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Evaluation of Quality of different Non-branded samples of Black Tea

Saeed Akhter,*Shahid Masood, **H.S. Jadoon, *Ijaz Ahmad, ***Zhou Fen and *A.M. Salariya

Food Technology Centre, PCSIR Laboratories Complex, Peshawar, Pakistan

*Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan

**Islamia College University, Peshawar, Pakistan

***Huizhou School of Guang Dong Province, Huizhou, P.R. China

Corresponding Author: shahidmasoodft@gmail.com

ABSTRACT

The present study was designed to evaluate the quality of black tea available in the markets of Peshawar, Pakistan and to detect adulteration in black tea. Seventeen non branded samples of black tea were analyzed for their quality evaluation. Moisture%, ash%, caffeine%, tannin%, water extract% and added colors were determined. Moisture contents ranged from 2.39 to 10.0%, ash 4.2 to 10.4%, caffeine 0.13 to 3.98%, tannin 0.04 to 6.83% and water extract 9.3 to 39.2% in different samples of black tea. It was found that five samples were of inferior quality due to low caffeine contents than the standard value prescribed by Pakistan Food Laws and Regulations. However, two samples were adulterated with added color. The samples containing less caffeine than standard also exhibited less tannin contents. Rest of all the samples were found fit for human consumption according to the Food Laws and Regulations set by Government of Pakistan.

Key words: Adulteration, quality evaluation, black tea, caffeine, tannin.

INTRODUCTION

Tea (*Camellia sinensis)* is one of the most frequently consumed beverages. About 20% of the world production is consumed as green tea (an extract from heated and dried tea leaves), whereas 80% is consumed as black tea (which is produced from leaves by enzymatic oxidation). Dried tea extract contains 25-40% polyphenols (Balentine, 1992). Tea is the most popular beverage next to water, consumed by over two-thirds of the world's population. It is processed in different ways in different parts of the world to give green, black or oolong tea (Gupta *et. al.*, 2002).

In black tea, most of the catechins are oxidized to thearubigens and theaflavins, which give characteristic red-brown color. Both green tea and black tea also contain flavonol glycosides (quercetin and kaempferol) (Balentine, 1992). Highly significant decrease in fasting serum glucose (18.4%) and triglyceride levels (35.8%), significant decrease in LDL/HDL plasma cholesterol ratio (16.6%) and non significant increase in HDL plasma cholesterol levels (20.3%) was observed as a result of black tea consumption in a normal population. Black tea contributes to a decrease in cardiovascular risk factors and improves the overall antioxidant status in humans (Bahorun *et. al.*, 2012). Black tea polyphenols contribute to vascular health (Hodgson *et. al.*, 2013). Chinese dark teas (CDTs) have anti-obesity properties and decreases total serum cholesterol, triglyceride, low density lipoprotein cholesterol (LDL-C) by inhibiting the lipid absorption and biosynthesis. CDTs also possess antimicrobial, antioxidative and antimutagenic activities (Zhang *et. al.*, 2013).

Tea has many health benefits. Since ancient times, antipyretic, anti-inflammatory, antimicrobial and antioxidative properties of tea have been recognized. Black tea contains polyphenolic components like theaflavins (TF), thearubigins (TG) and catechins. The constituents present in black tea impart protection against androgen induced oxidative injury that may develop prostate cancer (Imtiaz *et. al.*, 2005). Shukla and Taneja (2002) evaluated the anticarcinogenic activity of aqueous black tea extract (ATE) and found that the percentage of mice having lung tumors was decreased after ATE administration

and significant decrease in the number of tumors/ mouse was also observed.

Black tea is a source of caffeine, a methylxanthine that stimulates the central nervous system, relaxes smooth muscle in the airways to the lungs (bronchioles) and acts on kidney as a diuretic (increasing urine). Hamer (2007) reviewed the beneficial effects of tea flavonoids on immune function. Customarily, tea was used to reduce toxins and to improve resistance against diseases. Tea extract has beneficial effects on fecal microflora balance (improving gut health) and antimicrobial effects in the upper respiratory tract (resistance against infections such as common cold).

Tea is subjected to many forms of sophistication including artificial coloring and facing, that is, the attachment of heavy bodies to the surface of tea to increase weight. In general, it may be assumed that coloring is intended to conceal damage or inferiority. Very often, tea leaves which have been damaged during processing are faced solely to improve their appearance (Wiley, 1919). Adulterated tea may contain chemicals or additives which do not belong to tea, exhausted tea leaves or color etc. Adulterants are generally added to reduce manufacturing costs and to deceive consumers. Adulteration and contamination in different food products including tea are constant threat to the health of common man in Pakistan. The types of impurities found in food items sold in the markets should be highlighted. Government should discourage food adulteration. Adulterated tea may cause health problems instead of providing health benefits. Therefore, the present study was conducted to evaluate the quality of black tea available in the markets of Peshawar, Pakistan.

MATERIALS AND METHODS

Tea Samples were collected from different tea dealers, distributors and customer's houses in Peshawar city, Khyber Pakhtunkhwa, Pakistan. All the samples were collected sealed in aluminum foil and stored in card board box. All the samples were protected from light and air till the completion of study. Samples were coded by allotting Serial No. from 1 to 17 as shown in Table 1.

Samples were analyzed for physicochemical tests like moisture %, ash %, caffeine%, tannin%, water extract and added color. All the tests were performed in Food Technology Center of PCSIR Laboratories Complex, Peshawar. The entire chemicals used were of analytical grade. Moisture% (Ch 30.1.32, method No. 925.19), ash% (Ch 30.1.25, method No. 920.100) and water extract (Ch 30.1.34, method No. 920.104) were determined according to their respective methods as described in AOAC (2005).

Caffeine content

The caffeine content was determined according to Method No. 925.16 (AOAC 2005). Power-Chesnut Method (14.019, AOAC, 10th Edition) was followed. Grinded sample (10 g, mesh size 30) was taken. Moistened with alcohol and extracted with alcohol for 8 hr in Soxhlet. Transferred extract with hot water to porcelain dish containing 10 g MgO suspension in 100 ml water. Evaporated slowly and stirred to dry. Rubbed residues with pestle to paste with boiling water, transferred with hot water to smooth filter. Collected filtrate in 250 ml flask washed with boiling water and made up volume. Added 20 ml H_2SO_4 (1+ 9) and boiled for 30 min. Cooled, filtered through double paper into separator, washed with small portion of H_2SO_4. Extracted with six 20 ml portion of $CHCL_3$. Washed and combined $CHCL_3$ extract in separator with 5 ml 1% KOH solution. Filtered $CHCL_3$ in Erlenmeyer flask. Washed KOH solution with 2 ten ml portion of $CHCL_3$, adding them to flask together with $CHCL_3$ washing of filter paper.Then evaporated on steam bath to small volume (10-15 ml). Transferred with $CHCL_3$ to weighed beaker, evaporated carefully, dry 30 min at 100°C and weighted. The purity of sample was tested of residue by detecting N_2 and multiplying by factor 3.464.

Tannin and coloring matter

Tannin was determined by the method as described by Ruck (1963). Sample (25 gm) was taken in 600 ml beaker, added 300 ml water and boiled for 1 hour, replaced the water lost by evaporation. After Cooling, transfered to 500 ml volumetric flask, made up volume and filtered through No. 4 Whatman paper. Filtrate (400 ml) was taken in 600 ml beaker, added 0.3 gm Powdered $CaC0_3$ and heated to boiling. Cooled, transferred to 500 ml volumetric flask, make up volume and filtered through No. 5 Whatman paper. Filtrate (200 ml) was taken in 2 L porcelain dish, added 800 ml water and 20 ml indigo solution. Titrated with 0.1 N $KMnO_4$ (until blue color changes to green and then golden yellow). The ml of KMn04 solution used "a". To the remaining filtrate added 1 gm carbon and shaking was done occasionally for 10 minutes. Then filtered through No. 5 Whatman paper and 200 ml filtrate was titrated with $KMnO_4$ solution as described above. The ml of $KMn0_4$ used "b."

1 ml. 0.1N $KMnO_4$ = 0.0035 gm Tannin

$$\% \text{ tannin and coloring matter} = \frac{(a - b) \times \text{normality of } KMnO_4}{\text{wt. of sample titrated}} \times 3.5$$

Table 1: Quality characteristics of non-branded black tea samples

Sample No.	Parameters (%)					Added Color
	Moisture	Total Ash	Caffeine	Tannin	Water Extract	
1	4.94	5.21	3.27	1.14	32.80	Present
2	2.40	10.4	3.73	2.76	37.70	Absent
3	5.20	5.10	3.68	1.25	33.50	Absent
4	7.29	5.35	3.94	1.70	33.20	Present
5	7.22	4.90	3.98	5.17	33.80	Absent
6	7.60	4.47	3.92	5.25	24.70	Absent
7	3.50	5.50	3.36	5.10	31.45	Absent
8	4.10	5.65	3.22	5.00	31.72	Absent
9	3.14	5.58	3.30	5.25	31.65	Absent
10	5.40	4.40	3.16	6.83	24.70	Absent
11	8.99	5.65	0.17	0.04	10.30	Absent
12	8.95	4.95	0.13	0.09	11.60	Absent
13	5.13	6.79	1.15	0.30	28.60	Absent
14	5.80	6.30	1.55	0.48	23.00	Absent
15	2.39	6.14	2.40	5.15	39.20	Absent
16	10.0	4.20	0.35	0.15	9.30	Absent
17	4.90	5.00	3.20	2.00	32.00	Absent
Standard	3.5-9.0	5-8	Min. 2	Min. 5	Min. 23	Absent

Min = Minimum

Color addition in tea sample

The presence or absence of added color was determined according to Ch 26.1.05, Method No. 930.17 (AOAC 2005).

RESULTS AND DISCUSSION

Seventeen samples of non branded black tea were collected from the local market of Peshawar, Khyber Pakhtunkhwa, Pakistan. Samples were analyzed for moisture%, ash%, caffeine%, tannin%, water extract and added colors. The results are shown in Table 1. Moisture contents ranged from 2.39 to 10.0%, ash 4.2 to 10.4%, caffeine 0.13 to 3.98%, tannin 0.04 to 6.83% and water extract 9.3 to 39.2% in different samples of black tea (Table 1).

Caffeine content

Caffeine percentage of five samples was found less than the standards values prescribed by Pakistan Pure Food Laws and Regulations (Awan, 2010). One cup of tea contains about 50 mg of caffeine, depending on the strength and size of cup (as compared to coffee, which contains 65 to 175 mg of caffeine/ cup). Margriet *et. al*. (2006) showed that functional ingredients in green tea, black tea and caffeine have the potential to produce significant effects on metabolic functions such as satiety, thermogenesis and fat oxidation.

It is recommended that daily intake of 200-300 mg of caffeine is safe, but 500-600 mg daily can cause a number of health problems (Anonymous, 2009). The American Congress of Obstetricians and Gynecologists (ACOG, 2010) concluded that caffeine consumption is safe up to 200 mg per day in pregnant women. However, one may collapse if consume too much caffine. It improves performance during sleep deprivation but may lead to insomnia (Snel and Lorist, 2011). Its excessive use is harmful to digestion and causes sleep disturbance. According to Rapuri *et al*, (2007) caffeine suppresses Vitamin D Receptor, causing osteoporosis.

Tannin contents

Tannin contents ranged from 0.04 to 6.83% (Table 1). Berketova and Kosheleva **(**2010) demonstrated that tannins ranged in tea from 0.91 to 7.07%. Tannin contents of the same samples (which were low in caffeine) were also found out of range from the standards of Pakistan Food Laws and Regulations. Tannins have shown potential antiviral (Lu *et al* 2004), antibacterial (Akiyama *et al* 2001) and antiparasitic effects (Koloziej and Kiderlen 2005). If tea contains exhausted tea leaves then tannin contents and yield of extract are decreased. However, low caffeine and tannin contents may be due to undesirable changes took place under all the sets of conditions studied (Cloughley, 1986), but the rate of

deterioration increased with increasing temperature and moisture content. High moisture content had a much greater deleterious effect than high temperature. Tea manufactured and stored during the hot and humid production season deteriorated more rapidly than those produced and stored during the milder conditions of off- season (Jayabalan *et. al*. 2008; Kaack and Christensen, 2008).

Results of color added

Two samples were adulterated with added color as shown in the Table 1. Peoples can not diagnose such type of adulteration because low quality of black tea and change in sense of consumer's taste is one of the reasons. Various kinds of tea are produced by the tea manufacturers to attract consumer opinion. However, rest of all the samples were found fit for human consumption. The quality parameters for these were in the range of standard values of Food Laws and Regulations. No sample was adulterated with impurities like wheat husk and bran or any foreign leaves.

Conclusion

It was concluded that five samples were of inferior quality due to low caffeine contents than the standard value prescribed by Pakistan Food Laws and Regulations. These samples also exhibited less tannin contents. Two samples were adulterated with added color. Adulterants are generally added to reduce manufacturing costs and to deceive consumers. Adulteration and contamination in different food products including tea are constant threat to the health of common man in Pakistan. Government should discourage food adulteration. Adulterated tea may cause health problems instead of providing health benefits.

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Application of edible coating for improving meat quality: A review

Muhammad Issa Khan, Muhammad Nawaz Adrees, Muhammad Rizwan Tariq and Muhammad Sohaib

National Institute Of Food Science & Technology, University of Agriculture Faisalabad-Pakistan

*Corresponding Author: drkhan@uaf.edu.pk

ABSTARCT

Edible coatings can improve the quality of fresh, frozen, and processed meat, poultry, and seafood products by retarding moisture loss, reducing lipid oxidation and discoloration, enhancing product appearance in retail packages by eliminating dripping, sealing in volatile flavors, functioning as carriers of food additives such as antimicrobial and antioxidant agents, and reducing oil uptake by battered and breaded products during frying. This paper reviews the application of various types of lipid, polysaccharide and protein-based edible coatings, as well as multicomponent edible coating systems, on meats, poultry, and sea foods.

Key words: Edible coating, Meat Quality, oxidative stability, cooking loss

INTRODUCTION

Edible coatings from polysaccharides, proteins, and lipids can extend the shelf-life of foods by functioning as solute, gas, and vapor barriers. Although use of edible coatings and films to preserve food quality is not a novel concept, research in this field at academic, government, and private industry laboratories has intensified recently. Factors contributing to renewed interest in development of edible coatings include consumer demand for high quality foods; food processors' needs for new storage techniques; environmental concerns over disposal of nonrenewable food packaging materials; and opportunities for creating new market outlets for film-forming ingredients derived from under-utilized agricultural commodities.

Film formation and properties for several polysaccharide, protein, and lipid substances have been reviewed (Kester *et al*., 1999). Commercial applications of edible films and coatings include fresh produce coatings from waxes, oils, resins, and sucrose fatty acid polyesters (Baldwin, 1994); collagen casings for sausages (Hood, 1987); chocolate coatings for confections (Alikonis, 2000); confectioner's glaze made from shellac (Biquet, 1998); corn zein-based coatings for nutmeats, candy, and pharmaceutical tablets (Andres, 1984); gelatin-based pharmaceutical coatings (Rose, 1987); and cellulose ether-based water soluble pouches for food ingredients (Anon, 1992). This overview discusses the rationale of using edible coatings on meats, poultry, and sea foods and summarizes research findings on the effectiveness of and the problems associated with various types of coatings.

Rationale of Using Edible Coatings on Meats, Poultry, and Sea foods

Almost any sector of the food industry could utilize appropriately formulated edible coatings to meet challenges associated with marketing safe, nutritious, stable, economical, and high quality foods. Particularly with regard to the meat, poultry, and fisheries industries, the following are potential benefits of using edible coatings:

(i) Moisture loss during storage of fresh or frozen meats leads to texture, flavor, and color changes, while also reducing saleable weight. Edible coatings with good moisture barrier properties could help alleviate the problem of moisture loss. For example, when meat is removed from vacuum packages, a 3–5% reduction in weight occurs due to moisture evaporation.

Application of coatings prior to vacuum packaging could prevent this moisture loss, thereby having a important economic impact by increasing saleable weight of products.

(ii) When fresh meat, poultry, or fish cuts are packaged in retail plastic trays, dripping of product juices occurs making such packages unattractive to consumers. Edi-ble coatings could hold in juices, prevent dripping, enhance product presentation, and eliminate the need for placing absorbent pads at the bottom of trays.

(iii) The rate of rancidity causing lipid oxidation and brown coloration-causing myoglobin oxidation in meats could be reduced by using edible coatings of low oxygen permeability, although not so low as to create anaerobic conditions.

(iv) Edible coating solutions, which have been heated just prior to application, could reduce the load of spoilage and pathogenic microorganisms and partially inactivate deteriorative proteolytic enzymes at the surface of coated meat, poultry, and fish cuts.

(v) Volatile flavor loss from, and foreign odor pick-up by meat, poultry, and seafoods could be restricted with edible coatings.

(vi) As an application of active packag-ing, edible coatings carrying antioxidants (e.g. toco-pherols) and/or antimicrobials (e.g. organic acids) can be used for direct treatment of meat surfaces, thereby delaying meat rancidity and discoloration, and reducing microbial loads.

(vii) Coatings applied on the surface of fish, poultry, and meat pieces prior to battering, breading, and frying, could improve the products' nutritional value by reducing oil uptake during frying. It is evident from the above that edible coatings could substantially improve the quality of meats, poultry, and seafoods

Types of coating

There are mainly three types of coating.

1. Lipid-based Coatings

2. Polysaccharide based coating

3. Protein based coating

1.1 Waxes, fats, and oils

Coating foods with fat, a practice known as 'larding', was used in 16th century England (Labuza and Contrer, 1993). Waxes (e.g. carnauba wax, beeswax, paraffin wax) and oils (mineral oil, vegetable oil) have been commercially used since the 1930s as protective coatings for fresh fruits and vegetables (Baldwin, 2002). In the 1950s, several meat processors in the U.S. were applying strippable coatings of petroleum derived microcrystalline wax on frozen meats, such as beef, veal, lamb, hamburger patties, and luncheon meats (Mcgrath, 2004).

Generally, wax coatings have been shown to be substantially more resistant to moisture transport than most other lipid or non lipid edible coatings (Watters and Brekke, 1998). However, wax-based, as well as fat- and oil-based, coatings present application (i.e. thickness and homogeneity control, greasy surface, cracking) and organoleptic (i.e. waxy taste, rancidity) problems (Guilbert, 2000). Few references can be found in the literature regarding the effectiveness of waxes, fats, and oils as protective coatings for meats. (Mcnally, 1992) dipped dressed whole chickens into molten wax, mineral oil, corn oil, or lard prior to freezing. Mineral oil and wax reduced moisture loss from the frozen birds more than corn oil or lard but not as much as cellophane bags . Freshly cut meats, on which protective coatings of molten fat (e.g. beef tallow, lard) droplets had been deposited, were superior to uncoated control samples in terms of color and moisture retention during storage at 2 to 4°C (LIEBERMAN and GILBERT, 2002). Frozen meats, poultry, and fish did not undergo substantial dehydration when coated in oil-in-water emulsions prepared at 60 to 80°C by blending an animal fat or vegetable oil with emulsifiers, water, and, optionally, seasoning and preservative agents. Substantial reduction in moisture uptake during stor-age of freeze-dried meats were reported when liquefied fat-based coatings, at a temperature in the range of 52 to 79°C, were sprayed on dehydrated meat pieces (Aydt et al., 1999). These coatings were composed of beef tallow, lard, a lactic acid–fatty acid triglyceride (e.g. glycerol lacto-palmitate), and a vegetable oil. The use of long chain (16 to 20 carbon atoms) saturated fatty alcohols or fatty acids as protective coatings to control moisture loss and freezer burn in refrigerated or frozen meats was suggested by Anderson (BRANDENBURG et al., 2003). These coatings were applied on meats prior to refrigerating or freezing in the form of aqueous, elevated temperature (50 to 90°C) emulsions of fatty alcohols or fatty acids and, optionally, an emulsifier. Reportedly, better results were obtained in frozen meats when the meat was first coated with ice prior to applying the fatty film and with refrigerated meats when the fatty film-forming material was applied as an emulsion in glycerin or water–glycerin (MCHUGH and KROCHTA, 2000). It was claimed that the inter-mediate hydrophilic layer of ice, glycerin, or water– glycerin formed between the film and the meat surface attracted polar groups of the fatty film-forming material while repelling its hydrophobic carbon chains. As a result, fatty molecules were properly aligned and compressed together and the moisture-retarding ability of the fatty film increased (Brandenburg et al., 2003).

1.2 Glycerides and acetylated glycerides

Monoglycerides (monoacylglycerols), diglycerides (dia-cylglycerols), and triglycerides (triacylglycerols) are the mono-, di-, and triesters, respectively, of glycerin with fatty acids (Nawar, 2000). Acetylated glycerides (acetoglycerides) can be prepared either through the reaction of glycer-ides with acetic anhydride or through the catalysed interesterification of a fat or oil with triacetin (Feuge, 1991). Both glycerides and acetylated glycerides have been utilized as coatings.

