcium and sodium were determined with Hitachi-Biochemical Automatic Analyser 7070 (Hitachi, Tokyo, Japan) as mentioned by Hagiwara *et al.* (2003).

3.12.2.8. Liver and Kidney Function Tests

Serum was also analyzed for liver functioning through determination of enzymes i.e. glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT) and alkaline phosphatase (ALP). Kidney function was also evaluated through serum urea and creatinine assesment (Beutler, 1982).

3.12.3. Organs Weight

Organs i.e. liver, heart, kidney, spleen, and small intestine were collected after dissection to determine the effect of test diets on organ weights of rats. The organs were properly cleaned and weighed on electronic balance. The intestine length was measured with the help of scale. The results were then expressed as organ to body weight ratios (g/100g of body weight).

3.13. Preparation of DMG-Wheat Flour Blends

DMG flour was further blended with all-purpose wheat flour (King Milling Co., Howell, Michigan, USA) at 5, 10, 15, 20, and 25% (w/w) levels to prepare composite flour samples (Table 3). The composite blends were mixed thoroughly by sieving and kept in woven polypropylene bags at room temperature.

Table 3. Treatments of DMGF-wheat flour blends

Treatments	Wheat flour (%)	DMG flour (%)
T_0	100	-
T_5	95	5
T_{10}	90	10
T_{15}	85	15
T_{20}	80	20
T_{25}	75	25

T_0 acts as control

3.14. Analyses of Flour Blends

Flour samples were analyzed for physical analysis, functional and rheological behavior as discussed below properties. All treatments were also analyzed for dough rheology flours henceforth:

3.14.1. Physical

Physical analysis of all treatments and DMG flour were carried out for Hunter color parameters ("L", "a", "b", Chroma and Hue angle) as described earlier. Bulk density was determined by putting known weight of sample in 100 mL cylinder and tapping gently on table as described by Okaka and Potter (1977). Chroma, hue angle and bulk density were calculated as follows:

$$\text{Chroma} = \sqrt{a^2 + b^2}$$

$$\textit{Hue angle}\,(h^o) = \tan^{-1}\left[\frac{b}{a}\right]$$

$$\text{Bulk Density} = \frac{\text{Weight of the sample}}{\text{Volume of the sample in cylinder}} \text{x} 100$$

3.14.2. Functional Properties

Functional properties such as water and oil absorption capacities, emulsifying and foaming properties, least gelation concentration and apparent viscosity of flour blends along with control and DMG flour were determined according to their respective procedures as described under.

3.14.2.1. Water and Oil Absorption Capacities

Water and oil absorption capacities were determined by the method of Sosulski *et al.* (1976). Flour samples were mixed with water and centrifuged (Model: Sorvall RC-5B; DuPont Instrument, Newtown, Conn., USA) for determination of water absorption capacity (WAC). Likewise oil absorption capacity (OAC) was determined by mixing flour samples with oil and centrifuging to calculate OAC as follows:

$$\text{Water/oil absorption capacity} = \frac{\text{Weight of water/oil absorbed by the sample}}{\text{Weight of the sample}} \text{x} 100$$

3.14.2.2. Emulsifying Properties

Emulsion activity and stability were determined by the method of Yasumatsu *et al.* (1972). Flour was mixed with water and corn oil and then centrifuged. The ratio of the height of emulsion layer to the height of liquid layer was noted to calculate emulsion activity. The emulsion was heated followed by cooling and centrifuging to determine emulsion stability.

$$\text{Emulsion Activity} = \frac{\text{Height of the emulsion layer}}{\text{Height of the liquid layer x weight of the sample}} \text{x} 100$$

3.14.2.3. Foaming Properties

Foaming capacity and stability were determined as described by Okaka and Potter (1977). Flour blend samples were mixed with water in a graduated cylinder and shaken till foaming occurred. The volume of foam formed was

expressed as foaming capacity and the reading was taken after one hour to determine foaming stability as % of the initial foam volume.

$$\text{Foaming capacity} = \frac{\text{Foam volume after whipping - Volume before whipping}}{\text{Volume of foam before whipping}} \text{x}100$$

$$\text{Foaming stability} = \frac{\text{Foam volume after one hour of whipping}}{\text{Foam volume after whipping}} \text{x}100$$

3.14.2.4. Least Gelation Concentration

The least gelation concentration was determined by the method of Sathe and Salunkhe (1981). Blend dispersions of 2% to 20% (w/v) were prepared in test tubes and heated in a water bath. The dispersions were then cooled to determine least gelation concentration based on visual observation. The results were expressed as no (–), complete (+), or partial (±) gelation.

3.14.2.5. Apparent Viscosity

Apparent viscosity of flour dispersions was determined using Brookfield DV-II viscometer (Brookfield Engg., Middleboro, Mass., USA) by preparing dispersions of 5, 10, 15 and 20 g flour in 100 mL water. The apparent viscosity was measured using spindle no. 2 at shear rate of 100 rpm at room temperature. The readings were recorded after 30 s of shearing time. Viscosity readings are relative units and, and were used to compare relative viscosity as an index.

3.14.3.Analysis of DMGF-Wheat Flour Doughs

Doughs were evaluated for textural and farinograghic studies as described below:

3.14.3.1. Texture

The texture analysis of dough under compression mode was performed using SMS Texture Analyzer, equipped with a 500-N load cell and a 35-mm cylindrical probe. The flour samples were kneaded with Hobart Mixer for 3 min by adding 60% water on flour weight basis. Dough samples for analysis were

prepared by sheeting with a rolling pin over a rectangular platform with frame of 10-mm height to form cylindrical doughs (10-mm x 35-mm). The samples were then subjected to uniaxial compression (Ravi and Sushelamma, 2004). A crosshead speed of 100 mm/min was used to compress the doughs to 50% of their original height. The parameters derived from the force-deformation curve included hardness, the maximum resistance for the compression peak (height of peak) and stickiness, the maximum value of the negative peak of the compression.

3.14.3.2. Farinographic Studies

The rheological behavior of control and blended flour doughs was determined with Brabender Farinograph (Model: 810114 Nr 071199, Brabender GmbH & Co. KG, Germany) equipped with 50-g bowl capacity. Dough characteristics like water absorption (WA), dough development time (DDT) and dough stability (DS) were determined from each farinogram following the protocol presented in AACC (2000).

3.15. DMGF-Wheat Flour Blends for Product Development

Pan breads and Sugar-snap cookies were prepared from the treatments (Table 3) and evaluated for physical parameters and sensory attributes. The objective was to determine the feasibility of DMG flour incorporation in the formulation of these products to improve the nutritional quality.

3.15.1. Preparation of Bread

Pan breads were prepared according to the method described in AACC (2000), considering the water absorption requirement of individual treatment (Appendix III). After preliminary evaluation, breads from control and four treatments (T_5, T_{10}, T_{15} and T_{20}) were prepared according to the modified AACC (2000) method, using formulations shown in Table 3. Doughs were prepared through mixing in Hobart Mixer adopting straight dough procedure. After 30 min of fermentation, doughs were punched and placed in greased pans. After 60

min of proofing, doughs were baked at 220 °C for 20 min. Bread loaves were cooled to room temperature packed in polyethylene bags before further quality evaluation.

3.15.1.1. Physical Analysis

Bread volume was determined by rapeseed displacement method. Loaf weight and density were also determined, while specific volume of the bread loaves was calculated according to method presented by Penfield and Campbell (1990). The color of the bread crumb was measured with Hunter Color Meter (Model: D25 L optical sensor, Hunter Associates Lab., Reston, Virginia, USA) for "L", "a", "b", Chroma and Hue angle. The maximum force expressed as the firmness of the bread in Newtons (N) was determined by Stable Micro System (SMS) Texture Analyzer (Model: TA-XT2*i*, Texture Technologies Corp., Scarsdale, New York, USA). Bread crumb color and firmness were determined according to protocols as described in preceding section (3.8).

3.15.1.2. Sensory Evaluation

Taste panel consisting of 12-members with previous experience in bakery products evaluation was recruited for sensory evaluation after their consent (Appendix IV). Sensory evaluation was done on a performa (Appendix V) having 15-cm unstructured scale for all the attributes as described in Appendix VI, according to the method of Stone and Sidel (2004). All evaluations were conducted under cool white incandescent light at room temperature in a single session. Evaluation and scoring was accomplished using protocol as described earlier for cake evaluation.

3.15.2. Preparation of Cookies

Sugar-snap cookies were prepared, from all the flour blends along with control, according to the method of AACC (2000) with some modifications. The ingredients were added as per sequence in Hobart Mixer and the contents were mixed to a homogeneous mass. The batter was rolled and cut with a 50-

mm dia cookie cutter. The cookies were baked at 205 °C for 15-18 min and cooled at ambient temperature. The cookies were then packed in polyethylene bags and kept at room temperature prior to further analysis.

3.15.2.1. Physical Analysis

The diameter (D) and thickness (T) of the cookies prepared from control and treatment samples were measured and spread factor was calculated through their respectives protocols described in AACC (2000).

Textural analysis, as force required to break individual cookies, was done through SMS Texture Analyzer. The sample cookie was placed under the three point bending/breaking probe and a crosshead speed of 100 mm/min was used for the test. Breaking force was determined for six replicates for all cookies.

The crust color of the sample cookies was measured with Hunter Color Meter taking 100 g sample and the Hunter parameters obtained were "L", "a" and "b".

3.15.2.2. Sensory Evaluation

The preliminary screening of cookies with all five DMG flour levels compared to control was done by a trained panel of 10-judges and cookies made with 3 levels (5, 10, and 15%) of DMG flour were selected for further consumer acceptance analysis. A 75-member consumer panel was recruited for sensory evaluation, using 9-point hedonic scale (Appendix VII). The panel comprised a broad cross-section of adult population (students, faculty and staff) at Michigan State University (MSU). All evaluations were conducted at room temperature on the same day under white lights in the Department of Food Science and Human Nutrition.

Cookies were presented in small cups, labeled with 3-digit random codes and the panelists were provided with distilled water and unsalted crackers to clean their mouths between samples. The cookie samples were presented in random order and panelists were asked to rate their acceptance for color, aroma, taste, crispiness, and overall acceptability. A score of 5 or below was considered a

limit of acceptability for all sensory attributes tested. Panelists were also asked to answer additional questions (Appendix VIII) on demographics and marketing of DMG-meal-fortified cookies.

3.16. Statistical Analysis

Data was obtained by applying completely randomized design (CRD) and the results were analyzed through analysis of variance technique (Steel et al., 1997) using Cohort version 6.1 (Co-stat 2003) to determine the level of significance. The separation of means or significant difference comparisons were done using Tukey's HSD test and DMRt. The statistical significance was defined *as* $P \leq 0.05$. Correlation analysis was also carried out in order to explicit interactions between different quality attributes of end products.

RESULTS AND DISCUSSION

In Pakistan, no authenticated research information is available regarding composition and nutritional profile of maize germs of locally grown hybrids. For the purpose, maize germs were separated from various hybrids and characterized for proximate composition, selected minerals, amino acid & fatty acid profiles and tocopherols. Based on compositional analysis, one hybrid germ (Pioneer 32-F-10) was selected for further analysis and product development. Maize germ oil (MGO) was extracted from selected hybrid, refined and analyzed for various physical and chemical characteristics. The MGO was evaluated for cake production through blending with normal shortening at different levels. Moreover, its defatted portion was processed to produce food grade meal and subjected to biological and safety evaluation using experimental animals i.e. Sprague Dawley rats. Defatted maize germ (DMG) meal was then blended with wheat flour in different combinations to prepare composite flour blends. The flour samples were evaluated for functional properties, textural analysis and rheological behavior. Flour blends were then used to develop value-added products like bread and cookies; analyzed for product suitability through physical and sensory quality. The data was statistically analyzed to check the level of significance. The results for all the parameters investigated are discussed hereafter:

4.1. Maize Germ Recovery

The germ is an important part of maize kernel, a rich source of nutrients and usually separated by wet-milling process (Johnson and May, 2003). The mean squares for germ recovery are mentioned in Table 4. The germ recovery

was significantly different among various hybrids. It is obvious from the results (Figure 1) that the highest germ yield/recovery (7.68±0.11%) was obtained from P-1 followed by commercial (7.50±0.20%) and P-2 hybrids (7.47±0.23%), while the lowest germ was recovered (6.31±0.16%) from R-1 maize hybrid. The germ recovery from P-1, P-2 and commercial hybrids differed non-significantly. The overall germ recovery for all the hybrids ranged from 6.31±0.16 to 7.68±0.11%.

Maize germ is generally separated (90%) through wet-milling process from kernels (Johnston and May, 2003). Germ is roughly 12% of the total maize kernel (Zayas, 1994) however, yield has always been lower than the stated value due to loss of nutrients and germ fractions during wet-milling process.

Johnston *et al.* (2005) studied the germ yield through modified maize processing techniques and reported that the quick germ process resulted in 6.50% of germ while the yield from the boiled maize was reported to be 6.34%. Parris *et al.* (2006) also observed similar trend i.e. 7.55%, which conforms to the results of present investigation. In another study, Singh and Eckhoff (1996) worked to investigate the effect of soak time, soak temperature and level of lactic acid on germ yields during wet-milling process of maize. They reported that the germ yield ranged from 4.03 to 6.83% while the highest germ yield was recorded after 12 hrs soak time at 59°C.

It is rather difficult to reproduce results of germ recovery at laboratory scale by using wet-milling process. In industry, the separation of germ is carried out with germ-cyclones; separating the parts by difference in density (Dowd, 2003). Germ-cyclones are used to separate germs at pilot-plant scale, but at laboratory scale germs are separated through skimming or flotation technique (Rubens, 1990).

Table 4. Mean squares for germ recovery from different maize hybrids

Source	df	Germ recovery
Maize hybrids	5	0.7398133**
Error	12	0.0524444
Total	17	

** = *P≤ 0.01*

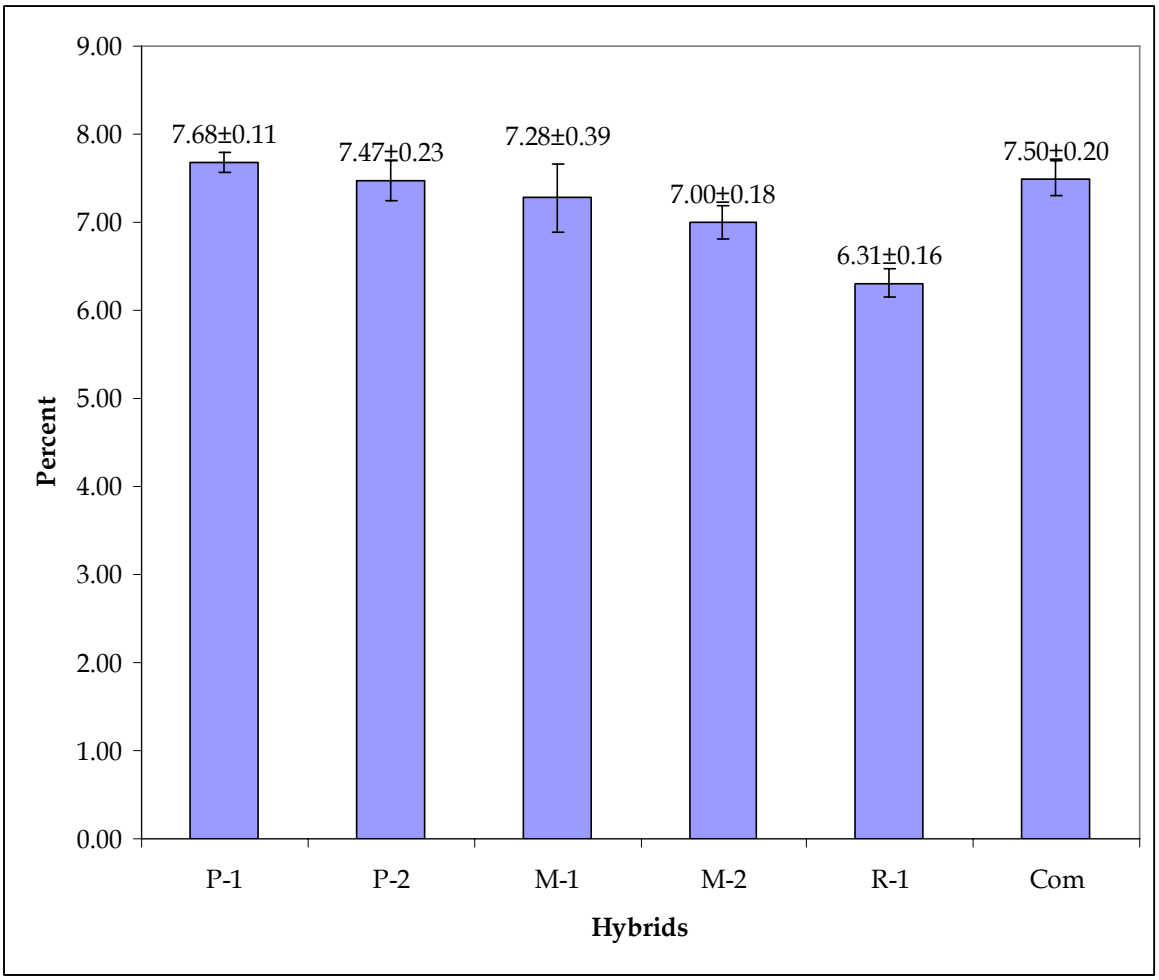

Figure 1. Germ recovery from different maize hybrids

P-1 = Poineer-32-F-10
P-2 = Poineer-32-B-33
M-1 = Monsanto-6142
M-2 = Monsanto-6525
R-1 = Rafhan-2331
Com = Commercial

4.2. Analysis of Germ

The knowledge of food composition is of prime importance for basic and applied research in the domain of food science. In most of the cases, it serves as

the basis for setting up of dietary standards and overall acceptability from the consumers' view point.

4.2.1. Proximate Composition

Proximate composition is an important criterion to have an idea about the overall composition and nutritional status of any ingredient intended for food use. So, the proximate composition of all the maize germ samples was determined and the data thus obtained was statistically analyzed. Mean squares for proximate composition of different germ samples are presented in Table 5. Proximate composition varied significantly among various maize germ samples for crude protein, crude fat, crude fiber, ash and NFE content. The mean values for these traits are given in Table 6.

Pioneer hybrid germs (P-1 and P-2) had significantly highest fat content than rest of the samples. The highest crude fat was present in P-1 hybrid (38.8±0.65%); while the range for this trait was from 32.1±1.15 to 38.8±0.65%. The germ samples contained crude protein ranging from 16.34±1.44 to 20.96±0.56%. P-1 hybrid had the highest protein content (20.96±0.56%) which was non-significantly different from P-2 (19.69±0.64%) and M-1 (19.10±1.5%), the lowest crude protein (16.34±1.44%) was present in commercial germ sample.

The present results are within the ranges as reported earlier by Johnston *et al.* (2005) for oil content (23.00-40.89%) and protein content (13.09-22.17%) during comparison of germ derived from different milling processes. The oil and protein contents from quick germ milling process were 36.43±0.46% and 21.36±0.69%, respectively, which is in harmony with the results of present investigation.

Table 5. Mean squares for proximate composition of maize germ samples

Source	df	Crude protein	Crude fat	Crude fiber	Ash	NFE
Germ samples	5	15.4159**	32.122**	2.0059**	3.4515**	83.673**
Error	12	0.98919	0.6231	0.0975	0.0152	1.2283
Total	17					

** = *P≤ 0.01*

Table 6. Proximate composition of maize germ samples

Germ samples	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	NFE (%)
P-1	20.96±0.56a	38.8±0.65a	2.73±0.26c	3.64±0.06c	33.86±7.86d
P-2	19.69±0.64ab	38.23±0.66a	2.63±0.58c	3.56±0.16c	35.89±1.03c
M-1	19.10±1.5abc	36.24±0.76b	3.87±0.22b	4.61±0.11b	36.10±0.91c
M-2	17.94±0.48bc	34.8±0.6c	3.47±0.26b	4.67±0.10b	39.10±2.06b
R-1	17.13±0.61c	33.15±0.76d	3.97±0.18b	4.94±0.14a	40.19±0.02b
Com	16.34±1.44d	32.1±1.15e	4.79±0.20a	3.08±0.12d	43.69±0.77a

Means sharing the same letter in a column are not significantly different

P-1 = Poineer-32-F-10
P-2 = Poineer-32-B-33
M-1 = Monsanto-6142
M-2 = Monsanto-6525
R-1 = Rafhan-2331
Com = Commercial

Oil and protein in maize germ are the two most important components (Johnston *et al.,* 2005) as the value of germ obtained from any process is directly related to these parameters (Ash and Dohlman, 2004; RFN, 2004). Maize germ, the only living part of the kernel is an excellent source of oil (50%) (CRA, 2006).

Montgomery *et al.* (2005) evaluated dried full-fat maize germ obtained through wet-milling and stated that the germ contained 45% lipid and 12% crude protein on dry weight basis. Kulakova *et al.* (1982) evaluated germs separated from three maize hybrids and reported that the fat content of wet milled maize ranged from 30.7-33.0%, while protein 20-21.1%. The present results are further supported by the findings of Singh *et al.* (2001), CRA (2006) and Ruan (2004).

The means for the crude fiber content of different germ samples (Table 6) revealed that the commercial maize germ contained the highest fiber content i.e. 4.79±0.20% followed by 3.97±0.18 and 3.87±0.22% in R-1 and M-1, respectively. The lowest (2.63±0.58%) crude fiber was observed in P-2. The highest concentration of ash was in R-1 (4.94±0.14%), followed by M-2 (4.67±0.10%) and M-1 germ (4.61±0.11%), whereas, the lowest ash content was recorded in commercial germ (3.08±0.12%). The ash content of various samples ranged from 3.08±0.12 to 4.94±0.14%. Commercial maize germ showed highest nitrogen free extract (NFE) (43.69±0.77%) followed by R-1 (40.19±0.02%) and M-2 (39.10±2.06%), the lowest NFE was found in P-1 germ sample (33.86±7.86%).

Gardner *et al.* (1971) reported similar results for fiber and ash contents for dry milled maize germ. In a report, Ruan (2004) stated that lightly toasted maize germs composed of 6.5% ash and 46.5% carbohydrates. The results concerning crude fiber in the present investigation are closely associated with the earlier findings of Kulakova *et al.* (1982). Later, Johnston *et al.* (2005) recorded similar observations for ash content (1.43 to 3.22%) of germs as found in present study. Crude fiber is represented by an insoluble but combustible organic residue, while ash content in food samples is characterized as inorganic portion left after

incineration. The ash content is generally considered as the indirect way for the estimation of minerals but it might not be essentially the same as present in original food (Kirk and Sawyer, 1991).

The significant results for proximate composition, in the present investigation, might be due to variation in the composition of various hybrids (Kulakova *et al.,* 1982) as some compositional differences can be anticipated owing to crop variety/hybrid, different climatic & soil conditions, agricultural practices and post-harvest handling. The more fiber percentage could be one of the possible reasons for lower oil and protein contents in commercial hybrid germ. Overall, Pioneer hybrid germs contained more fat and protein, the two most valuable components, than rest of the tested germ samples.

The results of present study elucidated that maize germ not only be used as a source of oil, but also a potential source of protein and fiber for the preparation of allied value-added products to improve the nutritional quality.

4.2.2. Minerals

Maize germ samples were analyzed for selected minerals and the data was subjected to statistical analysis; mean squares for potassium, phosphorus, magnesium, calcium and iron are presented in Table 7. There were significant differences among the germ samples for the listed minerals. The results regarding their respective mean values are presented in Table 8.

R-1 hybrid contained highest quantity of P, K, Mg, Ca and Fe (1.79±0.12 g/100g, 1.64±0.01 g/100g, 0.78±0.07 g/100g, 12.13±1.45 mg/100g, 14.46±1.45 mg/100g, respectively), whereas commercial germ contained the lowest mineral contents (1.06±0.05 g/100g, 1.19±0.03 g/100g, 0.43±0.04 g/100g, 7.13±0.83 mg/100g, 9.08±0.41 mg/100g, respectively).

Table 7. Mean squares for mineral composition of maize germ samples

SOV	df	Phosphorus	Potassium	Magnesium	Calcium	Iron
Germ samples	5	0.4443**	0.3844**	0.09123**	20.915**	29.818**
Error	12	0.0107	0.0019	0.0048	2.0912	1.6651
Total	17					

** = *P≤ 0.01*

Table 8. Mineral composition of maize germ samples

Germ samples	Phosphorus (g/100g)	Potassium (g/100g)	Magnesium (g/100g)	Calcium (mg/100g)	Iron (mg/100g)
P-1	1.34±0.13b	1.21±0.01c	0.57±0.05b	8.95±1.02b	10.67±1.02b
P-2	1.30±0.06b	1.19±0.04c	0.56±0.07b	8.79±1.4b	10.44±1.10b
M-1	1.71±0.05a	1.55±0.05b	0.74±0.09a	11.52±1.82ab	13.69±1.4a
M-2	1.68±0.13a	1.56±0.06b	0.77±0.05a	11.53±1.83ab	13.72±1.79a
R-1	1.79±0.12a	1.64±0.01a	0.78±0.07a	12.13±1.45a	14.46±1.45a
Com	1.06±0.05c	1.19±0.03c	0.43±0.04c	7.13±0.83c	9.08±0.41c

Means sharing the same letter in acolumn are not significantly different

The ranges for phosphorus, potassium, magnesium, calcium and iron were recorded as 1.06±0.05 to 1.79±0.12 g/100g, 1.19±0.03 to 1.64±0.01 g/100g, 0.43±0.04 to 0.78±0.07 g/100g, 7.13±0.83 to 12.13±1.45 mg/100g and 9.08±0.41 to 14.46±1.45 mg/100g, respectively.

Overall, the evaluated minerals were highest in R-1 germ whereas, commercial germ contained the lowest mineral contents. The higher mineral content in R-1 and the least in commercial germ are supported by the data for ash contents (Table 6) as there is linear relationship between ash and mineral contents. Though the data elucidated significant differences, yet all the germ samples were good source of tested minerals. Owing to better mineral profile, the maize germ has a potential to be used as source of respective minerals in food preparations.