According to Lovegren and Feuge, 1998 acetylated glycerol monostearate coatings were slightly more permeable to water vapor than poly-amide, ethylcellulose, and polystyrene films and sig-nificantly more permeable to water vapor than cello-phane and

polyethylene films. In terms of oxygen barrier ability, acetylated glycerol monostearate coat-ings were less permeable to oxygen than ethylcellulose and polystyrene films (Lovgren and Feuge, 2001). **(**Woodmansee and Abbott, 2004) dipped broiler legs scalded at 53 to 60°C into Myvacet acetylated monog-lycerides types 5-00 and 9-40 (2:1 mixture). In the Myvacet products the first number indicates degree of acetylation (e.g. 5 stands for 50% acetylation) while the second number is the iodine number. After storage at 4°C for 10 d, Myvacet-coated broiler legs had a weight loss due to dehydration of 4.2 to 6.3% as opposed to a weight loss of 15.1 to 30.2% for uncoated control broiler legs.

The coated broiler legs exhibited less skin darkening during storage than uncoated control samples (MCHUGH and KROCHTA, 2000). Saturated acetylated glyceride coatings in which the antibiotic chlortetracy-cline had been incorporated increased the time elapsing before microbial populations caused off-odors in fresh beef steaks stored at 5°C. Excellent moisture retention by coated steaks was also reported although the coating's high impermeability to oxygen resulted in meat discoloration during storage (HOUTS, 2001). According to (SKERRITT et al., 2002) found that Myvacet 7-00 and, to a lesser extend, Myvacet 7-15 acetylated monoglyceride coatings retarded moisture loss from fryer parts stored at 0.5°C for 28 d or at –18°C for 6 months. The greater effectiveness of the Myvacet 7-00 coating as a moisture barrier can be explained by the unsaturation (iodine number 15) of the Myvacet 7-15 product. In general, as unsaturation of the hydrocarbon chain increases, the water vapor permeability of lipid coatings increases as well.

The effect of acetylated monoglyceride coat-ings on organoleptic properties of broiler parts was investigated by (FAROUK, et al., 2005) .They coated samples for fresh storage (2°C for 1 or 2 weeks) with Myvacet 7-00, and samples for frozen storage (–18°C for 1 or 8 months) with Myvacet 7-15. Reportedly, after oven-roasting or deep-fat frying, coated broiler parts were equally acceptable in terms of flavor and juiciness scores, moisture content, and fat content, to the uncoated control samples which had been stored in polyvinylidene wraps (ZABIK, M.E. and L.E. DAWSON, 1999).

2. Polysaccharide-based Coatings

Film formation and properties of several polysaccha-ride materials such as starch and starch derivatives, alginates, cellulose derivatives, carrageenan, various plant and microbial gums, chitosan and pectinates have recently been reviewed by Nisperos Carriedo, 1994). In general, due to their hydrophilic nature, polysaccharide films generally exhibit limited water vapor barrier ability. However, certain polysaccharides, applied in the form of high moisture gelatinous coatings, can retard moisture loss from coated foods by functioning as sacrificing agents rather than moisture barriers (KESTER, 1999). Application of various types of polysaccharide-based protective coatings on meat products is discussed below

2.1 starch and starch derivatives

Amylose, the linear fraction of starch, is known to form coherent, relatively strong, free-standing films in con-trast to amylopectin films which are brittle and non-continuous (Zobel, 1998). Transparent, oil-impermeable films cast from water–butanol solutions of gelatinized amy-lose had very low oxygen permeabilities in dry condi-tions (Wolff *et al*., 1995). Presumably, films cast from ethanol– water dispersions of dimethyl sulfoxide-pretreated high amylose (71%) starch retained low oxygen permeability even at high RH (Mark *et al.*,1990).

Hydroxypropylated derivatives of high amylose starch made films with very poor moisture barrier abilities but with substantial oxygen barrier abilities of RH up to 78% (Jokay *et al.*, 1999). Presumably, such films could protect meat products during frozen storage and subsequently be dissolved during thawing and cooking. However, no references can be found in the literature assessing the effectiveness of hydroxypropylated high amylose starch films as protective coatings for frozen meat, poultry, and sea foods. In terms of food packaging applications, these films were primarily intended for use on frozen foods including frozen meat, poultry, and fish. Reportedly, Ediflex wraps were flexible, transparent, impermeable to oxygen, resistant to oil and grease, heat sealable, soluble in hot or cold water, and printable (HOUTS, 2001). Presumably, such films could protect meat products during frozen storage and subsequently be dissolved during thawing and cooking. However, no references can be found in the literature assessing the effectiveness of hydroxypropylated high amylose starch films as protective coatings for frozen meat, poultry, and seafoods (TAYLOR, 2005).

2.2 Alginates

Alginates, which are extracted from brown seaweeds of the Phaephyceaeclass, are the salts of alginic acid, a linear co polymer of D-mannuronic and L-guluronic acid monomers (Sanderson, 2001). Films produced by evaporation of water from a thin layer of alginate solution are impervious to oils and greases but, as with other hydrophilic polysaccharides, have high water vapor permeabilities (King, 1996). The ability of alginates to react with di- and trivalent cations is utilized in alginate film formation. Calcium ions, which are more effective than magnesium, manganese, aluminium, ferrous, and ferric ions as gelling agents (Allen, *et al*., 2004), 'bridge'

alginate chains together via ionic interactions, a phenomenon followed by inter chain hydrogen bonding .

Alginates have often been combined with regular and modified starches, oligosaccharides, or simple sugars in meat containing formulations. According to (Hartal , 1997), tearing strength of alginate coatings increased by adding maltose, lactose, and corn syrup of intermediate (Jokey et al., 1999) dextrose equivalent (DE). Nevertheless, when reducing sugars are included in coatings, non-enzymatic browning may occur during cooking (Earle, 1990). According to (Taylor, 2005) coated fresh beef steaks, pork chops, and skinned chicken drumsticks by immersion (1 s) in an aqueous solution of sodium alginate, or sodium alginate and regular corn starch, or sodium alginate and oxidized starch, followed by immersion in a 5 mol/L CaCl2 solution (1–2 s). After storing at 1°C and 85–95% RH for 1, 2, 4, and 7 d, product shrink was generally reduced by the coatings. Furthermore, all three coatings were equally effective in improving product texture, juiciness, and, in some cases, color, general appearance, surface texture, and odor of uncooked and cooked products. Similarly, (Hartal, 1994) reported significant improvements in moisture reten-tion, texture, and juiciness for chicken drumsticks coated with an alginate-intermediate DE corn syrup film or an alginate-intermediate DE corn syrup monoglyceride film prior to storing (1°C, 85–95% RH, 12 d). Despite the aforementioned improvements, an experi-enced sensory panel found the flavor of cooked beef steaks and pork chops which had been coated with alginate or alginate–starch films to be inferior to the flavor of uncoated samples (WILLIAMS et al., 2004). Also, free calcium and other metal cations used for fixing alginate coatings may increase proteolytic enzyme activity on meat surfaces by acting as enzyme activators. Sensory evaluation data showed that alginate coatings fixed in calcium propionate solutions had better flavor than coatings fixed in CaCl 2 solutions (Mark et al.,1990). However, because calcium propionate has weaker ionizing properties than CaCl2, immersion time in calcium propionate solution had to be longer to obtain coatings of similar strength to those fixed in a CaCl2 solution. A method for protecting seafood from dehydration and oxidation during frozen storage was described by Earle and Snyder (1998). Microorganisms and enzymes in the seafood were first inactivated by heating in water (e.g. at 70°C for 30 min); soaking in an aqueous solution containing chlorine; or soaking in a weak solution of acetic acid. Subsequently, the products were immersed in an aqueous dispersion of sodium alginate (0.02 to 0.15) and native starch, oxidized starch, or dextrins (0.98 to 0.85). For better results, a vegetable oil also was incorporated into the mixture. Gelling of the coating was accomplished by dipping in a 0.2 mol/L CaCl2 solution (1–2 min). It was claimed that shrimp, mack-erel, and kingfish treated in this manner retained their original flavor, texture, and color after frozen storage for 3 months (WANSTEDT et al., 2003). Earle and Snyder (1998) were awarded patents for a fresh meat coating process in which a sodium alginate–oligosaccharide solution and a CaCl 2 -thickening gum gelling solution were successively applied on fresh meat by spraying or dipping. Based on these two inventions, an edible alginate-based coating, Flavor-Tex, for use on meats, poultry, seafood, and other foods was developed by Food Research, Inc. (Tampa, FL) and marketed by D. H. McKee, Inc. (Tampa, FL) in the 1970s (MCCORMICK, 2005). Flavor-Tex's formulation included maltodextrin along with sodium alginate in the first solution and carboxymethyl cellulose along with CaCl2 in the second solution (STOLOFF, 2001). Alginate Flavor-Tex coatings applied on lamb carcasses stored at 4°C and on beef cuts stored at 5°C reduced moisture loss without significantly affecting total aerobic microbial counts on coated meat surfaces. Hydrochlorous acid incorporated into the alginate coating did not inhibit microbial growth on beef cuts. Sensory evaluation of cooked lamb and beef cuts that had been coated with Flavor-Tex did not reveal any significant differences in comparison with uncoated control samples (NATRAJAN and Sheldon, 2004). During frozen storage at –18°C, red snapper and silver salmon treated with Flavor-Tex alginate coatings and sealed in polyethylene bags had slightly greater mois-ture contents and developed less lipid oxidation (based on thiobarbituric acid assays) than uncoated controls in polyethylene bags (Mark et al.,1990). Trimethylamine (indicator of bacterial growth) concentration, hypoxanthine (indica-tor of nucleotide degradation) concentration, and aerobic plate counts were not significantly affected by Flavor-Tex coatings. In terms of frozen fish moisture content, there were no significant differences between Flavor-Tex coatings and ice glazes. However, Flavor-Tex coatings remained intact after thawing so that drip loss was reduced.WILLIAMS et al., 2004) studied the effects of alginate Flavor-Tex coatings on sensory attributes of raw and precooked (before or after coating) pork patties stored at –20°C while wrapped in polyethylene-coated freezer paper. Alginate-coated patties had improved texture, flavor, juiciness, and overall palatability over uncoated control samples. Moreover, warmed-over flavor (lipid oxidation) was eliminated in coated patties with no precooking and patties coated after precooking as judged by sensory scores and thiobarbituric acid values. In a recent study, (Hargens-Madsen, 2006) coated pre-cooked pork chops with CaCl2-gelled alginate–starch coatings. During storage at 4°C for 3, 6, or 9 d, the coatings did not retard moisture loss. However, sensory evaluation of microwave-heated pork chops showed that coated samples were juicier than uncoated control samples. Thiobarbituric acid values and sensory scores showed

that pork chops developed less lipid oxidation after refrigerated storage for 6 and 9 d when the natural antioxidant tocopherol was incorporated into the alginate–starch coatings. However, the trained sensory panel detected an off-flavor in pork chops treated with tocopherol-containing alginate–starch coatings. In this study by Hargens-Madsen pork chops gained almost 20% of their original weight from alginate– starch coatings (with or without tocopherol). From a practical standpoint, application of such thick coatings may be unrealistic.

2.3 Carrageenan

The polysaccharide gum carrageenan, a galactose polymer, is extracted from Irish moss (Chondrus crispus) and from other species of red seaweeds (Whistler and Daniel, 1991). Carrageenan is a complex mixture of at least five distinct polymers designated as é, ë-, ì-, í- and î-carrageenan (Whistler, 1991). Of these, mixtures of é-, ë- and ì-carrageenan are used in food applications . Gelation of é- and ë-carrageenan occurs with both monovalent and diva-lent cations, whereas ì-carrageenan is nongelling (Sanderson, 2002). Use of carrageenan coatings for prolonging frozen storage life of fatty fish has been proposed (Stoloff, 1989).

Coating mackerel fillets by dipping into aqueous carrageenan solutions (10 g/kg) prior to freezing and storing at –18°C prevented any major sensory changes for up to 5 months, whereas uncoated controls were found unacceptable after 3 months (Stoloff, 1989). Further delay of spoilage, until the seventh or eighth month of storage at –18°C, was noticed when antioxidants, gallic acid or ascorbic acid were added to carrageenan coating solutions (PEARCE and LAVERS, 2003). Lecithin was also claimed to reduce oxidation in meat when incorporated into carrageenan meat coatings. Pearce and Lavers studied the effect of applying a carrageenan coating prior to freezing on the shelf-life of defrosted whole chicken carcasses. Birds were coated by dipping into aqueous boiling (100°C) solutions containing carrageenan and potassium chloride in a 4:1 mixture (40 g/L). Reportedly, the refrigerated (4°C) shelf-life of defrosted coated birds, as judged by off-odor development, increased from 120 h for uncoated controls to 168 h. Shelf-life was further increased to 192 h when salt was incorporated into carrageenan coatings (Whistler, 1991).. Carrageenan coatings were also applied on poultry meat by (Jokey et al., 1999).Fresh chicken parts were dipped into a 40 g/L aqueous solution of carrageenan at 64°C. During storage at 2°C, shelf-life of coated poultry slightly increased. Spoilage was further retarded by incorporation of water soluble antibiotics (i.e. chlortetracycline, oxytetracycline) into carragee-nan coatings. In fact, inclusion of antibiotics into coatings was a more effective spoilage retardation method than dipping poultry into antibiotic–saline solutions (MEYER, et al., 2003). However, antibiotics are no longer approved for use on poultry meat as a preservative.

2.4 Agar

Agar is a gum derived from a variety of red seaweeds of the Rhodophyceaeclass and, like carrageenan; it is a galactose polymer (Sanderson, 2004). It forms strong gels characterized by melting points far above the initial gelation temperature (Whistler and Daniel, 1991). Similar to carrageenan coatings, agar coatings in which water soluble antibiotics (i.e. chlortetracycline, oxytetracycline) had been incorporated were effective in extending shelf life or poultry parts stored at 2°C (Meyer, *et al.*, 1998). Also, the storage life of beef steaks at 5°C was extended by 2 d by applying chlortetracycline-contain-ing, glycerin-plasticized agar coatings (AYRES, et al., 2004). However, despite their substantial thickness (0.8–0.9 mm), these agar coatings did not reduce moisture loss from meat as acetylated glyceride or ethylcellulose-based coatings did (36). Recently, Natrajan and Sheldon studied agar coatings as vehicles for addition of the bacteriocin nisin to fresh poultry. EDTA, citric acid, polyoxyethylene sorbitan monolaureate, and 500 íg/mL nisin were incorporated into agar coatings and applied on broiler skin samples contaminated with Salmonella typhimu-riumat a 1:2 weight ratio (coating:skin). Reductions in S. typhimurium populations of 1.8 to 4.6 log cycles were recorded after storage at 4°C for 96 h (Natrajan and Sheldon, 2004).

2.5 Dextran

Dextrans are microbial gums composed solely of á-D-glucopyranosyl units but with varying types and amounts of glycosidic linkages. Leuconostoc mesenteroidesand L. dextranicum are the micro-organisms usually employed in dextran biosynthesis from sucrose fermentation (Whistler and Daniel, 1991). Dextran coatings applied in the form of aqueous solutions or dispersions have been proposed for use on unpeeled shrimp , peeled shrimp , fish (Toulmin, 1996), and meat products such as ham, sausage, and bacon (Novak, 2000) to preserve their flavor, color, and freshness during refrigerated or frozen storage.

2.6 Cellulose ethers

Cellulose, the structural polysaccharide of plants, is composed of D-glucose units linked through â-1,4 glycosidic linkages. Native cellulose is a crystalline cold water-insoluble high molecular weight polymer (Ganz, 1998). The reactivities of the three hydroxyl groups at positions 2, 3, and 6 on the glucosyl units of cellulose are utilized for making useful derivatives.

Cellulose ethers are polymer substances obtained by partial substitution of hydroxyl groups in cellulose by

ether functions. Methylcellulose (MC), hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), and carboxymethylcellulose (CMC) are water soluble ethers possessing good film-forming properties (Felcht, 1999). Their relative hydrophilicities increase in the order of HPC<MC<HPMC<CMC . Ethylcellulose (EC) is another film-forming cellu-lose ether which, in contrast to the aforementioned ethers, is water insoluble (Krumel, and Lindsay, 2001).

3. Protein-based Coatings

Formation and properties of films from animal and plant proteins, such as collagen, gelatin, milk proteins, wheat gluten, soy protein, corn zein, and peanut protein, have recently been reviewed (Gennadios, 2003). Over-all, in a similar manner to polysaccharide films, protein films exhibit relatively high water vapor permeability values, i.e. approximately two to four orders of magnitude greater than those of conventional polymeric packaging materials such as polyethylene, polypropylene, polyester, and polyvinylidene chloride (Lieberman and Gilbert, 1994).. The limited resistance of protein films to water vapor transmission is attributed to the substantial inherent hydrophilicity of proteins and to the significant amounts of hydrophilic plasticizers, such as glycerin and sorbitol, incorporated into films to impart adequate flexibility. On the other hand, the good oxygen barrier properties in low relative humidity environments of films from collagen, wheat gluten, corn zein, soy protein, and whey protein have been documented by (MOREAU and ROSENBERG, 2002). A problem that may be encountered with protein-based coatings on raw meat, poultry, and seafood is the expected susceptibility of proteins to proteolytic enzymes present in these foods. Moreover, in terms of consumer acceptance and food labeling requirements, researchers investigating the application of protein-based coatings on meat and other food products need to consider the potential occurrence of individualistic adverse reactions to proteins. For example, food aller-gens have been identified among the protein fractions of milk, egg white, peanuts, soybeans, and rice. Certain individuals develop an adverse reaction to wheat gluten, and its gliadin fraction in particular, known as celiac disease or gluten-sensitive enteropathy or non-tropical sprue (Gennadios, 2003). Lactose intolerance exhibited by lactase deficient individuals is another, fairly common, dietary intolerance. Elevated levels of lactose, the carbohydrate contained in con-siderable amounts in bovine milk, are present in total milk proteins and in whey protein concentrates which may be employed in formation of edible coatings Krumel, and Lindsay, 2001).

3.1 Collagen

Production of collagen sausage casings from the regen-erated corium layer of food-grade beef hides is a well established technology and has been discussed else-where (Gennadios *et al*., 2003). As an alternative to preformed collagen casings, Unilever has developed a technology where the collagen casing is co-extruded around the sausage meat batter (Smits, 1991). The co-extrusion process is continuous and better controlled than the conventional batch process where meat batter is stuffed into pre-formed casings . Large processing plants with high volume lines use the co-extrusion technology for collagen casing production. Furthermore, use of pro-teins other than collagen, such as wheat gluten, corn zein, soy protein, peanut protein, and feather keratin, in manufacturing of sausage casings has been suggested (Jones and Whitmore, 2002).

Collagen-based edible coatings also have been pro-posed for use on meat products other than sausages. Jones and Whitmore described a method where ground collagen was mixed with an aqueous mixture of lactic acid and glyceraldehyde, heated to about 75°C, and neutralized to pH 7 to make a coating for hamburgers capable of withstanding cooking temperatures without melting. An edible collagen film (Coffi, Brechteen Co., Mt. Clemens, MI), intended for use on netted roasts, boneless hams, fish fillets, roast beef, and meat pastes, was commercialized in the U.S. in the late 1980s. According to the manufacturer, Coffi can reduce cook shrink, increase product juiciness, and allow for easy removal of elastic stretch netting after heat processing YOUNG et al., 2004. (SHEU and ROSENBERG, 2002) reported that both refrigerated and frozen/thawed round beef steaks wrapped in Coffi collagen film prior to standard retail packaging (perme-able film overwrap) or vacuum packaging exhibited significantly less fluid exudate than unwrapped controls. Moreover, based on thiobarbituric acid analysis and on instrumental (Hunter color meter) and sensory color evaluation, the collagen films had no significant effect on meat oxidation and color.

3.2 Gelatin

Film-forming applications of gelatin, which is derived by partial hydrolysis of collagen, in the pharmaceutical and food industries include microencapsulation of ingredients and manufacture of tablet and capsule coatings (Gennadios, 2003). Similar to other types of edible films, gelatin coatings have shown potential as carriers of antioxidants. Turkey steaks sprayed with an aqueous gelatin suspension of various antioxidants developed less lipid oxidation (lower peroxide values by 60–90%) in skin and meat fat than uncoated controls during frozen storage at –12°C for 6 months (Klose *et al.*, 1990)

3.3 Milk proteins

Edible films and coatings from casein, whey protein, and total milk proteins have been discussed in detail (Torres, 1994). Recent studies have combined case-inates with stearic acid for coating peeled carrots and with acetylated monoglycerides for coating zuc-chini . Whey protein-based coatings have been tested on breakfast cereals, raisins, frozen peas, and cheese pieces and as oxygen barriers on peanuts . Also, food additives have been microencapsu-lated in whey protein or in whey protein/carbohydrate wall systems (Klose *et al*., 1990).

Applications of coatings that combine acetylated mon-oglycerides with caseinates (or with whey protein isolate on frozen salmon pieces were discussed earlier. Whey protein isolate coatings alone, followed by an antioxidant (ascorbic acid and citric acid) spray did not affect moisture loss rate but delayed onset of lipid oxidation and reduced peroxide values in frozen King salmon samples (Earle, 1990).

3.4 Cereal proteins

Film formation from corn zein, the prolamin fraction of corn proteins, and from wheat gluten, a mixture of the prolamin and glutelin fractions of wheat proteins, has been studied extensively (Gennadios and Weller, 1991). Other cereal proteins, particularly the prolamin fractions, are also known film-formers. For example, recent studies have evaluated properties of films from sorghum kafirin and rice bran protein (Buffo and Weller, 1995). As mentioned, corn zein has been used in commercial coating formulations for shelled nuts, candy, and pharmaceutical tablets.

The use of corn zein as an edible coating or packaging film for cooked meat and poultry has recently been suggested. Corn zein coatings on precooked pork chops did not reduce moisture loss but decreased lipid oxidation (signifi-cantly lower thiobarbitouric acid values) after storage at 4°C for 6 and 9 d (Klose et al., 1990).

3.5 Oilseed proteins

Edible films and coatings from the globulin protein fractions of soybeans and peanuts have been reviewed (Coleman and Creswick, 2003). A process was described where dehydrated meat was stabilized with protein coatings, preferably made from mixtures of soy protein isolate and egg albumen (Folk, 1993). Lean meat was mechanically reduced into fibers and mixed in aqueous protein solutions. Formed slurries were extruded, cut in pieces, and heated to coagulate the protein.

Meat flavor was retained by the protein coatings while the product had good texture and rehydration characteristics. Use of soy protein as microencapsulating medium has been reported (Torres, 1994). Functionality of soy protein or peanut protein-based edible coatings on other food products has yet to be explored.

CONCLUSION

Several types of edible coatings have been applied on meats, poultry, and seafoods over the years. However, all of these systems presently have exhibited certain shortcomings and have not received substantial commercial acceptance. The numerous benefits to be afforded to food processors and consumers by effective edible coating formulations justify further research in this field. As more edible biopolymers are investigated for film formation and as new concepts related to multicomponent edible coating systems are developed, wide commercial exploitation of edible packaging for meats, poultry, and seafoods may be realized.

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Quality assessment of meat in relation to colour and muscle fiber types

Mubashera Anwer, Muhammad Issa Khan, Imran Pasha, Muhammad Rizwan Tariq and Muhammad Sohaib

National Institute of Food Science and Technology, University of Agriculture, Faisalabad

Corresponding author: drkhan@uaf.edu.pk

ABSTRACT

The current study was premeditated to evaluate meat quality based on its colour and fiber types. The meat samples were analyzed for its chemical constituents (ash, crude protein, crude fat, moisture) and quality parameters like pH, colour, and water holding capacity, cooking loss, drip loss and tenderness. The moisture content of meat cuts was found between 74.46-76.78%. The crude protein content was observed from 22.78-24.20%. The crude fat content was ranged from 5.70-9.76%. The ash content was 1.77-1.98%. The pH content was observed 5.80-5.90%. The color content was varied 39-42 for L*(lightness), 20-23 for a*(redness) and 5-7 for b*(yellowness). The cooking loss was found between 33-37%. The muscle fiber types are varied between three cuts, Type I was high in rib cut 62%, types IIA was high in round cut 24% and type IIB was high in sirloin cut 55%. Finally, Data obtained was subjected to statistical analysis.

Keywords: Meat, Meat quality, Muscle fiber, Meat color, Texture

INTRODUCTION

Meat is an important edible postmortem constituent originating from the live animals that are used as food by the humans. All muscle tissues of meat contain high amount of protein, and are considered as adequate source of vitamin $B_{6,}$ vitamin B_{12}, phosphorus, niacin, zinc, choline, riboflavin, selenium and iron. However they do not contain dietary fiber and are very low in carbohydrates. The meat fat content varies depending on the breed, species and the way of growth (1*)*. Pakistan 's total production of meat up to the year of 2011 was estimated 3095,000 tons, from which mutton and beef were 616,000 tons and 1,711,000 tons respectively (2).

Increased consumer awareness and demand about quality products in the recent past have urged the food manufacturers to produce homogeneous and high quality products. Similarly the meat quality has become an area of great importance and concern in the recent years (3). Chemical composition, sensory and technological attributes of meat affected by weight, sex, breed, environment and post-mortem factors, storage time and temperature (4, 5). Consequently, the main concern of user is inconsistency in quality characteristics of meat (6, 7). Quality of meat is most important for meat retailer and producer in order to meet the consumer's demand and applicable standard requirements for a consistent satisfactory product (8). As a result of multifaceted combination of visual appeal and eating satisfaction, consumer agrees to take the product. Particularly, flavor, juiciness and tenderness satisfy the eating properties due to influence on replicate purchases, these showing overall meat quality (9). Meat should appear good to consumers when they decide to buy it before satisfying their taste. Once the meat is bought, it must meet the expectations of juiciness, aroma, tenderness and flavor (10).

Meat colour is the first condition that consumer use to judge meat quality and acceptability (11). (12) Stated that colour is an important factor in selection of meat products. Meat colour is one of the most important for consumers are indication of an originality and uprightness. Consumers will often refuse products in which the colour varies from the predictable appearance. Therefore, colour is frequently used to determine economic value of food. A comprehensive understanding of the variation in quality properties associated to colour is important for further processing to decrease the potential

negative impact of meat color variation on further processed products (13). It is declared that the color is the main quality characteristic that responsible for shelf life of meat products. Retail sale enhanced as color of the product will be adequate.

The oxidation condition of the muscle pigment myoglobin tells the color of fresh meat (14). Three forms of myoglobin stays. In the reduced formed myoglobin is of purple color in the absence of oxygen. In the presence of oxygen, oxymyoglobin is formed, that has bright red color. The iron has in ferrous state in both these forms (15). The reduced-myoglbin and oxymyoglobin is convertible that depends on the concentration of oxygen (16). The color of meat is affected by chemical changes like oxidation, changes in pH, enzyme action, hydrolysis and protein denaturation.

Muscle fibers are polynucleated, elongated cells classified on their contractile and metabolic properties. Fibers are classified on the basis of stain reactions, as type I (slow-twitch oxidative) or β- red fibers, IIA (fast-twitch oxidative) or α-red fibers, and IIB (fast-twitch glycolytic) or α-white fibers. Muscles have more than 40% β- red fibers are red, more than 40% α-white fibers are white, and others are intermediate. Muscle fiber type composition is extremely variable and can be subjective by many extrinsic and intrinsic factors such as animal breed, class, selection intensity and post-slaughter processing (17).

The diversity of skeletal muscle can be attributed to the heterogeneous characteristics of the individual muscle fibers and the mosaic composition of the numerous fiber types (18, 19). Fiber type composition can vary significantly in different species and muscle types, depending on function (20). Moreover, there are many factors that contribute to fiber type variation, such as sex, age (21), breed (22), hormones (23), and physical activity (24). These fiber type variations differ according to their molecular, metabolic, structural, and contractile properties (25). Therefore, having an understanding of such muscle fiber characteristics is important for the study of overall muscle characteristics and subsequent meat quality.The present study was planned to assess the meat quality on the basis of muscle fiber types and color of meat with following objective; to assess chemical composition and quality of meat cuts in relation to meat color and muscle fiber types.

MATERIALS AND METHODS

Meat samples were obtained from local market and stored at 4^0C. Following chilling, all trim able fat and connective tissues were removed. Collected samples were subjected to various analyses with respect to their quality attributes.

Chemical composition:

Crude protein content was determined by kjeldahl method and moisture, ash and crude lipid according to (26).

Physicochemical analysis

The quality of meat samples was assessed by carrying out different physicochemical analyses: The pH of meat samples were measured by using pH meter following the method described by (27) with some modifications. The color of meat samples were determined by the L*(lightness), a* (redness), and b* (yellowness) using colorimeter by using method as describe by (28). The Water holding capacity of meat samples was determined by mixing meat with 0.6 M Nacl solution, place mixture in water bath. It was further centrifuged (4^0C) at 10,000 rpm for 15 min in refrigerated centrifugation machine. The supernatant was decanded and measured (29). The Cooking loss of meat samples were measured by placing meat in polythene bag and heated in water bath. Cookout was drained and cook mass was cooled and weighed (30). The Drip loss of meat samples was determined by method describe by (31). Meat sample were placed in refrigerator under polyethylene sealed cover. After 24 hour sample were wiped and dried. Weight of sample was drip loss of meat sample. The Tenderness of meat samples were determined by the method described by (28). Sample were packed in plastic bag and cooked in a water bath in 95^0C until core temperature reached 80^0C. Sample was cooled and weighed for thermal loss determination. Then Shear force of meat samples were determined by the method described by (28). It was determined as maximum force perpendicular to the fibers.

Histochemical analysis

The histochemical analyses were carried out for different meat samples to assess the fiber types. The ATPase activity was determined by the method described by (32) with slight modifications. After acid incubation slices were incubated in ATP solution. The muscle fiber diameter was determined as described by (33) with some modifications. A core of muscle tissue was fixed in formal saline for 24 h

and was blended at low speed for 30 sec. A drop of the homogenate was placed over a glass slide and observed under a microscope with calibrated micrometer. The diameter of fiber was measured.

Statistical analysis

The results obtained from different parameter were exposed to statistical analysis by following the respective methods described by (34).

RESULTS AND DISCUSSION

The results regarding mean values for chemical composition of meat cuts are given in Table 4.1. The moisture content varied non-significantly between 74.46-76.78% in different cuts of meat. The higher moisture content was found in round cut. The protein content varied significantly between 22.78-24.20%. Rib cut have higher content of protein. The fat content varied greatly significant between three cuts from 5.70 to 9.76%. Higher fat content were observed in round cut. The ash content varied significantly between 1.77-1.98%.

The results of current study are in near to the finding of (35), according to it the mean and standard deviation for moisture, fat and ash of beef round and chunk cuts were 72.28 ± 2.83, 6.86 ± 3.45, and 1.26 ± 0.28 mg/g, respectively. According to (36), Moisture, fat, protein and ash of Gluteus medius (GLM) muscle of sirloin were 73.86±0.40, 3.80±0.50, 20.86 ± 0.26 and 1.40± 0.02 respectively. Moisture, fat, ash and protein of Longissimus dorsi (LOD) muscle of rib were 72.47± 0.40; 5.45± 0.50; 1.17± 0.02; 20.87± 0.26. The results regarding mean values for physicochemical traits of meat cuts are given in Table 4.2. The pH values of three cuts varied non-significantly between 5.74-5.90. The highest pH (5.90) was observed in Round. (37), Mean pH values for the *longissimus dorsi* were decrease from 6.47 ± 0.21 to 5.46 ± 0.05 from 1st hour to 24th hour and for *triceps brachii* muscles decreases from 6.66 ± 0.20 to 5.54 ± 0.14. A significantly higher pH is recorded in comparision with heifers and young bulls of the *longissimus dorsi* (LL) and *semitendinosus* (ST) muscle. Similarly, pH have significant impact on ageing time in the muscles. As conditioning time was increased up to 48 hours pH decreased and then constant up to 48 hours. In LL muscle pH drop from 6.68 to 5.66 and in ST muscle from 6.65 to 5.60 during aging time 45 minutes to 14 days (38) The color L* varied highly significant among different cuts of meat animals from 39.12 to 42.44. The highest L*(42.44) content was observed in Round cut. The a* value varied highly significant among different cuts of meat animals from 20.66 to 23.44. The value of b* varied highly significant among different cuts of meat animals from 6.89 to 7.64. The highest b*(7.64) content was observed in Round. The results of current study are in line with the finding of (39) reported that color values of different muscles of round and rib cut. Longissimus dorsia, Psoas major and Semimembranosus muscles have 42.16±1.29, 19.40±1.04, 8.44±0.77; 40.15±1.61, 19.22±1.58, 7.91±1.32, and 37.51±1.06, 19.56±2.04, 7.65±1.38 values for L*, a* and b* respectively. The round and chunk cuts had color values (L*, a*, and b*) means and standard deviations as 41.06 ± 4.55, 29.57 ± 4.05, and 22.78 ± 4.32, respectively. Yield grade or quality grade has no effect the color values lightness, redness and yellowness as weight of carcass. For each of these properties (L*, a*, and b*) the value increased as weight of the carcass increased (35). (40) observed color vales of rib muscle cut LT *longissimus thoracis of* beef based on *L** (33.7), less red *a** (18.4) and *b** (6.6), which is near to current study. The cooking loss varied significantly among different cuts of meat animals from 33.88 to 35.77. The highest cooking loss (35.77) content was observed in Round. (41) reported that different muscles show significant differences in cooking loss. *Triceps brachii* and *ongissimus thoracis* show the lowest values, representing a better water holding capacity while *semitendinosus* muscle showed the highest losses. Related results were also found by (42) from Chianina cattle on the same muscles.

The results indicate that drip loss varied significantly among different cuts of meat animals from 4.55 to 5.38. The highest drip loss (5.38) content was observed in Sirlion. (39) observed drip loss (%) in different muscles of round and sirloin cut Longissimus dorsi 3.51±0.15, Psoas major 3.28±0.28 and Semimembranosus 3.25±0.54. According to (38) drip loss increases as the aging time increases, in LL muscle drip loss increases from 1.54 to 2.87 after 14 days and in ST muscle increase from 2.26 to 3.53%.

The water holding capacity varied non-significantly among different cuts of meat animals from 43.77 to 45.22. The highest (45.22) water holding capacity was observed in Round. Our results match with (35) findings weight loss due to centrifugation, the mean and standard deviation for expressible moisture of round and chunk muscle was 37.50± 5.15%. According to (36) mean values for water holding

capacity for GLM and LOD are 41.29 and 37.67 respectively.

Table 1: Mean Values Of chemical composition of meat cuts

Treatment	Moisture	Protein	Fat	Ash
T_1	76.78±1.54	22.78b±0.46	9.76a±0.15	1.86b±0.06
T_2	75.92±1.49	23.34b±0.47	6.40b±0.1	1.77c±0.12
T_3	74.46±1.52	24.20a±0.28	5.70c±0.1	1.98a±0.04

T1= round, T2=sirloin, T3=rib

Table 2: Mean Values of physicochemical traits of meat cuts

Treatment	pH	L*	a*	b*	Water holding capacity	Cooking loss	Drip loss	tenderness
T_1	5.90±0.115	42.44±0.85	23.44±0.47	7.64a±0.16	45.22±0.91	35.77a±0.72	4.96b±0.1	5.26±0.15
T_2	5.85±0.115	39.12±0.79	21.21±0.43	6.89b±0.18	43.77±0.88	34.64ab±0.7	5.38a±0.11	5.46±0.15
T_3	5.74±0.115	40.55±0.82	20.66±0.42	5.75c±0.12	44.55±0.9	33.88b±0.68	4.55c±0.1	4.80±0.1

T1= round, T2=sirloin, T3=rib

Table 3: Mean Values Of fiber types and fiber diameter of meat cuts

Treatment	Type 1	Type 11A	Type 11B	Fiber diameter
T_1	25.29b±0.51	24.50a±0.49	49.89b±1.0	55.44a±1.11
T_2	21.50c±0.43	22.50b±0.45	55.69a±1.12	53.77a±1.08
T_3	62.00a±1.24	19.69c±0.40	16.50c±0.33	50.58b±1.02

T1= round, T2=sirloin, T3=rib

tenderness varied significantly among different cuts

of meat animals from 4.80 to 5.46. The highest tenderness (5.46) was observed in Sirlion. (43) recommended the following categories for beef steaks on the basis of the W-B force: tender from 2.27 up to 3.58 kg, moderate 4.08–5.40 kg and tough 5.90–7.21 kg. According to this distribution, the ultimate LL and ST belongs to the tender category. (44) observed higher LD tenderness of meat obtained from the heifers and stored at 4°C.These authors declare that the aging time increased as shearing force W-B decreases. The force decreased as aging time increased, the force decreased from 7.21 to 3.80 kg in 2nd day and 14 days.

The results regarding mean values for fiber types and fiber diameter of meat cuts are given in Table 4.3. The results indicate that muscle fiber diameter varied significantly among different cuts of meat animals from 50.33 to 55.44. The highest tenderness (55.44) was observed in Round. (33) reported that fiber diameter and tenderness are negatively related. Accordimg to (39) In the PM (*Psoas major*) muscle longer sarcomere length with the lowest shear force WBSF, whereas shorter sarcomere length with highest WBSF was showed in SM (*semimembranosus*) muscle. Similar results were observed by (45). (46) also showed the relation of sarcomere length and shear force.

The results indicate that muscle fiber type 1 varied significantly among different cuts of meat animals from 21.50 to 62.0. The highest percentage of type1 (60.0) was observed in Rib cut. . The results indicate that muscle fiber type 11A varied significantly among different cuts of meat animals from 19.69 to 24.50. The highest percentage of type1 (24.50) was observed in Round. The results indicate that muscle fiber type 11B varied significantly among different cuts of meat animals from 16.50 to 49.89. The highest percentage of type1 (49.89) was observed in Round. The results of current study are in line with the finding of (39) that the SM muscles contain higher percentage of fiber type 11A and type 11B as compared to other muscles, though type 1 fiber were high in PM muscle. SM muscle had lower percentage of fiber type 11A and LD muscle had lower percentage of muscle fiber type 11A. According to (39) composition of LD muscle were type 1, 33.1 type 11A, 14.9 and type11B 52.6%, and ST contain type1 12.0, type 11A 27.3 and type 11B 61.8%. (47) observed different muscles of round, sirloin and rib cut. GLM muscle of sirloin cut consist of type 1 19.5, type 11A 24.9 and type 11B 55.6%. SM contain type1 24.3, type 11A 26.0 and type 11B 49.7%. LD muscle contain type 1 35, type 11A 21.8 and type 11B 43.2%.

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Nutritional and antioxidant profile of some selected Pakistani potato cultivars

Abdul Aziz[1], Muhammad Yasin*[1,2], Muhammad Atif Randhawa[1], Adeela Yasmin[1,3], Muhammad Ahmar Jahangir[1], Muhammad Sohail[1]

[1]National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan

[2]University College of Agriculture & Environmental Sciences, The Islamia University of Bahawalpur, Pakistan

[3]Department of Food Science and Human Nutrition, Government College University, Faisalabad, Pakistan

***Corresponding author:** yasinft_uaf@yahoo.com

ABSTRACT

Present researcher was designed to evaluate the nutritional and antioxidant activity of the some prominent potato cultivars. The mean indicated that potato contained appreciable amount of moisture (77.5 to 82.37%), ash (1.46 to 1.66%), crude protein (2.037 to 3.062%), crude fat (1.16 to 1.89%) and crude fiber (2.12 to 2.66%). However, specific gravity were in the ranged of 1.05 to 1.22. The antioxidant assays indicated that the highest total phenolic content, DPPH inhibition, FARP and ABTS values were observed in FD 8-3, followed by Cardinal whereas the lowest activity was noticed in FD 19-2. Conclusively, the consumption of the potato will provide nutritional worth along with antioxidant potential that might be helpful for proper functioning of the physiological systems of body.

Keywords: Potato, Proximate composition, Antioxidant potential, Potato quality

INTRODUCTION

Consumers have paid immense attention towards the benefits of nutrients and natural antioxidants in food, such as fruits and vegetables (Zhou and Yu, 2004). Nowadays, potato has become high yielding carbohydrate enrich vegetable containing phytochemicals and minerals contents throughout the world (Abbasi *et al.*, 2011; Andre, *et al.*, 2007). Furthermore, it is rich in antioxidants such as vitamin C, polyphenols (phenolcarboxylic acids), carotenoids and selenium (Lachman *et al.*, 2006) and α-tocopherol (Kalt, 2005). It is fourth most important food crop worldwide after maize, wheat and rice, with production of more than 323 million tons (Aziz *et al.*, 2012).

There number of investigations expounded that potato containing phytochemicals with high free radical scavenging activity that may helps to reduce the risk of chronic health diseases and age related neuronal degeneration (Teow *et al.*, 2007). It is well documented that principal components of potato that are responsible for antioxidant activity are phenolics such as chlorogenic acid, gallic acid, protocatechuic acid, caffeic acid and quercetin (Rodriguez de Sotillo *et al.*, 1994; Al-Saikhan *et al.*, 1995; Nara *et al.*, 2006). Other phenolics in potato include ferulic acid, *p*-coumaric acid as well as small amounts of rutin, quercetin, myricetin, kaempferol, naringenin and other flavonoids (Nara *et al.*, 2006). However, red fleshed potato has contained pelargonidin and peonidin-3-rutinoside-5-glycosides acylated with *p*-coumaric and ferulic acid (Reyes, 2005).

Therefore, owing to higher consumption of potato in daily life contributed is ranked third highest for providing total phenolic content among fruits and vegetables after orange and apple (Chun *et al.*, 2005). Previously extensive research indicates that fruit and vegetables enrich in antioxidants play an indispensable role against health associated discrepancies and reduced the risk of chronic diseases (Gundgaard *et al.*, 2002).

According to phenolics classification, potato is categorized in medium group among different vegetables. Thus, the utilization of potato in a daily diet provides not only nutritive value but also give antioxidants to the body for proper functioning. Earlier, it is reported that red fleshed potatoes has two to three times higher antioxidant activity as measure by various antioxidant assays than that of white fleshed due to synergistic effect of each anthocyanin pigment (Hayashi *et al.*, 2003; Kosieradzka *et al.*, 2004; Lukaszewicz and Szopa, 2005). Considering the above facts regarding potato nutritive value and antioxidant profile, present research project was designed to evaluate the locally grown potato cultivars for their nutritional and antioxidant profiling.

MATERIALS AND METHODS

Procurement of raw materials

Ten potato varieties were collect from the Horticulture Section of Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan (2007-08). The tubers were stored in the sacks after harvesting from the field. All the potato were cleaned with water manually to remove dust, field trashes, stones, damaged seeds and other foreign matters.