The present results are in agreement with those reported earlier by Barbieri and Casiraghi (1983). Inglett and Blessin (1979) also determined mineral content in defatted maize germ flour and reported 2.74 g/100g, 2.36 g/100g, 1.02 g/100g, 15 mg/100g, and 20 mg/100g of phosphorus, potassium, magnesium, calcium and iron, respectively. In another study similar results were reported by Garcia *et al.* (1972), while studying the mineral composition of different dry-milled maize and wheat germ samples. The high concentration of potassium in maize germ has great significance in the human diet as, normally, it is deficient in this mineral (Cuthbertson, 1989).

4.2.3. Amino Acid Profile

The shortage of food, protein and essential amino acids are amongst the major problems of human nutrition in developing countries like Pakistan. The nutritional quality of the diet can be enhanced by improving protein content and limiting amino acids especially lysine. Maize germ samples were evaluated for amino acid profile. Mean squares for essential and non essential amino acids are

presented in Tables 9 and 10. All the essential amino acids except methionine, trytophan and cystine showed significant variations with respect to maize germ samples (Table 9). Likewise, there were significant differences between non-essential amino acids, with the exception of proline. The mean values for essential amino acids of different maize germ samples are presented in Table 11.

The highest lysine content (5.79±0.14 g/100g) was found in P-1 hybrid germ followed by P-2 (5.21±0.05 g/100g) and commercial germ (5.01±0.09 g/100g), while the lowest concentration (4.60±0.28 g/100g) for lysine was recorded in M-1 hybrid germ. P-1 germ also contained the highest amount (3.34±0.08 g/100g) of isoleucine while the commercial hybrid germ had the lowest value (2.41±0.17 g/100g) for this amino acid.

Overall, the ranges for essential amino acids noted were: isoleucine 2.41±0.17 to 3.60±0.14 g/100g, leucine 6.54±0.12 to 7.99±0.16 g/100g, lysine 4.60±0.28 to 5.79±0.14 g/100g, threonine 3.20±0.24 to 4.07±0.04 g/100g, tryptophan 1.17±0.03 to 1.31±0.06 g/100g, valine 4.32±0.08 to 5.61±0.01 g/100g, methionine 1.73±0.66 to 2.35±0.07 g/100g, tyrosine 2.9±0.43 to 3.85±0.07 g/100g, phenylalanine 4.14±0.05 to 4.70±0.13 g/100g and cystine 1.55±0.21 to 1.86±0.08 g/100g. The lysine amino acid was found to be highest P-1 (5.79±0.14 g/100g) followed by P-2 (5.21±0.05 g/100g) and commercial germ (5.01±0.09 g/100g), while the least quantity (4.60±0.28) of lysine was observed in M-1. The significant highest amount (39.04±0.13 g/100g) of total essential amino acids (TEAA) was present in P-2 hybrid germ followed P-1 (38.35±0.18 g/100g), while the lowest content (33.3±0.08 g/100g) was observed in commercial germ. The TEAA contents in M-1 (37.71±0.08 g/100g), M-2 (37.44±0.15 g/100g) and R-1 (37.63±0.76 g/100g) germs were found to be non-significant.

Table 9. Mean squares for essential amino acids of maize germ samples

SOV	df	Isoleucine	Leucine	Lysine	Threonine	Tryptophan	Valine
Germ samples	5	0.3388**	0.569**	0.386**	0.4804**	0.0049^{ns}	0.3889**
Error	6	0.0160	0.035	0.020	0.0183	0.0055	0.0186
Total	11						

SOV	df	Valine	Methionine	Tyrosine	Phenylalanine	Cystine	TEAA
Germ samples	5	0.3889**	0.0843^{ns}	0.2522*	0.1153*	0.0263^{ns}	8.1694**
Error	6	0.0186	0.0834	0.0380	0.0142	0.0124	0.0168
Total	11						

* = *P≤ 0.05*
** = *P≤ 0.01*
ns = Non-significant

TEAA = Total essential amino acids

Table 10. Mean squares for non-essential amino acids of maize germ samples

SOV	df	Alanine	Arginine	Aspartic acid	Glutamic acid
Germ samples	5	0.1017*	0.3715*	0.6022**	0.2642**
Error	6	0.0141	0.0749	0.0268	0.0244
Total	11				

SOV	df	Histidine	Serine	Proline	Glycine
Germ samples	5	0.3274**	0.1326**	0.1045^{ns}	0.3537*
Error	6	0.0178	0.0101	0.0334	0.0637
Total	11				

* = *P≤ 0.05*
** = *P≤ 0.01*
ns = Non-significant

Table 11. Essential amino acids (g/100g protein) of maize germ samples

Amino acids	Germ samples					
	P-1	P-2	M-1	M-2	R-1	Com
Isoleucine	3.34±0.08ab	3.33±0.1ab	3.21±0.08bc	3.60±0.14a	2.99±0.16c	2.41±0.17d
Leucine	7.18±0.04b	7.27±0.13b	7.99±0.16a	7.29±0.16b	6.54±0.12c	6.59±0.36c
Lysine	5.79±0.14a	5.21±0.05b	4.60±0.28c	4.63±0.11c	4.95±0.04b	5.01±0.09bc
Threonine	4.03±0.10b	4.18±0.11b	4.05±0.07b	4.07±0.04b	4.73±0.16a	3.20±0.24c
Tryptophan	1.25±0.07	1.21±0.15	1.31±0.06	1.25±0.04	1.20±0.01	1.17±0.03
Valine	5.61±0.01a	5.24±0.09b	4.82±0.11c	5.06±0.06bc	5.26±0.28b	4.32±0.08d
Methionine	2.15±0.70	2.35±0.07	2.00±0.14	2.08±0.17	2.15±0.05	1.73±0.66
Tyrosine	3.20±0.14bc	3.73±0.11a	3.85±0.07a	3.55±0.08ab	3.58±0.09ab	2.9±0.43 c
Phenyl-alanine	4.15±0.07c	4.70±0.13a	4.14±0.05c	4.37±0.05bc	4.54±0.96ab	4.14±0.05c
Cystine	1.66±0.12ab	1.86±0.08a	1.75±0.07ab	1.55±0.21b	1.71±0.04ab	1.84±0.06ab
TEAA	38.35±0.18b	39.04±0.13a	37.71±0.08c	37.44±0.15c	37.63±0.76c	33.3±0.08d

Means sharing the same letter in a row are not significantly different.

P-1 = Poineer-32-F-10
P-2 = Poineer-32-B-33
M-1 = Monsanto-6142
M-2 = Monsanto-6525
R-1 = Rafhan-2331
Com = Commercial

Table 12. Non-essential amino acids (g/100g protein) of maize germ samples

Amino acids	Germ samples					
	P-1	P-2	M-1	M-2	R-1	Com
Alanine	6.80±0.14a	6.52±0.12ab	6.25±0.08b	6.75±0.07a	6.34±0.19b	6.67±0.04a
Arginine	8.35±0.07b	8.23±0.10b	8.24±0.06b	9.29±0.59a	8.94±0.01ab	8.46±0.29b
Aspartic acid	8.14±0.08b	9.00±0.014a	8.44±0.10b	8.14±0.13b	7.34±0.28c	8.46±0.18b
Glutamic acid	14.05±0.07c	13.85±0.07c	14.70±0.04a	14.45±0.35ab	14.76±0.02a	14.19±0.10bc
Glycine	6.55±0.07a	5.36±0.08b	6.00±0.42ab	5.65±0.35b	5.72±0.16b	5.57±0.21b
Histidine	3.13±0.10bc	3.17±0.07b	2.81±0.01cd	2.70±0.28d	2.89±0.06bcd	3.83±0.09a
Serine	5.04±0.05cd	5.54±0.13a	4.84±0.09d	5.23±0.08bc	4.97±0.04d	5.32±0.16ab
Proline	5.07±0.09b	5.22±0.26ab	5.40±0.28ab	5.27±0.10ab	5.07±0.16b	5.67±0.10a

Means sharing the same letter in a row are not significantly different

Mean values for non-essential amino acids of different hybrid germs are depicted in Table 12. Among non-essential amino acids, glutamic acid was the highest (13.85-14.76 g/100g) whereas, lowest content (2.70-3.83 g/100g) was recorded for histidine in all the tested samples. The ranges for non essential amino acids were: alanine 6.25±0.08 to 6.80±0.14 g/100g, arginine 8.23±0.10 to 9.29±0.59 g/100g, aspartic acid 7.34±0.28 to 9.00±0.014 g/100g, glutamic acid 13.85±0.07 to 14.76±0.02 g/100g, glycine 5.36±0.08 to 6.55±0.07 g/100g, histidine 2.70±0.28 to 3.83±0.09 g/100g, serine 4.84±0.09 to 5.54±0.13 g/100g and proline 5.07±0.16 to 5.67±0.10 g/100g.

Essential amino acids are required in sufficient amount in the diet and their contribution to the total protein content is important from nutritional viewpoint (Anjum *et al.,* 2005). Maize proteins are deficient in few essential amino acids like lysine and tryptophan but these deficiencies are mainly related to endosperm portion of the kernel (Khoi *et al.,* 1986). The amino acid profile of maize germs found to be nutritionally valuable exhibiting balanced protein with respect to essential amino acids and in agreement with FAO/WHO standards.

Maize germ meal has a potential for its applications in food products as albumin and globulin fractions represent the major portion of the germ proteins (Lawton and Wilson, 2003). Gupta and Eggum (1998) studied the amino acid profile of processed defatted maize germ and reported better protein quality i.e. 47 % albumin and its amino acids particularly lysine and tryptophan were comparable with that of the casein. Kulakova *et al.* (1982; 1983) also mentioned that maize germs contain biologically balanced proteins which are not inferior to egg albumin and casein.

Previously, all the essential amino acids, except isoleucine, were found to be comparable with the amino acid requirements of preschool children (5.5 g/100g protein) (Neilsen *et al.,* 1979). Inglett and Blessin (1979) evaluated composition of germ flour proteins from different mills and varieties; there were

little differences in amino acid content among different sources of maize germs and the lysine accounted for 5% of 17 amino acids recovered. Previously, Tsen *et al.* (1974) also reported that the proteins in the maize germ contain 6% lysine and balanced in remaining essential amino acids. Maize germ protein with lysine content (the first limiting amino acid in wheat), more than twice than that of wheat flour, is suitable for fortification in bread recipes.

Blessin *et al.* (1973) compared the amino acid profile of defatted maize germ flour with egg and concluded that maize germ flour contained 38.3% of total essential amino acids against 51.3% in egg. They further concluded that the maize germ protein compares favorably when individual amino acids are discussed as a part of total essential amino acids. Neilsen *et al.* (1973) also evaluated the amino acid profile of maize germ protein and compared with sodium casinate, a protein widely used and recognized as a reference protein. They described that maize germ protein was significantly higher in sulfur-containing amino acids while other essential amino acids were either equal or slightly lower as compared to Na-casinate. Being a rich source of balanced protein, maize germ holds a potential as an intervention against protein malnutrition.

4.2.4. Fatty Acid Profile

Fatty acid composition is of prime importance while studying the composition, nutritional quality and oxidative stability of lipids in any product, intended for food use. Statistical analysis depicted that all the fatty acids were affected significantly due to source of maize germ (Table 13).

Table 13. Mean squares for fatty acid profile of maize germ samples

SOV	df	C14	C16	C18:0	C18:1	C18:2	C18:3
Germ samples	5	0.1289**	7.1949**	0.3737**	45.40**	52.489**	3.9114**
Error	6	0.00305	0.0584	0.0132	0.6870	0.7416	0.0351
Total	11						

** = *P≤ 0.01*

C14 = Myristic acid
C16 = Palmitic acid
C18:0 = Stearic acid
C18:1 = Oleic acid
C18:2 = Linoleic acid
C18:3 = Linolenic acid

Table 14. Fatty acid profile (g/100g) of maize germ samples

Fatty acids	Germ samples					
	P-1	P-2	M-1	M-2	R-1	Com
C14	0.05±0.01d	0.06±0.01cd	0.17±0.04c	0.19±0.05c	0.35±0.04b	0.70±0.11a
C16	11.19±0.10d	8.53±0.15e	11.26±0.34c	12.37±0.23b	10.21±0.25d	14.00±0.29a
C18:0	0.60±0.02c	1.79±0.01a	1.71±0.20ab	1.43±0.06b	1.59±0.19ab	2.53±0.01ab
C18:1	29.85±0.28b	32.10±0.85bc	39.91±1.024a	31.52±0.98c	33.86±0.67b	26.24±0.62e
C18:2	56.33±0.22a	55.49±0.65a	44.16±1.055c	50.79±0.65b	50.14±0.51b	53.3±0.95b
C18:3	1.67±0.10d	1.81±0.12d	2.58±0.32ab	2.78±0.17ab	3.01±0.14a	2.33±0.29b

Means sharing the same letter in a row are not significantly different.

Fatty acid profiles of different maize germ samples are shown in Table 14. Amongst fatty acids, linoleic acid (C18:2) was in highest quantity (44.16±1.055 to 56.33±0.22 g/100g), followed by oleic acid (26.24±0.62 to 39.91±1.024 g/100g) and palmitic acid (8.53±0.15 to 14.00±0.29 g/100g) in all the germ samples while myristic acid (C14) was found to be the lowest (0.05±0.01 to 0.70±0.11 g/100g). P-1 germ sample contained highest amount (56.33±0.22 g/100g) of linoleic acid followed by P-2 (55.49±0.65 g/100g) and commercial germ sample (53.3±0.95 g/100g) whereas, the lowest amount (44.16±1.055 g/100g) was found in M-1 hybrid germ sample. Regarding oleic acid (C18:1), M-1 contained the highest quantity (39.91±1.024 g/100g) followed by R-1 (33.86±0.67 g/100g), and M-2 (32.10±0.85 g/100g) hybrid germs while the lowest content (26.24±0.62 g/100g) was present in commercial germ. The ranges for these fatty acids were: myristic acid (C14) 0.05±0.01 to 0.70±0.11 g/100g, palmitic acid (C16) 8.53±0.15 to 14.00±0.29 g/100g, stearic acid (C18:0) 0.60±0.02 to 2.53±0.01 g/100g, oleic acid (C18:1) 26.24±0.62 to 39.91±1.024 g/100g, linoleic acid (C18:2) from 44.16±1.055 to 56.33±0.22 g/100g and linolenic acid (C18:3) 1.67±0.10 to 3.01±0.14 g/100g.

It is obvious from the results that the germ samples contained highest amount of polyunsaturated fatty acids followed by monounsaturated, while saturated fatty acid contents were the lowest in all tested samples. The present results are within the recommended ratios of respective fatty acids as proposed by Codex Alimetarius.

Present results are in consistency with the earlier findings reported by Leibovitz and Ruckenstein (1983) for the fatty acid profile of maize germ oil obtained from different regions of South Africa and USA. They stated that the triglyceride structure of maize germ oil contained no such triglyceride molecule that consisted of completely saturated fatty acids however, a small quantity of disaturated triglycerides was found. On an average, the triglyceride structure of maize germ oil can be represented as: triglycerides with all saturated fatty acids

(S3) = 0%, triglycerides with two-saturated and one-unsaturated (S2U) = 2.2%, triglycerides with one-saturated and two-unsaturated (SU2) = 40.3%, triglycerides with all unsaturated fatty acids (U3) = 57.5%. The findings of the present investigation are also corroborated with EFSA (2005); maize germ oil contains 14% saturated, 30% monounsaturated and 56% polyunsaturated fatty acids.

In another trial conducted by Rudan-Tasic and Klofutar (1999) found that refined maize germ oil contains 10-15% saturated, 20-35% monounsaturated and 50-60% of polyunsaturated fatty acids. Lemcke-Norojarvi *et al.* (2001) also reported the similar proportions of fatty acids for corn oil. CRA (2006) published typical fatty acid profile of refined corn oil as linoleic acid 54-60%, linolenic 1%, palmitic acid 11-13%, stearic acid 2-3% and oleic acid 25-31%

Corn oil can play a vital role in the diet being a rich source of essential fatty acids (polyunsaturated fatty acids) especially linoleic acid; help to regulate blood cholesterol and elevated blood pressure (Hauman, 1985; Dupont *et al.*, 1990). Hegsted *et al.* (1993) reported that saturated fats raise, polyunsaturated fats lower and monounsaturated fats have no effect on blood cholesterol concentration. Corn oil, readily available and high in polyunsaturated fatty acids, is considered as a standard against which other oils are compared to asses their cholesterol lowering capacities. Jacono and Dougherty (1993) concluded that corn oil based diet reduced the blood pressure about 12% in men and 5% in women, with elevated blood pressure.

According to the National Research Council and Food and Agriculture Organization/World Health Organization recommendations; about 2-4% of energy should be taken from essential fatty acids with an additional 3% of energy for pregnant and breast feeding women. Thus, a tablespoon of maize germ oil in diet can satisfy the essential fatty acids requirements of healthy child or adult (CRA, 2006).

4.2.5. Tocopherols

Alpha and gamma forms are tocopherol isomers of vitamin E group; a fat soluble vitamin which exhibits antioxidant activity both in oil and physiological system. Mean squares for α- and γ-tocopherol contents are presented in (Table 15). Concentration of both tocopherols varied significantly among various hybrid germs.

Mean values for tocopherols are presented in Table 16. The maximum α-tocopherol content (9.68±2.52 mg/100g) was found in P-2 followed by P-1 (7.41±0.45 mg/100g) and M-1 hybrid germs (6.65±0.35 mg/100g). The lowest α-tocopherol (4.87±0.56 mg/100g) was present in commercial germ sample. In case of γ-tocopherol, significantly highest amount (37.83±0.39 mg/100g) was present in P-2 germ sample and the lowest (29.52±0.23 mg/100g) in commercial germ sample. The α-tocopherol ranged from 4.87±0.56 to 9.68±2.52 mg/100g whereas the range of γ-tocopherol was 29.52±0.23 to 37.83±0.39 mg/100g.

The results for tocopherols from present investigation are also supported with the earlier findings by Lemcke-Norojarvi *et al.* (2001) who observed tocopherol content in maize germ oil. They reported 21.3 mg/100g α-tocopherol and 94.1 mg/100g γ-tocopherol. It is worth mentioning that the oil is about 40% of the total germ portion thus the values of current study are in conformity on germ weight basis with those reported by Lemcke-Norojarvi *et al.* (2001). The lower oil content and high amount of fiber in commercial maize germ might be a possible reason for significant low level of tocopherol in the respective sample.

Table 15. Mean squares for tocopherols of maize germ samples

SOV	df	Alpha tocopherol	Gamma tocopherol
Germ samples	5	16.458**	45.331**
Error	12	1.231	0.1849
Total	17		

** = *P≤ 0.01*

Table 16. Tocopherols of maize germ samples

Germ samples	Alpha tocopherol (mg/100g)	Gamma tocopherol (mg/100g)
P-1	7.41±0.45b	35.51±0.79b
P-2	9.68±2.52a	37.83±0.39a
M-1	6.65±0.35bc	33.25±0.42c
M-2	4.63±0.61c	33.27±0.21c
R-1	5.84±0.16bc	31.03±0.25d
Com	4.87±0.56c	29.52±0.23e

Means sharing the same letter in a column are not significantly different.

P-1 = Poineer-32-F-10
P-2 = Poineer-32-B-33
M-1 = Monsanto-6142
M-2 = Monsanto-6525
R-1 = Rafhan-2331
Com = Commercial

Vegetable oils contain several minor compounds including antioxidants showing beneficial physiological properties (Deckere and Korver, 1996). Tocopherols are known to be key chain-breaking antioxidant thus preventing the promulgation of oxidative stress, in biological membranes (Ricciarelli *et al.,* 2001). Maize germ oil is among the rich sources of tocopherols, which is preferably retained in the plasma as compared to other family members of vitamin E. Tocopherols and tocotrienols are also helpful in cholesterol lowering or preventing cardiovascular maladies (Lloyd *et. al.,* 2000; Birringer *et al.,* 2002).

Compositional analysis of germ samples depicted its importance being good source of nutrients. Maize germ samples contained high amount of oil rich in essential fatty acids and tocopherol contents. Germ samples were also found to be good source of quality protein with balanced amino acid profile. Additionally, minerals like phosphorus, potassium, magnesium and iron were also present in substantial amount in various hybrid germs.

Based on the results of compositional profile and germ recovery, P-1 (Pioneer-32-F-10) germ was selected and used further for the preparation of value-added products. For the purpose, oil was extracted from the respective germ and used in cake formulation, whereas its defatted portion was evaluated through *in-vivo* efficacy study and then for product suitability.

4.3. Characteristics of Maize Germ Oil (MGO)

Edible oils are key components of human diet and impart characteristic flavor and texture to foods. Physical and chemical properties of oil are important considering its processing functionality, nutritional quality and storage stability. MGO characteristics are affected by variety/hybrid, germ separation techniques, quality of germ, method of extraction, processing & storage conditions and degree & method of refining. Food oils in general have noticeable variations in their composition; it is difficult to establish fixed values for chemical and

physical characteristics. The MGO from selected hybrid germ (P-1) was extracted, refined and evaluated for various physical and chemical characteristics.

4.3.1. Physical Parameters

Physical characteristics of MGO i.e. color, flavor/odor, specific gravity and refractive index are shown in Table 17. MGO showed agreeable flavor and pale yellow color. The specific gravity was noted to be 0.923±0.001 and the refractive index measured at room temperature was 1.472±0.001.

Color of oil reflects the degree of refining and is an important criterion for its intended use in food formulations. The flavor of the oil is also key property, which is subjective to temperature, moisture, air in contact, light and presence of antioxidants. Specific gravity is a good indicative of purity of oil. It is dependant on the number of double bonds and the chain length of the fatty acids. At any given temperature specific gravity increases as the mean molecular weight decreases (higher saponification value) with increase in degree of unsaturation (higher iodine value). Refractive index of oil increases with increase in the number of double bonds (iodine value). In general, the refractive indices of oils relate to the degree of unsaturation in a linear way (Rudan-Tasic and Klofutar, 1999).

The results for specific gravity and refrective index are closely associated with those reported by Rudan-Tasic and Klofutar (1999) for maize germ oil. CRA (2006) also published typical values for physical parameters of MGO: color; pale yellow, specific gravity; 0.922-0.928, referective index; 1.470-1.474, and flavor; slight corn, slight nutty/buttery.

Table 17. Physical and chemical characteristics of maize germ oil

Characteristic	Value
Color	Pale yellow
Flavor	Agreeable
Specific gravity	0.923±0.001
Refractive index	1.472±0.001
Iodine value (g/100g)	113.67±1.53
Saponification value (mg KOH/g)	192.67±2.08
Acid value (mg KOH/g)	0.33±0.001
Peroxide value (meq/Kg)	1.69±0.03
Free fatty acids (g/100g)	0.033±0.01

4.3.2. Chemical Parameters

The MGO was evaluated for iodine value, saponification value, acid value, POV and free fatty acids. The mean values for all these traits are presented in (Table 17). The iodine value, saponification value, acid value, peroxide value, free fatty acids were found to be 113.67±1.53 g/100g, 192.67±2.08 mg KOH/g, 0.33±0.001 mg KOH/g, 1.69±0.03 meq/Kg and 0.033±0.01 g/100g, repectively.

Iodine value is used to assess degree of unsaturation of fatty acids and indicator of oxidative stability. The higher iodine value represents the greater degree of unsaturation. Saponification value gives the idea of molecular weight of fatty acids present in oil; higher saponification value corresponds to lower molecular weight of fatty acids. Saponification value of MGO shows that fatty acids present in MGO have high number of carbon atoms.

The acid value is an indirect measure of free fatty acids content present in oil/fat and hence the index of freshness. Humidity and temperature result in increased acid value due to hydrolysis of glycerides. Higher acid value gives an idea about increased susceptibility of oils to rancidity. The oils intended for dietary purposes should not contain high free fatty acids.

Presence of free fatty acids in oils/fat is not desirable because they render unpleasant odor and deteriorate the quality of the product. Peroxide value is used to measure the oxidative rancidity of oil and is one of the most important parameters to evaluate the degree of deterioration of lipids. Peroxides and hydro-peroxides produced due to oxidation of oil/fat undergo break down to produce bad smelling aldehydes, ketones and acids. Fresh refined oils should have zero POV but for acceptable storage stability, the POV should be less than 5 mmol/Kg of sample (Rudan-Tasic and Klofutar 1999).

Chemical characteristics of germ oil are in agreement with the published

data for these indices. According to the list of primary specifications for corn oil laid down by the Committee on Food Chemicals Codex of the National Academy of Sciences/National Research Council, free fatty acids should not be more than 0.1% and POV not more than 10 meq/Kg (FCC, 2003). Present results are in conformity with these specifications.

The findings of present research are also within the ranges given by Corn Refiners Association for chemical and physical characteristics of corn oil (CRA, 2006). Leibovits and Ruckenstein (1983) also reported that the iodine value of crude maize germ oil is 110-125 g/100g. They further showed that free fatty acids and POV values of refined maize oil samples ranged from 0.02-0.03% and 0.0-0.1 meq/Kg, respectively. The findings of present investigation for POV, iodine value, acid value and saponification value are also in line with the earlier results reported by Rudan-Tasic and Klofutar (1999).

4.4. Maize germ oil (MGO) for Cake Development

Maize germ oil from selected hybrid was blended with normal shortening for the preparation of value-added products. For the purpose, cakes were prepared and subjected to physical and sensory quality evaluation.

4.4.1. Physical Characteristics

Mean squares for physical parameters of cakes including loaf volume, specific volume, weight and crumb hardness are shown in Table 18. The statistical results depicted that cake volume and specific volume were affected significantly, whereas treatment did not show any momentous effect on weight and crumb hardness.

Table 18. Mean squares for physical parameter of MGO fortified cakes

SOV	df	Volume	Specific volume	weight	Crumb hardness
Treatments	5	191.38889**	0.0169389**	1.2288889^{ns}	5.903999 ^{ns}
Error	12	4.6111111	0.0015667	0.8616667	2.0946953
Total	17				

** = *P≤ 0.01*
ns = Non-significant

Table 19. Physical parameters of maize germ oil (MGO) fortified cakes

Treatments	Volume (cc)	Specific volume (cc/g)	Weight (g)	Crumb hardness (N)
T_0	281.3±1.2c	3.29±0.02d	85.5±0.9	21.45±2.13
T_{20}	282.3±0.6c	3.31±0.05cd	85.2±1.5	21.28±1.61
T_{40}	284.0±1.7c	3.29±0.01d	86.3±0.6	22.96±1.07
T_{60}	292.3±4.5b	3.37±0.04bc	86.7±0.3	24.00±1.03
T_{80}	295.7±1.2b	3.42±0.06ab	86.6±1.2	24.07±1.45
T_{100}	300.7±1.2a	3.47±0.01a	86.7±0.6	24.54±1.07

Means sharing the same letter in a column are not significantly different.

T_0 = Normal shortening
T_{20} = MGO 20%
T_{40} = MGO 40%
T_{60} = MGO 60%
T_{80} = MGO 80%
T_{100} = MGO 100%

Means of cake physical characteristics are given in Table 19. Cake volume increased gradually with the addition of maize germ oil (MGO) in formulation and highest cake volume was recorded in T_{100} (100% MGO). Cake volume was improved from 281.3±1.2 cc for normal shortening to 300.7±1.2 cc for 100% MGO fortified cakes. Maize germ oil showed positive effect on specific volume; increased from 3.29±0.02 to 3.47±0.01 cc/g as the MGO level was increased from 0 to 100%.

Though, crumb hardness increased with increasing level of MGO, but the values were non-significant. Cake weights did not depict variations with MGO fortification. Ranges for weight and crumb hardness were 85.2±1.5 to 86.7±0.6 g and 21.28±1.61 to 24.54±1.07 N, respectively. Overall, cakes prepared from MGO addition exhibited increased volume, specific volume and crumb hardness.

The results for hardness and volume are also supported by the findings of Jacob and Leelavathi (2007); studied effect of fat type on cookie quality. They reported that oil increased resistance during mixing as compared to bakers fat resulting in comparatively harder texture. The product prepared with oil addition also resulted in increased spread ratio. Fat is one of the basic components of cake formulation and acts as lubricant providing plasticity to the dough (Maache-Rezzoug *et al.,* 1998). Oil in shortening has a key role in adequate aeration (O'Brien, 2004) resulting increased volume.

4.4.2. Crumb Color

Mean squares for Hunter color values of cakes prepared with different levels of maize germ oil (MGO) are given in Table 20.

Table 20. Mean squares for hunter color values of MGO fortified cakes

SOV	df	L	a	b	Chroma	Hue angle
Treatments	5	1.984*	1.07975**	0.701417*	0.50635^{ns}	8.2369975**
Error	18	0.588889	0.029861	0.22625	0.2200917	0.2283431
Total	23					

* = *P≤ 0.05*
** = *P≤ 0.01*
ns = Non-significant

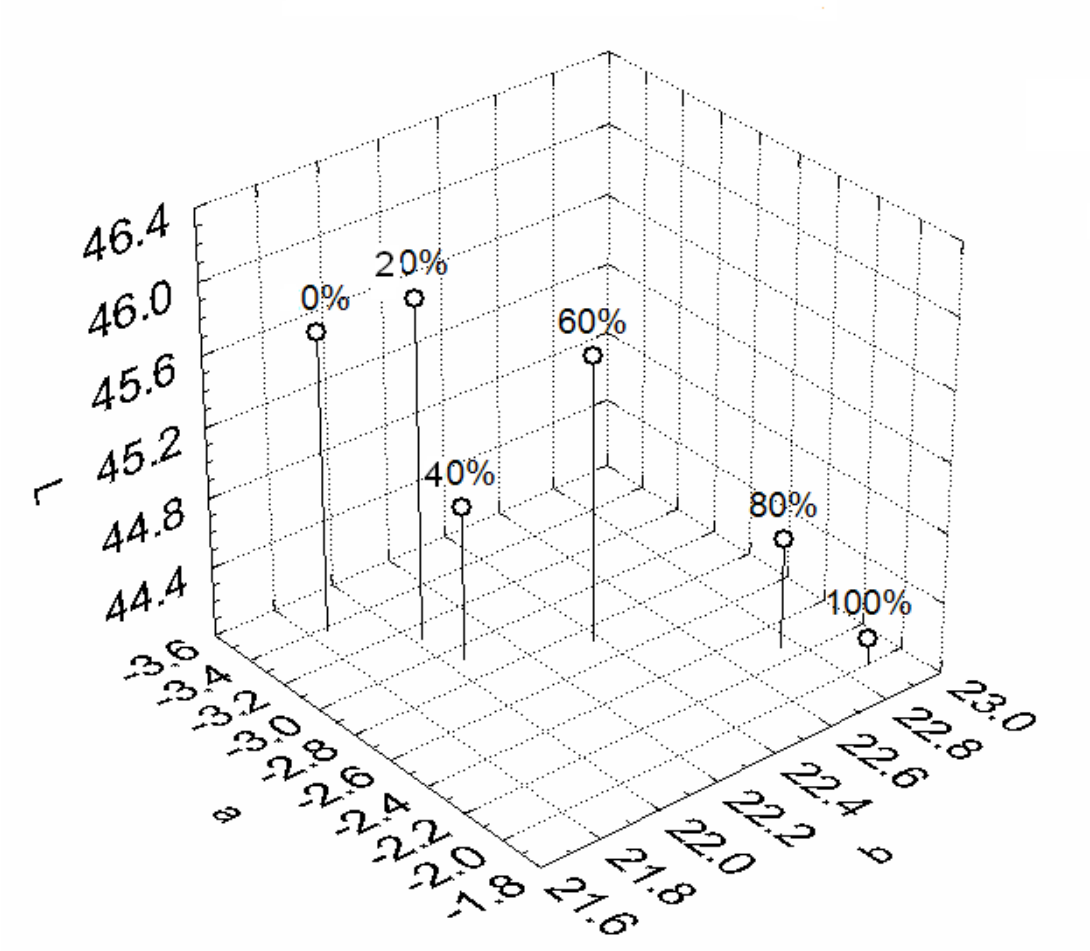

Figure 2. Hunter color "L", "a" and "b" values of MGO fortified cakes

"L" = Lightness
"a" = Redness
"b" = Yellowness

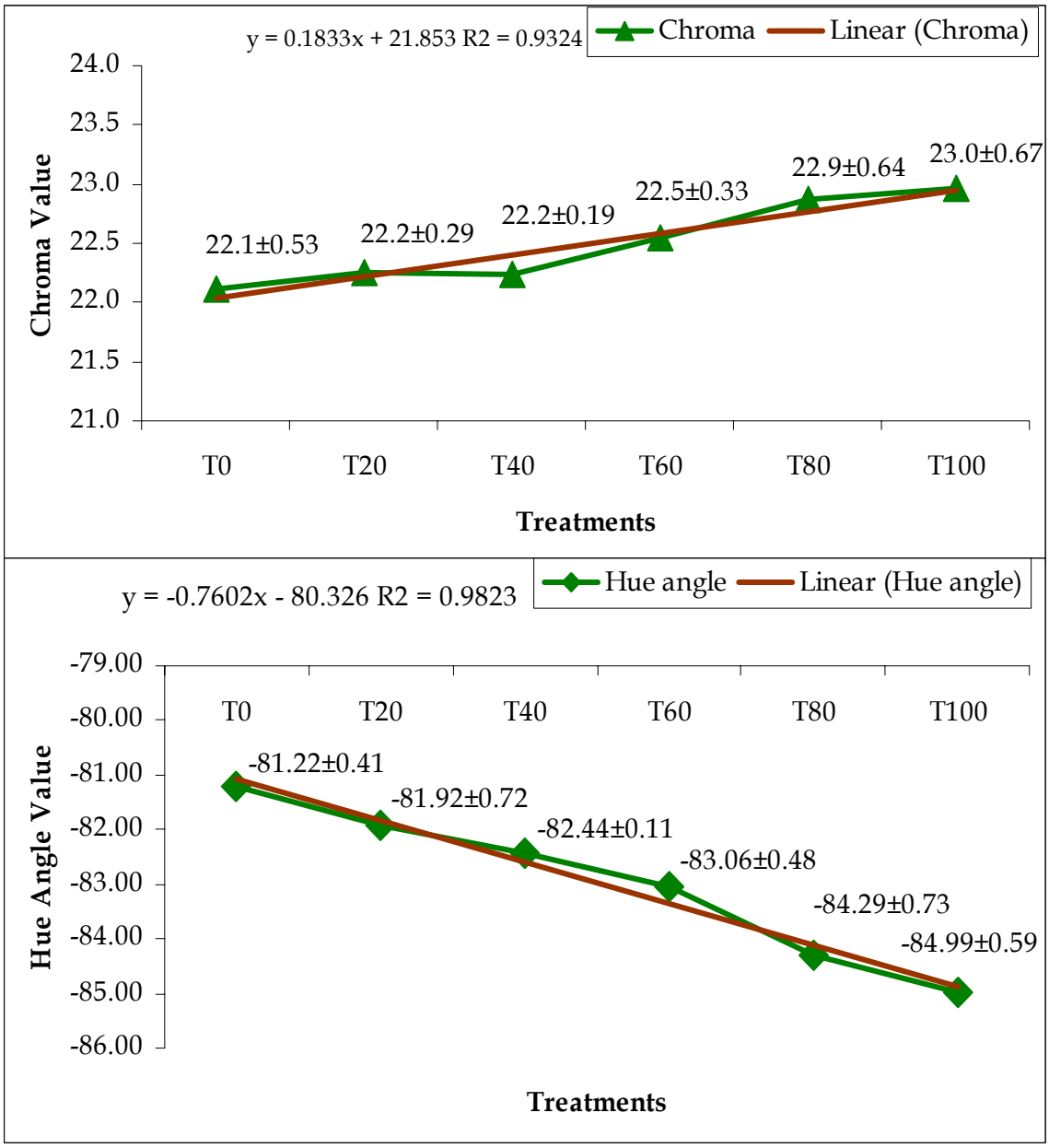

Figure 3. Chroma and hue angle values of MGO fortified cakes

Redness (“a”), hue angle, lightness (“L”) and yellowness (“b”) were significantly affected by MGO blended treatments whereas chroma was found to be non-significant. Mean values for color parameters i.e. “L”, “a” and “b” are illustrated in Figure 2.

It is evident from results that MGO addition resulted in decreased greenness (“-a”), lightness (“L”) and increased yellowish color (“b”) of cake crumb. The normal shortening cake was lighter in color, as depicted by higher “L” value, that decreased with MGO addition. Hunter color “b” values increased significantly with MGO addition in cake formulation, representing an increased yellowness of the cake crumb.

The values for chroma and hue angle also increased momentously by adding up of MGO in cakes’ formulation (Figure 3). Chroma and hue angle values ranged from 22.1 to 23.0 and -81.22 to -84.99, respectively These results clearly indicated that the addition of MGO significantly decreased lightness and increased yellowness of the crumb. The results are supported by the fact that MGO was pale yellow in color and the replacement of shortening with MGO imparted yellowish crumb color.

4.4.3. Sensory Evaluation

Mean squares for sensory evaluation scores of cakes prepared from various treatments are illustrated in Table 21. Sensory scores for overall quality and texture softness depicted significant variation with that of treatments, while cells uniformity, color, flavor and taste showed non-significant differences with treatments. The means for sensory scores are presented in Table 22.

Table 21. Mean squares for sensory scores of MGO fortified cakes

SOV	df	Cells uniformity	Texture softness	Color	Flavor	Taste	Overall quality
Treatments	6	6.5138889^{ns}	7.1555556*	1.2888889^{ns}	2.6888889^{ns}	2.0888889^{ns}	3.2472222*
Error	65	4.6780303	2.3964646	2.15152	2.3510101	3.1136364	1.0012626
Total	71						

* = *P≤ 0.05*
ns = Non-significant

Table 22. Sensory scores of MGO fortified cakes

Treatments	Crust color	Cells uniformity	Texture softness	Aroma	Taste	Overall quality
T_0	12.8±1.4	12.3±1.5	13.0±1.5a	11.5±1.4	11.3±1.7	11.1±1.1c
T_{20}	12.4±1.6	12.0±1.5	12.0±1.8abc	12.0±1.5	11.3±1.7	11.7±1.2bc
T_{40}	11.8±1.5	12.5±1.6	11.5±1.7bc	11.8±1.5	11.8±1.9	11.8±1.0abc
T_{60}	12.3±1.3	10.9±2.4	12.8±1.4ab	12.3±1.3	12.1±1.9	12.6±0.8a
T_{80}	12.3±1.1	11.1±2.6	11.6±1.2bc	12.8±1.5	11.7±1.6	12.2±1.0ab
T_{100}	12.2±1.7	10.8±2.9	11.0±1.7c	12.5±1.9	10.9±1.8	12.2±0.9ab

Means sharing the same letter in a column are not significantly different.

Overall quality increased with the level of MGO in cake formulation and significantly highest score (12.6±0.8) was recorded for T_{60} (60% MGO) that non-significantly differed from 80% and 100% MGO fortified cakes. Lowest scores (11.1±1.1) for overall acceptability were assigned to control cakes; improved non-significantly up to 40% MGO addition. Scores for texture softness decreased with MGO blending levels from 13.0±1.5 to 11.0±1.7. The scores for texture softness are supported by the objective data for texture hardness, as there is an inverse correlation between these traits.

The ranges for crust color, cells uniformity, aroma and taste were 12.2±1.7 to 12.8±1.4, 10.8±2.9 to 12.3±1.5, 11.5±1.4 to 12.8±1.5 and 10.9±1.8 to 12.1±1.9, respectively. It can be noted that T_{60} (60% MGO) was awarded maximum scores for overall quality and taste, the two most important parameters. It is concluded that MGO blending with normal shortening in cake formulation improved the sensoric and physical attributes of finished product. In some other studies normal shortening was also successfully replaced with oils to improve the nutritional quality of bakery products e.g. wheat germ oil, rice bran oil and sunflower oil (Anjum *et al.,* 2008; Sharif *et al.,* 2003; Jacob and Leelavathi, 2007).

Edible oils are vital, serving as important ingredient of many foods by imparting characteristic flavor and texture to finished food products (Rudan-Tasic and Klofutar, 1999). Maize germ oil contains 30% monounsaturated fatty acids and 56% polyunsaturated fatty acids (EFSA, 2005); can play a vital role in the diet being a rich source of essential fatty acids that help to regulate cholesterol and blood pressure (Hauman, 1985; Dupont *et al.,* 1990).

Consequently, maize germ oil incorporation in cake formulation potentially satisfies the requirements for essential fatty acids (CRA, 2006). Maize germ oil is amongst the rich sources of tocopherol, preferably retained in the plasma as compared to other members of vitamin E. Tocopherols and

tocotrienols are also helpful in cholesterol lowering or preventing cardiovascular maladies and oxidative stress (Lloyd *et. al.,* 2000; Birringer *et al.,* 2002; Ricciarelli *et al.,* 2001). Thus, MGO incorporation in cake recipe can be helpful for the improvement of end product quality.

4.5. Chemical Composition of DMG Flour

Defatted maize germ (DMG) portion, left after oil extraction from selected hybrid (Pioneer-32-F-10), was processed to produce food grade meal and evaluated for its proximate composition, dietary fiber and selected minerals. Mean values for chemical composition of DMG flour are given in Table 23.

The proximate composition of DMG flour was: 32.19±0.84% crude protein, 1.26±0.11% crude fat, 4.32±0.59% crude fiber, 5.49±0.61% ash, and 56.74±0.55% NFE. The analysis revealed that DMG flour was excellent source of nutrients especially protein (32.19±0.84%) and dietary fiber (31.87±2.98%). DMG flour showed potential for macro-minerals like phosphorus (2.15±0.14 g/100g), potassium (1.96±0.16 g/100g) and magnesium (0.89±0.10 g/100), and micro-minerals i.e. iron (17.46±1.12 mg/100g).

These findings are consistent with previously reported results for DMG flour; 24.8-31.7% protein, 0.3-1.7% crude fat and 3.3-6.9% crude fiber and 3.79-8.42% ash (Nielsen *et al.,* 1979; Barbieri and Casiraghi, 1983). Many other researchers have reported the nutrient-dense properties of DMG flour: balanced proteins, fiber and minerals (Lawton and Wilson, 2003; Zayas and Lin, 1989; Johnston and Singh, 2004). Inglett and Blessin (1979) investigated the chemical composition of Defatted maize germ flour and concluded that it contained 2.74% phosphorus, 2.36% potassium, 1.02% magnesium, 15 mg/100g calcium and 20 mg/100g iron.

Table 23. Chemical composition of Defatted maize germ flour

Parameters	Values	Parameters	Values
Crude protein (%)	32.19±0.84	Phosphorus (g/100g)	2.15±0.14
Crude fat (%)	1.26±0.11	Potassium (g/100g)	1.96±0.16
Crude fiber (%)	4.32±0.59	Magnesium (g/100g)	0.89±0.10
Ash (%)	5.49±0.61	Calcium (mg/100g)	13.98±0.78
NFE (%)	56.74±0.55	Iron (mg/100g)	17.46±1.12
Dietary fiber (%)	31.87±2.98		

In an earlier study, commercial dry-milled defatted germ samples were evaluated for mineral composition; contained significant amounts of potassium, phosphorus, magnesium and iron (Garcia *et al.,* 1972) that are in conformity with the present results.

The results for higher dietary fiber of defatted maize germ in present study are supported by Ruan (2004); stated that lightly toasted full-fat maize germ contains 26% dietary fiber. Dietary fiber in food represents the portion of diet which is indigestible by enzymes in gastrointestinal tract of humans (Bermink, 1994), and remains to be important component of diet with lot of allied health benefits. Cereals such as wheat, corn and oats have traditionally been used to uplift the fiber content of food products (McKee and Latner, 2000).

Overall, Defatted maize germ flour possessed good nutritional profile that explicit its potential to be used in food preparations as a functional ingredient.

4.6. Efficacy Studies

Considering, the nutritional potential of defatted maize germ (DMG) meal, as it is evident from its chemical composition; it was evaluated for *in-vivo* protein quality and safety in experimental male Sprague Dawley rats.

4.6.1. Protein Quality

Protein in diet, supplies different proportions of amino acids; required for growth and maintenance. Mean squares for the protein quality parameters; true digestibility (TD), net protein utilization (NPU), biological value (BV), net protein ratio (NPR) and protein efficiency ratio (PER) are presented in Table 24. All the quality traits were significantly affected by the test diets. Protein quality parameters are illustrated in Figure 4 and 5.

It is evident from the results that casein diet caused significantly higher values for all the parameters followed by Defatted maize germ flour, while the

lowest values were recorded for wheat based diet. DMG flour diet resulted in 87.10±0.78% TD, 76.70±1.25% NPU, 88.06±o.67% BV, 5.12±0.21 NPR and 2.15±0.03 PER in Sprague Dawley (SD) rats, that were significantly higher than that of wheat based diet (74.17±0.75% TD, 47.39±1.12% NPU, 63.89±1.34% BV, 3.62±0.19 NPR and 1.41±0.04 PER). DMG flour diet resulted in lower but comparable values for protein quality as compared to casein.

With respect to essential amino acids, casein has balanced profile and is considered as a standard against which other proteins are evaluated. Protein quality values are also supported by the results of amino acid profile of DMG flour as discussed in previous section. Wheat bears poor quality proteins as it is deficient in essential amino acids especially lysine (Anjum *et al.,* 2005); resulted in lower values for protein quality parameters.

These findings are also in harmony with earlier findings of Gupta and Eggum (1998) who reported 87.10% BV, 75.60% NPU, and 86.80% TD. They also mentioned that DMG flour has quality protein with balanced amino acid profile, comparable to casein and other standard proteins. Pogna *et al.* (1994) stated that the nutritional quality of wheat can be improved through supplementation of limiting amino acids especially lysine.

Keeping in view the importance of germ, Arshad *et al.* (2007) evaluated wheat flour supplemented with defatted wheat germ (DWG) for biological assay compared to casein; reported progressive improvement in protein quality parameters with DWG supplementation in wheat flour. Maize germ protein has balanced amino acid composition, exhibiting conformity with FAO/WHO standards (Kulakova *et al.,* 1982; Barbieri and Casiraghi, 1983).

Table 24. Mean squares for *in-vivo* protein quality parameters of test diets

SOV	df	TD	NPU	BV	NPR	PER
Diets	2	291.86333**	1289.8744**	746.02333**	3.0763444**	0.9820778**
Error	6	0.6922222	1.4111111	1.8422222	0.0281111	0.0012111
Total	8					

** = *P≤ 0.01*

TD = Total digestibility
NPU = Net protein utilization
BV = Biological value
NPR = Net protein ratio
PER = Protein efficiency ratio

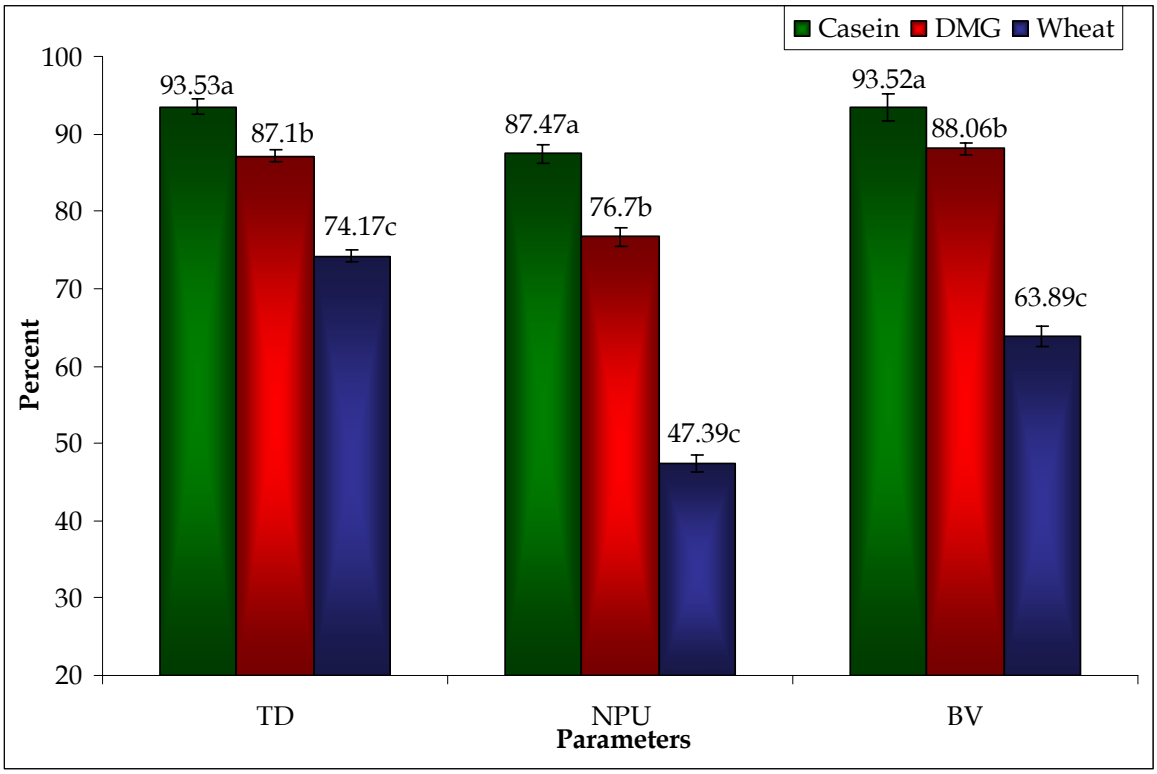

Figure 4. *In-vivo* True digestibility, net protein utilization and biological value of test diets

Mean bars sharing the same letter for individual parameter are not significantly different.

TD = True digestibility
NPU = Net protein utilization
BV = Biological value

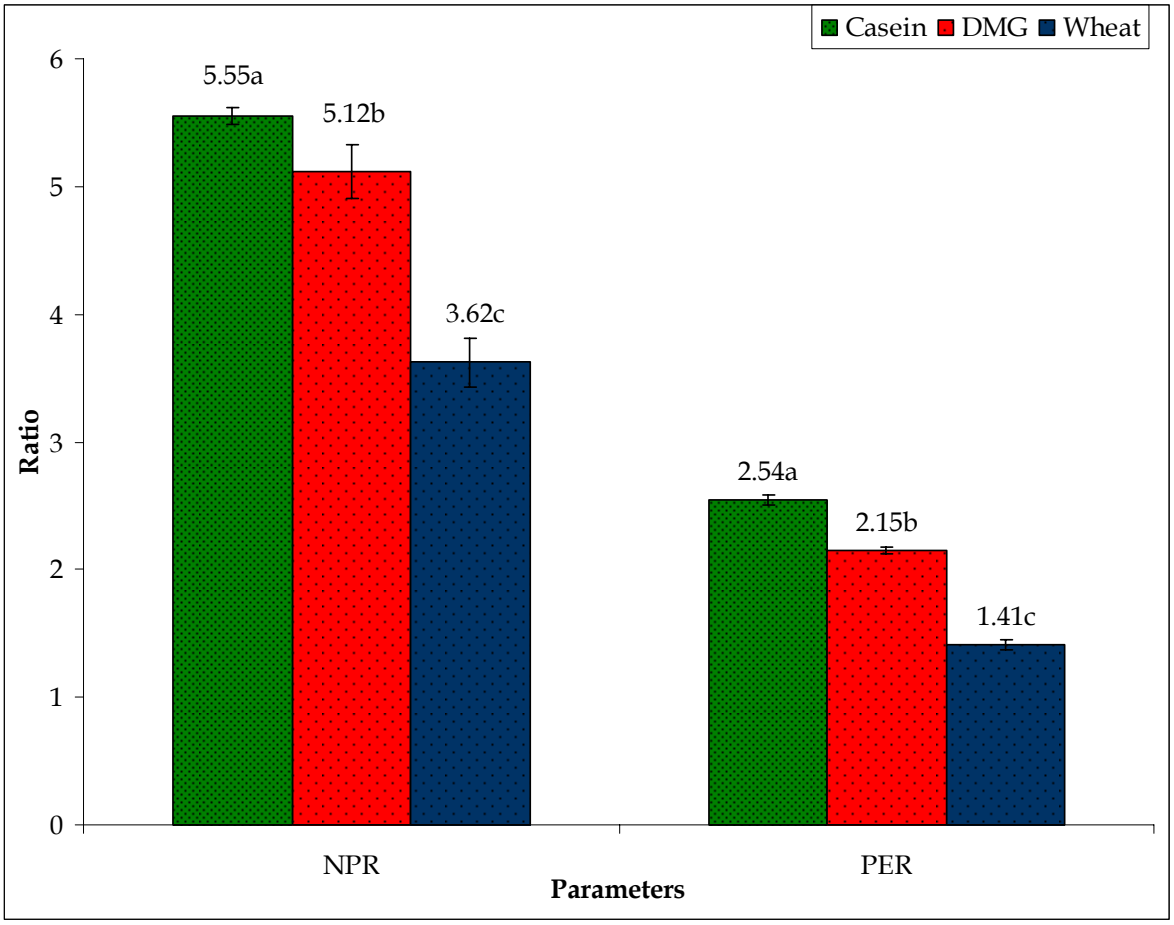

Figure 5. *In-vivo* net protein ratio and protein efficiency ratio of test diets

Mean bars sharing the same letter for individul parameter are not significantly different.