(i) Specific Gravity of potato

The specific gravity was determined as the weight of 10 cleaned tubers both in air (W_1) and completely immersed in a container of water (W_2) were calculated by the following formula (Elfaki and Abbsher, 2010).

$$\text{Specific gravity} = \frac{(W_1)}{(W_1) - (W_2)}$$

Where
W_1=Wt. of tubers in the air

W_2= Wt. of tubers in water

Proximate composition

(i) Moisture Contents of potato

The moisture content of flesh and peels of each variety was determined using air forced draft oven at 70±5 °C temperature till the sample weight becomes constant according to the method of AOAC (2006). The moisture was calculated according to the following formula:

$$\text{Moisture \%} = \frac{\text{Loss in Weight}}{\text{Wt. of fresh sample (g)}} \times 100$$

(ii) Ash content of potato

The ash content of each sample was determined by incinerating of dry sample in Muffle furnace at a temperature of 550-600°C for 5 to 6 hours by using protocol as described in AOAC (2006). The following formula was used to calculate the ash content.

$$\text{Ash content (\%)} = \frac{\text{Loss in ash}}{\text{Wt. of fresh sample (g)}} \times 100$$

iii) Crude Protein of potato

The nitrogen present in each sample was estimated by using Kjeldahl`s method as mentioned in AOAC (2001). Sample (5.0 g) was digested with concentrated H_2SO_4 in the presence of digestion mixture (K_2SO_4, $CuSO_4$, $FeSO_4$, with 100:10:5 parts respectively). The digested sample was then filtered and volume was made to 250 mL. The 10 mL of diluted sample was distilled with 40% NaOH. Ammonia gas (NH_3) was liberated and absorbed into 4% boric acid which was then titrated with N/10 H_2SO_4 to light pink color as an end point. A factor of 6.25 was applied for the conversion of percent nitrogen into percent crude protein. Following equation was used to calculate % nitrogen:

Crude fat of potato

Soxtec System (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) was used to determine the crude fat content in potato samples using hexane as a solvent according to the guidelines illustrated in AOAC (2006).

Crude fiber of potato

Crude fiber content of fat free samples was determined by digesting initially with 1.25% H_2SO_4 for 30 min followed by 1.25% NaOH solution for same time in Labconco Fibertech (Labconco Corporation Kansas, USA) apparatus as per procedure described in AOAC (2006).

Antioxidant assays

Sample preparation

The samples were washed, cut into 2 cm slices and steamed at 100 °C for 15 min to prevent browning of flesh. Samples were cooled, cut into 2 cm^3 cubes, lyophilized and ground to fine powder. The flour was stored in a polyethylene resealable bag at 4 °C for further analysis.

(i)Total phenolic content of potato

Extraction of phenolic compounds: Five grams of potato flour were mixed with 40 mL methanol for 24 h in orbital shaker at 250 rpm. The resultant suspension was filtered through Whatman No. 1 filter paper and finally diluted to 210 mL with methanol. Sample solutions were stored at 4 °C in amber color media bottles and used for subsequent analyses.

Determination of total phenolic content: Total phenolics of potato sample were estimated through modified method of Slinkard and Singleton (1997). Briefly, two hundred microliters of the sample was added in 1.4 mL distilled water and subsequently 100 µL of Folin-Ciocalteu was added. After gentile mixing of 2 min, 300 µL of 20% Na_2CO_3 solution was added and resultant adduct was allowed to react

for 2 h. The absorbance of color mixture was recorded at 765 nm UV–Vis Spectrophotometer. Calibration curve was prepared by using gallic acid solutions (10–400 µg/mL). Results were expressed as mg gallic acid/100g fresh sample

(ii) DPPH radical scavenging activity of potato

DPPH radical scavenging activity of potato extracts was determined using the method described by by Huang *et al*. (2005b). Sample (1 mL) was mixed with same volume of methanolic DPPH solution (0.0012M). The mixture was kept for 30 min under dark place for the completion of reaction. Free radical scavenging activity of the potato was measured by recording the absorbance of reaction mixture at 517 nm using UV–Vis Spectrophotometer. Percent inhibition was calculated using the following equation.

$$\text{Inhibition (\%)} = [100 \times (A_{\text{blank}} - A_{\text{sample}}) \div A_{\text{blank}}]$$

(iii) ABTS reducing activity assay of potato

For the estimation of ABTS assay, Pennycooke *et al.,* (2005) protocol was used. The weighted quantity (54.2 mg) of ABTS was dissolved in 5mM phosphate buffer (pH 7.0) and activated its radical activity by addition of 1 g of MnO_2 with infrequent stirring and time of activation 30 min. The resulted adduct was centrifuged at 7000*g* for 5 min and passed through filtrate having pore size 25 µm. Afterwards, solution was diluted with phosphate buffer for adjusting the absorbance at 0.700 ± 0.01. Sample (5 µL) was added and stay for reaction completion for 20 min. Absorbance of the solution was recorded at 734 nm.

(iv) Reducing power of potato

Fe-reducing power of the potato was estimated by following the method described by Singh and Rajini (2004). One milliliter of 0.2 M phosphate buffer having pH 6.6 and 1 mL of 1% (w/v) $K_3Fe(CN)_6$ were mixed with 1 mL aliquot of the extract. The resultant mixture was incubated for 20 min at 50 °C. Thereafter, 1 mL trichloroacetic acid (10%, w/v) was added to the mixture. The resulting adduct was centrifuged at 3000 rpm for 10 min at 25°C. One mL of supernatant was mixed with same volume of distilled water and 0.2 mL of 0.1% (w/v) $FeCl_3$ solution. The absorbance was read at 700 nm using 1 UV–Vis Spectrophotometer. Standard cure was plotted by determining the reducing power of α-tocopherol (20–100 ppm).

Statistical analysis

The data obtained for each parameter were subjected to statistical analysis using Completely Randomized design (CRD) technique and level of significance was determined by using least student according to the methods described by Steel *et al.* (1997).

RESULTS AND DISCUSSION

Moisture content of potato

The mean values for moisture content in the flesh of different potato varieties in Table 1 showed significant variations. The results showed that FD1-8 (82.37%) had the highest moisture content in the flesh of potato while FD40-10 (77.5%) had lowest moisture content. The SH-5, FD35-36 and FD8-3 had almost the same moisture contents and Diamant, FD 40-10 and FD19-2 exhibited the statistically at par moisture contents. The results of present investigation is in accordance with the earlier findings of Sotelo and Serrano, (2000), they reported that the moisture of various tested potato are in ranged of 82 to 87%. Later, Naz *et al*. (2011) documented that Pakistani potato contained 73.17 to 81.44% moisture content grown in Abbottabad. One of their peers, Abbasi *et al*. (2011) evaluated the six verities of potato cultivated in district Okara and reported the moisture content ranged from 74.15 to 77.05%.

Ash content of potato

The results revealed that the ash content varied significantly among different potato varieties. The maximum ash content was observed in SH-5 (1.66%), followed by Diamant (1.62%) and FD 1-8 (1.62%) whilst the lowest content was noticed in FD 19-2 (1.46%) that are statistically equivalent to FD 35-36 (Table 1).

The present study results are in harmony with the earlier findings of Abbasi *et al.* (2011), they expounded that the ash content of potato grown in district Okara varied from 1.89 to 3.30%. Similarly, Naz *et al.* (2011) reported ash content of different Pakistani potato cultivars that differed from 3.67 to 5.18% on dry weight basis. Earlier, Javid *et al.* (1995) investigated the proximate composition of Diament, Cardinal, Lale-e-Faisal and Desir. They reported that ash content was varied from 1.0 to 2.0% in tested potato cultivars.

The differences in ash content among potato cultivars attributed might be due to their genotype. It has been reported that ash content of potato varieties also influenced by non-genetic factors like soil, climatic conditions and use of fertilizer etc (Abbasi *et al.,* 2011).

Crude Protein of potato

The perusal of the results inferred that the protein content was varied significantly in different potato varieties (Table 1). The protein content varied from 2.16 to 3.15%. The results showed that maximum protein was found in the potato variety Cardinal (3.15%). However, mean values of Cardinal is statistically at par with the value FD 35-36, FD 8-3, FD 40-10 and FD 19-2. The lowest protein was exhibited by the potato variety FD 8-1 (2.16%).

The instant results are synchronized with earlier findings of Javid *et al.* (1995), they reported that protein content of potato cultivars ranged from 1.20 to 1.64%. Afterwards, Naz *et al.* (2011) delineated that protein content was varied from 0.95 to 1.39% dry weight basis in tested potato cultivars. In contrary, higher protein content was reported by Abbasi *et al.* (2011) that were in ranged 9.88 to 11.86% on dry weight basis. The variations in the proximate composition might be due to genetic and non-genetic factors.

Crude fat of potato

Crude fat explicated non-significant differences among all selected potato cultivars. Results in Table 1 exhibited that crude fat contents varied from 1.16±0.006 to 1.89±0.006%. The maximum fat content (1.89±0.006%) was noticed in Diamant whereas minimum value was observed for this parameter in Cardinal (1.16±0.006%). Previously, Abbasi *et al.* (2011) reported that fat content was varied from 0.80 to 1.29% of six potato cultivars grown in district Okara. One of their peers, Naz *et al.* (2011) observed fat content of potato grown in Abbottabad were in the ranged of 0.41 to 1.19%. Earlier, Javid *et al.* (1995) reported the value of fat content of potato cultivars *i.e.* Desir (0.77 to 0.78%), Lale-e-Faisal (0.55 to 0.57%), Diamant (0.57 to 0.58%) and Cardinal (0.66%).

Crude fiber of potato

Crude fiber exhibited non-momentous variations among tested potato verities. It is evident from Table 1 that maximum crude fiber content (2.66±0.013%) was presented by FD 40-10 whereas FD 1-8 indicated the minimum value for crude fiber (2.12±0.010%). Earlier reported value of crude fiber by Naz *et al.* (2011) are in accordance with present study result that crude fiber content of potato cultivars grown in Pakistan are in the ranged of 1.99 to 3.59%. The present results are further strengthening by the findings of Abbasi *et al.* (2011), they documented that the crude fiber content varied from 6.43 to 7.83% on dry weight basis.

Specific Gravity of potato

Specific gravity of a potato is directly proportional to its starch concentration. The perusal of the results inferred that the specific gravity varied significantly among potato cultivars from 1.22 to 1.05 (Table 1). The results showed that maximum specific gravity was found in FD40-10 (1.22) while lowest were exhibited by Diamant (1.05). Earlier, Abbasi *et al.* (2011) investigated six cultivars of potato for their physic-chemical analysis and reported that specific gravity of tested cultivars were in the ranged of 1.081 to 1.01.

Antioxidant activity of potato

The total phenolic content of selected potato cultivars were expressed as mg gallic acid equivalent (GAE)/100g fresh weight (FW) and presented in Table 2. The tested potato cultivars showed significantly ($P < 0.05$) differences among each other in terms of total phenolic contents. Total phenolic content of potato are in ranged from 17.8±0.81 to 34.0±1.65mg GAE/100g FW. Among the potato cultivars, FD 8-3 (34.0±1.65mg GAE/100g FW) exhibited maximum total phenolic contents followed by Cardinal (31.2±1.34mg GAE/100g FW) whereas, minimum value was observed in FD 19-2 (17.8±0.81mg GAE/100g FW). This variations may be endorsed to genotypes and harvest location that influence the accumulation of phenolic compounds by synthesizing different quantities and/or types of phenolics (Lachman *et al.*, 2008; Sulc *et al.*, 2008). Earlier, Karadeniz *et al.* (2005) reported that the phenolic content of potato are 32.44 ± 6.07 mg GAE/100 g. Previously, Al-Saikhan *et al.* (1995) also delineated that potato contains 11.41–27.47 mg GAE/100 g total phenolic contents. In contrary, Kaur and Kapoor (2002) and Vinson *et al.* (1998) documented higher total phenolic contents as 231.46 ± 9.73 mg GAE/100g FW and 100.37 ± 66.35 mg GAE/100g FW, respectively compared to the present study results. Generally, it is consider that edible part of potato accounts 40% of the total phenolic content (Chu *et al.*, 2002), while amount of conjugated phenolics in potato is 57.9±13.4% (Vinson *et al.*, 1998). The tendency of phenolic compounds to accumulate in the peel is cause of low-phenolic content in the flesh of potato (Reyes, 2005; Nara *et al.*, 2006).

DPPH radical scavenging activity

The mean pertaining to DPPH radical scavenging activity exhibited the maximum value (37.54±1.67%) for FD 8-3 followed by Cardinal (34.98±1.73%), whereas FD 19-2 showed minimum value 19.86±0.99% inhibition. Previously, it was investigated that the radical scavenging activity of the

Table 1: Proximate composition and specific gravity of some selected potato cultivars

Potato Cultivar	Moisture	Ash	Crude protein	Crude fat	Crude fiber	Specific gravity
SH-5	81.64±2.61^{B}	1.66±0.008^{A}	2.477±0.012 ^{BC}	1.21±0.005	2.33±0.011	1.22±0.005^{A}
Diamant	77.75±2.01^{E}	1.62±0.007^{B}	2.477± 0.011^{BC}	1.89±0.006	2.47±0.012	1.16±0.004^{AB}
Cardinal	79.9±2.22^{D}	1.58±0.007^{C}	3.353±0.016 ^{A}	1.16±0.006	2.45±0.010	1.16±0.004^{AB}
FD 35-36	81.32±2.76^{BC}	1.49±0.006^{FG}	3.062± 0.015^{AB}	1.29±0.004	2.35±0.011	1.15±0.004^{B}
FD 8-3	81.51±2.45^{BC}	1.5±0.007^{EF}	3.062± 0.014^{AB}	1.45±0.005	2.41±0.010	1.14±0.003^{BC}
FD 8-1	79.5±2.16^{D}	1.53±0.007^{DE}	2.037± 0.012^{C}	1.65±0.005	2.55±0.012	1.13±0.003^{BC}
FD 40-10	77.5±2.00^{E}	1.52±0.008^{DEF}	3.207± 0.014^{A}	1.88±0.006	2.66±0.013	1.13±0.003^{BC}
FD 1-8	82.37±2.53^{A}	1.62±0.007^{B}	2.185± 0.013^{C}	1.34±0.005	2.12±0.010	1.12±0.04^{BC}
FD 19-2	77.8±2.26^{E}	1.46±0.006^{G}	3.062±0.014^{AB}	1.78±0.006	2.56±0.012	1.08±0.002^{CD}
FD 3-9	81.14±2.47^{C}	1.55±0.007^{CD}	2.185± 0.013^{C}	1.44±0.004	2.15±0.010	1.05±0.002^{D}

Letter containing different letter in a column varied significantly

Table 2: Antioxidant potential of some selected Pakistani potato cultivars

Cultivar	Total phenolic content (mg of GAE/100gram of FW)	DPPH (% inhibition)	FARP (mg ascorbic acid/100g FW)	ABTS^{+} (mg ascorbic acid/100 FW)
SH-5	24.0±1.10^{C}	26.43±1.32^{C}	46.09±1.99^{C}	57.12±2.77^{C}
Diamant	21.3±1.07^{C}	22.67±1.12^{C}	42.45±2.01^{C}	51.33±2.45^{C}
Cardinal	31.2±1.34^{B}	34.98±1.73^{B}	51.23±2.34^{B}	62.25±3.01^{B}
FD 35-36	26.7±1.30^{C}	29.34±1.40^{C}	47.76±2.12^{C}	59.51±2.78^{C}
FD 8-3	34.0±1.65^{A}	37.54±1.67^{A}	56.32±2.76^{A}	69.61±3.12^{A}
FD 8-1	19.9±0.99^{CD}	22.13±1.01^{CD}	41.91±2.10^{CD}	49.21±2.19^{CD}
FD 40-10	20.1±0.91^{CD}	23.87±1.11^{CD}	45.85±2.09^{CD}	53.73±2.51^{CD}
FD 1-8	18.5±0.89^{D}	22.03±1.00^{D}	42.37±1.99^{D}	51.03±2.43^{D}
FD 19-2	17.8±0.81^{D}	19.86±0.99^{D}	37.49±1.78^{D}	45.44±2.07^{D}
FD 3-9	22.7±1.09^{C}	26.37±1.22^{C}	47.55±2.22^{C}	57.52±2.55^{C}

Letter containing different letter in a column varied significantly

potato sample (on a µg phenolic content basis), is higher than that of α-tocopherol (Lee *et al.,* 2004), which accounts for its higher-EC50 value. There is evidence that hydrogen abstraction is only a marginal pathway in the reaction between antioxidant and DPPH (Prior *et al.,* 2005).

ABTS reducing activity assay

The mean pertaining to ABTS ranged from 69.61±3.12 to 45.44±2.07 mg ascorbic acid/100 FW in tested potato cultivars. The maximum value for ABTS 69.61±3.12 mg ascorbic acid/100 FW was observed in FD 8-3 followed by Cardinal 62.25±3.01 mg ascorbic acid/100 FW however FD 19-2 showed the minimum value 45.44±2.07 mg ascorbic acid/100 FW.

Earlier, it is proven that color of flesh has significant influence on antioxidant activity of the potato. Moreover, the red potato has higher antioxidant activity which is investigated through ABTS than that of white and yellow-fleshed. The red fleshed potato has more antioxidant activity (359.38 mg ascorbic acid Eq/kg of FW) that is four time higher than yellow or white fleshed cultivars (82.83 mg ascorbic acid Eq/kg FW). The variations among the tubers antioxidant activity was might be due to colored of potato flesh that contained varying quantity of anthocyanin (Lachman *et al.* 2008, 2009).

Reducing power of potato

The value of FRAP among all the selected potato cultivars ranged from 56.32±2.76 to 37.49±1.78 mg ascorbic acid/100g FW. The cultivar FD 8-3

expressed the maximum value of FRAP (56.32±2.76 mg ascorbic acid/100g FW) followed by Cardinal (51.23±2.34 mg ascorbic acid/100g FW). However, the FD 19-2 exhibited minimum value of FRAP (37.49±1.78 mg ascorbic acid/100g FW).

Previously, it was expounded that the activity of ethanolic and aqueous potato extract has activity 62.3% and 62.5%, respectively (Kaur and Kapoor, 2002). Afterwards, Karadeniz *et al*. (2005), reported similar activity (70%) of the potato extracts 70% for same sample weight. Numerous investigations reported that potato has applicable amount of antioxidant that possess significant inhibition ability (Al-Saikhan *et al.* 1995; Karadeniz *et al.*, 2005).

CONCLUSIONS

The proximate composition indicated that potato contained crude protein (2.037 to 3.062%), crude fat (1.16 to 1.89%), crude fiber (2.12 to 2.66%) and ash (1.46 to 1.66%). Nonetheless, specific gravity values were varied from 1.05 to 1.22. The antioxidant assays revealed that the maximum total phenolic contents, DPPH inhibition, FARP and ABTS values of tested ten potato cultivars were noticed in FD 8-3, followed by Cardinal however the minimum values of aforementioned parameters were recorded in FD 19-2.

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Optimization of Cultural Conditions for Xylanase Biosynthesis by *Aspergillus niger* Using Sugarcane bagasse

Zulfiqar Ahmad[1], Masood Sadiq Butt[2], Muhammad Tahir Nadeem[3], Muhammad Yasin[1,2]

[1]University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Bahawalpur

[2]National Institute of Food Science and Technology, University of Agriculture, Faisalabad

[3]Food Science, Nutrition and Home Economics, Government College University, Faisalabad

Corresponding Author: zulfiqar2233@gmail.com

ABSTRACT

In present research exploration, sugar cane bagasse an indigenous waste/by product carbon source was tested as substrate for optimum biosynthesis of xylanase by *Aspergillus niger* using the submerged fermentation technique. The for xylanase production, thee concentration levels (2.5, 3.0 and 3.5%) of sugar cane bagasse, four different fermentation temperatures (*i.e.* 25.0, 27.5, 30.0 and 32.5^{o}C) and four initial pH levels (5.0, 5.5, 6.0 and 6.5) were used for the culture medium for a period of 24, 48, 72, 96 h. The results revealed that the *Aspergillus niger* exhibited maximum enzyme activity (44.00±1.45) at 3.0 % sugarcane bagasse concentration followed by 42.00±0.29 and 39.07±0.55 at 3.5 and 2.5 % concentration respectively at 30^{o}C, 5.5 pH after a period of 72 h of incubation. The comparison of the effect of various initial pH levels of culture medium exhibited that pH 5.5 had most potent role in xylanase synthesis as compared to different other pH levels. Time scale analysis revealed that fermentation period of 72 h was the most suitable for obtaining maximum enzyme activity. Moreover a temperature of 30^{o}C was found to be optimum for higher yields of xylanase. Conclusively, for optimum production of xylanase production 3.0% sugarcane bagass was used in the culture medium having pH 5.5 for the period of 72 h at 30^{o}C.

Keywords: Xylanase, *Aspergillus niger*, Sugarcane bagasse, Optmization, culture media

INTRODUCTION

Xylanase is an extracellular enzyme being used in poultry diets for feed utilization and various food industries for processing of food (Walk *et al.*, 2011). The enzyme holds potential for the degradation of plant cell wall materials and liberates useful nutrients by hydrolyzing non-degradable hemicellulose fibers (Bertichini, *et al.*, 2009a; Angel, 2010). Furthermore, it hydrolyses the highly polymerized β-1, 4 D-xylosidic linkages and substituted β-1, 4 D-xylobiose, -xylotriose and -glucucoronosyl residues. Thus, microbial xylanase (β-1, 4 D-xylan xylanohydrolase) is extensively used in various industries including food, feed, textile, pulp, paper processing units (Leisola *et al.*, 2002; Walk *et al.*, 2011). On account of beneficial role of xylanase, different methodologies are being used for their maximum biosynthesis (Saleem and Akhtar 2002; Chithra and Muralikrishna, 2008; Pal and Khanum, 2011). During the last few decades, great interest has been developed in xylan and its hydrolytic enzymatic complex due to their multidimensional role in fermentation, for application in bread production and in animal and poultry feed preparation (Kongbuntad *et al.*, 2004b). The depolymerization action of xylanase results in the conversion of polymeric substances into xylo-oligosaccharides and xylose (Subramaniyan and Prema, 1998; Bajpai, 1999; Omar *et al.*, 2008).Various sugars such as xylose, xylobiose and xylo-oligomers can be synthesis by the enzymatic hydrolysis of xylan and by ethanolic extraction (Jaddou *et al.*, 1986).