NPR = Net protein ratio
PER = Protein efficiency ratio

Kulakova *et al. (*1983) evaluated germ protein and concluded that the total

protein and its basic alkali-soluble fractions were comparable to egg albumin and casein. The albumin and globulin fractions constitute the major part of the germ protein. Good quality protein in DMG flour depicts its potential to be used in array of food formulation for human consumption (Gupta and Eggum, 1998; Lawton and Wilson, 2003).

4.6.2. Safety Evaluation

Evaluation through animal modeling to asses the safety of potential non-conventional food ingredients is imperative before they are incorporated in food formulations. Safety evaluation of DMG flour was accomplished through efficacy studies in Sprague Dawley (SD) rats to determine the impact of test diets on serum biochemistry and organ weights.

4.6.2.1. Serum Proteins

Mean squares for serum protein chemistry are given in Table 25; showed no momentous effect of treatments and study period on serum protein and allied fractions. A steady trend in rats was observed in case of serum total proteins, albumin, globulin and albumin/globulin ratio fed on DMG and basal diets throughout the study period (Table 26).

The results for serum total protein, albumin, globulin and A/G ratio of rats fed on basal and DMG flour diets ranged from 6.19±0.42 to 6.58±0.35, 3.15±0.14 to 3.32±0.23, 3.02±0.12 to 3.15±0.14 and 1.02±0.08 to 1.05±0.11 g/dL, respectively. The results of present investigation are in agreement with the experimental results performed by Zhou and Han (2006) through parallel trials on male and female SD rats, to evaluate the safety of protein. They observed protein values ranging from 65.8±3.3 to 67.2±4.6 g/L and A/G ratio of 0.98±0.05 to 1.07±0.11 during a thirty day feeding trial.

Table 25. Mean squares for serum protein chemistry

SOV	df	Protein	Albumin	Globulin	A/G ratio
Diets	1	0.1606008^{ns}	0.01012^{ns}	0.0241968 ^{ns}	0.00076003^{ns}
Period (days)	2	0.1538068^{ns}	0.0457401^{ns}	0.0141584 ^{ns}	0.0011152^{ns}
Interaction (diets x days)	2	0.0011001^{ns}	0.0000801^{ns}	0.0030817 ^{ns}	0.000265^{ns}
Error	24	0.1404812	0.0364994	0.0336679	0.0038574
Total	29				

ns = Non-significant

Table 26. Serum protein chemistry

Parameter	Diet	Study Period			
		15 days	30 Days	45 Days	Mean
Protein (g/dL)	Basal	6.19±0.42	6.25±0.39	6.41±0.42	6.28
	DMG	6.32±0.51	6.39±0.46	6.58±0.35	6.43
Albumin (g/dL)	Basal	3.15±0.14	3.21±0.17	3.29±0.13	3.22
	DMG	3.19±0.17	3.25±0.16	3.32±0.23	3.25
Globulin (g/dL)	Basal	3.02±0.12	3.07±0.07	3.13±0.09	3.07
	DMG	3.11±0.08	3.13±0.16	3.15±0.14	3.13
A/G Ratio	Basal	1.04±0.03	1.05±0.09	1.05±0.08	1.05
	DMG	1.02±0.08	1.04±0.1	1.05±0.11	1.04

Petterino and Argentino-Storino (2006) also reported similar findings while feeding SD rats with controlled diet for a period of thirteen weeks to establish a clinical chemistry reference data for researchers and regulatory agencies.

4.6.2.2. Urea and Creatinine

Mean squares for serum urea and creatinine concentration are mentioned in Table 27. Moreover, their respective mean values are presented in Table 28. Results for serum concentration of urea and creatinine were non-significant with respect to diets and study period.

The serum values for urea and creatinine in rats fed on basal and DMG flour diets for a period of 45 days ranged from 13.84±1.48 to 14.10±1.12 mmol/L and 0.32±0.03 to 0.34±0.03 mg/dL, repectively.

Petterino and Argentino-Storino (2006) also observed similar values for these parameters while conducting pre-clinical toxicity study with Sprague Dawley (SD) rats. Malley *et al.* (2007) observed non-significant pattern for creatinine values, ranging from 0.39-0.43 mg/dL for female rats and 0.32-0.33 mg/dL for male rats, while studying sub-choronic feeding of transgenic maize in SD rats. Kidney function analysis was also performed by Farag *et al.* (2006) through creatinine determination during the study.

4.6.2.3. Liver Enzymes

Statistical results depicted non-significant differences with test diets, whereas study period showed significant impact on serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), while non-significant effect on alkaline phosphatase (ALP). Mean values of AST, ALT and ALP of rats fed on DMG flour and basal diets are presented in Table 28.

Increased enzymes concentrations were noted from commencement to the end of study from 40.14 to 46.265, 87.04 to 94.65 and 147.77 to 151.91 U/L, for

ALT, AST and ALP, respectively. The possible reason for this uplift might be due to aging of rats with the study period. Nevertheless, all the values were within safe limits.

He *et al.* (2008), while comparing two types of maize grains through 90 days trial on Sparague Dawley (SD) rats observed a range of 41.50±4.79 to 55.80±10.03 and 80.60±13.04 to 105.9±28.90 U/L for ALT and ALP, respectively. The results remained non-significant within the control and test group. Similar trends were also observed by MacKenzie *et al.* (2007) while, testing safety of transgenic maize grain using SD rats. They reported AST values within the range of 86±12-101±27 U/L and ALP from 91±19 to 112±25 U/L.

4.6.2.4. Serum Lipid Profile and Glucose

Mean squares for serum lipid profile and glucose depicted that cholesterol, triglycerides and LDL were significantly affected with test diets whereas HDL and glucose were non-significantly different with respect to diets and study period. Mean square values for lipid profile and glucose concentrations are presented in Table 29.

DMG flour diet showed favorable impact on serum lipid profile in SD rats. Cholesterol, triglyceride and LDL concentration decreased up to 6.80, 12.45 and 16.19%, respectively with DMG flour diet compared to that of basal diet (Figure 6). Likewise, serum glucose also decreased 6.50% after 45 days of DMG feeding. Good cholesterol (HDL) content in serum increased (5.55%) with DMG diet compared to control diet at the end of trial (Figure 7). In the present study, serum glucose concentration in rats fed on DMG diet decreased from 114.60±1.60 to 107.78±5.60 mg/dL.

Table 27. Mean squares for serum kidney and liver function tests

SOV	df	Urea	Creatinine	ALT	AST	ALP
Diets	1	0.0188501^{ns}	0.00034^{ns}	0.2255067^{ns}	47.37382^{ns}	31.51465^{ns}
Period (days)	2	0.1267671^{ns}	0.0000585^{ns}	93.649997**	145.37894*	43.413632^{ns}
Interaction (dietsxdays)	2	0.0022202^{ns}	0.0000852^{ns}	4.3433668^{ns}	5.513575^{ns}	2.397665
Error	24	0.6926853	0.000395	6.2931485	29.009699	78.399746
Total	29					

* = *P≤ 0.05*
** = *P≤ 0.01*
ns = Non-significant

ALT = Alanine aminotransferase
AST = Aspartate aminotransferase
ALP = Alkaline phosphatase

Table 28. Serum kidney and liver function tests

Parameter	Diet	Study Period			
		15 days	30 Days	45 Days	Mean
Urea (mmol/L)	Basal	13.89±1.01	14.03±1.56	14.10±1.12	14.01
	DMG	13.84±1.48	13.95±1.90	14.08±1.81	13.96
Creatinine (mg/dL)	Basal	0.33±0.03	0.34±0.02	0.34±0.03	0.34
	DMG	0.32±0.03	0.33±0.04	0.34±0.03	0.33
ALT (U/L)	Basal	40.95±2.89	42.78±3.94	46.21±3.23	43.31
	DMG	39.34±2.23	43.76±3.84	46.32±3.11	43.14
	Mean	40.15c	43.27b	46.27a	
AST (U/L)	Basal	88.31±6.05	93.33±7.63	95.16±9.06	92.27
	DMG	85.78±8.47	89.34±6.07	94.14±8.34	89.75
	Mean	87.05b	91.34ab	94.65a	
ALP (U/L)	Basal	146.33±7.32	148.32±9.49	151.43±12.57	148.69
	DMG	149.21±9.05	150.62±10.70	152.40±9.23	150.75

Means sharing the same letter in a row are not significantly different.

Table 29. Mean squares for serum lipid profile and glucose

SOV	df	Cholesterol	Triglyceride	HDL	LDL	Glucose
Diets	1	222.7524*	969.57675**	21.0841^{ns}	176.671**	188.993^{ns}
Period (days)	2	29.16951^{ns}	89.98675^{ns}	0.17396^{ns}	14.9142**	93.9316^{ns}
Interaction (diets x days)	2	2.1791701^{ns}	24.36175^{ns}	0.65108^{ns}	1.4005^{ns}	24.4912^{ns}
Error	24	32.807026	48.677642	5.12038	4.30158	47.7322
Total	29					

* = *P≤ 0.05*
** = *P≤ 0.01*
ns = Non-significant

HDL = High density lipoprotein
LDL = Low density lipoprotein

Table 30. Serum lipid profile and glucose

Parameter	Diet	Study Period			
		15 days	30 Days	45 Days	Mean
Cholesterol (mg/dL)	Basal	98.00±3.02	97.66±2.04	95.70±2.72	97.12a
	DMG	93.25±2.11	92.57±1.30	89.19±0.79	91.67b
Triglyceride (mg/dL)	Basal	121.00±4.03	119.40±0.87	118.10±0.13	119.50a
	DMG	112.49±2.50	108.50±2.43	103.40±6.34	108.13b
HDL (mg/dL)	Basal	37.77±0.31	37.15±0.60	37.26±0.15	37.39
	DMG	38.87±0.89	39.01±1.23	39.33±1.25	39.07
LDL (mg/dL)	Basal	36.03±1.13	36.63±0.78	34.82±0.83	35.83a
	DMG	31.88±1.42	31.86±0.31	29.18±0.77	30.97b
	Mean	33.96a	34.25a	32.00b	
Glucose (mg/dL)	Basal	118.10±1.90	115.82±1.90	115.27±2.10	116.40
	DMG	114.60±1.60	109.75±2.10	107.78±5.60	110.71

Means sharing the same letter in a column or row are not significantly different.

The ranges for triglycerides, cholesterol, and LDL were from 103.40±6.34 to 121.00±4.03 mg/dL, 89.19±0.79 to 98.00±3.02 mg/dL and 29.18±0.77-36.03±1.13 mg/dL, respectively. Nagaoka *et al.* (1991), while studying the effect of dietary protein on plasma and liver lipids in experimental rats, concluded that casein and whey proteins had a propensity for increase in HDL contrary to soy proteins. Serum HDL levels of 41 and 42.54 mg/dL have been reported in rats by Khosla *et al.* (1995) and Rehman *et al.* (2001).

Cholesterol has the ability to deposit in blood channels thus narrowing them which may lead to plaque development. The cholesterol itself is not so critical as are the lipoproteins; cholesterol transport facilitators. High density lipoproteins are more efficient in transporting blood cholesterol compared to low density lipoprotein (Awan 1993; Pitt *et al.,* 1999). Increased level of HDL has the tendency to lower cholesterol by transporting it to liver where it is metabolized. Decreased total cholesterol and increased HDL/LDL ratio are indicatives of improved serum lipid profile (Pedersen *et al.,* 1998; Robins *et al.,* 2001; Butt *et al.,* 2007). In the present study, improvement in serum lipid chemistry possibly is due to higher dietary fiber content in DMG flour diet as compared to basal diet.

4.6.2.5. Serum Electrolytes

Mean squares for serum electrolytes (Table 31) depicted non-significant differences in sodium and potassium due to test diets and study period on, while calcium was significantly affected by the sources of variance (study period).

Serum calcium content improved with study period in SD rats, while serum sodium and potassium electrolytes showed non-momentous trend (Table 32). Sodium, potassium and calcium ranged from 146.03±0.50 to 149.50±1.50 mmol/L, 6.15±0.27 to 6.42±0.45 mmol/L and 8.49±0.20 to 9.64±0.36 mg/dL, respectively.

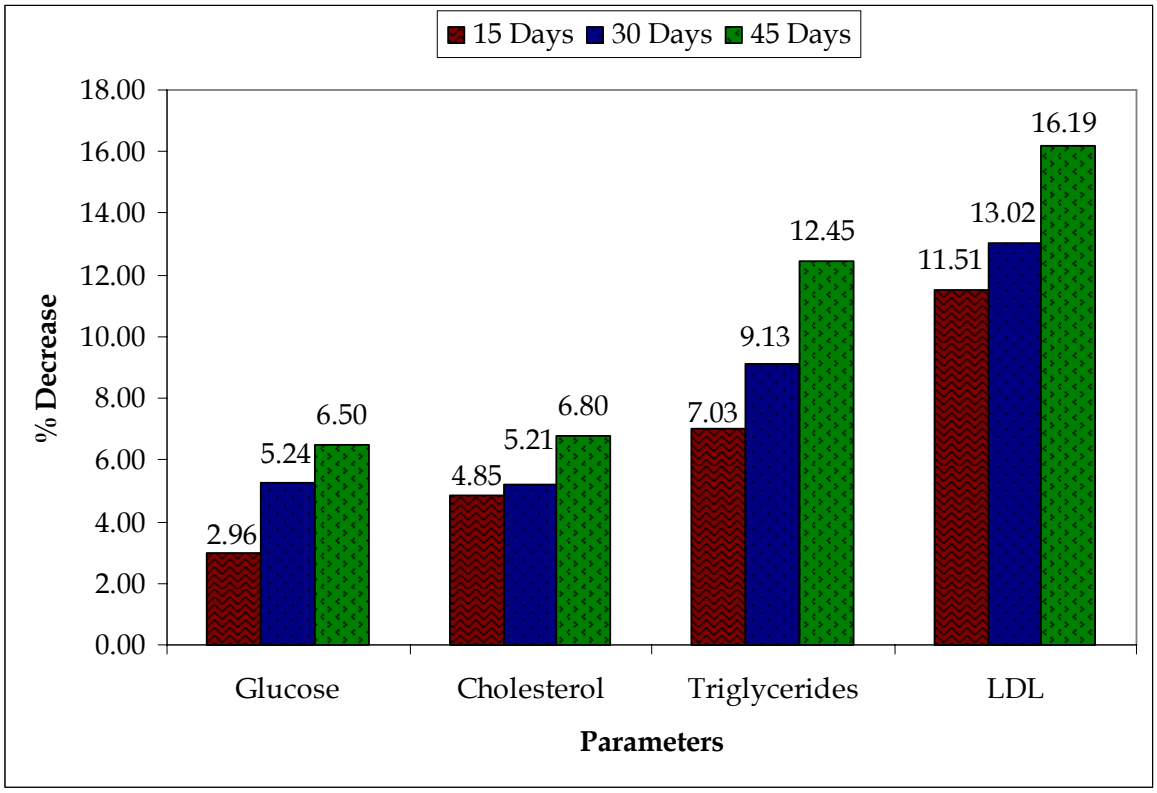

Figure 6. Percent decrease of glucose, cholesterol, triglycerides and LDL in DMG compared to basal fed rats.

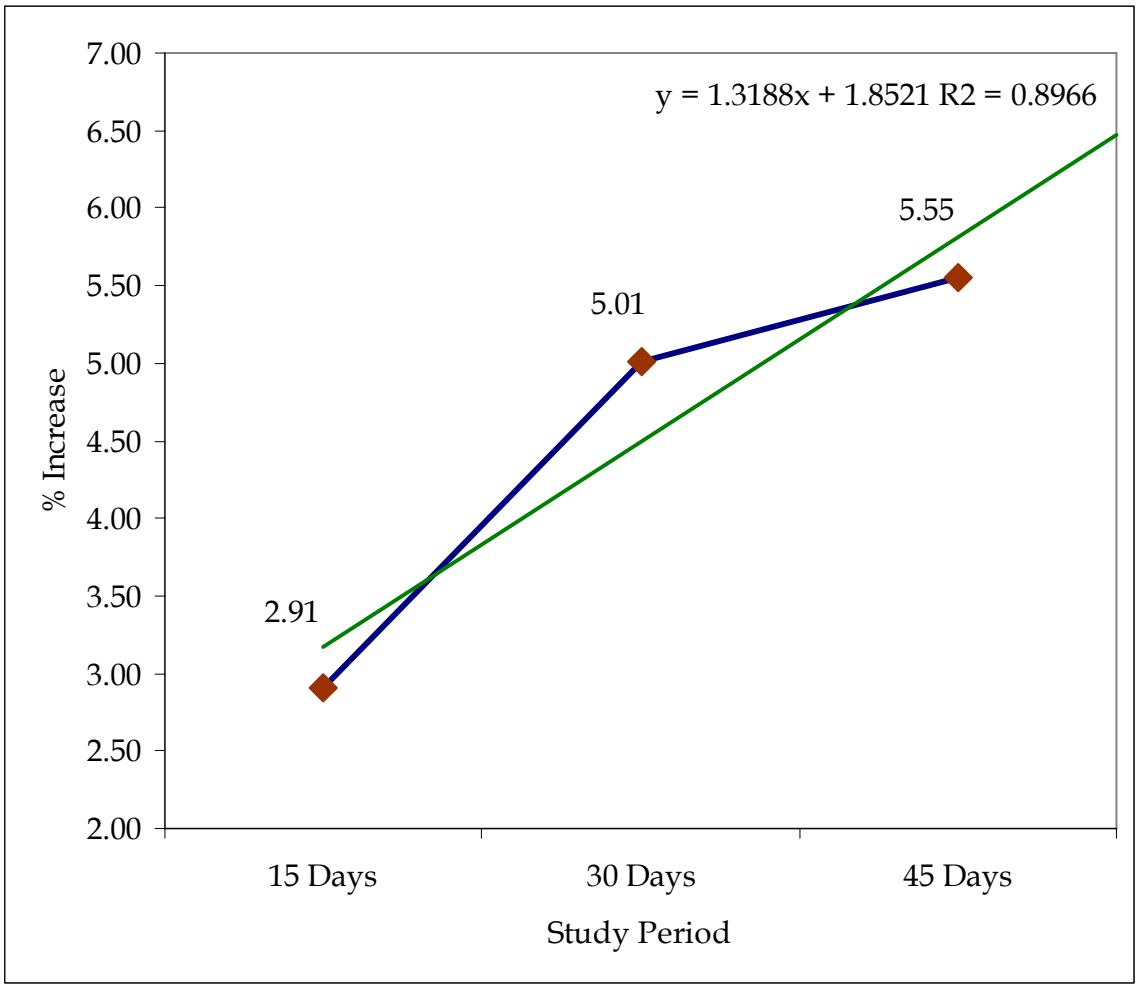

Figure 7. Percent increase of HDL in DMG compared to basal fed rats

Sodium, potassium and calcium are the main electrolytes/cations found in serum. Any fluctuation in the electrolyte balance can be an indicator of some metabolic turmoil. The results for serum electrolytes are in accordance with those reported by Malley *et al.* (2007) in a sub-chronic feeding study of maize grain in SD rats. Appenzeller *et al.* (2008) also conducted sub-chronic feeding study of soybean in rats and reported 10.7 to 11.0 mg/dL, 146.5 to 148.7 mmol/L and 6.01-6.70 mmol/L of calcium, sodium and potassium, respectively. Hagiwara *et al.* (2003) also presented blood biochemistry data in SD rats fed on diet containing annatto for 13 weeks and reported calcium concentration from 9.19±0.33 to 9.53±0.27 mg/dL and non-significant differences in sodium & potassium concentration.

4.6.2.6. Organ Weights

Organ weights including liver, heart, kidney, spleen and intestine were not affected with diets, study period and their interaction (Table 33). The only exception was intestine length, which varied momentously with diets and study period. The results for organ weights of rats fed are shown in (Table 34).

Mean intestine length (0.934 m) of rats fed on DMG flour was significantly higher than those fed on basal diet (0.885 m). Mean intestine length of rats after 15 days of study period was 0.881 m that significantly increased to 0.954 m at the end of study. The ranges for organ weights were: liver 2.824±0.027 to 3.036±0.075 g, heart 0.370±0.020 to 0.387±0.010 g, kidney 0.684±0.003 to 0.733±0.044 g, spleen 0.158±0.006 to 0.175±0.006 g, intestine weight 7.256±0.110 to 7.788±0.166 g.

The results of present investigation are closely associated with Appenzeller *et al.* (2008); reported liver, heart, kidneys and spleen weights as 2.722±0.135 g, 0.321±0.020 g, 0.780±0.050 g and 0.146±0.015 g, respectively in male Sprague Dawley rats fed on control diet.

Table 31. Mean squares of serum electrolytes

SOV	df	Sodium	Potassium	Calcium
Diets	1	0.2609601^{ns}	0.0963333^{ns}	1.7234437^{ns}
Period (days)	2	0.5478^{ns}	0.0048001^{ns}	5.5057968**
Interaction (diets x days)	2	28.29626^{ns}	0.0463637^{ns}	0.2776368^{ns}
Error	12	67.140515	0.1874145	1.1842997
Total	17			

* = *P≤ 0.05*
** = *P≤ 0.01*
ns = Non-significant

Table 32. Serum electrolytes

Parameter	Diets	Study period			
		15 days	30 Days	45 Days	Mean
Sodium (mmol/L)	Basal	147.65±2.35	146.03±0.50	149.50±1.50	138.73
	DMG	148.50±0.50	149.20±1.80	146.04±2.00	138.91
Potassium (mmol/L)	Basal	6.28±0.37	6.15±0.27	6.30±0.40	6.24
	DMG	6.30±0.10	6.42±0.45	6.35±1.05	6.36
Calcium (mg/dL)	Basal	8.49±0.20	9.14±0.35	9.64±0.36	9.09
	DMG	8.65±0.05	9.05±0.05	9.14±0.33	8.95
	Mean	8.57b	9.10a	9.39a	

Means sharing the same letter in a column or row are not significantly different.

Previously, in a research trial, Malley *et al.,* (2007) also reported similar findings for organ to body weight ratios of Sprague Dawley rats that differed non-significantly with test diets. The current organ to body weight ratios correlate with some previous studies conducted on experimental rats modeling (Hagiwara *et al.,* 2003; Jia *et al.,* 2005).

The increase in intestine length might be due to more fiber content in DMG flour diet. Fiber in diet increases food transit time in intestine thereby resulting in increased intestine length. An increase in intestine length (10%) has also been reported by Frias and Sgarbieri (1999) with fiber enriched diet. Johnson *et al.* (1984) also observed a significant increase in small intestinal length of rats fed on fiber supplemented diets.

Defatted maize germ (DMG) meal resulted in higher values for protein quality parameters as compared to wheat based diet in Sprague Dawley (SD) rats. In the experimental, the *in-vivo* protein quality of DMG flour was comparable with that of casein. Favorable influence of DMG flour was observed on serum biochemical profile like cholesterol, triglycerides, HDL, LDL, and glucose. The results for liver & kidney functioning tests and serum protein chemistry of DMG flour diet were non-significant as compared to basal diet and were within the safe limits. Serum calcium status improved with study period whereas sodium and potassium concentration were non-significant irrespective of diets and study period. Organ weights of rats fed on DMG and basal diet showed non-significant variations except for intestine length. It is concluded that Defatted maize germ flour possessed good quality proteins and bears a potential to be incorporated in food formulations for value-addition.

Table 33. Mean squares for organ weights of rats

SOV	df	liver	Heart	kidney	Spleen	Intestine weight	Intestine length
Diets	1	0.01555^{ns}	0.00114^{ns}	0.003224^{ns}	0.000074^{ns}	0.05633^{ns}	0.01845**
Period (days)	2	0.08199^{ns}	0.00000003^{ns}	0.000757^{ns}	0.0000420^{ns}	0.53568^{ns}	0.01549**
Interaction (diets x days)	2	0.00452^{ns}	0.0000394^{ns}	0.001542^{ns}	0.000020^{ns}	0.00384^{ns}	0.00022^{ns}
Error	24	0.03159	0.000510	0.0017958	0.000099	0.19666	0.00292
Total	29						

* = *P≤ 0.05*
** = *P≤ 0.01*
ns = Non-significant

Table 34. Organ weights of rats

Parameter	Diets	Study period			
		15 days	30 Days	45 Days	Mean
Liver (g/100g)	Basal	3.033±0.057	2.954±0.104	2.889±0.055	2.959
	DMG	3.036±0.075	2.879±0.077	2.824±0.027	2.913
Heart (g/100g)	Basal	0.387±0.010	0.384±0.034	0.383±0.002	0.385
	DMG	0.370±0.020	0.373±0.003	0.374±0.002	0.372
kidney (g/100g)	Basal	0.703±0.062	0.733±0.044	0.694±0.049	0.710
	DMG	0.696±0.056	0.684±0.003	0.688±0.007	0.689
Spleen (g/100g)	Basal	0.165±0.001	0.175±0.006	0.160±0.007	0.166
	DMG	0.164±0.007	0.169±0.006	0.158±0.006	0.164
Intestine weight (g/100g)	Basal	7.256±0.110	7.432±0.171	7.693±0.056	7.460
	DMG	7.300±0.081	7.553±0.042	7.788±0.166	7.547
Intestine length (m)	Basal	0.909±0.067	0.920±0.025	0.974±0.032	0.885b
	DMG	0.854±0.046	0.865±0.075	0.935±0.058	0.934a
	Mean	0.881b	0.893b	0.954a	

Means sharing the same letter in a column or a row are not significantly different.