The manipulation of biotechnological techniques has played an important role in the potential utilization agricultural waste materials including sugar cane bagasse, wheat bran, corn cobs and rice bran. There is an increasing gist to utilize such neglected materials in the production of enzymes which can be employed further in food processing (Mohammadi *et al.*, 2006; Okafor *et al.*, 2007). Various organisms have different potential for the syntheses of enzymes among them fungi are the most common source of hemicellulases like xylanases and glucanases. Previously, different microorganisms including the strains of *Aspergillus nidulans* (Pinaga *et al.*, 1994; Ganga *et al.*, 1998), *Aspergillus Kawachii* (Ito *et al.*, 2000), *Penicillium sp.* (Fadel and Fouda, 1993; Gasper *et al.*, 1997), *Trichoderma reesei* (Liu et al., 1999), *Streptomyces* (Patel *et al.*, 1994; Kansoh and Gammel, 2001) and *Bacilus pumilus* (Rashid, 1999) have been exploited for xylanase production. In present study, Aspergillus *niger* was used for the synthesis of xylanase which generally grows on most of the food and waste materials, stored grains, compost piles, and exhibits saprophytic mode (Chen *et al.*, 1999; Wu *et al.*, 2000; Haq *et al.*, 2002). Therefore, the present study was designed for the synthesis of xylanase by *Aspergillus niger* using sugarcane bagasse an organic waste materials at different levels of substrate, pH, temperature and time.

MATERIALS AND METHODS

Procurement of Substrates

Sugarcane bagasse used as a substrate was obtained from Crescent Sugar Mills, Faisalabad. The substrate was dried, ground to 40 mm mesh and treated with 2.0% NaOH. The prepared sample was stored in air tight containers for further utilization in xylanase biosynthesis.

Fermentative organism

The pre-isolated and purified culture of the fungus *Aspergillus niger* was obtained from the Biotechnology Laboratory of NIFSAT (National Institute of Food Science and Technology), University of Agriculture, Faisalabad.

Growth on Potato dextrose agar for sporulation

Aspergillus niger was cultivated on the Potato dextrose agar and the spores were stored for the utilization of organism in different trials. The sporulation medium having pH (6.0) was prepared and autoclaved at 121^{o}C for 15 minutes under 1.1 kg/cm^2 pressures. After that the medium was transferred aseptically to pre-sterilized cotton plugged test tubes. The tubes were inoculated with *Aspergillus niger* and incubated at 30°C for 3 days for spores production (Asghar *et al.,* 2000).

Preparation of inoculums

The culturing medium was prepared and kept at 37°C and sterilized by autoclaving (Juhasz *et al.,* 2003). The culture from the sporulation medium was transferred to the inoculation medium in 500 mL conical flask by using inoculation loop under aseptic conditions. The inoculated medium was incubated at 37°C in an orbital shaker at 130 rpm for 3 days.

Enzyme production

After 72 hours of incubation, 3% of the inoculum was added to each fermentation flask (250 mL) for xylanase synthesis. The optimization of various culture conditions such as pH, temperature of incubation and period of fermentation was carried out.

Carbon source and culture conditions

Sugarcane bagasse was used separately at different concentrations (2.5, 3.0 and 3.5%) as carbon source. The fermentation for each trial was conducted for a period of 96 hours. Xylanase biosynthesis was carried out at different pH values (5.0, 5.5, 6.0, and 6.5), temperature (25, 27.5, 30 and 32.5°C) and different time intervals (24, 48, 72 and 96 h).

Sample harvesting and enzyme assay

After specified intervals of incubation, the biomass was filtered through filter paper. The filtrate was centrifuged at 10,000 rpm for 15 min at 10°C in the centrifuge (Sigma Laborzentrifugen (3K30) D-37520, Osterode–am-Harz, Germany) to remove the spores and mycelia. The supernatant was carefully collected and stored at refrigerated temperature in sterilized glass bottles. The filtrate was tested for xylanase activity; determined at 55°C using 0.6% (w/v) oat spelt xylan (sigma) at pH 6.0. Reducing sugars were measured using DNS method (Miller, 1959; Carmona *et al.,* 1998). The color intensity was estimated at 550 nm using spectrophotometer (CECIL CE 7200). Enzyme activity was expressed as IU/mL.

RESULTS AND DISCUSSION

The statistical analysis revealed that different pH levels, various incubation periods and different temperature of incubation at various concentrations (2.5, 3.0 and 3.5%) of sugarcane bagasse significantly affected the xylanase synthesis.

The mean values (Fig. 1) demonstrate the time scale production of xylanase by *A. niger* at different temperature (25, 27.5, 30.0 30.5°C), various pH levels at 2.5 % sugarcane bagasse concentration. The graphical illustration revealed increased enzyme production with the passage of time up to 72 h at all pH values afterwards a decreasing trend was observed in the xylanase biosynthesis. At the beginning (Fig. 1) the fungus exhibited least activity of xylanase and minimum value (2.99±0.06) was observed when the fermentation was carried out at pH 6.5 after 24 h. However, with increase in the temperature of incubation there was a gradual increase in xylanase biosynthesis up to 30.0^{o}C. After 48h of incubation the maximum xylanase activity (26.97±0.50) was observed when the fermentation was carried out at 32.5^{0}C and 5.5 pH of the culture medium followed by the enzyme activity of 26.00±0.70 at pH of 6.0 and 30^{o}C.Nonetheless, after 72 h of fermentation period the highest xylanase activity (39.07±0.55) was noted when the initial pH of the culture medium was kept at 5.5 and the temperature of incubation was 30.0^{o}C. A decrease in xylanase activity was recorded when the time of fermentation was extended more than 72 h and a minimum enzyme activity (6.94±0.08) after 96 h of incubation was calculated when the pH of culture medium was kept at 6.5 and 25.0^{o}C.

The time scale production of xylanase (Fig. 2) shows when the fermentation was carried out at 3.0 % concentration of sugarcane bagasse under different conditions of temperature and pH. The highest yield (44.00±1.45) of xylanase was noted after 72 h fermentation at a pH of 5.5 under the temperature of 30.0 C. however, fluctuation in the temperature than 30.0^{o}Ccaused significant declined in xylanase synthesis was observed. Likewise, a decrease or increase in initial pH of the culture medium than 5.5 resulted in reduced

xylanase synthesis at all temperatures and fermentation periods.

The means for xylanase activities (Fig. 3) illustrated that fermentation at the highest concentration of sugar cane bagasse (3.5 %) at the initiation of study, the fungus was least able to produce xylanase. Whereas, the time scale analysis showed increasing trend in the biosynthesis of sylanase with the higher fermentation time and maximum production was recorded at 72 h. After 24 h of incubation, the minimum (2.10±0.06) xylanase activity was noted at a temperature of 25°C and 6.5pH, whereas maximum xylanolytic activity (10.00±0.42) was observed at 30°C and 5.5pH. In xylanase synthesis by *A. niger* at 3.5% of carbon source, the highest xylanase activity (42.00±0.29) was noted when the fermentation was carried out for 72 h at a temperature of 30.0°C and 5.5 pH. The further increase in fermentation period resulted in decreased xylanase activities at all temperatures and pH values of the culture medium.

A. niger is a mesophile fungus; it gives maximum output at 30°C, whereas elevated temperature conditions result in harmful impact on its activity causing lower yields of xylanase. Furthermore, lower concentration (2.5%) of sugarcane bagasse resulted in less significant xylanase biosynthesis due to less accessibility of nutrients by the fungus, while the higher concentrations of substrate (3.5%) also gave lower enzyme yields due to a little oxygen supply in the dense medium while the concentration of 3.0% showed a better role due to the provision of adequate nutrients and oxygen availability.

Regarding the time of fermentation, it was found that in the beginning of trials the fungus was in lag phase so was not able to produce satisfactory yields of the xylanase. At 72 h of incubation, the organism was most energetic and produced the highest enzyme activities, nontheless, after 72 h the xylanase activities was lowered due to exhaustion of the nutrients and loss of enzyme activities by the proteolytic enzymes present in the fermentation medium.

The findings of present investigation are similar with the results of Duenas *et al.* (1995), they documented that the optimum temperature for *Aspergillus* to exhibit the highest xylanase synthesis is 30°C. One of their peers, Ilieva *et al.* (1995) also reported the highest activities of xylanase at 30°C. Later, Chen *et al.* (1999) delineated that under shaking conditions of *A. niger* shows maximum activity of xylanase when the fermentation is carried out at 28-32°C for 60 h. Afterwards, Haq *et al.* (2004) also reported the optimum xylanase synthesis by a mutated strain of *Aspergillus niger* required temperature was 30°C. Earlier, Gouda (2000) studied *A. tamarii* for xylanase production under both solid state and submerged fermentation conditions and inferred that the 30°C temperature is optimum for maximum enzyme recovery during submerged fermentation.

Regarding the optimum period of fermentation, the findings of current study are in line with the results reported by Camacho and Aguilar (2003), that *Aspergillus spp.* exhibited maximum xylanase activity at 72 h of fermentation in wheat bran used as substrate as a source of carbon. Similarly, Cai *et al.* (1997) and Senthilkumar *et al.* (2005) reported the highest xylanase activities at 72 h of incubation. However, present study shows some differences with the work of Palma *et al.* (1996); they reported 96 h of incubation as optimum time period for xylanase synthesis by *A. niger*, whereas Gawande and Kamat (1999) found 48 h of incubation is a best suitable for xylanase biosynthesis (26.7 IU/mL). The reason for these differences may be an indigenous strain of *A. niger* and substrate.

While dealing with pH of culture medium, it was found that pH of 5.5 is optimum for xylanase biosynthesis; these findings are in close conformity with the results reported by Gokhale *et al.* (1991) who reported a pH of 3.0-3.5 as optimum for xylanase synthesis by *A. niger*. The results of current study are also in harmony with the findings of Haq *et al.* (2004) who reported a pH of 5.0 as optimum for xylanase synthesis by a mutated strain of *A. niger*. Earlier, Marquez *et al.* (1999) also reported the pH 5.5 as best suitable for maximum xylanase biosynthesis by *A. niger*.

Conclusion

In conclusion, *A. niger* was synthesized the highest xylanase biosynthesis at 72 h of incubation, 5.5 pH and 30°C. The enzyme production gradually increased with the passage of incubation time. However, after 72 h a decreasing trend was noted in xylanase synthesis that may be due to the depletion of nutrients from culture medium that resulted in negative effect on the growth of organism.

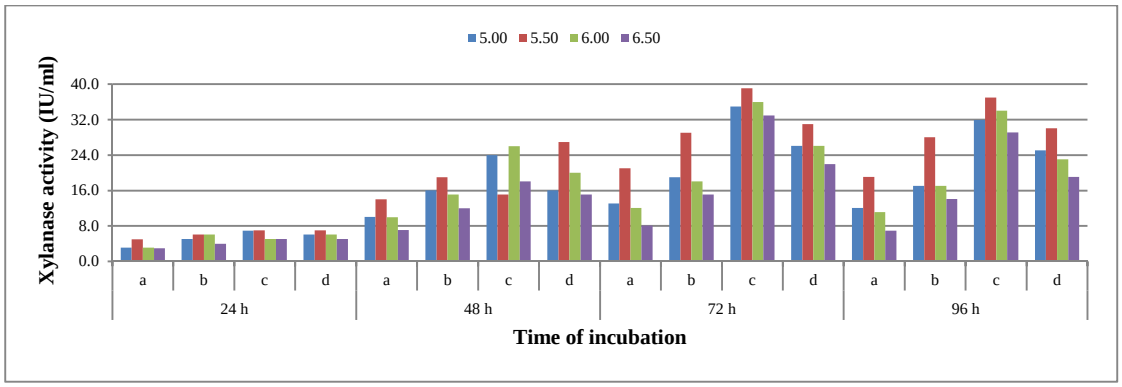

Fig 1:Xylanase biosynthesis at 2.5 % of sugarcane bagasse (a,b,c and d express 25.0, 27.5, 30.0 and 32.5 ^{o}C respectively)

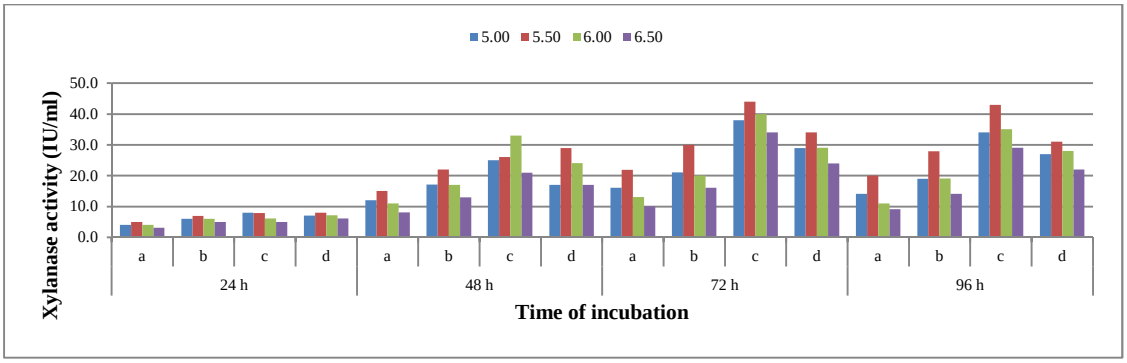

Fig 2: Xylanase biosynthesis at 3.0 % of sugarcane bagasse (a,b,c and d express 25.0, 27.5, 30.0 and 32.5 ^{o}C respectively)

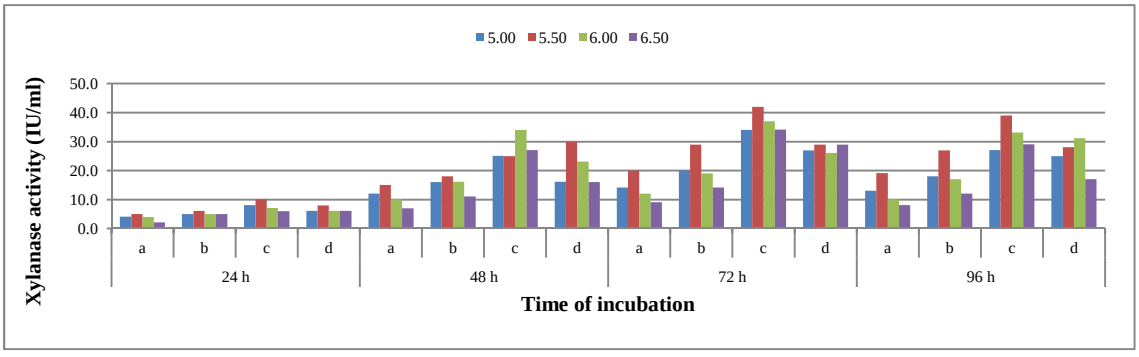

Fig 3: Xylanase biosynthesis at 3.0 % of sugarcane bagasse (a,b,c and d express 25.0, 27.5, 30.0 and 32.5 ^{o}C respectively)

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Rheological and functional properties of pumpkin wheat composite flour

Imran Pasha, Qurratul Ain Bashir Khan, Masood Sadiq Butt and Muhammad Saeed

National Institute of Food Science and Technology, University of Agriculture Faisalabad

Corresponding author: ipasha2001@yahoo.com

ABSTRACT

Pumpkin powder is a nutritional supplement and is known for large, surprising, increase in organoleptic properties and loaf volume of bread. Purpose of this study was to determine the functional, physico-chemical and rheological properties of pumpkin wheat composite flour. Wheat flour was supplemented with four different concentrations of pumpkin flour (control, 5%, 10% and 15%). The chemical analysis of treatments revealed that the moisture contents were affected highly significantly. The moisture content in control, 5%, 10% and 15% pumpkin wheat composite flour is 10.43, 11.05%, 13.00 and 14.17% respectively. It is obvious from the data that water absorption capacity ranged from 55.333 to 57.5 ml and mixing time of treatments ranged from 2.77 to 3.67. In mixographic study, peak height showed highly significant result and mean value ranged from 55.50 to 59.30 BU. In case of sensory evaluation, incorporation of pumpkin flour recorded highest scores for all quality attributes of substitution 5%, slightly higher than that control treatment. The addition of 5% pumpkin flour resulted in bread with higher loaf volume and that bread has more acceptability.

Keywords: Pumpkin, Organoleptic Properties, Rheological Properties, Composite Flour,Physico-chemical Properties

INTRODUCTION

Pumpkin belongs to genus *Cucurbita* of the family *Cucurbitaceae* is one of the largest families of vegetable kingdom. They are widely grown and consumed in many tropical and sub-tropical countries around the world (Juna *et al.*, 2006). They come under the classification of highly perishable food (Doymaz, 2004). Trace elements such as Zinc, Vitamins such as carotenoids, tocopherol and other substances like proteins, phytosterols, Polly-unsaturated fatty acids and antioxidants are naturally present in pumpkin that can be important to human health. (Phillips *et al.*, 2005; Applequist *et al.*, 2006; Glew *et al.*, 2006; Ryan *et al.*, 2007; Sabudak, 2007; Stevenson *et al.*, 2007).

Pumpkin flour due to its dark yellow-orange color, highly pleasing flavor and sweet taste is more likely to be used in bakery. Processed pumpkin flour has increased shelf-life. Pumpkin wheat composite flour can be used as natural color additive in many food products, such as pasta, instant noodles, sauces, spice and soups. Pumpkins are considered to be a rich source of pectin, carotene, minerals, vitamins and dietary fiber (Djutin, 1991). Protein from seed of pumpkin contains a reasonably well balanced composition of amino acid contents with greater level of lysine (Longe *et al.*, 1983). Pumpkin seed flour has been used as protein supplement in many local foods and gained popularity. (Giami and Bekebain, 1992; Sunday and Issac, 1999). Pumpkin seed proteins can increase in-vitro protein digestibility of bread (El-Soukkary, 2001).Hence composite flour made by adding pumpkin flour can improve bread's nutritional quality (Ptitchkina *et al.*, 1998). In many countries, pumpkin pulp and seeds being boiled and consumed as a vegetable, are also processed into flour or fermented product and utilized as flavoring ingredient, protein supplementation and functional agent in many local foods. (Achinewhu, 1987; Banigo and Akpapunam 1987; Barber *et al.*, 1989). Wheat flour bread represents the chief source of carbohydrate for a good number of the people in many countries (Giami *et al.*, 1999). Pumpkin wheat composite flour improves the texture, nutritional value and color of different bakery products and other products of food. It is feasible to produce bread with good nutritional value and sensory characteristics from pumpkin flour supplementation to wheat flour. Addition of 5% pumpkin flour gives better results of overall rheology.

MATERIAL AND METHODS

The available variety of pumpkin was procured from local market and wheat variety ARRI 11 was procured from Ayub Agricultural Research Institute, Faisalabad. A representative sample of hundred grams of wheat variety was taken and thousand kernel weight was recorded. The test weight of the wheat variety was recorded by using Schoper Chondrometer (OHAVS; Chicago) and was tempered to 14% moisture level. The tempered wheat was milled by Brabender Quadrumate Senior Mill (C.W. Brabender Instruments, Inc) to obtain different milling fractions i.e. break, reduction flour, bran and shorts. Then the fractions obtained were weighed and their percentage was calculated on the basis of total material recovered according to AACC (2000).Straight grade flour was obtained by blending the break roll flour and reduction flour fractions. The supplementation was made of wheat flour with four different levels (Control, 5%, 10%, 15%) of

pumpkin flour. The pumpkin composite flour was evaluated for moisture, crude protein, crude fat, crude ash and crude fiber according to their respective methods as described in AACC (2000). Nitrogen free extract (NFE) content was calculated according to the following expression.

NFE = 100 – (Moisture% + Protein% + Fat% + Fiber% + Ash%)

The pumpkin wheat composite flour samples were evaluated for rheological properties by using Farinograph and Mixograph by following the methods given in AACC (2000). The physical dough properties of different wheat flour blends containing pumpkin flour in different concentrations were determined by using Farinograph (Brabender D-4100 SEW; Germany) according to the procedure described in AACC (2000). The farinograms were interpreted for different characteristics like water absorption, dough development time, dough stability, mixing tolerance index and softening of the dough.The pumpkin wheat composite flour was run through Mixograpgh (National NSI-33R) to assess mixing properties of the dough i.e. mixing time and peak height percentage, by following the method described in AACC (2000). The supplementation was made of wheat flour with four different levels (Control, 5%, 10% and 15%) of pumpkin flour and bread was prepared with straight dough procedure as given in AACC (2000) method No. 10-10. Sensory Evaluation of bread was performed by using the procedure of Lawless and Heymann (1998).

Statistical analysis

To determine the level of significance of the data, each parameter was subjected to statistical analysis (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

The main objective of this research was to make different level of supplementations of pumpkin flour in wheat flour and elucidate its effects on the bread. Results of the present study included nutritional quality of pumpkin wheat composite flour, its rheological and functional properties. Bread was prepared with composite flour and also evaluated for different physical characteristics, sensory properties and textural attributes.