4.7. Defatted Maize Germ-Wheat Flour Blends

Defatted maize germ (DMG) meal was blended with wheat flour at various levels and evaluated for physical, functional and rheological properties. The knowledge of these properties is essential for successful incorporation of novel or un-conventional ingredient in food preparations.

4.7.1. Hunter Color

Color depicts first impression for the acceptance of any product by consumers. Mean squares for Hunter color values of different treatments of DMGF-wheat flour blends along with control (T_0) are shown in Table 35. Color lightness ("L"), yellowness ("b") and chroma were significantly affected with treatments whereas, DMG flour level in flour blends did not show any significant impact on "a" and hue angle values.

The means for Hunter color parameters of all the treatments along with DMG flour are given in Table 36. DMG flour showed values of 54.85±0.35, 0.60±0.22, 11.43±0.15, -88.96±0.16 and 11.44±0.96 for color parameters of "L", "a", "b", hue angle and chroma, respectively. Wheat flour (T_0) was whiter than all other treatments as indicated by the higher "L" values (62.08±0.46) that decreased with DMG flour augmentation. However, lowest "L" value (58.53±0.13) was recorded for T_{25} (25% DMG flour). As compared to control (wheat flour), the DMG flour addition in the range of 5-25%, resulted increased yellow tint (higher "b" values in positive range), whereas "a" values (red-to-green) exhibited vague response.

Hue angle (h°), represented by the ratio of arctan (b/a), is a good indicator of changes in color by a single value. Hue angle is the attribute of color perception by means of which an object is judged to be red, yellow, green, blue or purple, while chroma is the attribute of color perception that expresses the degree of departure from the grey color of the same lightness (Little, 1975).

Table 35. Mean squares for Hunter color values of DMGF–wheat flour blends

SOV	df	"L"	"a"	"b"	Hue angle	Chroma
Treatments	5	10.199604**	0.0154167^{ns}	3.1844167**	3.1956742^{ns}	3.13564**
Error	18	0.1049653	0.1129167	0.0293056	8.8017569	0.0374
Total	23					

** = *P≤ 0.01*
ns = Non-significant

DMGF = Defatted maize germ flour

Table 36. Hunter color values of DMGF–wheat flour blends

Treatments	"L"	"a"	"b"	Hue angle	Chroma
T_0	62.08±0.46a	-0.7±0.45	5.33±0.15f	-82.64±4.73	5.38±0.19e
T_5	61.74±0.21ab	-0.8±0.18	6.18±0.19e	-82.60±1.83	6.23±0.17d
T_{10}	61.55±0.48b	-0.9±0.48	6.68±0.22d	-82.63±3.94	6.74±0.26c
T_{15}	59.20±0.27c	-0.7±0.31	6.95±0.24c	-84.10±2.37	6.99±0.26c
T_{20}	59.03±0.22c	-0.8±0.26	7.48±0.05b	-84.09±1.97	7.52±0.07b
T_{25}	58.53±0.13d	-0.8±0.21	7.78±0.10a	-84.50±1.45	7.81±0.11a
DMGF*	54.85±0.35	0.60±0.22	11.43±0.15	-88.96±0.16	11.44±0.96

Means sharing the same letter in a column are not significantly different.
*Data given for reference only; not compared with flour blends.

T_0 = All-purpose wheat flour
T_5 = DMGF 5%
T_{10} = DMGF 10%
T_{15} = DMGF 15%
T_{20} = DMGF 20%
T_{25} = DMGF 25%

The DMG flour had a hue angle of –88.96 indicating more yellowish in color compared to wheat flour (–82.51), which was also supported by higher chroma value (11.44) of DMG flour. Chroma values increased from 6.23 to 7.81, while hue angle was –82.62 to –84.49 up to 25% levels of DMG flour in flour blends, indicating more yellowish color.

4.7.2. Bulk Density

Mean square for bulk density of flour blends is expressed in Table 37. It can be seen that bulk density was significantly affected by treatments. Mean values of bulk density are graphically illustrated in (Figure 8).

Bulk density of DMG flour (0.43 g/mL) was significantly less than that of wheat flour (0.62 g/mL). The DMG flour addition (5-25%) resulted in decreased bulk density in flour blends. Overall, bulk density ranged from 0.55±0.01 to 0.62±0.001 in all flour blends. The lower bulk density in case of DMG flour can be attributed to defatting process which results in porous texture of the defatted product (Akpata and Akubor, 1999). Thus, DMG flour can potentially be beneficial for addition in many foods, especially weaning formulations where lower bulk density is a desirable factor.

4.7.3. Water and Oil Absorption Capacities

Water and oil absorption capacities are amongst the important functional properties for additives supplemented in food systems. Water and oil absorption were significantly affected with treatments (Table 37). The mean values for water and oil absorption capacities are shown in Table 38. DMG flour showed excellent water holding property (363.3±5.43%) with 427% higher water absorption capacity as compared to wheat flour (85.0±1.70%).

Table 37. Mean squares for bulk density, water and oil absorption of DMGF-wheat flour blends

SOV	df	Bulk density	Water absorption capacity	Oil absorption capacity
Treatments	5	0.0027167**	1469.7024**	484.76716**
Error	12	0.00010556	8.4450889	10.658033
Total	17			

** = *P≤ 0.01*

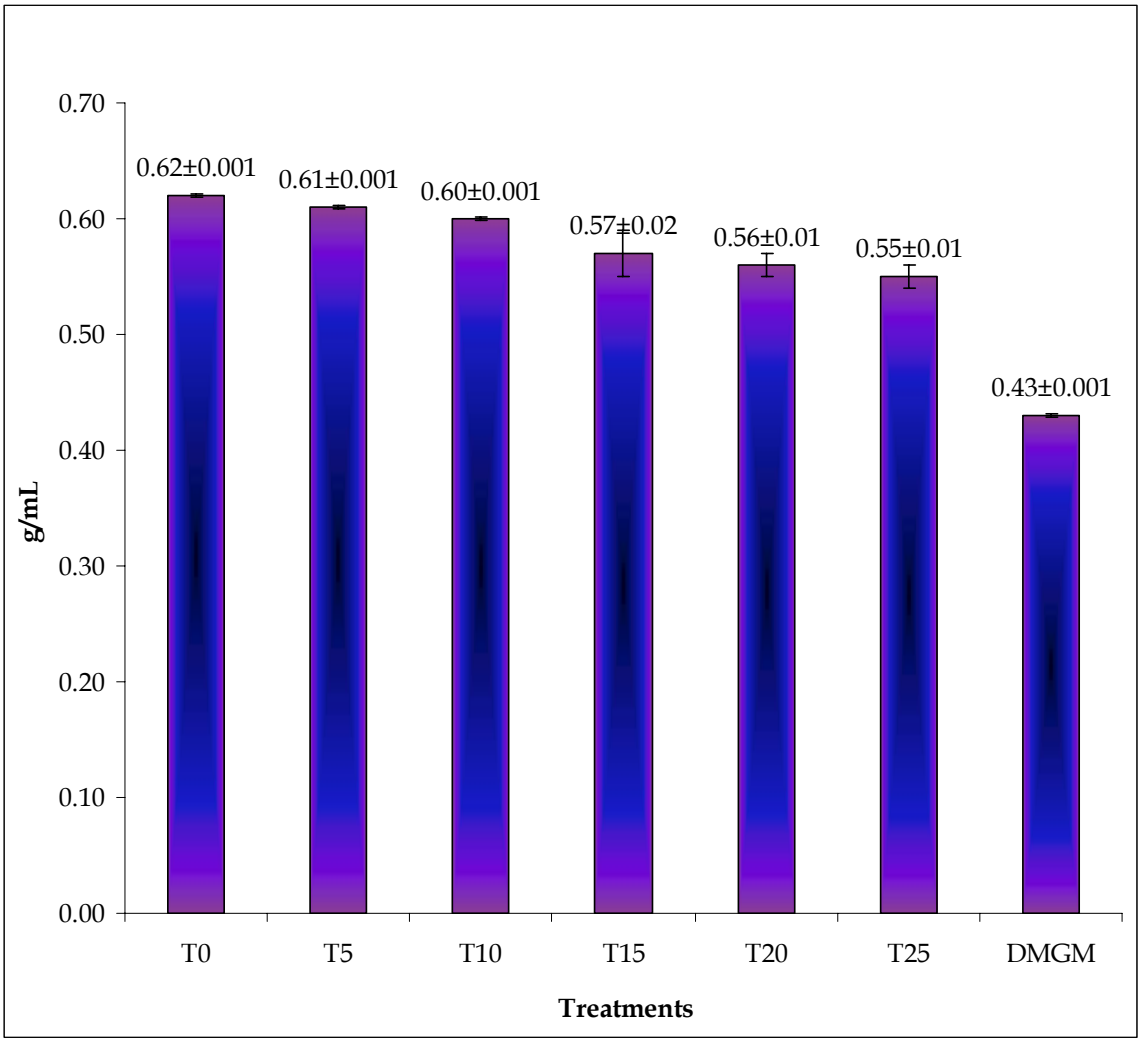

Figure 8. Bulk density of DMGF-wheat flour blends

The oil absorption capacity of DMG flour was noted to be 180.0±4.73% that was more than twice than that of wheat flour (T_0). Therefore water and oil absorption capacities of flour blends were improved at all levels of DMG flour addition. The water absorption capacity increased from 85.0±1.7% to 143.3±3.34% up to 25% DMG flour (T_{25}) level in flour blends, while oil absorption capacity ranged from 75.0±1.70% for control to 111.7±1.65% for T_{25}.

Higher degree of water absorption in DMG flour versus wheat flour could be attributed to higher protein content (Zayas and Lin, 1989), and probably the germ defatting process itself. The results regarding water absorption capacity depicted that DMG flour-added blends can be useful in bakery products to improve dough handling properties.

The amount of water present in processed products will depend on the extent to which the dry ingredients absorb or adsorb water under various environmental conditions (Phillips and Sternberg, 1979). Nielsen *et al.* (1979) reported water-binding capacity of 420-690% for corn germ meal. Maize germ protein flour, rich in starch, has also been reported to stabilize emulsions by absorbing or binding excess water, enabling more water to be added (Bhattacharya and Hanna, 1985; Luallen, 1985).

Oil absorption capacity also increased significantly with increasing levels of DMG flour in the flour blends. Present results for oil absorption capacity of DMG flour are in close agreement to those reported previously (Lin and Zayas, 1987; Huang and Zayas, 1991), in sausage batters with added maize germ protein flours. They reported increased water-holding capacity and decreased cooking losses of sausage batters supplemented with maize germ protein flours, most likely by binding fat and water to increase product yields. Owing to these characteristics, DMG flour can be introduced in food formulations, where an improvement in water and oil holding capacities is required.

Table 38. Water and oil absorption capacities of DMGF–wheat flour blends

Treatments	Water absorption capacity (%)	Oil absorption capacity (%)
T_0	85.0±1.70f	75.0±1.70e
T_5	96.7±3.35e	90.0±3.30d
T_{10}	105.0±1.70d	96.7±3.35c
T_{15}	113.3±3.35c	101.7±5.00bc
T_{20}	133.3±3.35b	103.3±3.35bc
T_{25}	143.3±3.34a	111.7±1.65a
DMGF*	363.3±5.43	180.0±4.73

Means sharing the same letter in a column are not significantly different.
*Data given for reference only; not compared with flour blends

4.7.4. Emulsion Properties

Emulsifying properties of an un-conventional food are important to assess its potential use in different food formulation as a functional or stabilizing agent. Statistical analysis indicated that there were significant variations among different flour blend samples regarding emulsion capacity and stability (Table 39). Mean values of flour blends along with DMG flour are indicated in (Table 40). The DMG flour was shown to possess 63.6±1.21% emulsion capacity with 58.5±0.98% emulsion stability.

The emulsion capacity of wheat flour (50.0±0.80%) increased gradually by the addition of DMG flour; nevertheless, this increase was significant at 10% or more DMG levels. It was observed, as compared to control (wheat flour), the emulsion stability of flour blends increased significantly at all DMG flour levels. Emulsion capacity ranged from 50.0±0.80 to 54.7±0.60% whereas, the range for emulsion stability was recorded from 47.4±0.70 to 51.6±1.25%.

These results for emulsifying properties corroborated to those reported for DMG flour previously (Lin and Zayas, 1987a). Maize germ proteins have been reported to improve not only emulsifying capacity but also emulsion stability which in turn improve texture (Huang and Zayas, 1991) by forming stable gels. The relatively higher emulsion properties of DMG flour could be attributed to high soluble protein content (Lawton and Wilson, 2003) that form the protective barrier around fat droplets thus preventing their coalescence (Kinsella, 1976). The DMG flour, with high emulsion activity and stability, has a potential to be used as an ingredient in processed bakery and meat products, and as a stabilizing agent in the colloidal foods, thus having potential importance in industrial processing.

Table 39. Mean squares for emulsion and foaming properties of DMGF–wheat flour blends

SOV	df	Emulsion capacity	Emulsion stability	Foaming capacity	Foaming stability
Treatments	5	9.857**	6.837**	35.772486**	127.87325**
Error	12	1.2125	0.7858333	0.8671833	3.0546667
Total	17				

** = *P≤ 0.01*

Table 40. Emulsion properties of DMGF–wheat flour blends.

Treatments	Emulsion capacity (%)	Emulsion stability (%)
T_0	50.0±0.80c	47.4±0.70c
T_5	51.6±1.30bc	49.3±0.70b
T_{10}	53.4±1.55ab	50.0±0.20ab
T_{15}	53.8±0.60a	50.6±1.15ab
T_{20}	54.4±1.35a	51.2±0.90a
T_{25}	54.7±0.60a	51.6±1.25a
DMGF*	63.6±1.21	58.5±0.98

Means sharing the same letter in a column are not significantly different
*Data given for reference only; not compared with flour blends

4.7.5. Foaming Properties

Mean squares for foaming capacity and stability of treatments and DMG flour are given in Table 39. It was observed that both foaming capacity and stability were significantly affected among different treatments. DMG flour had foaming capacity 19.7±0.23% and foaming stability 90.0±2.13%, whilst wheat flour possessed 33.7±1.40% and 21.2±0.59% of foaming capacity and stability, respectively (Table 41). The highest foaming capacity was noted in case of wheat flour which decreased with DMG flour addition whereas an opposite trend was found in case of foaming stability, which improved with DMG flour augmentation from 21.2±0.59 to 38.4±2.45%.

The decrease in foaming capacity was significant at or above 10% DMG flour level. Defatting process and higher mineral & fiber contents of DMG flour might possibly be the contributors towards lower foam forming ability in DMG flour fortified blends. Vani and Zayas (1995) reported that maize germ protein flour had lower foaming capacity than that of wheat germ and soy flours at 1% concentration level. The foaming stability of DMG flour was 90.0% versus 21.2% for wheat flour in present study. As expected, DMG flour addition resulted a significant increase in the stability of foam in flour blends, but only at 15% or higher levels.

4.7.6. Least Gelation Concentration

Least gelation concentration is the amount in percentage of flour dispersion which after certain treatment makes complete gel. The results for least gelation concentration of different flour dispersions of all the treatments are shown in Table 42.

Table 41. Foaming properties of DMGF–wheat flour blends.

Treatments	Foaming capacity (%)	Foaming stability (%)
T_0	33.7±1.40a	21.2±0.59d
T_5	33.4±0.40a	23.6±2.75d
T_{10}	29.9±1.40b	24.3±1.00d
T_{15}	27.9±0.85bc	29.0±1.00c
T_{20}	26.4±0.60cd	33.2±1.55b
T_{25}	25.7±0.20d	38.4±2.45a
DMGF*	19.7±0.23	90.0±2.13

Means sharing the same letter in a column are not significantly different
*Data given for reference only; not compared with flour blends

Table 42. Least gelation concentration of DMGF–wheat flour blends.

Treatments	Concentration of flour blends in the dispersion (%)									
	2	4	6	8	10	12	14	16	18	20
T_0	–*	–	–	–	±	±	+	+	+	+
T_5	–	–	–	±	±	±	+	+	+	+
T_{10}	–	–	–	±	±	+	+	+	+	+
T_{15}	–	–	±	±	±	+	+	+	+	+
T_{20}	–	–	±	±	+	+	+	+	+	+
T_{25}	–	±	±	±	+	+	+	+	+	+

*Gelation levels: None (–), complete (+), partial (±)

Gelling power of the flour dispersions increased with the level of DMG flour in flour blends. The highest gelling power was observed in T_{25} (25% DMG flour). The partial gelation in T_{25} was observed at 4% flour suspension, whereas above 8% flour dispersion resulted in complete gelling. The lowest gelling properties were noticed in case of T_0 (100% wheat flour), where complete gelation occurred at or above 14% flour dispersion. In general, the complete or partial gelling was observed at 10% or higher dispersion concentration of flour blends (Table 42). At lower concentrations (~6%), only partial gelling was noticed at >15% DMG flour in flour blends.

Gelling power is reported to increase with defatting process (Akpata and Akubor, 1999). Protein gels are aggregates of denatured molecules and defatting process might have resulted in higher concentration of denatured protein in DMG flour thereby, resulting in more gelling power.

Considering the results, it is obvious that DMG flour is effective for adding up in various preparations as a gelling agent. The present findings are also supported by Sathe *et al.* (1982); reported the DMG flour could be a valuable additive in foods like puddings and sauces that require thickening and gelling.

4.7.7. Apparent Viscosity

Mean squares for apparent viscosity of treatments at different dispersion levels are presented in Table 43. Apparent viscosity was significantly affected by treatments, dispersions and their interaction. Mean values for this parameter of various treatments at different flour dispersions are illustrated in Figure 9.

As expected, the apparent viscosity increased with DMG flour and flour dispersions; ranged from 12.5 to 36.0 units.

Table 43. Mean squares for apparent viscosity of DMGF-wheat flour blends dispersions

SOV	df	Viscosity
Treatments	5	16.298667**
Dispersions	3	1337.2815**
Interaction (treatment x dispersions)	15	5.9534815**
Error	48	0.1883333
Total	71	

** = *P≤ 0.01*

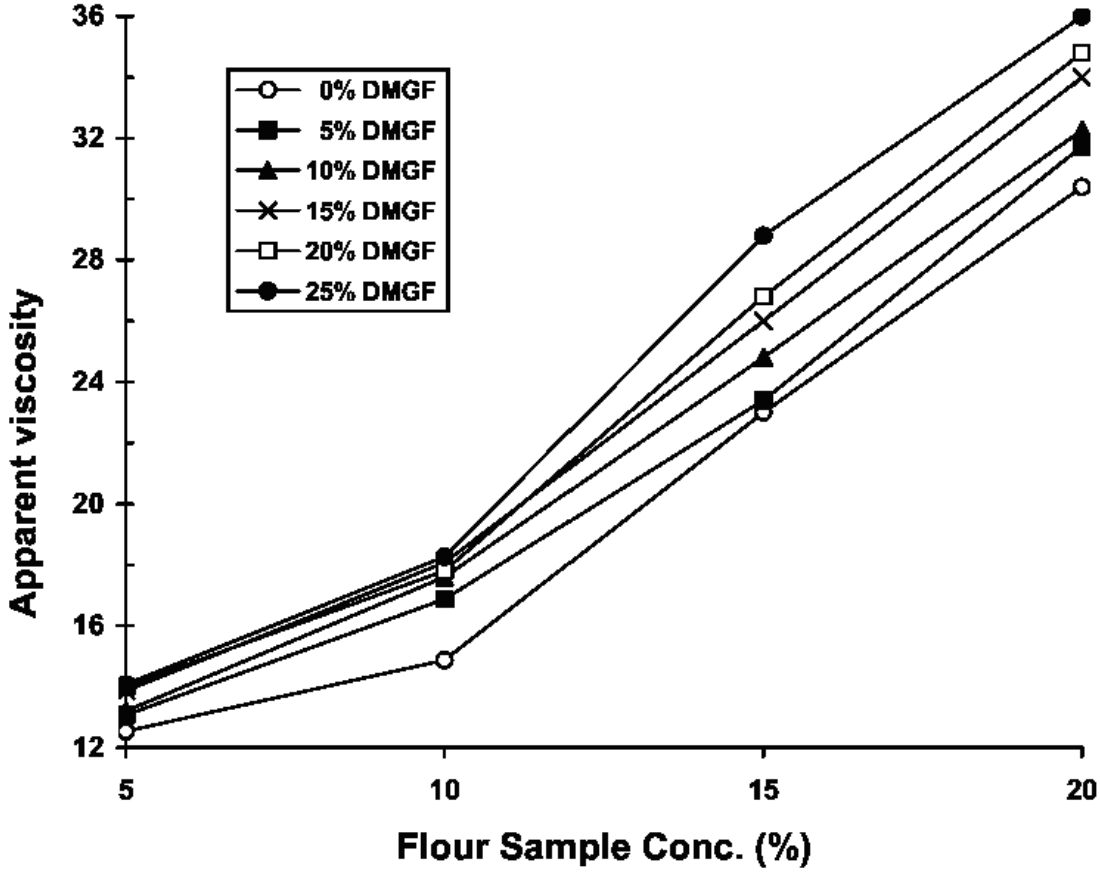

Figure 9. Apparent viscosity of DMGF-wheat flour blend dispersions

The highest apparent viscosity was recorded (36.0 units) for T_{25} (25% DMG flour) followed by T_{20} (20% DMG flour) and T_{15} (15% DMG flour) at 20 g/100mL flour dispersion, whereas, the apparent viscosity in T_0 (wheat flour) at the same dispersion concentration was 30.4 units.

The significant interaction values between treatments and flour dispersions can be assigned to variable DMG flour levels in different flour blends contributing higher levels of water absorption capacities. Another possible reason for increase in viscosity of blends could be the presence of high insoluble matter like fiber in the dispersions with higher DMG flour levels; similar trend was also reported by Marsh *et al.* (1980) for tomato concentrate. The concentration of the insoluble suspended matter has been shown to have momentous effect on the viscosity and type of viscous flow (Bourne, 2002).

4.7.8. Dough Texture

As discussed in the methodology, water added to prepare the dough was kept constant at 60%, on flour wt. basis; appropriate amount of water is needed to provide the desired dough consistency. Mean square for force-deformation curve recorded for doughs prepared from different flour blends is presented in Table 44. Force-deformation curve was significantly affected at all levels of DMG flour addition. The values for this parameter are depicted in Figure 10.

The control dough (wheat flour) had a hardness value of 7.56 N, which increased significantly to 84.6 N, when the DMG flour level increased to 25%. The steep increase in hardness values could be again attributed to the high water absorption capacity of DMG flour. Likewise, stickiness also increased significantly from –0.372 to –4.610 N when the DMG level was 25% in the dough.

Table 44. Mean squares for force deformation curve of DMGF-wheat flour blends

Source	df	Force Deformation Curve
Treatments	5	2787.6946**
Error	12	6.4405069
Total	17	

** = *P≤ 0.01*

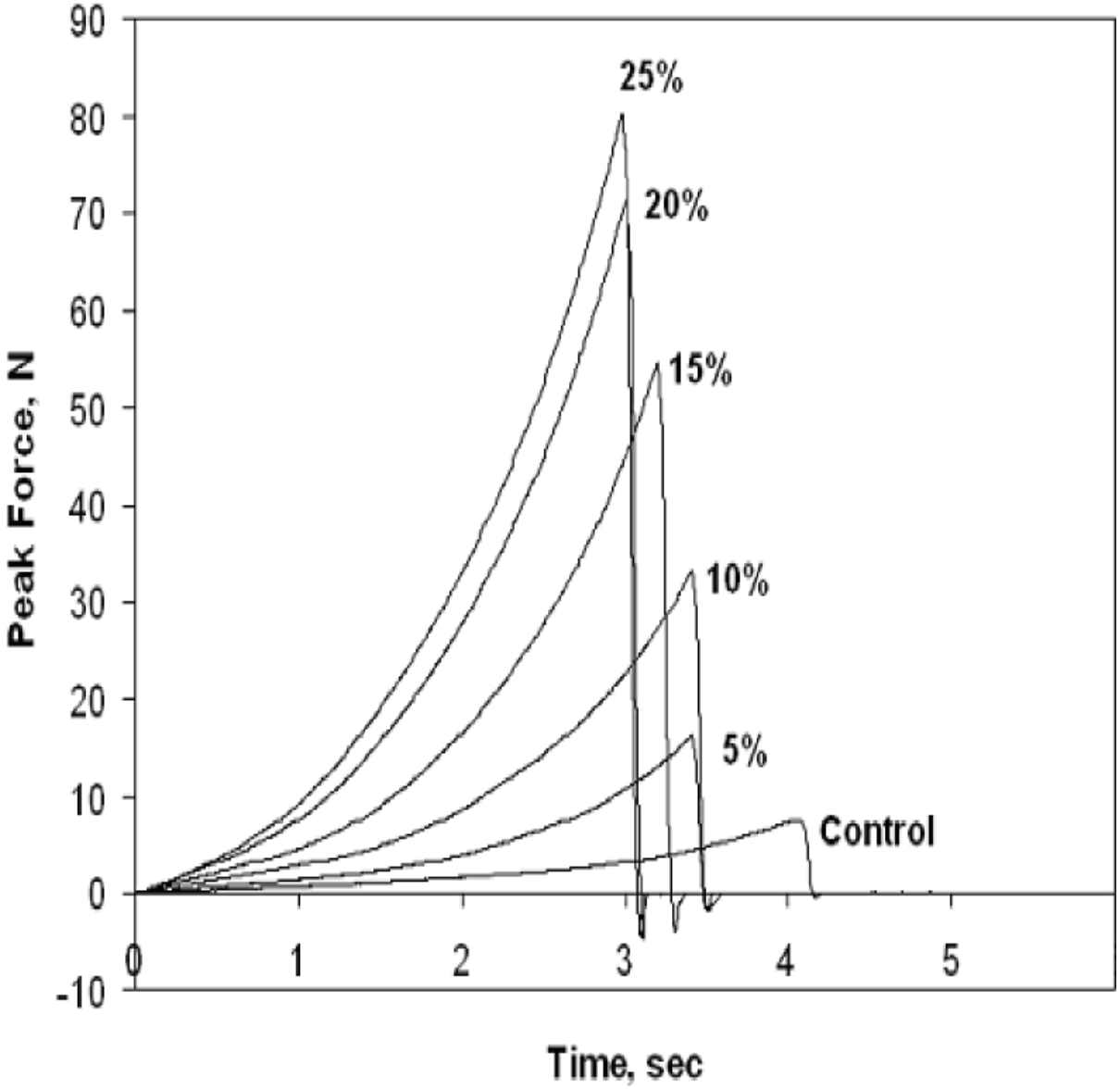

Figure 10. Force-deformation curves of dough made from DMGF-wheat flour blends

Control = All-purpose wheat flour

The increase in stickiness is probably due to the low oil content of DMG flour, and increased inter-particle friction. The viscoelastic properties that affect dough machinability depend strongly on water distribution in the dough (Ruan *et al.,* 1999). Many researchers have reported that the mobility of water in food systems can be studied by measuring the protons spin–spin relaxation time (Ruan *et al.,* 1999; Ablett, 1992). In the wheat flour dough system, water interacts with gluten and starch to form a continuous network with dispersed particles, which are responsible for dough elasticity and extensibility (Grant *et al.,* 1999; Letang *et al.,* 1999). The properties of the dispersed starch phase, the continuous gluten phase and interaction between the components contribute to the dough viscoelasticity. Rolée and Le Meste (1999), studied the change of storage modulus (G') in wheat starch preparations at different water content; observed an increase in initial modulus when moisture content is decreased.