Physical characteristics of wheat

1. Test weight

The mean of the values for test weight shows that ARRI 11 wheat variety exhibits 79 kg hl^{-1} test weight. It wasreported that test weight ranged from 66.20 to 80.20 kg hl^{-1} in 130 hard red spring wheat varities (Klava, 2004).Pasha *et al.*, (2009) reportedthat test weight was in range 64.00 to 79.50 kg hl^{-1} in different wheat varities which were grown during two crop years.

2. Thousand Kernel weight

The mean results for 1000 kernal weight have been presented in wheat variety ARRI 11 was calculated to be 41 g. Pasha *et al.*, (2009) studied different wheat varieties and reported that thousand kernel weight was found in range of 26.02 to 49.03 g of flour.

Chemical characteristics of pumpkin wheat composite flour

The mean values of moisture content, protein content, fiber content, fat content, ash content and NFE of different treatments having different level of substitution of pumpkin flour in wheat flour are shown in table 1 respectively. Mean values for moisture content ranged from 10.43 to 14.17 and these results are in accordance with the findings of Ptitchkina *et al.*, (1998) who found that moisture content of the bread went up after the addition of pumpkin powder at concentration up to 10g/kg flour. The result of protein content is in agreement with the results of Ermakov, (1987); Ensminger *et al.*, (1994). Crude fiber content was ranged from 0.42 to 2.51% in the flour of different wheat varieties (Butt *et al.*, 2004). Fat has been reported to affect the baking quality of flour possibly due to surfactant effects and reaction with protein (Branlard and Dardevet,, 1985). Zahoor (2003) and Ahmad (2001) reported variation in fat content from 1.09 to 2.52% in some Pakistani wheat varieties. Ash content and NFE results are similar to Ahmad (2001), Butt (1997), See *et al.*, (2007) respectively.

Comparison between pumpkin flour (PF) and Pumpkin pulp (PP

The chemical analysis of pumpkin flour and pumpkin pulp are shown in table 2. Results indicated that pumpkin flour was lower in moisture (10.67%) and higher in protein, fat, ash, fiber, carbohydrate content than pumpkin pulp. These results are similar to Pongjanta *et al.*, (2006).

Table 1: Chemical characteristics of pumpkin wheat composite flour

Treatments	Moisture	Protein	Fiber	Fat	Ash	NFE
T_0	10.43^{c}	13.33^{a}	0.53^{b}	2.33^{a}	1.40^{c}	71.57^{a}
T_1	11.05^{c}	11.77^{ab}	2.41^{ab}	2.10^{ab}	1.87^{b}	70.80^{ab}
T_2	13.00^{b}	10.50^{b}	2.62^{a}	1.67^{bc}	2.25^{a}	68.32^{bc}
T_3	14.17^{a}	10.10^{b}	2.89^{a}	1.47^{c}	2.45^{a}	67.14^{c}

T_0=Control, T_1=Pumpkin flour 5% wheat flour 95%, T_2 =Pumpkin flour 10% wheat flour 90%, T_3 = Pumpkin flour 15% wheat flour 85

Table 2: Chemical analysis of pumpkin flour and pumpkin pulp

Treatments	Moisture	Protein	Fiber	Fat	Ash	NFE
PP	90.50^{a}	9.68^{a}	0.84^{a}	0.83^{a}	5.35^{a}	72.64^{a}
PF	10.67^{b}	0.9^{b}	0.58^{b}	0.20^{b}	0.77^{b}	6.96^{b}

Table 3: Farinographic and Mixographic characteristics of bread

Treatments	Water absorption	Dough development	Dough stability	Mixing tolerance index	Softening of dough	Mixing time	Peak height
T_0	55.33^{c}	5.77^{a}	11.50^{a}	40.67^{a}	120.33^{a}	3.67^{a}	59.30^{a}
T_1	56.33^{b}	5.47^{a}	11.00^{a}	40.00^{a}	118.00^{ab}	3.50^{a}	58.00^{ab}
T_2	57.17^{ab}	5.57^{b}	12.00^{b}	39.50^{ab}	117.67^{ab}	2.83^{b}	57.00^{bc}
T_3	57.50^{a}	5.50^{a}	12.70^{b}	38.50^{b}	116.00^{b}	2.77^{b}	55.50^{c}

Table 4: External characteristics of bread

Treatments	Volume of bread	Crust color	Symmetry of form	Evenness of bake	Character of crust
T_0	7.00^{a}	7.00^{a}	2.80^{a}	2.90^{a}	2.90^{a}
T_1	8.00^{b}	7.00^{a}	2.80^{a}	2.80^{a}	2.80^{a}
T_2	7.10^{bc}	6.90^{b}	2.60^{a}	2.60^{a}	2.60^{a}
T_3	6.90^{c}	6.62^{a}	2.20^{a}	2.20^{a}	2.20^{a}

Table 5: Internal characteristics of bread

Treatments	Grain of bread	Crumb color	Aroma of bread	Taste of bread	Mastication of bread	Texture of bread
T_0	7.50^{a}	8.10^{a}	8.00^{a}	12.60^{a}	8.00^{a}	12.20^{a}
T_1	7.50^{a}	8.00^{a}	7.70^{a}	12.60^{a}	7.60^{a}	12.20^{a}
T_2	7.40^{ab}	7.60^{a}	7.50^{ab}	11.00^{b}	7.20^{a}	11.90^{a}
T_3	6.80^{b}	6.90^{b}	6.90^{b}	10.80^{b}	6.40^{b}	10.80^{b}

1. **Farinographic studies**

The mean values for water absorption, dough development time, dough stability, mixing tolerance index and softening of dough are given in table 3. Water absorption and softening of dough are increased with an increase in protein content as well as improvement in gluten quality (Matz, 1972). Water absorption value of flour for ARRI 11 wheat variety is 53.33 ml. Dough development time is an indication of protein quality and strength of flour (Pyler, 1988). Dough stability also increased with protein quantity and gluten quality Kovacsa *et al.,* (2004). The mixing tolerance index in all varieties was decreased with decrease of protein.

2. Mixographic studies

The mean values for mixing time and peak height are also shown in table 3. The decrease in mixing time was might be due to decreased level of gluten content in composite flour as compared to the control. PH ranged from 55.50 to 58.00 BU in composite flour and is lower than the PH value of the controlled (59.30 BU). These results are in accordance with the findings of Malomo *et al.,* (2011).

Sensory Evaluation

1. External characteristics of bread

The analysis of variance for volume of breads, crust color, symmetry of form, evenness of bake and character of crust prepared from different treatments have been shown in table 4. The analysis showed that there is a significant interaction among the effect of wheat varieties and treatments in case of volume of bread and crust color while non-significant interaction in case of symmetry of form, evenness of bake and character of crust. These results were similar with the findings of ptitchkina *et al.,* (1998).

2. Internal characteristics of bread

The mean scores for grain of bread, crumb color, aroma, taste, mastication and texture of bread are shown in table 5. The significant interaction was observed among treatments in all cases of internal characteristics of bread e.g. grain, crumb color, aroma, texture etc. The results of present research were similar to the results of Ptitchkina *et al.,* (1998) and El Demery (2011).

CONCLUSION:

From the results it can be concluded that it is feasible to produce bread with good nutritional value and sensory characteristics from pumpkin flour supplementation to wheat flour. The addition of 5% pumpkin flour resulted in bread with high loaf volume and good overall acceptability.

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A comprehensive review on wheat flour dough rheology

Muhammad Rizwan Amjid[1], Aamir Shehzad[1,*], Shahzad Hussain[2], Muhammad Asim Shabbir[1], Moaazam Rafiq Khan[1], Muhammad Shoaib[1]

[1]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

[2]Department of Food Science and Nutrition, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia

[*]**Corresponding Author:** aamir1326@yahoo.com

ABSTRACT

The applications of rheology to the main processes encountered during bread making (mixing, fermentation and baking) are reviewed. Factors affecting dough rheology and influences of various additives on the rheological properties of flour doughs are illustrated and the component interactions are emphasized. The most commonly used rheological test methods and their relationships to product functionality are reviewed. Rheological testing has become a powerful and preferred approach for examining the structure and the fundamental properties of wheat flour doughs because of its characteristic and sensitive response to the structure variation of wheat flour doughs. It is shown that the most commonly used method for rheological testing of doughs, shear oscillation dynamic rheology, is generally used under deformation conditions inappropriate for bread making and shows little relationship with end-use performance. The frequency range used in conventional shear oscillation tests is limited to the plateau region, which is insensitive to changes in the HMW glutenin polymers thought to be responsible for variations in baking quality. Molecular size and structure of the gluten polymers that make up the major structural components of wheat are related to their rheological properties via modern polymer rheology concepts. Interactions between polymer chain entanglements and branching are seen to be the key mechanisms determining the rheology of HMW polymers.

Introduction – what is rheology?

Rheology can be defined as the study of how materials deform, flow or fail when force is applied. The name is derivated from Greek word: rheos, meaning the river, flowing, streaming. Therefore rheology means "flow science". Rheological investigations not only include flow behaviour of liquids, but also deformation behaviour of solids. Normally, to measure rheological properties, the material is subjected to a controlled, précised and quantifiable distortion or strain over a given time and the material parameters such as stiffness, modulus, viscosity, hardness, strength or toughness are determined by considering the subsequent forces or stresses (Dobraszczyk and Morgenstern, 2003).

Food rheology focuses on the flow properties of single food components, which might already display a complex rheological response function, the flow of a composite food matrix, and the effect of processing on the food structure and its properties. For processed food the composition and the addition of ingredients to obtain a certain food quality and product performance requires deep rheological understanding of single ingredients their relation to food processing, and their final discernment (Fischer and Windhab, 2011).

Rheology is another valuable tool that gives a quantitative measure for the amount of stress in the dough, which is closely related to the quality of the molecular gluten network (Bloksma and Bushuk 1988b). Rheological measurements on dough are used to define its physical properties. The primary objectives of rheological measurements are:

- To get a quantitative description of the material's mechanical properties.
- To gain information related to the molecular structure and composition of the material.
- To characterise and guess the material's performance during processing and for quality control (Dobraszczyk, 2003).

Rheological measurements are an important tool to aid in process control and process design, it tells us how dough will behave under a given set of conditions and can be used to describe and guess its performance during practical processing (Scott and Richardson, 1997), e.g., during mixing, sheeting (Love *et al.*, 2002; Morgenstern *et al.*, 2002; Binding *et al.*, 2003), proofing (Shah *et al.*, 1999), and baking of dough (Fan *et al.*, 1994). Moreover, it can also be related to product functionality. Many rheological tests are used to predict end product quality such as mixing behaviour, sheeting and baking performance (Dobraszczyk, 2004a). In order to examine process conditions and expect product performance and consumer acceptance, rheological instrumentation and measurements have become essential tools in analytical laboratories (Herh *et al.*, 2005). Herh *et al.*, (2000) studied that in predicting storage and stability measurements and in understanding and designing

texture, knowledge of the rheological and mechanical properties of different food systems is important.

Rheological properties should be independent of size, shape and how they are measured; in other words, they are worldwide, rather like the speed of light or density of water, which do not depend on how much light or water is being measured or how it is being measured. It would be encouraging to know that the stiffness of bread or viscosity of dough measured in a laboratory in Faisalabad (Pakistan) will be the same measured in any laboratory in the world, even if they are measured using different tests, sample sizes or shapes. In short, the rheological approach is that the properties that are measured are reproducible and can be compared between different samples, test sizes and shapes, and test methods (Dobraszczyk, 2004b).

Full understanding of the rheological behaviour of flour dough is of great importance from the practical point of view. Dough rheology directly affects the baking performance of flours, and rheological analyses have been made in order to optimize dough formulation. Although dough rheology has long been investigated, there remains a significant lack of understanding. This lacks of progress is due to the complexity of this biological system (Masi *et al.*, 2001).

Historical Background

Humankind has always been a perceptive feel for rheological testing, e.g. in physical and visual evaluations of material properties such as hardness, stiffness, flexibility, and viscosity, and their relation to end-use quality characteristics. People often naturally measure the quality of solid foods by gently squeezing them, or liquid viscosity is measured by gently rotating the liquid in its container. These intuitive measurements gradually became formalised into quantitative descriptions of material properties by scientists such as Newton (1687), Boyle (1662), Pascal (1663), Hooke (1678), Young (1807) and Cauchy (1827) (Tanner and Walters, 1998).

Modern rheology as an independent discipline can be dated back to 1929, when The Society of Rheology was set up by a number of scientists working in matching fields to secure an absolute standard for viscosity, and the name rheology was suggested by Bingham and Reiner to describe the study of flow and deformation of all forms of matter. The targets of rheologists are measurement, characterization and interpretation of the flow and deformation behaviour of materials. Since then rheology has developed quickly as a science and contributed to a number of applications such as colloids, suspensions and emulsions, polymer processing, extrusion and polymer modelling. Recent developments in polymer rheology have established a quantitative link between the molecular size and structure of polymers to their rheology and end-use performance (de Gennes, 1979; Doi and Edwards, 1986).

Rheological measurements are more and more being used as rapid, sensitive indicators of polymer molecular structure and forecasters of end-use performance and are being applied to bread doughs as indicators of the gluten polymer molecular structure and predictors of its functional behaviour in breadmaking (Marin and Montfort, 1996).

Rheological measurements

There are many test methods used to measure rheological properties. It is not feasible to explain all the available testing methods here, and referred to general reviews of rheology (Ferry, 1980; Barnes *et al.*, 1989; Whorlnow, 1992), rheological testing of foods (Sherman, 1970; Carter, 1990; Rao and Steffe, 1992; Dobraszczyk and Vincent, 1999; van Vliet *et al*., 1992) and cereal products (Bloksma and Bushuk, 1988a; Faridi and Faubion, 1986; Faridi and Faubion, 1990; Muller, 1975). It is common to classify rheological techniques according to the type of strain imposed: e.g. compression, extension, shear, torsion, and also the relative magnitude of the imposed deformation, e.g. small or large deformation. The main techniques used for measuring cereal properties have conventionally been divided into descriptive empirical techniques and fundamental measurements (Dobraszczyk, 2004b).

1. **Descriptive empirical rheological measurements**

Within the cereals industry there has been a long history of using descriptive empirical measurements of rheological properties, with instruments such as the penetrometer, texturometer, consistometer, amylograph, farinograph, mixograph, extensigraph, alveograph, various flow viscometers and fermentation recording devices (Muller, 1975) and (Shuey, 1975) (Table 1).

Empirical tests are easy to carry out and are often used in practical factory situations, providing data that are useful in assessing performance during processing and for quality control. The instruments are often vigorous and capable of resisting demanding factory environments, and do not require highly skilled or technically trained personnel. Simply because they do not provide data in fundamental units does not mean that these tests are valueless: in fact, they have provided a great deal of information on the quality and performance of cereal products such as consistency, hardness, texture, viscosity, etc. However, these measurements are not strictly `rheological' tests since:

- The sample geometry is variable and not well defined.
- The stress and strain states are uncontrolled, complex and non-uniform.
- It is not possible to define any rheological parameters such as stress, strain, strain rate, modulus or viscosity.

Therefore, these tests are entirely descriptive and dependent on the type of instrument, size and geometry of the test sample and the specific conditions under which the test was performed. For example, empirical tests have been used to characterise the behaviour of bread doughs during processing, such as the Farinograph and Mixograph. The problem with the use of these instruments for rheological studies is that we cannot define the stress on the sample at any moment of time during the test. For example, in a mixograph bowl, only a small part of the dough is in contact with a pin at any given time, and the shape of the sample (dough) changes in a very complicated and unpredictable ways. Thus, it is impossible to determine the stress on the dough, as we do not know the geometry of our test piece. As a result, the measurement made using a mixograph are valid only for the mixograph, and measurements made using the farinograph are relevant only to the farinograph. Moreover many of these are used as `single point' tests, where a single parameter is often arbitrarily selected from a whole range of data acquired during the test as, for example, in selecting the peak torque from a mixing trace and then using this to correlate with performance. This neglects a large part of the recorded data, and is appropriate only to the set of conditions under which that test was performed and is generally not applicable to any other deformation conditions (Dobraszczyk and Schofield, 2002; Wikstrom and Bohlin, 1996). Since dough experiences a wide range of conditions of stress states and strain rates during processing and baking, and the rheological properties of dough are dependent both on time and strain, there is often a difference between such single point type tests and actual performance on the plant, where conditions of strain and strain rate may be poorly defined and very different from those in the laboratory test (Bloksma, 1990a; Stojceska *et al.*, 2007). While this may give satisfactory correlations with a textural or processing parameter, it is impossible to compare results between different testing machines, or to extrapolate the results to other deformation conditions (Dobraszczyk, 2004b).

Most food materials are viscoelastic and therefore their properties depend on how quickly the test is performed (the strain rate or frequency). This is important in many aspects of dough processing: if the dough is deformed quickly, such as in mixing or sheeting, then the rheological properties of the dough will be very different when measured at the typically slower rates of deformation found in conventional testing machines. Alternatively, during processing dough will experience strains very different in magnitude and nature than those generally available in a rheological test. Many food processes operate under extensional flow, while most rheological tests on foods are performed in shear. Tests under only one particular set of conditions of rate, temperature and strain will almost certainly not be applicable to another set of deformation conditions. What is necessary is to define the set of deformation conditions that the food endures in practice and perform tests under similar conditions (Dobraszczyk and Morgenstern, 2003).

2. Fundamental rheological measurements

Fundamental rheological tests determine well-defined physical properties independent of size, shape and how they are measured, and can be used for process design calculations and to model complex processing situations not amenable to direct measurement. Problems encountered with such fundamental tests are: complex instrumentation which is expensive, time consuming, difficult to maintain in an industrial environment and requires high levels of technical skill; often inappropriate deformation conditions; difficulty in interpretation of results; and slip and edge effects during testing (Dobraszczyk, 2003).

The main types of fundamental rheological tests used in cereal testing are: (i) dynamic oscillation, (ii) creep and stress relaxation, (iii) extensional measurements, (iv) flow viscometry (Dobraszczyk, 2004b) (Table 1).

Factors affecting dough rheology

There are a wide variety of substances added to the dough mix that might be generally classed as processing aids and which may have secondary effects on dough rheology. Stear (1990) gives a useful summary of these. But here, our main concern is those substances that have a primary effect on rheology and throw light on the factors controlling the response to the input of mechanical energy.

The substances of interest are listed below:

- Water
- D_2O (deuterium oxide – heavy water)
- Esterifying agents for glutamine residues
- Urea
- Salts
- Agents affecting disulfide bonding
- The protein subunits present

Water is of course a prerequisite for making dough: water plasticises dough, and the control of water content is of critical importance in mixing. It

determines the ratio of loops to trains and hence the ability of the dough to be extended and to resist extension (Belton, 2003). The actual level of hydration in dough is quite low; typically the level of added water to flour is in the order of 0.6 g of water per gram of flour. Since the intrinsic level of water in the flour is of the order of 14 %, the total water is about 0.75 g per gram. If the water is equally partitioned between the components of the flour this will mean that there is about 0.75 g of water per gram of gluten. In molecular terms this means that there will be about 5.5 water molecules per amino acid residue. This represents a highly concentrated protein system. Results reported using nuclear magnetic resonance (NMR) to measure the amount of mobile protein, in preparation of high-molecular-weight (HMW) subunits of gluten (Belton *et al.*, 1994), indicates that in this region of water to protein ratio, the quantity of mobile material is highly sensitive to water content (Fig. 1). In breadmaking, the water content of dough is thus chosen to be in a region where small changes in water content are likely to make a large change in the behaviour of the proteins.