Studies on water distribution in dough are complex because of the presence of numerous components such as starch, gluten, lipids and other flour constituents at different physical states and added fat.

4.7.9. Farinographic Studies

Dough rheological behavior is mainly affected due to protein quantity and quality of flour. The rheological characteristics are the source for understanding the dough handling behavior in bakery. Consequently, dough properties play a key role in quality of finished products. Mean squares for water absorption (WA), dough development time (DDT) and dough stability (DS) of flour blend samples are given in Table 45. Significant variations were observed among various flour samples for these traits.

Table 45. Mean squares for water absorption, dough development time and dough stability of DMGF-wheat flour blends

SOV	df	Water absorption	Dough development time	Dough stability
Treatments	5	272.32**	2.0038**	1.631**
Error	12	0.6539	0.0993	0.059
Total	17			

** = *P≤ 0.01*

Table 46. Water absorption, dough development time and dough stability of DMGF-wheat flour blends

Treatments	Water absorption (%)	Dough development time (min)	Dough stability (min)
T_0	61.04±0.57f	4.73±0.23d	5.17±0.24a
T_5	67.24±0.46e	5.70±0.23c	5.07±0.10ab
T_{10}	72.41±1.24d	6.18±0.53bc	4.68±0.16b
T_{15}	76.17±0.41c	6.57±0.31bc	4.05±0.18c
T_{20}	82.61±1.15b	6.45±0.33ab	3.67±0.34cd
T_{25}	86.63±0.60a	7.08±0.10a	3.42±0.34d

Means sharing the same letter in a column are not significantly different.

T_0 = All-purpose wheat flour
T_5 = DMGF 5%
T_{10} = DMGF 10%
T_{15} = DMGF 15%
T_{20} = DMGF 20%
T_{25} = DMGF 25%

Means for water absorption, dough development time and dough stability of flour blend samples are indicated in Table 46. Water absorption and dough development time increased while dough stability decreased with augmentation of DMG flour in blends. Highest values for water absorption and dough development time were noted in T_{25} i.e. 86.63±0.60% and 7.08±0.10 min, respectively followed by T_{20} and T_{15} while lowest values were observed in T_0 i.e. 61.04±0.57% and 4.73±0.23 min, respectively. The highest dough stability was found in case of T_0 (5.17±0.24 min) not significantly different from T_5 (5.07±0.10 min) whereas minimum value was observed in T_{25} (3.42±0.34 min). The ranges for water absorption, dough development time and dough stability as derived from the farinograms were 61.04±0.57 to 86.63±0.60%, 4.73±0.23 to 7.08±0.10 min, and 3.42±0.34 to 5.17±0.24 min, respectively.

The results of present investigation regarding water absorption, dough development time and dough stability are in conformity with the earlier findings of Shahzadi (2005). She reported 60.22 to 62.00% water absorption, 4.95-7.20 min dough development time and 4.10-5.90 min dough stability of composite flour samples during 60 days of storage. They blended wheat flour with guar gum, chickpea and lentil to prepare composite flour blends and noted that water absorption and dough development time increased while dough stability decreased with the incorporation of non-wheat flours in wheat flour blends. The findings of rheological behavior are also in accordance with the results presented by Abdel-Kader (2000); studied farinographic behavior of decorticated cracked broad beans-wheat flour blends.

The significant increase in water absorption with the blending levels of DMG flour might be attributed to enhanced protein contents in flour blends. Defatting process during DMG flour preparation resulting in more porous structure; can be a key contributor to improve water absorption capacity.

Increase in total protein with decrease in gluten protein coupled with more water absorption and higher viscosity in DMG-wheat composite flours result in increased time to develop dough with optimum consistency (Shahzadi *et al.,* 2005). Diminishing of gluten protein with DMG flour fortification might also be responsible for decreased dough stability as gluten content of flour is directly correlated to this parameter. The rheological behavior is also supported by the results for water absorption capacity, viscosity and textural analysis of different flour blends as discussed in preceding section.

4.8. DMGF-Wheat Flour Blends for Product Development

Physical, functional and rheological properties of DMGF-wheat flour blends depicted its worth for value-added baked products. Thus, DMGF-wheat flour blends were used to develop bread and cookies.

4.8.1. Bread

Based on the results of some preliminary trials, four treatments of DMGF-wheat flour blends (T_5, T_{10}, T_{15} and T_{20}) along with control were selected for bread production.

4.8.1.1. Physical Characteristics

Mean squares for different bread physical parameters are shown in Table 47. The statistical results for loaf volume, specific volume and loaf weight revealed significant variations due to treatments. Means for physical characteristics of bread are given in Table 48.

Bread loaf volumes decreased significantly, from 963.8±3.75 to 709.0±3.77 cc, with a progressive increase of DMG flour level from 0 to 20%; similar effect was observed on bread specific volume with DMG flour addition.

Table 47. Mean squares for physical parameters of DMGF-fortified breads

SOV	df	Volume	Specific volume	Weight	Crumb hardness
Treatments	4	33260.929**	1.2999567**	71.280667**	347.36746**
Error	10	24.525333	0.00062667	0.2646667	1.8600695
Total	14				

** = *P≤ 0.01*

Table 48. Physical parameters of DMGF-fortified breads

Treatments	Loaf volume (cc)	Specific volume (cc/g)	Loaf weight (g)
T_0	963.8±3.75a	5.4±0.01a	179.9±1.10d
T_5	956.3±3.75a	5.3±0.03a	179.7±0.15d
T_{10}	928.0±4.82b	5.0±0.03b	187.0±0.25c
T_{15}	869.5±7.55c	4.6±0.04c	190.2±0.05a
T_{20}	709.0±3.77d	3.8±0.02d	188.2±0.15b

Means sharing the same letter in a column are not significantly different.

T_0 = All-purpose wheat flour
T_5 = DMGF 5%
T_{10} = DMGF 10%
T_{15} = DMGF 15%
T_{20} = DMGF 20%

Highest specific volume (5.4±0.01 cc/g) was recorded for T_0 (100% wheat flour bread) that was non-significantly different from 5% DMG flour fortified bread (5.3±0.03 cc/g), whereas significantly lowest specific volume was noted for T_{20} (3.8±0.02 cc/g). Weight of DMG flour fortified breads increased with 15% DMG flour addition. The values for loaf weight ranged from 179.7±0.15 to 190.2±0.05 g.

The decrease in loaf volume and specific volume was expected, as the amount of gluten, which imparts higher volume, was decreased as a result of gluten-free DMG flour in bread formulation. Partial replacement of wheat flour with non-glutenous flour has been shown to result in lower baked volumes; as reported by Banks *et al.* (1997) who observed a significant (10%) decrease in baked volume of muffins made with added defatted soy flour. Addition of DMG flour had a significant effect on loaf specific volumes with decrease from 3.8 to 5.4 cc/g in 20% DMG flour bread. Increased loaf weight for DMG flour fortified breads can be theorized for higher water absorption and retention capacities of DMG flour; resulting increased yield of the finished product (Brown and Zayas, 1990).

4.8.1.2. Crumb Color

Mean squares for crumb color of bread are shown in Table 49. The DMG flour addition resulted in significant variation in all Hunter crumb color parameters of bread i.e. "L", "a", "b", chroma and hue angle values.

It is evident from the results that DMG flour addition resulted in increased reddish ("a") & yellowish color ("b") with decreased whiteness ("L") (Figure 11). The 100% wheat bread was lighter in color, as depicted by higher "L" value (50.90). Generally, lightness "L" values decreased with DMG flour addition, however, the decrease was significant only at levels > 10%.

Table 49. Mean squares for Hunter crumb color parameters of DMGF-fortified breads

SOV	df	"L"	"a"	"b"	Chroma	Hue angle
Treatments	4	30.95375**	0.95175**	1.7145**	1.58587**	24.331318**
Error	15	0.3428333	0.0241667	0.064	0.0641317	0.5014567
Total	19					

** = *P≤ 0.01*

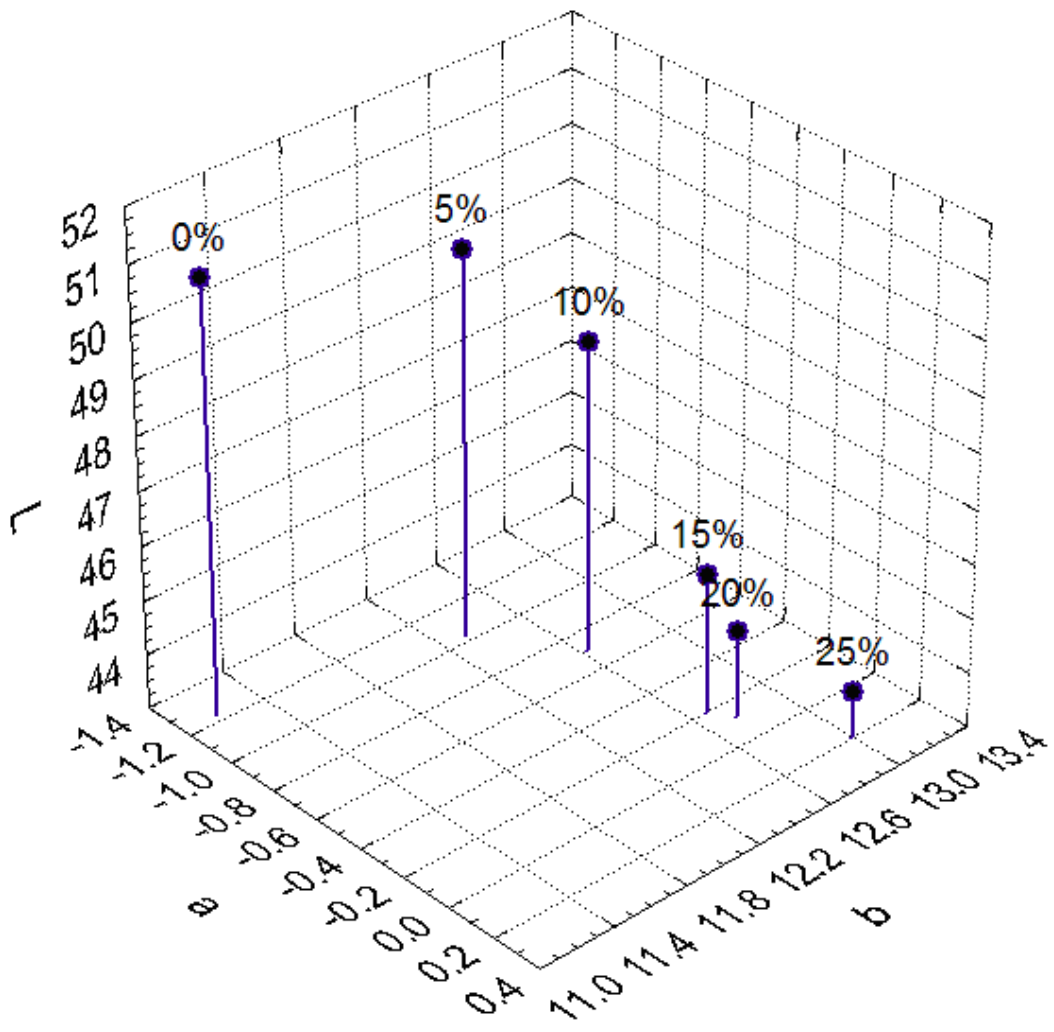

Figure 11. Hunter crumb color "L", "a", "b" values of DMGF-fortified bread

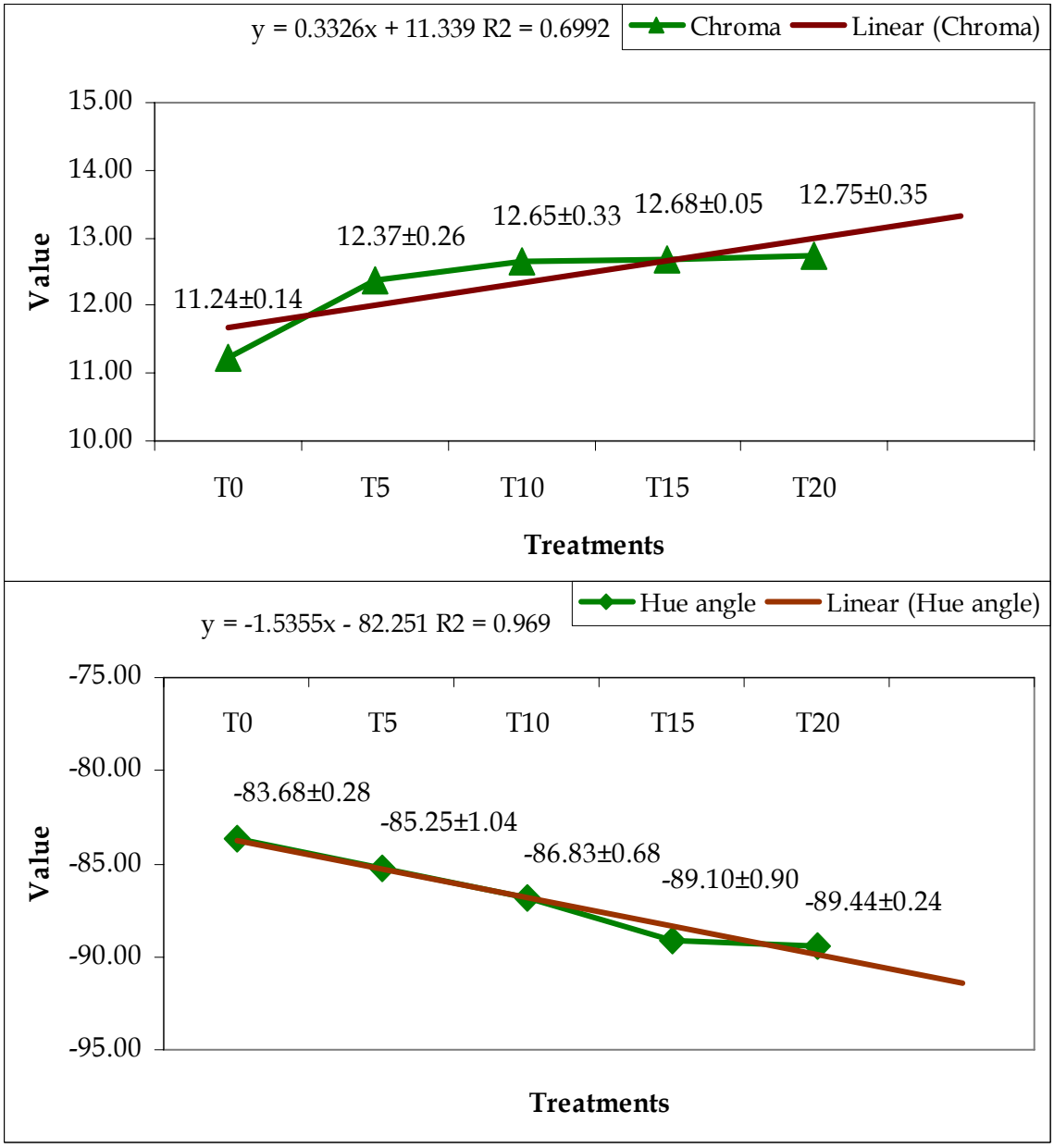

Figure 12. Chroma and hue angle values of DMGF-fortified bread

Hunter color "a" and "b" values increased momentously with DMG flour

addition in bread formulations, the only exception being 5% DMG flour level where "a" values were not different than that of control. Increasing "b" values represent an increase in yellowness of the crumb, as evident from "b" values of 11.18 in control versus 12.75 in 20% DMG flour added bread.

Greene and Benjamin (2004) also observed that bread "L" values decreased with decrease in sweet potato, whereas, whole-wheat flour showed an inverse effect. The lower "L" values in this study might possibly be due to Maillard browning and caramelization, which are influenced by the distribution of water and the reaction between reducing sugars and amino acids (Kent and Evers, 1994). In another study, Banks *et al.* (1997) reported that muffins containing partially defatted soy flour were lighter and redder in color than control. Koca and Anil (2007) reported that the crumb "L" and "b" values decreased and "a" values increased with flaxseed in bread formulation. Similar trend was observed by Alpaslan and Hayta (2006).

The values for chroma and hue angle also increased significantly with the addition of DMG flour in bread (Figure 12). These results clearly indicated that addition of DMG flour significantly decreased the lightness and increased yellowness of the bread crumb. These results are further supported by Hunter color values of DMG flour and DMG flour-wheat flour blends mentioned in previous section, where similar trend was observed.

4.8.1.3. Crumb Texture

The texture was significantly affected by the addition of DMG flour in bread recipe (Table 47). Hardness values increased by the progressive increase of DMG flour, from 32.84 N for control to 61.58 N for 20% DMG flour supplemented bread (Figure 13).

Figure13. Texture/firmness of DMGF-fortified bread crumb

Addition of DMG flour influenced the crumb texture by retaining more moisture and modifying the quantity and quality of protein in the wheat-DMG

flour bread. Furthermore, high levels of DMG flour addition led to lack of uniform crumb texture. Although, higher levels of DMG resulted in firmer bread (Figure 13), nonetheless bread having up to 15% DMG flour was found to be acceptable.

Murase *et al.* (2001) found that lack of uniform structure changes the textural characteristics, mainly firmness of bread. Alpaslan and Hayta (2006) reported that ground flaxseed, soy and corn flours had a significant effect on the textural parameters of bread. The rye flour addition was also shown to increase bread firmness from 0.61 to 14.83N (Esteller and Lannes, 2008).

Many factors are responsible to bring such sort of changes in bread texture, like amount of water in dough, differences in water retention capacities of wheat and DMG flours and possibly some changes in the protein content. The addition of DMG flour probably influenced all the aforementioned factors. Due to the high water absorption capacity, DMG addition affected the water requirement of the dough and its consistency. The high protein content of Defatted maize germ flour also affected the polymerization of proteins, resulting in more plasticized dough; eventually increased hardness of the crumb.

Evaluation of texture by instrumental and sensory analysis is important for product development (Dubost *et al.,* 2003). Texture analysis is one of the tests used to measure firmness, compressibility, cohesiveness and springiness of bread (Sidhu *et al.,* 1997). Instrumental evaluation of bread texture parameters that are analogous to the subjective method by touch or mouthfeel have been proven to correlate well with sensory measurements (Esteller *et al.,* 2004).

Table 50. Mean squares for sensory scores of DMGF-fortified breads

SOV	df	Crust color	Crumb color	Cell uniformity	Firmness	Aroma	Mouth feel	Overall acceptability
Treatment	4	200.731**	46.783167**	56.76275**	36.628083**	27.743333**	71.054375**	111.81942**
Error	55	1.7553788	4.1224848	2.6663788	3.0754848	2.7250758	2.3767386	2.3798485
Total	59							

** = *P≤ 0.01*

4.8.1.4. Sensory Evaluation

Mean squares for sensory evaluation scores of breads prepared from different treatments are presented in Table 50. The sensory scores for all parameters were statistically significant with respect to DMG flour-wheat flour treatments as illustrated in Figure 14.

In general, no significant differences were observed for sensory attributes of crumb color, cells uniformity, aroma, firmness and mouthfeel in breads up to 15% DMG flour addition. Overall, the DMG flour addition at 20% showed negative impact on most of the sensory characteristics. A detailed discussion of selected sensory attributes follows:

Crust color scores decreased significantly with DMG flour addition; effect was more pronounced in 20% DMG flour bread. The desirable crust color of bread is "Golden brown;" which became darker brown with subsequent DMG flour additions. Banks *et al.* (1997) reported that soy flour containing muffins crusts were darker and more uneven than that of the control. The change in crust color may be attributed to Maillard reaction between reducing sugars and proteins (Raidi and Klein, 1983). In addition to wheat protein, DMG flour contributed high protein content, thus leading to more favorable conditions for non-enzymatic Maillard reactions. Esteller and Lannes (2008) observed, during baking, amount of water on the dough surface quickly decreased providing favorable conditions for browning reactions resulting in darker brown color. Dhingra and Jood (2002) reported non-significant changes in crust color of breads prepared with 10% non-wheat flours.

The scores for crumb color also decreased significantly from 12.18 to 7.33 when 20% DMG flour was added to bread. Typical bread has white or pale yellowish color depending on the flour extraction rate and color pigments present.

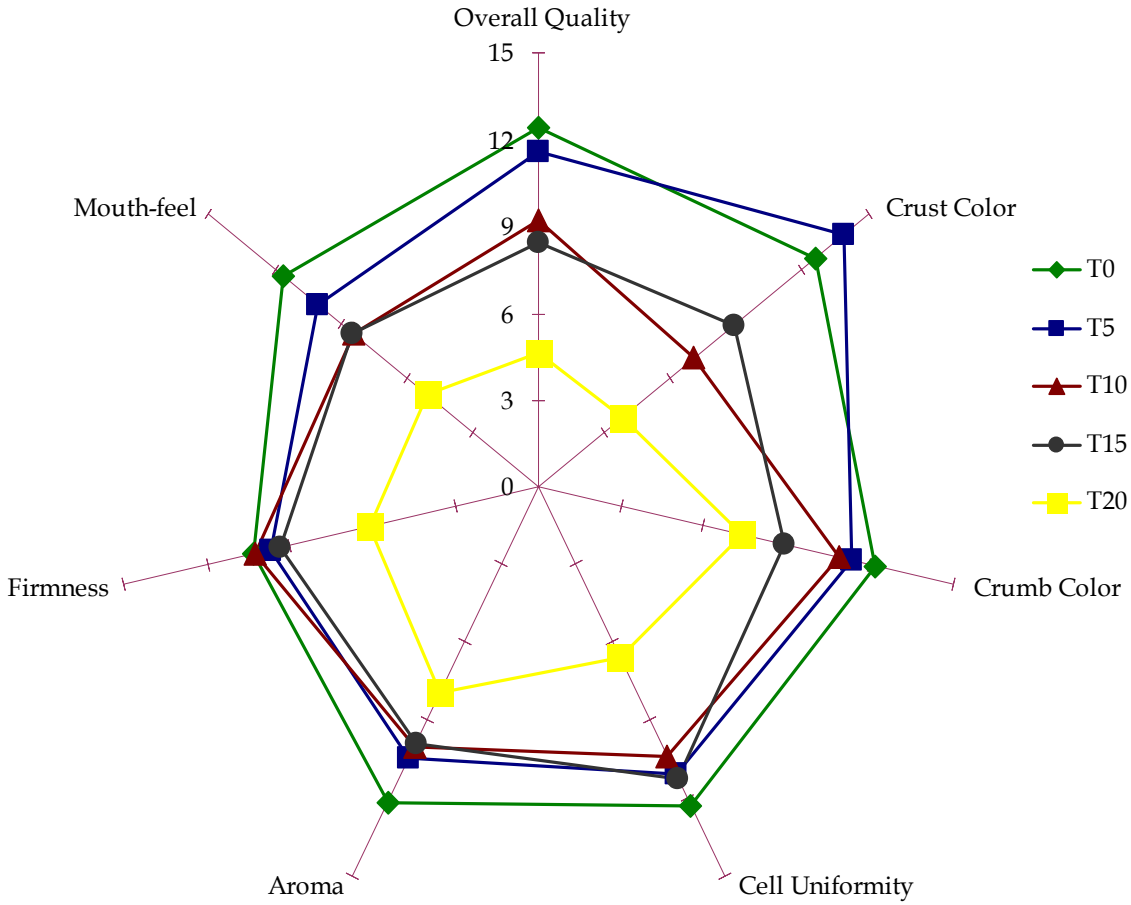

Figure 14. Effect of DMG flour addition on the sensory evaluation scores of bread

T_0 = All-purpose wheat flour
T_5 = DMGF 5%
T_{10} = DMGF 10%
T_{15} = DMGF 15%
T_{20} = DMGF 20%

Koca and Anil (2007), based on their results, suggested that bread containing up to 20% flaxseed flour could be baked with acceptable sensory quality. Hall and Johnson (2004) reported mean acceptability scores for bread with 10% Australian sweet lupin flour above 10; corresponding to "higher than acceptable" on the line scale used for sensory evaluation. According to Flander *et al.* (2007) sensory crumb properties of the oatmeal bread were mainly affected by ingredients, whereas processing conditions showed their main effect on crust properties and richness of the crumb flavor.

Cells uniformity, except for bread with 20% DMG flour, was not affected significantly, as the scores slightly decreased from 12.23 (control) to 11.03 in 15% DMG bread. The DMG flour addition probably modified the water absorption thus increasing interactions between fiber hydroxyl groups and hydrogen bonding.