Changing H_2O to D_2O has the effect of strengthening the dough (Tkachuk and Hlynka, 1968). This must indicate a role for hydrogen bonding in the dough, as the hydrogen bonds formed by D_2O are significantly stronger than those formed by H_2O and there seem to be no other significant differences between the two isotopic forms that could have an effect. Whereas strengthening hydrogen bonds strengthens the dough, treatment to esterify glutamines residues, thus removing their hydrogen bonding ability weakens the dough (Beckwith *et al.*, 1963; Mita and Matsumoto, 1981). The indication of this effect is that the glutamine amino side chains are involved in some hydrogen-bonding network that is important in controlling dough

Table 1: Rheological methods used for cereal products

Methods	Products	Property measured	References
Empirical Methods:			
Mixers: Farinograph, Mixograph, Reomixer	Dough	Mixing time/torque Apparent viscosity	(Mani *et al.*, 1992)
Extensigraph	Dough	Extensibility	
TAXT2/Kieffer RIG	Dough, Gluten	Extensibility	(Mani *et al.*, 1992)
Alveograph	Dough, Gluten	Biaxial extensibility	(Bonet *et al.*, 2006)
Amylograph RVA	Pastes, Suspensions	Apparent Viscosity, Gelatinisation temperature	
Consistometer	Sauces, Fillings	Apparent viscosity	(Bonet *et al.*, 2006)
Flow cup	Fluids, Sauces, Batters	Apparent viscosity	
Falling ball	Fluids	Apparent viscosity	
Flow viscosimeters	Fluids, Pastes	Apparent viscosity	
Fermentometers	Dough	Height, Volume	
Penetrometers	Semi-solid foods, Gels	Firmness, Hardness	
Texturometer, TPA	Solid foods	Texture, Firmness	
Fundamental methods:			
Dynamic oscillation, Concentric cylinders, Parallel plates	Fluids, Pastes, Batters, Doughs	Dynamic shear moduli, Dynamic viscosity	(Rouille *et al.*, 2005)
Tube viscometers: Capillary, Pressure, Extrusion, Pipe flow	Fluids, Sauces, Pastes, Dough	Viscosity, In-line viscosity	(Rouille *et al.*, 2005)
Transient flow: Concentric cylinders, Parallel plates	Semi-solid viscoelastic Material	Creep relaxation, Moduli and time	
Extrusion: Uniaxial, Biaxial, Dough inflation system, Lubricated compression	Solid foods, Doughs	Extensional viscosity, Strain hardening	(Dobraszczyk, 2004a)

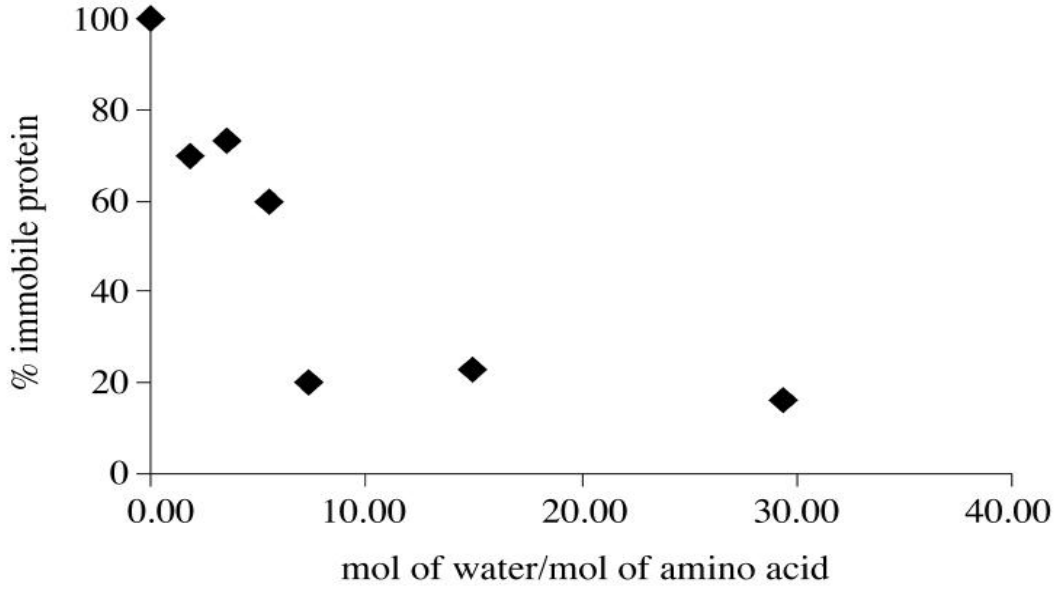

Fig. 1: A plot for the variation in the mobile fraction of high molecular weight subunits with water content

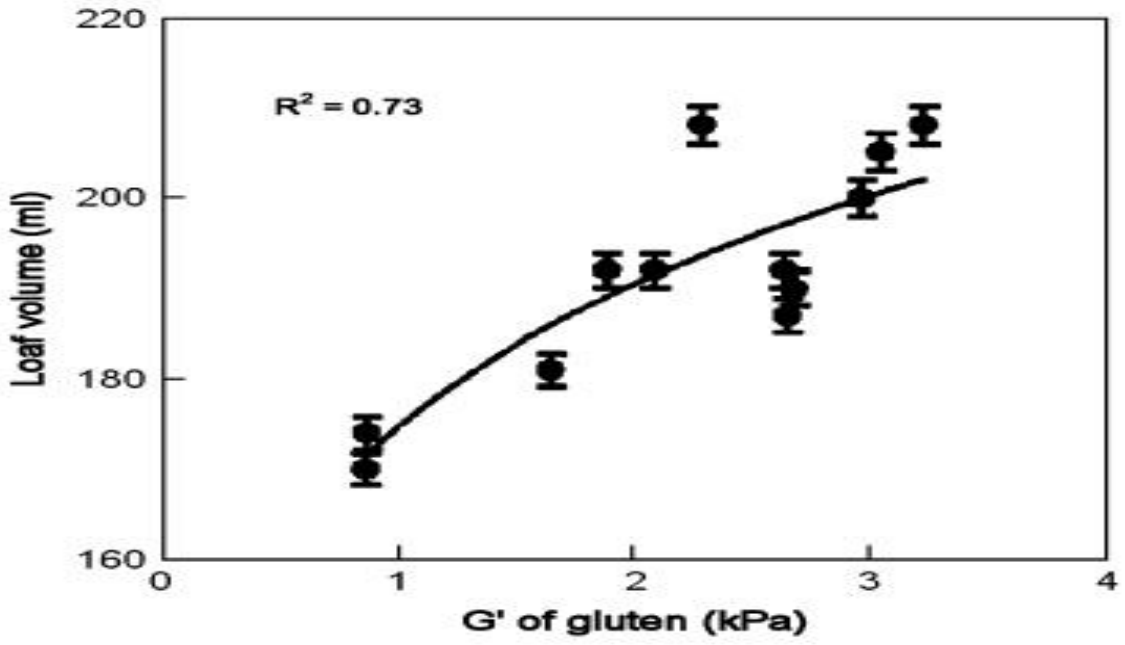

Fig. 2: Relationship between G of gluten (stress 25 Pa, frequency 1 Hz) and loaf volume for flours

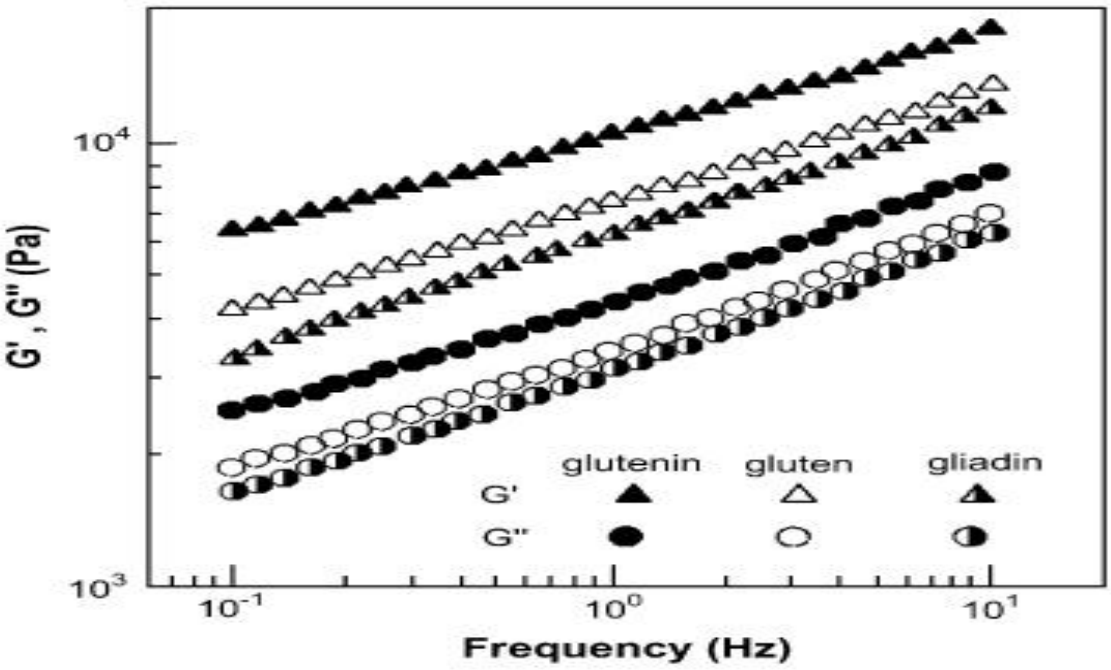

Fig. 3: Dynamic responses of durum dough enriched with 2 % gluten, gliadin and glutenin, respectively (Edwards *et al.*, 2001)

rheology. In a similar manner, the weakening effects of urea on dough rheology (Wrigley *et al.*, 1998) have been interpreted as being due to disruption of hydrogen bonding.

The molecular effects of salts can be quite subtle and the details of the mechanisms of the interactions of salts with proteins are not completely understood. Salts can affect both hydrogen bonding and protein solubility, both of which will affect the cohesiveness of the

For metal chloride salts, the gluten strength is increased with the charge density of the metal ion. Since generally higher charge densities result in a more hydrogen bonded water structure, this may be taken to imply that increasing the hydrogen bonding capacity of the solvent increases the gluten strength. Conversely, the extraction data for a series of sodium salts showed that the greater the capacity of the counter-ion to break down hydrogen bonding structure, the more it facilitated protein extraction. Apart from the obvious effects of shielding electrostatic charge interactions, the role of salts in protein is difficult to understand and much discussion has gone on in the literature. However spectroscopic results on gluten at constant water content (Wellner *et al.*, 2003) indicate that for the series NaCl, NaBr and NaI, increasing counter-ion size, and hence water structure breaking capacity, cause an increase in the amount of beta turn present and the

system. Eliasson and Larsson (1993) have reviewed the effects of salts on the behaviour of dough. The addition of sodium chloride to dough influences gas retention, increases the time to optimum dough development and increases the stability of the dough. These effects may arise from a variety of causes not directly linked to the interactions of the proteins. There may be effects on enzymes and yeast; however, more extensive studies have shown that both gluten strength (Preston, 1989) and extractability of proteins (Preston, 1985) are modified by the addition of salts.
amount of mobile protein present. This result is consistent with those of Preston (1985, 1989).

The role of disulfide linkages in the control of dough rheology is of the utmost importance. If disulfide bonds are reduced by a chemical agent, such as dithiothreitol, a dramatic reduction in dough strength is observed (Wrigley *et al.*, 1998) which is recovered on re-oxidation. The additions of various oxidizing and reducing agents that can affect the interchange of disulfide bonds also have major effects (Eliasson and Larsson, 1993). The actual mode of action of the various agents that can affect both the interchange among, and the number of disulfide bonds, is not entirely clear till now (Weegels *et al.*, 1994). However, their effect is profound. Indeed, the role of disulfide interchange in dough rheology has led Bushuk (1998) to remark that "The importance of the disulfide interchange reaction in the development and stress relaxation of bread doughs cannot be overemphasised".

The role of the nature of the various protein subunits in dough rheology and loaf quality has been the subject of intensive research. Gliadins are generally agreed to contribute to the viscous nature of the dough and glutenins to the elastic nature of the dough. Of the glutenins the most important are the HMW subunits even though they only constitute 12 % of the total flour proteins or 1-1.7 % of the flour dry weight (Shewry *et al.*, 2001).

Wheat grains and rheology

The physicochemical and rheological properties of flour differ significantly among wheat varieties which have far reaching effects on the end use quality of wheat. The rheological characteristics of flour vary between varieties (Stathopoulos *et al.*, 2008). Actual quality of wheat is the summation of effects of soil, climate and seed stock on the wheat plant and kernel components. The wheat grains are milled into flour and used in different end use products. The quality of the end product depends upon quality of wheat grain. The wheat suitable for one particular use may have certain properties that are totally unsatisfactory for other use (Faridi *et al.*, 1989; Anjum *et al.*, 2008).

Wheat flours from various classes and cultivars display great diversity in their functional properties. The variations in functional properties of a wheat cultivar are attributed largely to its gluten quality and quantity (Rao *et al.*, 2000). The farinograph test is one of the most commonly used flour quality tests in the world. The results are used as parameters in formulation to estimate the amount of water required to make dough, to evaluate the effects of ingredients on mixing properties, to evaluate flour blending requirements and to check flour uniformity. The results are also used to predict processing effects, including mixing requirements for dough development, tolerance to over-mixing and dough consistency during production. Farinograph results are also useful for predicting finished product texture characteristics. For example, strong dough mixing properties are related to firm product texture. Brabender farinograph has been used to predict doughing properties of flours. Dough is prepared during mixing and resistance to shear is recorded. Graph for strong wheat flour gives high water absorption, shows rapid development and minimal breakdown. Weak wheat flours also exhibit rapid development but their breakdown is greater as well as has low water absorption capacity. Farinographic water absorption of flour provides an indication of the potential of the protein molecules to absorb moisture (Dobraszczyk and Salmanowicz, 2008).

Dough stability is defined as the time difference between the point where the top of the curve first intercepts the 500 BU line and the point where the top of the curve leaves the 500 BU line (Sim *et al.*, 2011). Dough development time is the time from water addition to the flour until the dough reaches the point of the greatest torque. During this phase of mixing, the water hydrates the flour components and the dough is developed (Fu *et al.*, 2008). Mixing time define as the time in minutes taken by the curve to reach the peak. Peak height is the height attained by the curve at peak in cm as measured from the center of the peak to the base line and mixing tolerance measured as the angle in degrees formed by the ascending and descending curves at the apex, located in the center of the curve (Singh *et al.*, 2003).

Gluten is rich in gliadins and glutenins. Dynamic rheological parameters of glutens are able to indicate the wheat quality. Glutens from poor quality reconstituted using glutens of different wheat cultivers and a content source of starch and water soluble (Khatkar and Schofield, 2002a). Wheat are rheologically characterized as less elastic and more viscous than those from good quality wheat (Khatkar *et al.*, 1995). Glutens from good bread making wheat are cross linked in a higher degree so that the frequency dependence of G′ is smaller than that of glutens from poor bread making wheat (Janssen *et al.*, 1996b). Storage and loss moduli G′ and G″ of glutens show significant positive correlations with loaf volume (Khatkar *et al.*, 2002). Especially, G′ of gluten doughs can be directly related to the bread making performance, explaining 73 % of variation in loaf volume (Fig. 2) (Khatkar and Schofield, 2002a). The tan δ values of glutens are ranked as weak glutens > strong glutens > extra strong glutens while the G′ and G″ values show the reverse tendency. The weak glutens especially undergo a substantial structural change from solid-like to liquid-like behaviours with increasing frequency while the strong glutens maintain their elastic characters to a great extent (Khatkar, 2004).

Wheat flour water absorption 57.5 % in farinograph is observed. Canadian wheat cultivars have 60.7-65.9 % water absorption, 2.25-13 minutes development time and 5-25 minutes stability time in farinograph (Indrani and Rao, 2007). Water absorption of different wheat varieties were ranges from 58.1-66.4 % and the dough development time with average value of 6 minutes (Hruskova *et al.*, 2006). In another experiment, wheat with 53.6 % water absorption, 1.53 minutes dough development time and 1.40 minutes dough stability is determined (Paraskevopoulou *et al.*, 2010). Studies

showed that the Irish, Greek and Canadian wheat varieties had water absorption, dough development time and dough stability ranges from 50.7-61.5 %, 1.5-6 minutes 1-5 minutes respectively. In 2010, it is reported that the Irish wheat varieties had the water absorption, development time and stability ranges from 50-65.5 %, 1.5-6 minutes and 1-9 minutes respectively (Ktenioudaki *et al.,* 2010). Mixograph test was the best predictors for chewiness and firmness because it is simple, requires relatively small sample size and the results obtained are highly correlated with sensory data. It is the most useful test to predict the end use quality (Kovacs *et al.,* 1997). In mixograph, the mixing time varies from 2.3-7.9 minutes and peak dough resistance from 52.3-65.2 AU showed by Rao *et al.,* (2000). The values for dough development time and dough stability decreased with reduced protein content, but the value of mixing tolerance index increased (Fu *et al.,* 2008).

Both quantity and quality of protein influence water absorption (Kenny *et al.,* 2001; Akubor and Ukwuru, 2003; Paraskevopoulou *et al.,* 2010). Hefnawy *et al.,* (2012) reported that the increase in protein content increased the water absorption. Water absorption is an important characteristic of the wheat flour and in Indo-Pakistan wheat varieties ranged from 60-76 % water absorption (Sila, 2010).

Composite flour technology and rheology

There is a growing interest in fortifying wheat flour with high lysine material, such as dry beans to improve the essential amino acid balance of baked food products. Using composite flours may be advantageous in developing countries where adequate technology for the production dry protein concentrates/isolates is not available or affordable in order to utilize the bean proteins. Also the development of such blends could lead to improved utilization of indigenous food crops in countries where import of wheat flour is a necessity and dry bean production is more than adequate. Increasing levels of cowpea flour in the blends affected most dough properties and resulted in changed farinograph and extensograph characteristics, mainly by increased water absorption. Increased water absorption of wheat-bean composite flours may provide more water for starch gelatinization in the doughs during baking and may prevent stretching and tearing of gluten strands (Hallen *et al.,* 2004).

In the case of wheat dough, rheological analysis has been successfully applied as indicator of the molecular structure of gluten and starch, and as predictors of their functionality in baking performance (Collar and Bollain, 2005; Bollain *et al.,* 2006). Despite gluten free matrixes are structurally different than gluten dough; rheological assessment of the gluten free matrixes might give an indication of its further functionality. The cohesiveness was significantly affected only by soybean protein content (Marco and Rosell, 2008). Legumes such as soybean and chickpea proteins shows higher emulsifying activity and emulsion stability (Tömösközi *et al.,* 2001). They are used in food technology for supplying desirable functional properties such as emulsification, fat absorption, moisture holding capacity, thickening, and foaming (Marco and Rosell, 2008). The addition of 20 % soy flour to wheat produced a significant positive effect on the emulsifying activity of the samples (Ahn *et al.,* 2005).

Farinograph characteristics of flour blends showed that as the proportion of soy flour increased there was a slight increase in water absorption and decrease in dough stability. The results showed that incorporation of soy flour increased the water absorption capacity. At 20 % level of soy flour the water absorption was 77 % and at 40 % level, it was 80 %. The stability of the dough was found to decrease from 4.5 to 3.0 minutes when the soy flour content increased from 20 to 40 %. Dough development time and mixing tolerance index remained almost same for all flour blends that are 4.5 minutes and 110 BU respectively (Senthil *et al.,* 2002). In another trail as the percentage of soy flour increased from 5 to 10 %, water absorption increase from 57.2 to 57.9 %. As the level of flour blends in composite doughs increased, farinograph absorption and mixing tolerance index increased, but mixing time and dough stability decreased (Doxastakis *et al.,* 2002).

Rheological characteristics in different wheat varieties of Pakistan showed 55.20- 62.13 % water absorption, 3.33-16.42 minutes dough stability time and 3.58-9.92 minutes dough development time (Huma, 2004). Further studies reported that water absorption of wheat is 61.24 %. As percentage of chickpea increase, water absorption increase. Water absorption in 10 % chickpea is higher than 7.5 % and 5 % that is 67.85, 67.45 and 66.85 % respectively. Similarly increased in the dough development time and dough stability time as the concentration of chickpea increased. While rheological behaviour of the composite flours prepared by blending commercial wheat flour with lentil, chickpea and guar gum showed decrease in water absorption and increase in dough development time in a storage period of 60 days (Shahzadi *et al.,* 2005). In contrast, studied showed that at 5, 10, 15, 20 and 30 % replacement of chickpea with wheat the water absorption decreased from 62, 60, 57, 56.6 and 53.3 % respectively. In replacement of corn flour at the same percentage the water absorption increased from 63.3,

76.6, 83.3, 90 and 93.3 % respectively (Gujral and Pathak, 2002).

Ingredients and rheology

Rheological testing, especially in the linear viscoelastic region, has been used to follow the structure and properties of doughs and to study the functions of dough ingredients (Janssen *et al.,* 1996a). This testing simultaneously measures the viscous and elastic characters of dough expressed in storage and loss moduli, G′ and G″, and loss tangent tan δ. It is generally found that doughs made from good quality flour have tan δ values lower than doughs made from poor quality flour. The magnitude of modulus at intermediate and high strains is in the order of extra strong > strong > medium > weak (Safari-Ardi and Phan-Thien, 1998). Nevertheless, dynamic rheological tests on flour dough fail to predict the baking potential of wheat cultivars (Autio *et al.,* 2001).

Influence of water

Dough is a macroscopically homogeneous mixture of starch, protein, fat, salt, yeast, and other components. At optimum mixing, the dough is fully hydrated and has the highest elasticity. Water plays an important role in determining the viscoelastic properties of dough. Both G′ and G″ decrease as water content increases. The dynamic viscoelastic behaviour
of flour doughs can be understood by taking into account the dual role of water that behaves as inert filler reducing the dynamic properties proportionally and as a lubricant enhancing the relaxation (Masi *et al.*, 1998).

Influence of starch

Starch, making up ~80 % of wheat flour on dry basis, is able to form a continuous network of particles together with the macromolecular network of hydrated gluten. These two independent networks and their interaction give rise to the rheological properties of doughs. Though the interaction plays an important role, the relative contributions of the two sources are difficult to resolve. The component interactions depend on stress level. The starch – starch interactions dominate over protein – protein interactions at low stresses while the protein – protein interactions play dominant role at large deformations (Khatkar and Schofield, 2002b). The nonlinear rheological behaviour of starch is largely responsible for the behaviour of dough (Watanabe *et al.*, 2002).

In starch/gluten blend with constant water content, G′ increases rapidly with increasing protein content. The reconstituted doughs behave qualitatively like flour doughs with comparable compositions. When starch granules are apparently homogeneously dispersed in the gluten network, increasing starch content gives rise to an increase in G′ value (Watanabe *et al.,* 2002) thus enhancing the elasticity (Edwards *et al.,* 2002). Flour doughs cannot be viewed simply as a concentrated suspension of starch granules in hydrated gluten matrix. Mixing starches from different wheat cultivars into dough with constant gluten content leads to large rheological differences, indicating an active role of starch (Petrofsky and Hoseney, 1995).