Skrbic and Filipcev (2008) reported that 12-16% sunflower seed flour addition significantly reduced the crumb elasticity and also affected the shape of the breads yielding flatter products. Esteller and Lannes (2008) suggested that amount of water lost could be correlated to porosity as a function of time. They also showed that non-uniform crumb structure of bread comprised of wide distribution of cell sizes, with some regions showing large numbers of small cells while others contain only few large cells. The inter-connected pores influence heat, contributing to the transport of moisture across the porous medium during baking. Any changes in bread porosity affect the bulk density, water-holding capacity, and thus modify the dough spread and liquid uptake.

Firmness is another major sensory perception contributing significantly towards bread quality. Firmness values showed decreasing order in DMG flour-added breads, though non-significantly up to 15%. Koca and Anil (2007) observed non-significant differences in crust color, crumb grain and structure,

flavor, taste and overall acceptability between control and flaxseed added breads. The addition of active soybean flour and ascorbic acid resulted in softer and springier breads than control (Junqueira *et al.,* 2008).

Addition of DMG flour at ~ 15% did not affect the bread aroma and mouthfeel characteristics. The 20% DMG flour appeared to have negative effect on sensory quality, however, it is possible to overcome some of such negative perceptions by optimizing the formulations and/or changing processing conditions.

4.8.1.5. Correlation Between Bread Quality Attributes

In order to determine interactions between different quality attributes, a correlation analysis was done, as shown in Table 51. Defatted maize germ (DMG) meal levels in bread formulation showed significant positive correlation with "a" (0.983), Hue Angle (0.983), and SMS texture hardness (0.990) whereas, negative correlation was observed with overall quality (-0.967), crumb color (-0.972), aroma (-0.946), "L" (-0.0977), mouth feel (-0.954), volume (-0.895) and specific volume (-0.947).

Bread overall quality was linearly correlated with "L" (0.937) crust color (0.935), crumb color (0.960), cell uniformity (0.907), aroma (0.954), mouth feel (0.980), volume (0.962) and specific volume (0.987), while overall quality exhibited significant inverse correlation with "a" (-.925), Hue Angle (-0.911) and SMS texture hardness (-0.993).

In case of SMS texture hardness, significant and positive correlation was established with "a" (0.957) and hue angle (0.950). An opposite correlation was recorded between SMS texture hardness and "L" (-0.960), crust color (-0.913), crumb color (-0.972), aroma (-0.962), mouth feel (-0.977), volume (-0.938) & specific volume (-0.975).

Regarding physical parameters, the loaf specific volume was negatively

correlated with loaf weight (-0.763) with momentous positive correlation with volume (0.987). Loaf specific volume was also directly correlated with "L" (o.947), crust color (0.892), crumb color (0.976), cell uniformity (0.891), aroma (0.918), firmness (0.918) and mouth feel (0.974), while inversely correlated with "a" (-0.921) and hue angle (-0.894).

Instrumental color parameters "L", "a", "b", chroma and hue angle were highly correlated with each other; as these are basically inter-related. The bread crust color was highly correlated with crumb color (0.889) and showed a significant positive correlation (0.935) with overall quality. Similarly, crumb color and mouthfeel shared highly significant positive relationship (0.969); same was observed for cells uniformity with firmness (0.933), mouthfeel (0.937), volume (0.926) and aroma (0.927). Moreover, aroma showed a positive relationship with mouthfeel, volume and firmness, with correlation values of 0.978, 0.903 and 0.892, respectively.

The diminishing impact of DMG flour levels on loaf volume and specific volume of bread with non-glutenous flour has been reported by Banks *et al.* (1997). Koca and Anil (2007) reported a positive influence of flaxseed on "a" values in bread formulation. The linear effect of DMG flour on texture hardness/firmness was supported by Esteller and Lannes (2008); found similar effect with rye flour addition in bread production. Instrumental evaluation of bread texture parameters have been verified to associate well with sensory measurements (Esteller *et al.*, 2004). Correlation between uniform structure and the textural characteristics, mainly firmness has also been reported by Murase *et al.* (2001)

Table 51. Correlation coefficients of quality attributes of DMGF-wheat flour bread

	DMGF Levles	Hunter"L"	Hunter"a"	Hunter "b"	Chroma	Hue Angle	Overall quality	Crust color	Crumb color	Cell uni-formity	Aroma	Firm-ness	Mouth feel	Volume	Weight	Specific volume	SMS Texture
DMGF Levles	1																
L	-0.977**	1															
a	0.983**	-0.995**	1														
b	0.881	-0.890*	0.827	1													
Chroma	0.846	-0.749	0.798	0.998**	1												
Hue angle	0.983**	-0.981**	0.995**	-0.895*	-0.898*	1											
Overall quality	-0.967**	0.937*	-0.925*	-0.765	-0.734	-0.911*	1										
Crust color	-0.870	0.819	-0.821	-0.676	-0.632	-0.811	0.935*	1									
Crumb color	-0.972**	0.982**	-0.964**	-0.762	-0.738	-0.945*	0.960**	0.889*	1								
Cells uniformity	-0.806	0.732	-0.702	-0.629	-0.616	-0.686	0.907*	0.827	0.829	1							
Aroma	-0.946*	0.873	-0.873	-0.867	-0.855	-0.879*	0.954*	0.846	0.921*	0.927*	1						
firmness	-0.794	0.789	-0.737	-0.521	-0.508	-0.698	0.878	0.710	0.888*	0.933*	0.892*	1					
Mouth feel	-0.954*	0.918*	-0.901*	-0.778	-0.759	-0.888*	0.980**	0.854	0.969**	0.937*	0.978**	0.932*	1				
Volume	-0.895*	0.896*	-0.857	-0.610	-0.584	-0.822	0.962**	0.846	0.953*	0.926*	0.903*	0.967**	0.970**	1			
Weight	0.877	-0.886*	0.919*	0.775	0.732	0.927*	-0.798	-0.814	-0.791	-0.483	-0.701	-0.452	-0.706	-0.650	1		
Specific volume	-0.947*	0.947*	-0.921*	-0.685	-0.653	-0.894*	0.987**	0.892*	0.976**	0.891*	0.918*	0.918*	0.974**	0.987**	-0.763	1	
SMS Texture	0.990**	-0.960**	0.957*	0.825	0.797	0.950*	-0.99**	-0.913*	-0.972**	-0.873	-0.96**	-0.848	-0.977**	-0.938*	0.837	-0.975**	1

* = *P≤ 0.05*

** = *P≤ 0.01*

ns = Non-significant

Overall, the DMG flour addition showed a negative impact on most of the sensory scores but the quality remained within acceptable limits up to 15% DMG blending level. Non-wheat flour in bread formulation has been shown to increase color darkness in baked products (Banks *et al.,* 1997). Correlation between sensory properties and ingredients in formulation has also been reported by Flander *et al.* (2007).

4.8.2. Cookies

Cookies were prepared from DMGF-wheat flour blends and subjected to physical and sensorial evaluation to determine the suitability of DMG flour for cookies development.

4.8.2.1. Physical Characteristics

Mean squares for different physical parameters are given in Table 52. Physical characteristics like thickness, diameter and spread factor were affected significantly by treatments. Means of various physical characteristics of cookies are depicted in Table 53.

Diameter of cookies decreased significantly from 5.94 to 5.63 cm prepared up to 25% DMG flour added flour blends, whereas, thickness of the cookies increased with same fortification level (1.28 cm to 1.38 cm). Though significantly different from control, there were no significant differences in the values obtained for spread ratio of cookies prepared from flour blends with 5, 10, 15 or 20% DMG flour. The spread ratio decreased from 46.54 for control (wheat flour) cookies to 40.72 for 25% DMG flour-fortified cookies. The diameter, thickness and spread factor ranged from 5.63±0.04 to 5.98±0.01 cm, 1.28±0.02 to 1.38±0.01 cm and 40.72±0.42-46.54±0.64, respectively.

Table 52. Mean squares for physical characteristics of DMGF-fortified cookies

SOV	df	Diameter	Thickness	Spread factor
Treatments	5	0.0680**	0.0066**	13.829**
Error	18	0.0015	5.8472	0.4848
Total	23			

** = *P≤ 0.01*

Table 53. Physical characteristics of DMGF-fortified cookies

Treatments	Diameter (cm)	Thickness (cm)	Spread factor	Breaking force (N)
T_0	5.94±0.03a	1.28±0.02c	46.54±0.64a	12.1±2.4c
T_5	5.98±0.01a	1.38±0.01a	43.26±0.33b	14.8±6.0bc
T_{10}	5.86±0.07b	1.34±0.04b	43.68±0.88b	16.5±1.3bc
T_{15}	5.82±0.03b	1.32±0.03b	43.92±1.05b	18.8±2.4ab
T_{20}	5.72±0.02c	1.32±0.02b	43.22±0.58b	22.1±4.5a
T_{25}	5.63±0.04d	1.38±0.01a	40.72±0.42c	22.6±3.9a

Means sharing the same letter in a column are not significantly different.

T_0 = All-purpose wheat flour
T_5 = DMGF 5%
T_{10} = DMGF 10%
T_{15} = DMGF 15%
T_{20} = DMGF 20%
T_{25} = DMGF 25%

The present results for spread ratio and diameter were also in close agreement to those reported for cookies prepared by wheat-defatted wheat germ flour (Arshad *et al.,* 2007), wheat–cowpea bean flour (McWatters *et al.,* 2003) and wheat–soybean flour blends (Shrestha and Noomhorm, 2002). It has been suggested that spread ratio is affected by the competition among flour or any other ingredients for the available water during dough mixing (Fuhr, 1962). DMG flour has higher water retention capacity and more fibrous portion; resulting in lower spread ratio.

The textural analysis of cookies exhibited non-significant changes in the values for breaking force up to 5, 10, or 15% DMG flour level. However, this force increased significantly at higher levels of fortification; increase in force was 12.1 N for control to 22.6 N with 25% DMG flour fortification level (Table 53).

DMG flour has higher water and oil absorption than wheat flour (Luallen, 1985), resulting in a drier dough, might be one of the reasons for increased cookies hardness or breaking force.

In general, cookies made with added DMG flour resulted in firmer texture, increased thickness, reduced diameter and lower spread ratio; these effects were more pronounced with increasing level of DMG flour addition.

4.8.2.2. Hunter Crust Color

Mean squares for Hunter crust color parameters of cookies are presented in Table 54. The DMG flour addition in cookie formulation resulted in significant variation in all Hunter crust color parameters i.e. “L”, “a”, “b”, chroma and hue angle.

Table 54. Mean squares for Hunter crust color parameters of DMGF-fortified cookies

SOV	df	"L"	"a"	"b"	Chroma	Hue angle
Treatments	5	51.828**	0.3384*	11.707**	10.977477**	14.635028**
Error	18	0.1382	0.0854	0.0379	0.0396417	1.3280514
Total	23					

** = *P≤ 0.01*

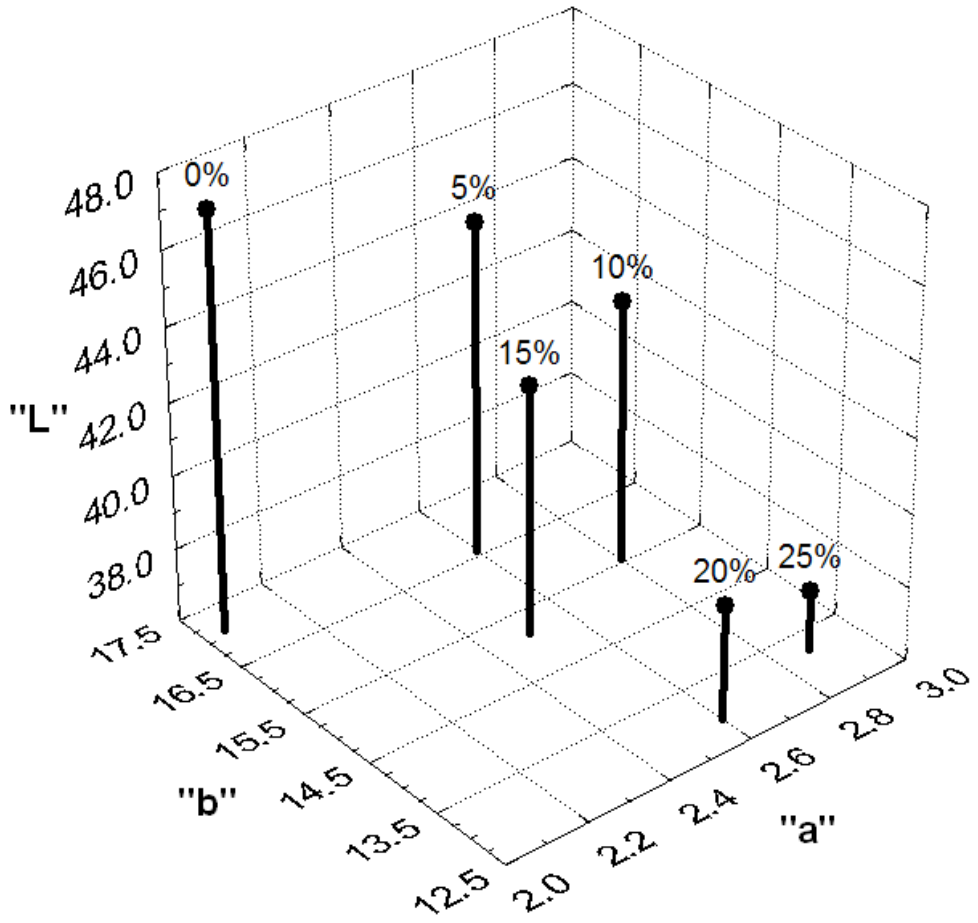

Figure 15. Hunter crust color "L", "a", "b" values of DMGF-fortified cookies

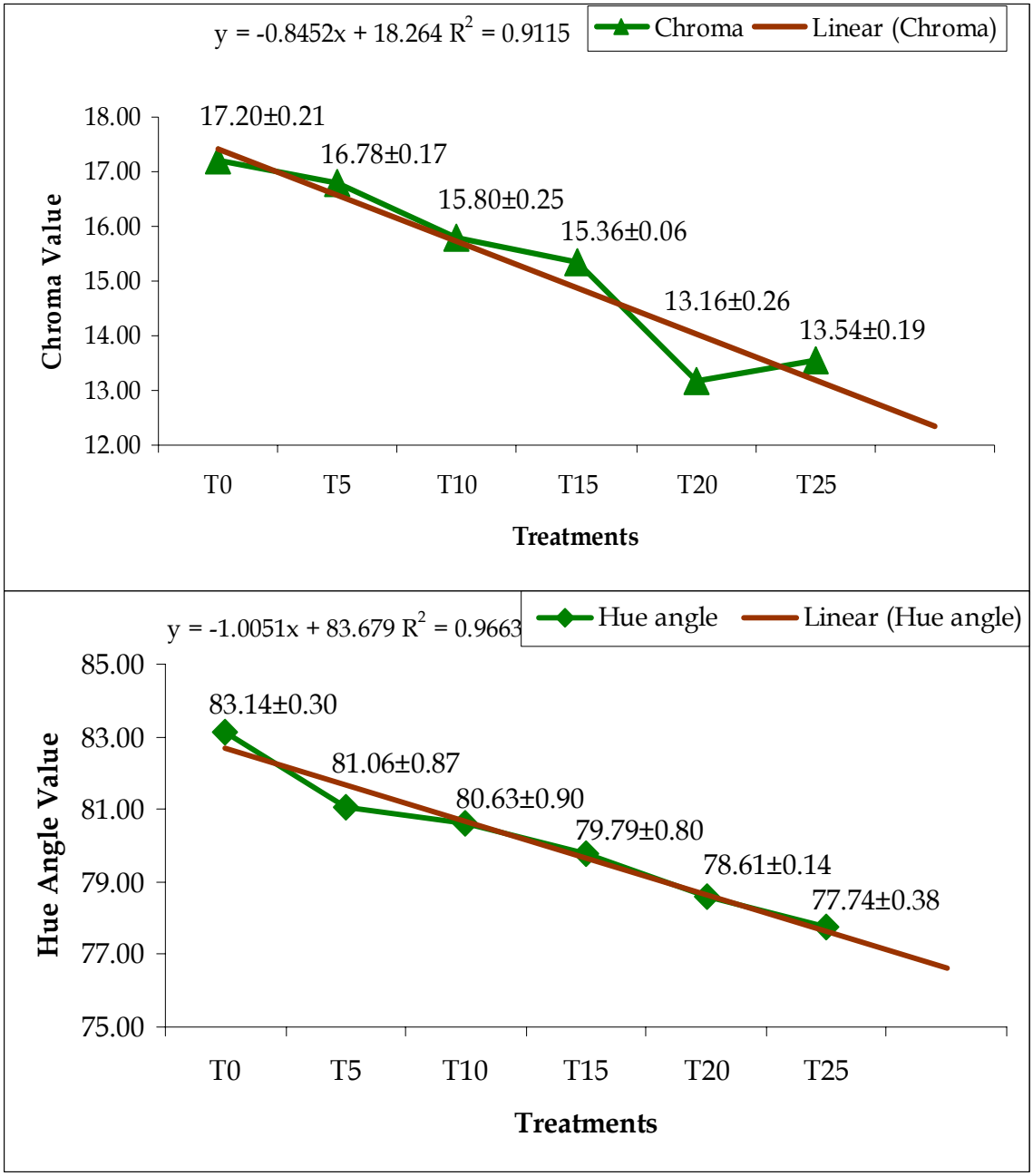

Figure 16. Chroma and hue angle values of DMGF-fortified cookies

It is evident from results that DMG flour addition resulted in increased reddish ("a") and decreased yellowish ("b") color and lightness ("L") (Figure 15).

The highest "L" value was recorded in case of control cookies (47.28) that decreased as concentration of DMG flour increased and the lowest "L" value (37.63) was noted for cookies prepared from T_{25} (25% DMG flour); whereas, an opposite trend was observed for "a" values, that were lowest in control and increased with DMG flour augmentation.

DMG flour concentration also showed significant effect on chroma and hue angle values. Chroma increased from 17.20 to 13.16 whereas, hue angle decreased from 83.14 and 77.74 (Figure 16). Overall, DMG flour resulted in more reddish, less yellowish and lighter color cookies. Though, all the parameters were significant yet values were not wider enough from control. The results are also supported by the fact that sensory panel did not observe any significant variation up to 20% level of DMG flour addition.

The increase in reddish tint while decrease in whiteness in crust color of DMG flour added cookies can be attributed to more less white color of DMG flour as compared to all purpose wheat flour. Zayas and Lin (1989) reported that DMG proteins were less in lightness and more in redness as compared to soy protein. Greene and Benjamin (2004) also observed decrease in "L" with increasing level of whole-wheat flour in baked product.

4.8.2.3. Sensory Evaluation

Mean squares for sensory scores of different treatments are presented in Table 55. The sensory scores for color, flavor, taste and overall acceptability varied significantly with treatments, while scores for crispiness were not significant with DMG flour addition. The mean values of sensory scores are mentioned in Table 56.

Table 55. Mean squares for small-panel sensory scores of DMGF-fortified cookies

SOV	df	Color	Aroma	Taste	Crispiness	Overall acceptability
Treatment	5	6.496667*	7.55**	11.81667**	2.1466667^{ns}	13.136667**
Error	54	2.29075	1.4351852	1.4351852	1.1851852	0.8388889
Total	59					

* = *P≤ 0.05*
** = *P≤ 0.01*
ns = Non-significant

Table 56. Small-panel sensory evaluation scores of DMGF-fortified cookies

Treatments	Color	Aroma	Taste	Crispiness	Overall acceptability
T_0	6.6±1.51a	6.3±1.06a	6.8±1.32a	7.1±1.20	6.9±0.88ab
T_5	6.6±1.26a	6.5±1.18a	7.0±1.33a	7.5±0.85	7.5±0.85a
T_{10}	6.9±0.99a	6.4±1.26a	6.6±0.97a	7.8±0.42	7.3±0.82a
T_{15}	6.8±1.23a	6.0±1.15a	5.9±0.99a	7.5±0.71	6.3±1.06b
T_{20}	5.4±2.01ab	4.6±1.17b	4.7±1.34b	6.9±1.43	5.2±1.03c
T_{25}	5.0±1.83b	4.7±1.34b	4.5±1.18b	7.0±1.49	4.7±0.82c

Means sharing the same letter in a column are not significantly different

For the sensory attributes of color, aroma, taste, and overall acceptability, cookies prepared from either 5 or 10% added DMG flour were not significantly different with that of control (Table 56). Cookies prepared from flour blends containing 20 and 25% DMG flour produced cookies with significantly lower scores for all sensory attributes. Although, the color darkened somewhat with DMG flour addition, as it was evident from lower Hunter "L" values (Figure 15), yet the panelists did not notice any major difference in sensory color up to 20% DMG level.

Cookies prepared from DMG flour resulted in firmer texture (Table 53), yet the panelists gave higher scores for crispiness up to the fortification level of 15%. Overall acceptability scores for cookies prepared with 5 or 10% DMG flour were higher than that of control and 15% DMG flour cookies. It is also worth mentioning that DMG flour in cookie formulations did not exhibit any momentous effect on sensory scores for all parameters except for overall acceptability up to 15% DMG flour addition.

Huang and Zayas (1991) isolated a number of compounds from corn germ flours, which cause off-flavor. However, acceptable limit of DMG flour supplementation might be enhanced by masking its particular flavor or aroma with certain treatments like heat application (McWatters, 1985).

4.8.2.4. Acceptability Studies

Analysis of variance for consumer acceptability depicted that aroma, taste, crispiness and overall acceptability of cookies were significantly affected with treatments, whereas color scores showed non-significant differences (Table 57). It is evident from the Figure 17, that most of the scores for all sensory attributes prepared from 10 or 15% DMG flour were lower than that of control and 5% DMG flour, yet all the scores were >5, which showed their acceptability.

Table 57. Mean squares for consumer acceptability scores of DMGF-fortified cookies

SOV	df	Color	Aroma	Taste	Crispiness	Overall acceptability
Treatment	3	3.38667^{ns}	20.1689**	24.6789**	8.32889**	25.0167**
Error	296	2.19514	2.4192	2.4253	1.92486	1.8804
Total	299					

** = *P≤ 0.01*
ns = Non-significant

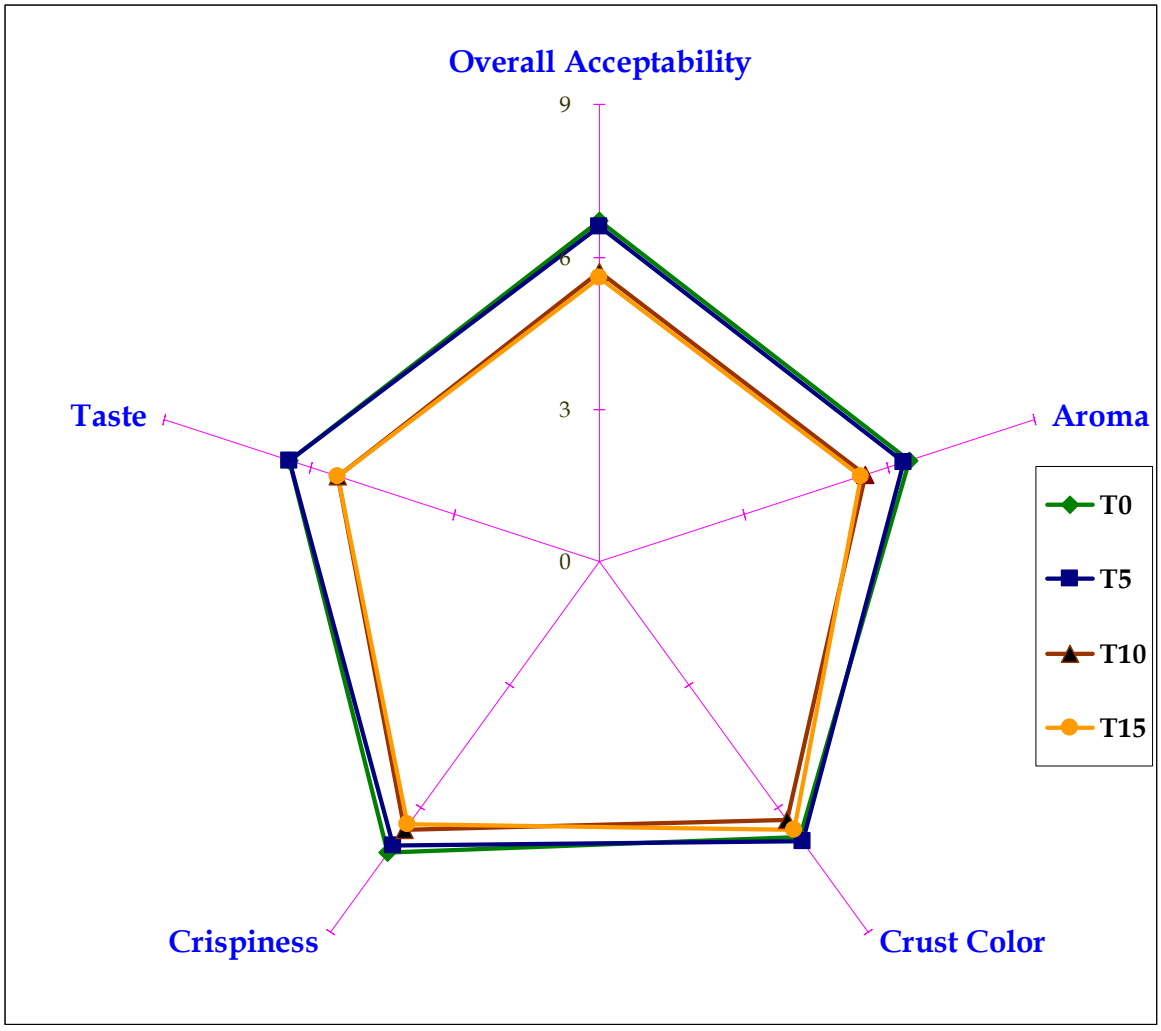

Figure 17. Consumer acceptability scores of DMGF-fortified cookies

T_0 = All-purpose wheat flour
T_5 = DMGF 5%
T_{10} = DMGF 10%
T_{15} = DMGF 15%

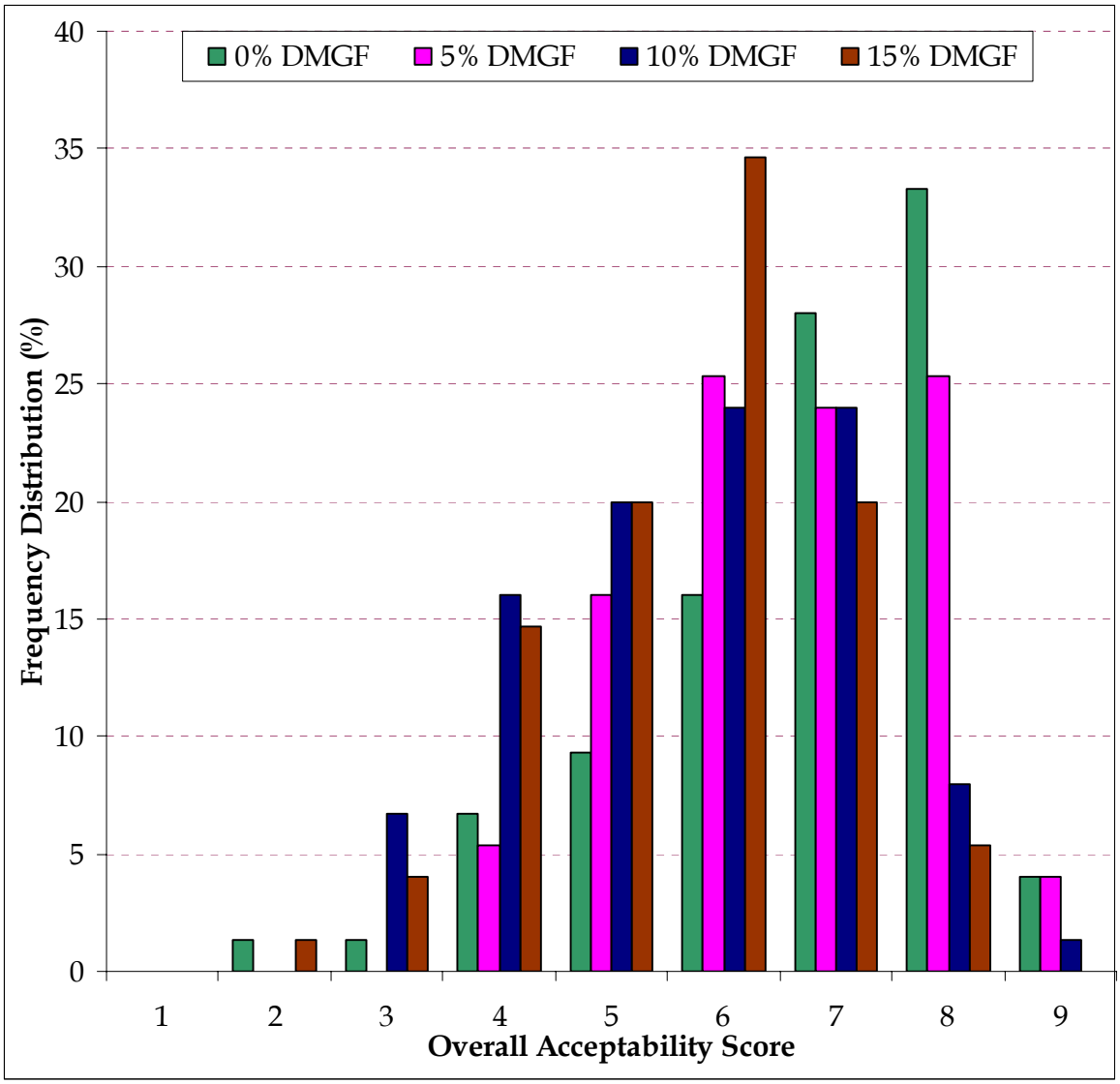

Figure 18. Frequency distribution of consumer acceptability scores of DMGF-fortified cookies

The results of crispiness support the data on objective measurement of texture, as breaking force (Table 53). As the level of DMG flour fortification increased, scores for cookie crispiness decreased accordingly. Buck *et al.* (1987) studied sensory properties of cookies fortified with DMG flour; reported that acceptable cookies with good aroma and crispiness could be prepared with flour blends having 20% DMG flour.

The frequency distribution of overall acceptability scores can reveal some additional information about sensory results (Temelli *et al.,* 2004). Figure 18 shows the frequency distribution of overall acceptability scores given by 75-member consumer panel for cookies made with or without added DMG flour.

It is interesting that as high as 78.61, 57.33, and 60.00% of panelists rated cookies made with 5, 10 and 15% DMG flour, respectively, at a score of 6 or higher; whereas, score of 4 or below, these numbers were 5.33, 22.67, and 20.00%, respectively. Thus, fortification of wheat flour up to 15% DMG flour produced cookies with high acceptability.

Consumer panelists' response with demographic split, whether to purchase DMG flour-fortified cookies, if available commercially is shown in Table 58. It is clear from the results that consumers' response to purchase Defatted maize germ flour fortified cookies was generally very positive. For example, 66% males and 62% female respondents said that they would prefer to purchase cookies containing DMG flour if available in market; the number for both genders combined was 64%. On the basis of age groups, 41–50 years, followed by 21–30 and 31–40 years olds, were the groups with the three highest affirmative responses. The highest positive response (71%), considering annual income level, was shown by US $ 21,000 to 30,000 income group, on the other hand graduates and professionals were the highest in numbers (26 out of 36), willing to purchase DMG flour fortified cookies.

Table 58. Consumer response, whether to purchase DMGF-fortified cookies, if available commercially

Demographic split	Affirmative response		Negative response		No answer	
	(n)	(%)	(n)	(%)	(n)	(%)
Gender						
Male *(n=38)*	25	66	12	32	1	3
Female *(n=37)*	23	62	8	22	6	16
Age (Yr)						
<20 *(n=9)*	5	56	3	33	1	11
21-30 *(n=27)*	18	67	7	26	2	7
31-40 *(n=12)*	8	67	2	17	2	17
41-50 *(n=13)*	9	69	3	23	1	8
51-60 *(n=14)*	8	57	4	29	2	14
>60 *(n=0)*	0	0	0	0	0	0
Income (US $)						
<20K *(n=35)*	24	69	10	29	1	3
21-30K *(n=7)*	5	71	2	29	0	0
31-40K *(n=9)*	6	67	2	22	1	11
41-50 *(n=5)*	0	0	2	40	3	60
51-60K *(n=7)*	4	57	1	14	2	29
>60K *(n=10)*	8	80	2	20	0	0
Education Level						
High school *(n=9)*	6	67	3	33	0	0
Ass. degree *(n=11)*	6	55	5	45	0	0
Bachelor *(n=18)*	10	56	5	28	3	17
Grad./professional *(n=37)*	26	70	7	19	4	11

SUMMARY

Present project was planned to characterize maize hybrid germs and explore its worth in value-added baked products. Germs were separated from six promising indigenous maize hybrids namely Poineer-32-F-10 (P-1), Poineer-32-B-33 (P-2), Monsanto-6142 (M-1), Monsanto-6525 (M-2), Rafhan-2331 (R-1) & commercial and evaluated for chemical composition. On the basis of germ recovery and compositional assay, one best hybrid germ; Pioneer-32-F-10 (P-1) was selected. Oil and defatted portion of the selected germ were analyzed for further use in baked products including cakes, cookies and bread. The significant outcomes of the present research are summarized hereafter.

The germ recovery differed significantly among various hybrids. The highest germ yield (7.68±0.11%) was recovered from P-1 followed by commercial (7.50±0.20%) and P-2 (7.47±0.23%), while the lowest germ (6.31±0.16%) was obtained from R-1 maize hybrid.

Proximate composition differed significantly among various germ samples. Overall, all the germs were found to be good source of crude fat (32.1±1.15 to 38.8±0.65%) and crude protein (16.34±1.44 to 20.96±0.56%). However, the crude fiber and ash contents ranged from 2.63±0.58 to 4.79±0.20% and 3.08±0.12 to 4.94±0.14%, respectively.

Momentous differences were also observed among the germ samples for various minerals like phosphorus, potassium, magnesium, calcium and iron. The evaluated minerals were highest in R-1 germ (1.79±0.12 g/100g, 1.64±0.01 g/100g, 0.78±0.07 g/100g, 12.13±1.45 mg/100g, 14.46±1.45 mg/100g, respectively) whereas, commercial germ contained the lowest minerals:

phosphorus 1.06±0.05 g/100g, potassium 1.19±0.03 g/100g, magnesium 0.43±0.04 g/100g, calcium 7.13±0.83 mg/100g and iron 9.08±0.41 mg/100g).

All the essential amino acids except methionine, trytophan and cystine showed significant variations with respect to maize germ samples. Likewise, there were momentous differences between non-essential amino acids, with the exception of proline. The highest lysine content (5.79±0.14 g/100g), a limiting amino acid, was found in P-1 followed by P-2 (5.21±0.05 g/100g) and commercial germ (5.01±0.09 g/100g), while the lowest concentration (4.60±0.28 g/100g) for lysine was recorded in M-1 hybrid germ. The P-1 germ also contained the highest amount (3.34±0.08 g/100g) of isoleucine while the commercial hybrid germ had the lowest value (2.41±0.17 g/100g) for this amino acid. The amino acid profile of maize germs found to be nutritionally valuable exhibiting balanced protein with respect to essential amino acids, and in agreement with FAO/WHO standards.

The fatty acids were affected significantly due to source of maize germ. Amongst fatty acids, linoleic acid (C18:2) was in highest quantity (44.16±1.055 to 56.33±0.22 g/100g), followed by oleic acid (26.24±0.62 to 39.91±1.024 g/100g) while myristic acid (C14) was found to be the lowest (0.05±0.01 to 0.70±0.11 g/100g). The results elucidated that the germ samples contained highest amount of polyunsaturated fatty acids (46.74-58.00%) followed by monounsaturated (26.24-39.91%), while saturated fatty acid contents were the lowest (10.38-17.23%) in all tested samples. Nevertheless, the results were within the recommended ratios of respective fatty acids as proposed by Codex Alimetarius.

Significant variations were exhibited by various hybrid germs for the concentration of α- and γ-tocopherols. The α-tocopherol ranged from 4.87±0.56 to 9.68±2.52 mg/100g whereas, the range of γ-tocopherol was 29.52±0.23 to 37.83±0.39 mg/100g.

Maize germ oil (MGO) from selected hybrid germ (P-1) had agreeable flavor, distinct pale yellow color, 0.923±0.001 specific gravity and 1.472±0.001

refractive index. The iodine, saponification, acid and peroxide values were found to be 113.67 g/100g, 192.67 mg KOH/g, 0.33 mg KOH/g and 1.69 meq/Kg, respectively whereas free fatty acids were recorded as 0.033 g/100g. The results of physico-chemical characteristics in the present research are corroborated with the ranges given by Corn Refiners Association.

Cakes prepared from various MGO levels (0, 20, 40, 60, 80 and 100%), showed significant variations in physical characteristics like volume and specific volume; increased gradually with the addition of MGO in formulations. MGO addition resulted in decreased greenness ("-a"), lightness ("L") and increased yellowish color ("b") of cake crumb. Sensory scores for overall quality and texture softness depicted significant variations, while cells uniformity, color, flavor and taste explicated non-significant differences with treatments. The cake prepared from T_{60} (60% MGO) was assigned maximum scores for overall quality. It was observed that MGO blending with normal shortening in cake formulation improved the physical and sensory attributes of finished product.

Defatted maize germ (DMG) portion, left after oil extraction from selected hybrid (P-1) was processed and evaluated for proximate components, dietary fiber and minerals. The resultant DMG meal contains crude protein 32.19±0.84%, crude fat 1.26±0.11%, crude fiber 4.32±0.59%, ash 5.49±0.61% and NFE 56.74±0.55%. It also showed potential for minerals like phosphorus (2.15±0.14 g/100g), potassium (1.96±0.16 g/100g), magnesium (0.89±0.10 g/100), calcium (13.98±0.78 mg/100g) and iron (17.46±1.12 mg/100g) nonetheless, dietary fiber was observed to be 31.87±2.98%.

Protein quality traits were affected by the test diets in Sprague Dawley rats. In the experimental, *in-vivo* protein quality of DMG meal was: 87.10±0.78% TD, 76.70±1.25% NPU, 88.06±o.67% BV, 5.12±0.21 NPR and 2.15±0.03 PER were significantly higher than that of wheat-based diet. Favorable impact of DMG meal on serum biochemical profile was observed; cholesterol, triglyceride, LDL

and glucose levels decreased up to 6.80, 12.45, 16.19 and 6.50%, respectively with DMG meal diet as compared to basal diet. The results for liver and kidney functioning tests of rats fed on DMG meal diet were found to be non-significant and within the safe limits. Serum calcium status improved with study period, whereas sodium and potassium concentration were non-significant irrespective of diets and study period. Organ weights of rats fed on DMG and basal diet showed non-momentous variations except for intestine length. In general, defatted maize germ meal possessed good nutritional profile that explicates its potential to be used for product value-addition.

Defatted maize germ (DMG) meal in wheat flour blends influenced physical, functional and rheological characteristics. The DMG meal in the range of 5-25% in flour blends resulted decrease in bulk density and foaming capacity whereas an increase in water & oil absorption capacities, emulsion properties, foaming stability and gelling behavior was observed. The apparent viscosity increased with increasing concentration of DMG meal and flour dispersions. Force-deformation curve was also affected significantly at all levels of DMG meal thus resulting in increased hardness and stickiness. Farinographic properties including water absorption and dough development time increased while dough stability decreased with augmentation of DMG meal.

Bread loaf volumes and specific volume decreased with progressive increase of DMG meal. Moreover, the DMG meal addition resulted in increased reddish (“a”) & yellowish color (“b”) with decreased lightness (“L”). Crumb hardness/firmness increased by the DMG meal addition, from 32.84 N for control to 61.58 N for 20% DMG meal supplemented bread. Sensory scores for all parameters of bread were statistically significant with respect to DMG meal-wheat flour treatments. Collectively, no significant differences were observed for sensory attributes like crumb color, cells uniformity, aroma, firmness and mouthfeel in breads up to 15% DMG meal addition. Correlation co-efficients

revealed the existence of high association between different bread quality traits. Overall, the DMG meal addition indicated that it should be added up to 15% for protein and fiber enriched bread preparation.

Cookies prepared from DMGM-wheat flour blends resulted in firmer texture, increased thickness, reduced diameter and lower spread ratio; the effects were more pronounced with increasing level of DMG meal addition. Moreover, an increase in reddish & yellowish color with decrease in lightness of crust color was observed. Most of the scores for sensory attributes prepared from 10 or 15% DMG meal (>5) were lower than that of control and 5% DMG meal that differed non-significantly with each other. It is interesting that as high as 78.61, 57.33, and 60.00% of consumer panelists rated cookies made with 5, 10 and 15% DMG meal, respectively, at a score of 6 or higher. Thus, suggesting fortification of DMG meal up to 15% in wheat flour to prepare cookies with high acceptability. Consumer response to purchase defatted maize germ meal fortified cookies was very positive e.g. 64% of the respondents said that they would prefer to purchase cookies containing DMG meal if available in market.

It is concluded from the present research exploration that Pioneer 32-F-10 (P-1) hybrid has higher germ yield which in turn possess better nutritional profile than rest of the maize hybrid germs. Its defatted portion bears a potential to cope with protein deficiencies in communities at risk through value-added products. Information derived from present investigation can also be helpful for the researchers for better understanding of the compositional, functional and nutritional status of maize germ and its role for product value-addition.

CONCLUSIONS

- All maize germ samples tested were found to be having good nutritional profile especially with reference to amino acid and fatty acid composition

- Germ from maize hybrid, Pioneer 32-F-10 (P-1), was selected as the best germ sample on the basis of germ yield and chemical composition and used for product value-addition

- Maize germ oil was successfully used to develop cake without deteriorating the sensory quality

- Defatted maize germ possessed comparable biological assay with that of casein and was found to be safe through animal modeling studies

- Defatted maize germ was successfully incorporated in bread and cookies on 15% flour weight basis

RECOMMENDATIONS

- Maize hybrid, Pioneer 32-F-10 (P-1) should be recommended for cultivation in order to achieve higher oil yields

- Solvent extraction technique should be encouraged to improve the recovery & quality of oil and defatted meal

- In Pakistan, palm oil should be replaced in food preparations with maize germ oil bearing potential health claims

- Utilization of defatted maize germ meal should be advised for human consumption in order to ameliorate food security situation

- Maize germ meal should be recommended in the diet of protein malnourished communities to cope with the deficiency of essential nutrients

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APPENDIX 1

Sensory evaluation of cakes fortified with maize germ oil

Time. ……. Date……..

Instructions

1. **Read carefully the description of individual attributes**
2. **Rate your acceptance by putting cross (X) on lines for all the samples.**
3. **Don't disturb the order of the samples.**

Crust color **Intensity of "golden brown" color, typical of cake crust**

Not at all intense Very intense

330: _____

371: _____

439: _____

737: _____

850: _____

973: _____

Cells Uniformity **The uniformity of air cells (porosity) present in cake crumb**

Not at all uniform Very uniform

330: _____

371: _____

439: _____

737: _____

850: _____

973: _____

Texture Softness: **Resistance offered by the "compactness of crumb" by finger**

Not at all soft Very soft

330: _____

371: _____

439: _____

737: _____

850: _____

973: _____

Aroma: **Degree of aroma intensity associated with typical cake**

Not at all intense Very intense

330: _____

371: _____

439: _____

737: _____

850: _____

973: _____

Taste: **Intensity of perceived taste of a typical cake slice**

Not at all intense Very intense

330: _____

371: _____

439: _____

737: _____

850: _____

973: _____

Overall quality: **Overall impression of the cake based on all attributes**

Highly unacceptable Very acceptable

330: _____

371: _____

439: _____

737: _____

850: _____

973: _____

Appendix II

Composition of vitamin and mineral mixture

Minerals		Weight (g)	Vitamins	Weight (g)
Calcium citrate		308.2	Thiamin hydrochloride	0.060
$Ca(H_2PO_4)_2\ H_2O$		112.8	Riboflavin	0.200
H_2HPO_4		218.7	Pyridoxin hydrochloride	0.040
HCl		124.7	Calcium pentothenate	1.200
NaCl		77.0	Nicotinic acid	4.000
$CaCO_3$		68.5	Inositol	4.000
$MgCO_3.\ Mg(OH)_2.\ 3H_2O$		35.1	p-aminobenzoic acid	12.000
$MgSO_4$ anhydrous		38.3	Biotin	0.040
Ferric ammonium citrate	91.41		Folic acid	0.040
$CuSO_4.\ 5H_2O$	5.98		Cyanocobalamin	0.001
NaF	0.76	16.7	Choline chloride	12.000
$MnSO_4.\ 2H_2O$	1.07		Maize starch	966.419
$KAl(SO_4)_2.\ 12H_2O$	0.54			1000.00
KI	0.24			
	100.00	1000.00		

Appendix III

Recipe for bread formulations

Ingredients	Wheat-DMG meal treatments				
	T_0	T_5	T_{10}	T_{15}	T_{20}
Wheat Flour (g)	100	95	90	85	80
DMG Meal (g)	0	5	10	15	20
Sugar (g)	5	5	5	5	5
Shortening (g)	3	3	3	3	3
Salt (g)	1	1	1	1	1
Dry Yeast (g)	3	3	3	3	3
Water (mL)	60	67	73	79	84

APPENDIX V

Sensory evaluation of bread fortified with defatted maize germ flour

Time. ……. Date……..

Instructions

4. **Read carefully the description of individual attributes**
5. **Rate your acceptance by putting a cross (X) on 15-cm line for all the samples.**
6. **Don't disturb the order of the samples.**

Crust color: **Intensity of "golden brown" color, typical of white bread**

Not at all intense Very intense

210: _____

261: _____

329: _____

627: _____

740: _____

Crumb color: **Intensity of "whitish" / "creamish" color typical of white bread**

Not at all intense Very intense

210: _____

261: _____

329: _____

627: _____

740: _____

Cells Uniformity: **The uniformity of air cells (porosity) present in the crumb**

Not at all uniform Very uniform

210: _____

261: _____

329: _____

627: _____

740: _____

Aroma: **Degree of aroma intensity associated with typical white bread**

Not at all intense Very intense

210: _____

261: _____

329: _____

627: _____

740: _____

Firmness: **Resistance offered by the "compactness of crumb" by finger feel**

Not at all soft Very soft

210: _____

261: _____

329: _____

627: _____

740: _____

Mouthfeel: **Intensity of perceived taste of a typical white bread slice**

Not at all intense Very intense

210: _____

261: _____

329: _____

627: _____

740: _____

Overall quality **Overall impression of the bread based on all attributes**

Highly unacceptable Very acceptable

210: _____

261: _____

329: _____

627: _____

740: _____

APPENDIX VI

Description of sensory attributes used for the bread evaluation

Attributes	Description of Attributes
Crust color	Intensity of "golden brown" color, typical of white bread
Crumb color	Intensity of "whitish"/"creamish" color typical of white bread
Cells Uniformity	The uniformity of air cells (porosity) present in the crumb
Aroma	Degree of aroma intensity associated with typical white bread
Firmness	Resistance offered by the "compactness of crumb" by finger feel
Mouthfeel	Intensity of perceived taste of a typical white bread slice
Off –flavor	Intensity of any off flavor, e.g. oily, oxidized, rancid, or pulse-like
Overall quality	Overall impression of the bread based on above attributes

APPENDIX VII

Sensory evaluation of cookies fortified with defatted maize germ flour

Time. ……. Date……..

Instructions

Chew a sample of cookie and score for color, flavor, taste, crispness, texture and overall acceptability using the following Scale:

1=dislike extremely
2=dislike very much
3=dislike moderately
4=dislike slightly
5=neither like nor dislike
6=like slightly
7=like moderately
8=like very much
9=like extremely

Please Note:

1. **Before proceeding to the next sample, rinse mouth with water.**
2. **Make inter-comparison of the samples and record the score.**
3. **Don't disturb the order of samples.**

Character	**117**	**651**	**695**	**751**
Color				
Flavor				
Taste				
Crispness				
Overall acceptability				
Any Additional Comments►►	**117**	**651**	**695**	**751**

Cookies Ingredients

Wheat Flour, Defatted Maize Germ Flour, Sugar, Shortening, Baking powder, Eggs, Vanilla Extract

APPENDIX VIII

Questionnaire for cookie consumer panel

▼Please answer these additional questions▼

1. Your Gender: Male ☐ Female ☐

2. Your Age Group (yrs) : < 20 ☐ 21-30 ☐ 31-40 ☐
41-50 ☐ 51-60 ☐ >60 ☐

3. Your Income Group ($) : <20,000 ☐ 20,001-30,000 ☐ 30,001-40,000 ☐
40,001-50,000 ☐ 50,001-60,000 ☐ >60,000 ☐

4. Your Education Level: High School ☐ Associate Degree ☐
Bachelor Degree ☐ Graduate/Professional Degree ☐

5. Would you like to purchase these protein and fiber enriched cookies if available in the market?
Yes ☐ No ☐

Thank You!

APPENDIX IV

Consent Form: *Defatted Maize Germ Meal Fortified Bread/cookies*

Dear Participant:

Several Michigan State University researchers are investigating consumer perceptions of cookies made with added defatted maize germ (DFMG) flour. We would like you to take about 15 minutes (including the time you spend reading this letter) to help us evaluate DFMG Cookies. We are asking for volunteers, 18 years or older, taste these samples. *If you have a known food allergy or sensitivity to* corn products, eggs, wheat flour, *please do not volunteer for this study.*

If you meet the above requirements, we would like you to look at, taste and answer questions related to the product quality. If you agree to taste these and provide your evaluation based on the survey questionnaire, please sign the consent form below. You will be given an ice cream coupon or food treat that are worth less than $5 for your evaluation and completion of the survey.

If you believe there is a potential of an allergic reaction upon sniffing and tasting**, notify the on-site sensory evaluation coordinator and/or principle investigator immediately. You will be released from participating in this study. Please note if you are injured as a result of your participation in this research project, Michigan State University will assist you in obtaining emergency care, if necessary, for your research related injuries. If you have insurance for medical care, your insurance carrier will be billed in the ordinary manner. As with any medical insurance, any costs that are not covered or in excess of whatever are paid by your insurance, including deductibles, will be your responsibility. Financial compensation for lost wages; disability, pain or discomfort is not available. This does not mean that you are giving up any legal rights you may have.**

Your response is anonymous and we have no way to connect you, as an individual, to this completed survey form. However, we do depend upon you taking the time to honestly respond to the questionnaire. You are free to not answer any question you choose, but please try to answer every question. We are not able to use incomplete responses nor are we able to provide the incentive for incomplete responses.

If you have any questions during your reading this consent form, or during or after your participation, please do not hesitate to contact the on-site sensory evaluation leader and/or the principle investigator. Feel free to contact Muhammad Siddiq or Janice Harte, the principle investigators, via phone at 355 8474 or siddiq@msu.edu or harteja@msu.edu for any inquiry you might have due to your participation in our study.

In case you have questions or concerns about your role and rights as a research participant, please feel free to contact Dr. Peter Vasilenko, Ph.D., Director of Human Research Protections, (517) 355-2180, fax (517) 432-4503, e-mail irb@msu.edu, mail 202 Olds Hall, Michigan State University, East Lansing, MI 48824-1047.

PLEASE NOTE UPON YOUR SIGNING THIS CONSENT FORM, YOU VOLUNTARILY AGREE TO PARTICIPATE IN OUR STUDY. YOUR SIGNATURE INDICATES YOU HAVE READ THE INFORMATION PROVIDED ABOVE AND THAT YOU HAVE HAD AN ADEQUATE OPPORTUNITY TO DISCUSS THIS STUDY WITH THE PRINCIPLE INVESTIGATOR AND HAVE HAD ALL YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION. YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM WITH YOUR SIGNATURE FOR YOUR RECORDS UPON YOUR REQUEST.

SIGNED

_____

DATE_____