Influence of proteins

The protein content of flours shows an inverse relationship with G′ and G″ up to ~14 % protein (Khatkar, 2005). Gluten contributes to the viscoelastic properties of dough to varying degrees depending on its source differing with both gliadin/glutenin ratio and LMW-GS (Edwards *et al.*, 2001; Edwards *at el.*, 2003). Gliadin enhances viscous flow of dough. An addition of 2 % gliadin results in increased dough extensibility and tan δ as compared to gluten and glutenin additions. Glutenin addition, on the other hand, results in more elastic dough in comparison with gluten and gliadin additions (Fig. 3) (Edwards *et al.*, 2001). Addition of glutenins at constant protein basis contributes to the dough strength with marked differences among donor cultivars (Edwards *et al.,* 2003). Increasing the glutenin/gliadin ratio improves maximum shear viscosity and dough strength (Uthayakumaran *et al.*, 2000).
Both low molecular weight glutenin subunits (LMW-GS) and high molecular weight glutenin subunits (HMW-GS) contribute to overall dough strength but LMW-GS enrichment improves the elasticity by introducing greater number of physical crosslinks (Edwards *et al.,* 2001). The source of LMW-GS influences the viscoelastic characteristics of doughs while source of HMW-GS does not show such an effect (Edwards *et al.,* 2003).

Influence of other additives

Rheological properties of materials depend on the structure and also on the arrangement of ingredients and the forces between them (Singh *et al.*, 2003). The importance of the soluble fraction of flour in determining the rheological properties of dough subjected to large deformations and its possible consequence for breadmaking performances was demonstrated by measuring shear and extensional viscosities of native wheat flour and reconstituted doughs using creep-recovery tests and lubricated squeezing flow tests (LSF). The viscosity plateau decreases with increasing additions of soluble fractions. They showed poor discriminating properties compared

with results of lubricating squeezing flow tests (Rouille *et al.,* 2005). Dairy ingredients are added to bakery products to increase nutritional and functional properties. Dynamic oscillation testing determined the effects of the ingredients on fundamental rheological properties. Adding 4 % sodium casienate (SC) decreased resistance to extension, while adding 4 % whey protein concentrate (WPC) increased extensibility (Kenny *et al.,* 2001). Functional food additives such as surfactants are widely used to improve the quality of bread. The percent water absorption increased significantly with the addition of surfactants (mono-diglyceride and lecithin) alone or in combination. Moreover, the overall dough rheological characteristics and baking quality improved and further these surfactants retarded the rate of staling in bread (Azizi *et al.,* 2003). The performance of different fat replacers at various levels (Inulin powder, Inulin gel and Simplesse) in wheat bread and dough compared to a control containing block fat was examined. Empirical and fundamental rheological tests were carried out on the doughs. The addition of inulin gel was found to increase water absorption. Moreover complex modulus for doughs containing fat was significantly lower than the doughs containing the fat replacers. The addition of simplesse and inulin increased the dough complex modulus significantly (O'Brien *et al.,* 2003).

Dynamic rheological testing has become a powerful and preferred approach for examining the structure and the fundamental properties of wheat flour doughs and proteins because of its characteristic and sensitive response to the structure variation of wheat flour doughs and proteins (Song and Zheng, 2007). Addition of carbohydrates such as arabinoxylans, β-glucans (Izydorczyk *et al.,* 2001), carrageenan, alginate (Howell *at el.,* 1998) and guar gum (Yu and Ngadi, 2006) improve the functional properties of wheat bread through associative interactions with gluten proteins that significantly increases G′ of doughs at the same water content.

Defatting improves protein interaction thus increases G′ and G″ significantly (Georgopoulos *et al.,* 2006). Addition of nonpolar lipids to the defatted flour at their natural level might partially restore the rheological behavior while higher levels of addition have no further effect. On the other hand, addition of polar lipids has a more pronounced beneficial effect (Papantoniou *et al.,* 2004). Addition of water-solubles dramatically shortens the optimum mixing time of the reconstituted flour and decreases G′ of the resultant dough (Miller and Hoseney, 1999).

Enzymes are used in baking to improve dough handling properties and the quality of baked products. Glucose oxidase (GO) is an enzyme with oxidizing effect due to the hydrogen peroxide released from its catalytic reaction. A reinforcement or strengthening of wheat dough and an improvement of bread quality can be obtained with the addition of GO, although inverse effects were obtained when excessive enzyme levels were added. The GO treatment modified gluten proteins (gliadins and glutenins) through the formation of disulfide and non-disulfide crosslinks. Excessive addition of GO produced an excessive crosslinking in the gluten network, responsible of the negative effect on the breadmaking properties. Rheological characteristics such as water absorption and dough tolerance showed a significant enhancement when added the higher concentration of glucose oxidase. Thus, the addition of GO promotes an increase in dough stability when over-mixing (Bonet *et al.,* 2006).

Processing conditions and rheology

Dough processing is an important factor determining the quality of bread. The most important mechanical steps in industrial dough processing are kneading, extrusion, and molding. In all of these processing steps, considerable changes in the structure and properties of the dough can occur. On a laboratory-scale level, these (structural) effects are well characterized but, so far, a little data is available for large-scale industrial dough processing line. The molecular and microstructural changes that can take place during the kneading step revealed that the dough shows a well-developed gluten network with a homogeneous dispersion of starch particles (at optimum kneading time). After the extrusion step (a sheeting procedure), the structure of the dough becomes coarser and the dough gluten network is oriented and partially disrupted. This is accompanied with an increase in both rheological stress and water mobility. After molding, the network structure is restored and both the rheological stress and the mobility of water decrease. These findings helps in optimization of industrial dough processing lines (Esselink *et al.,* 2003).

Dough rheological techniques are frequently used for the analysis of wheat flour baking value. When dough is subjected to mechanical perturbation it shows viscoelastic behaviour. That is, the mechanical force applied to the dough results in dimensional changes that are partially but not fully reversed when the force is removed. The observation of a maximum of resistance during the mixing process implies that the dough stores some of the mechanical energy expended as elastic potential energy. (Hruskova *et al.,* 2006). The distinctive rheological features of dough can predict

about their expected behaviour under various processing conditions that in turn may help to select suitable raw materials and their proportions, and to decide the appropriate process equipment. As a result, the quality of the finished product including texture, and hence, consumer acceptability is affected. The role of water content in this condition plays an important role as it acts as a plasticizer that affect the rheological behaviour markedly (Bhattacharya *et al.,* 2006).

Among the cereal flours, only wheat flour can form three-dimensional viscoelastic dough when mixed with water. Characterization of rheological properties of dough is oppressive in predicting the processing behaviour and in controlling the quality of food products. Farinograph, mixograph and extensograph are the most common empirical instruments used for characterizing dough rheology (Song and Zheng, 2007) and in evaluating the performance during processing and for quality control. Tests based on these instruments are useful for providing practical information for the baking industries while they are not sufficient for interpreting the fundamental behaviour of dough processing and baking quality (Dobraszczyk, 2003). The water absorption capacity of flour often defines its quality and its tendency to form viscoelastic dough. The hydration of flour is severe in the food industry, because it affects its functional properties and the quality of cooking products (Berton *et al.,* 2002).

It is studied that mild heating improves the strength of substandard bread flour such as soft wheat flour. Heating soft wheat flour at 80 °C for 15 min. improved its bread-making potential (Gelinas *et al.,* 2001). The gluten fractions in the different wheat varieties varied in the proportion of HMW glutenins and LMW gliadins. The fractions containing a higher proportion of HMW glutenins were associated with a predominantly elastic character, whereas fractions containing mostly gliadins exhibited a viscous-like behaviour. The frequency dependent rheological behaviour of fractions containing HMW proteins was less susceptible to heat, and their elastic character was maintained after heating, whereas the rheology of intermediate fractions and fractions containing mostly gliadins was more susceptible to heating, indicating a rapid change from viscous to elastic behaviour after heating. Moreover gluten was easier to extract and its texture was slacker after heating, it significantly increased dough-mixing stability and development time (Gelinas and McKinnon, 2004; Stathopoulos *et al.*, 2006).

Rheological effect on mixing

Mixing is a critical operation in food processing where, apart from the obvious function of mixing ingredients, the structure of the food is often formed. It is well known that in order to optimize bread quality, mixing must be stopped at the correct level of mechanical input. The actual process called mixing in reality has two separate processes going on within it: one is the homogenization of the various ingredients of the dough, which is a true mixing process, and the other is the development of dough structure by the mechanics of mixing energy into the system. Although the former is of vital importance, it is a process common to most food preparation processes; it is the latter process that demonstrates the uniqueness of wheat flour dough. As mechanical energy is put into the dough, its resistance to extension increases and then after some critical point decreases again. Optimum bread quality is achieved by choosing to stop mixing at the appropriate point on the mixing curve (usually close to, but not at, the maximum resistance) (Belton, 2003). For example, in the production of batters, pastes and doughs, the nature of the mixing action results in the hydration of flour particles leading to development of the viscoelastic properties gluten matrix and also incorporates air, which has a major effect on their rheology and texture (Dobraszczyk and Morgenstern, 2003; Singh *et al.*, 2003; Dobraszczyk *et al.,* 2006).

Most of the studies on doughs have been on the relationships between mixing, rheology and baking performance, because rheological changes occur in the gluten viscoelastic network during mixing and have importance for product quality. There is an intimate relationship between mixing, aeration and rheology: the design and operation of the mixer will develop texture, aeration and rheology to different extents (Campbell and Shah, 1999), and conversely the rheology of the food will affect the time and energy input required to achieve optimal development. This is seen in the great variety of mixers used in the food industry and the fact that certain mixers are required to produce a desired texture or rheology in a food (Campbell, 1995).

Studies on the rheology of mixing have focused on a number of areas: (i) the effects of mixer design and operation on the development of rheology and texture; (ii) empirical measurement of rheology during mixing from mixer torque or power consumption; (iii) effect of rheology on mixing patterns and performance; and (iv) simulation and prediction of mixing flow deformation patterns as functions of mixer geometry and rheology. Despite the obvious importance of mixing in the development of rheology and texture in doughs, there is very little information in the literature on these changes

during the different stages in the mixing process. Most work has either concentrated on empirical measurement of mixer motor torque, voltage or power consumption during mixing as a qualitative indication of changing rheology, or measurement of rheological changes at some time after mixing. Problems associated with these approaches are: failure to take into account motor and drive losses, frictional and surface effects between the dough and the mixer, varying signal damping and data acquisition rates, effects of aeration on rheology, and rheological relaxation effects. Since dough is a viscoelastic material which shows rapid relaxation after deformation, which varies between different flours, such measurements are not ideal and run the risk of giving misleading information. Nevertheless, much useful information has been obtained about the effect of mixing on gluten structure, rheology and baking performance (Weegels *et al.*, 1996; Skerrit *et al.*, 1999). Extensive work on dough mixing has shown that mixing speed and energy (work input) must be above a certain value to develop the gluten network and to produce satisfactory breadmaking (Kilborn and Tipples, 1972), and an optimum in work input or mixing time has been related to optimum breadmaking performance (Skeggs, 1985), which varies depending on mixer type, flour composition and ingredients (Mani *et al.*, 1992). For example, mixing doughs by elongational flow in sheeting to achieve optimum development required only 10-15 % of the energy normally used in conventional high speed shear mixers (Kilborn and Tipples, 1974), suggesting that much higher rates of work input can be achieved due to the enhanced strain hardening of doughs under extension. Numerous studies have shown that rheological measurements after mixing parallel changes in mixer torque and power consumption (Mani *et al.*, 1992; Zheng *et al.*, 2000; Anderssen *et al.*, 1998), especially if rheological measurements are made under large, non-linear deformation conditions closer to those experienced in the mixer (Mani *et al.*, 1992; Hwang and Gunasekaran, 2001). Recent studies have suggested that qualitative elongational rheological information during mixing can be derived directly from the torque/power consumption of a dough mixer (Gras *et al.*, 2000).

Extensive work on dough mixing has shown that mixing speed and energy (work input) must be above a certain value to develop the gluten network and to produce satisfactory breadmaking, and an optimum in work input or mixing time (peak development) has been related to optimum bread-making performance, which varies depending on mixer type, flour composition, and ingredients. If dough is under-mixed or mixed well beyond its peak development, then bread of inferior quality is produced. Kilborn and Tipples in a series of papers from 1972-77 investigated factors affecting dough development. Their results indicated that: (i) for a given flour, there is a minimum mixing speed and energy input (the critical mixing speed or energy) below which development could not be achieved, resulting in a loaf of poor volume, colour, and texture; (ii) the total energy input required for peak development differs between flour types; and (iii) both the total energy required and the critical mixing speed for a given flour differ between mixers with different mixing actions. Moreover both aeration and rheological characteristics of dough are dependent on both the total work input and the work input rate (Chin and Campbell, 2005).

Rheological effect on proofing, baking and final texture of bread

Proofing (fermentation) is an important step in the breadmaking process, where the expansion of air bubbles previously incorporated during mixing provides the characteristic aerated structure of bread, which is central to its appeal (Dobraszczyk *et al.*, 2000). Dough expansion during fermentation process (proofing) is greatly influenced by main components of flour and rheological properties of dough. Basically it depends on the optimum development of the gluten proteins network into a cohesive dough mass, encapsulating starch granules and other filler materials or components and air nuclei (Bloksma, 1990b). Although fermentation is clearly important in breadmaking, most rheological tests are performed on doughs without yeast and at room temperature. Few studies have been made on the changing rheological properties during fermentation and baking. Direct rheological measurements have been made on yeasted bread doughs (Kilborn and Preston, 1981), cake batters (Massey, 2002; Sahi, 1999), sour doughs (Wehrle and Arendt, 1998), and cracker sponge and dough (Oliver and Brock, 1997). Such measurements suffer from the problem of the evolving gas volume and metabolites from fermentation confounding the rheological data. The decrease in density as a result of increasing gas volume would be expected to have the effect of decreasing modulus and viscosity, but the compressibility of air may counteract this effect, especially at higher gas volumes and low densities where the moduli of the solid and gas phases converge, such as in cake batters, where shear modulus is directly related to the air content (Massey, 2002). Fermentation metabolites such as lactic and acetic acid may also exert rheological effects through changes in pH (Wehrle *et al.*, 1997).

Other approaches have been to measure the increase in height or volume of the fermenting product using

devices such as the rheofermentometer or risograph, but these provide no direct information about the rheology of the material, since they do not measure force or deformation per change in unit dimensions. Changes in aeration have been predicted from modelling the increase in dough height (Shah *et al.,* 1999), or by directly measuring internal gas pressure during fermentation (Matsumoto *et al.,* 1975). Another approach has been to prevent fermentation by inactivating the yeast by freezing and thawing (Newberry *et al.,* 2002), or by mixing under oxygen to rapidly saturate the yeast activity (Chamberlain and Collins, 1979).

During proof and baking the growth and stability of gas bubbles within the dough determines the expansion of the dough and therefore the ultimate volume and texture of the baked product (He and Hoseney, 1991). The limit of expansion of these bubbles is related directly to their stability, due to coalescence and the eventual loss of gas when the bubbles fail. The rheological properties of the expanding bubble walls will therefore be important in maintaining stability against premature failure during baking, and also in relation to gas cell stabilization and gas retention during proof, and thus to the final structure and volume of the baked product (Dobraszczyk *et al.,* 2000). The relevant rheological conditions around an expanding gas cell during proof and baking are biaxial extension, large strain, and low strain rate. Any rheological tests which seek to relate to baking performance should therefore be performed under conditions similar to those of baking expansion. Methods such as bubble inflation and lubricated compression offer the most appropriate method for measuring rheological properties of doughs. The major advantage of these tests is that the deformation closely resembles practical conditions experienced by the cell walls around the expanding gas cells within the dough during proof and oven rise, i.e., large deformation biaxial extension can be carried out at the low strain rates and elevated temperatures relevant to baking (Dobraszczyk *et al.,* 2003).

Recent work has shown that bread doughs exhibit strain hardening under large extensional deformations, and that these extensional rheological properties are important in baking performance (van Vliet *et al.,* 1992; Dobraszczyk and Roberts, 1994; Janssen *et al.,* 1996b; Dobraszczyk, 1997; Wikstrom and Bohlin, 1999; Dobraszczyk *et al.,* 2003). Strain hardening allows the expanding gas cell walls to resist failure by locally increasing resistance to extension as the bubble walls become thinner, and provides the bubble walls greater stability against early coalescence and better gas retention. It is therefore expected that doughs with good strain hardening characteristics should result in a finer crumb texture (e.g., smaller gas cells, thinner cell walls, and an even distribution of bubble sizes) and larger baked volume than doughs with poor strain hardening properties. It has been shown that good breadmaking doughs have good strain-hardening properties and inflate to larger single bubble volume before rupture, whereas poor bread-making doughs inflate to lower volumes and have much lower strain hardening (Dobraszczyk and Roberts, 1994; Dobraszczyk, 1997). Loaf volume for a number of commercial white flour doughs has been related directly to the failure strain and strain hardening properties of single dough bubbles measured at elevated temperatures in biaxial extension (Dobraszczyk *et al.,* 2003). Strain hardening and failure strain of cell walls were both seen to decrease with temperature, with cell walls in good breadmaking doughs remaining stable and retaining their strain hardening properties to higher temperatures (60 °C), whilst the cell walls of poor bread-making doughs became unstable at lower temperatures (45-50 °C) and had lower strain hardening. Bubble wall stability is increased to progressively higher temperatures with increasing baking volume, allowing the bubbles to resist coalescence and retain gas for much longer. Bubble wall instability in poorer breadmaking varieties occurs at much lower temperatures, giving earlier bubble coalescence and release of gas, resulting in lower loaf volumes and poorer texture (Dobraszczyk *et al.,* 2003).

The steaming of wheat flour for various periods weakened the gluten network structure whilst Prakash and Rao, (1999) have studied the effects of heat processing of cereal grains on the paste viscosity of cereal flours.

End product quality and rheology

The link between dough rheology and baking quality is long established, mainly due to empirical evidence from manual assessments such as kneading or stretching of dough by bakers after mixing. However, the results from conventional descriptive methods and fundamental rheological studies on doughs have often given disappointing correlations with baking quality, mainly because the deformation conditions in these tests are very different than those occurring during proof and baking.

Dough rheology is of considerable importance in bread and biscuit manufacturing as it influences the machinability of dough and the quality of end product (Indrani and Rao, 2007). Dough is the intermediate product between flour and biscuits. Dough which is too firm or too soft will not process satisfactorily on the

appropriate dough forming equipment and will not yield a suitable product. Doughs that are too strong do not allow proper development of the bubbles and result in the formation of dense, unpalatable loaves of small volume, while doughs that are too weak cannot retain the bubbles and result in large holes in the loaf or in the collapse of the loaf. It is reported that dough consistency influences the quality of biscuits (Manohar and Haridas Rao, 2002; Angioloni and Collar, 2008).

The quality of baked products is governed by rheological properties of dough (Stathopoulos *et al.*, 2006). This preceding rheological evaluation of the dough is good indicator of dough handling properties. Dough rheology characterization is an important parameter in the evaluation of biscuit wheat quality and indicates dough handling properties and the tendency of the dough to contract (Pedersen *et al.*, 2004). Several methods including mixograph, farinograph and extensograph are used for characterization of the rheological properties of biscuit dough (Ross *et al.*, 2004) and proteins present in wheat flour governed these rheological properties. Molecular size and structure of the gluten polymers that make up the major structural components of wheat are related to their rheological properties via modern polymer rheology concepts. Interactions between polymer chain entanglements and branching are seen to be the key mechanisms determining the rheology of HMW polymers. These structural and rheological properties of the insoluble polymer fraction are mainly responsible for variations in baking performance (Dobraszczyk, 2004a).

The rheological characterization of wheat flour dough is necessary for the successful manufacturing of bakery products because of its influence on mechanical handling and quality characteristics of the finished products (Agyare *et al.*, 2005) The suitability of wheat flour for the production of different baked products like breads, cakes, biscuits and chapattis depends primarily on particular rheological properties of dough such as water absorption, dough stability, strength, extensibility, elasticity etc. (Karaoglu, 2011). During breadmaking, rheological properties of dough change at every stage of breadmaking process. When the dough is mixed in a high speed mixer, it is converted into an elastic and coherent mass due to high stress conditions prevailing at this speed (Stojceska *et al.*, 2007). Rheological behaviour is associated directly with textural qualities such as mouth feel, taste and shelf stability (Herh *et al.*, 2000).

Conclusion

It can be concluded from the available literature that the dynamic rheological technique of frequency sweep under small deformations is highly promising for elucidating the structure of wheat proteins and the processibility of wheat flour dough. These studies demonstrate that the component interactions are fairly important for determining the rheological behaviours of gluten and flour doughs. It would appear that for HMW polymers such as gluten, large deformation extensional rheological properties are more sensitive to changes in polymer entanglements and branching than small deformation dynamic shear properties, based on sound polymer physics principles and experimental data. Insoluble HMW glutenins have been shown to be best related to variations in baking quality, and to the presence of long relaxation times, indicating entanglements of the HMW polymers. Strain hardening, which has been shown to be a sensitive indicator of entanglements and long-chain branching in HMW polymers, is seen in large extensional deformation of doughs and glutens, and is well related to bubble wall stability, long relaxation times and to variations in baking performance amongst different wheat varieties.

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