FACTORS INFLUENCING QUALITY AND NUTRITIONAL VALUE IN CHAPATIES

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DEDICATED
TO
MY MOTHER AND LATE FATHER

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CONTENTS

ABSTRACT

Chapati, an unleavened flat bread, is a staple in the diet in Pakistan. Wheat in the form of chapati can contribute as much as 90 % of the total dietary energy intake to the rural population and generally provides more than half dietary energy and protein. Such a heavy dependence on this cereal food has led to protein malnutrition due to wheat proteins being deficient in lysine, an essential amino acid. The aim of this study was to produce savoury chapati with enhanced nutritional value.

Attitudes to a variety of savoury flavours were determined in consumers of Pakistan and in immigrants to Glasgow. Chicken and meaty were most and cheesy least preferred. Amongst chapati quality characters, nutritional value was rated highest followed by flavour. These studies suggested that consumers favoured a product with appropriate flavour, a soft texture and of high nutritional value.

Typically chapaties are prepared with wholemeal flours with medium rheological optima. Doughs of British wheat varieties Fresco and Galahad (33+67) and Mercia and Galahad (50+50) had moderate stickiness, high sheeting ability, and were low in shrinkage after sheeting. Such suitable mixtures yielded flours suitable for chapati production.

Wheat protein profiles were determined by SDS-PAGE

electrophoresis and relationships between proteins and rheological properties determined with partial least squares regression (PLS2). Dough development time was positively correlated with aggregated values of high molecular weight (HMW) and low molecular weight (LMW) glutenins, ratio of polymeric to monomeric and LMW glutenins, and negatively correlated with the total of gliadin, globulin and albumin fractions. Dough stability showed negative and tolerance index positive correlations with gliadin. Resistance to extension was associated with dough stability and inversely with gliadin content. Such studies reveal that glutenin contributes strength and gliadin imparts weakness to flour.

Response surface methodology, used to optimise chapaties, revealed that a successful savoury product could be produced by incorporating 1.5 to 2.0 % yeast extract without any deleterious effect on dough physical characteristics. Yeast extract enhanced the concentrations of protein from 13.1 % to 14.4 % in chapati. In addition to enhanced nutritional value, yeast extract imparted a desirable savoury flavour to chapati.

1. INTRODUCTION

1.1 Chapaties and flat breads

Chapaties are steam-leavened flat breads common to India, Pakistan and parts of Africa (Ebeler and Walker, 1983; Rao et al., 1986a; Bass and Caul, 1972; Rahotra, 1979). In Pakistan the most commonly consumed least expensive products are chapaties and roties, using almost 80 % of the total wheat production. These are the primary, and cheapest, sources of protein and calories in the diet (Anjum et al., 1991). The terms "chapati" and "roti" are used synonymously for unleavened breads. In Punjab and Sindh provinces chapati and roti doughs are unleavened while in Baluchistan and Frontier provinces fermented roties are prepared. A coarse whole wheat flour (atta) is always used in these products. When mixed with water (Kent, 1975; Swaranjeet et al., 1982), the resulting dough is rolled into thin sheets and cooked on a hot surface (chapati) or baked in a mud oven (roti) (Kent, 1975) to produce a puffed ball (Chaudhry and Muller, 1976). Chapati is generally prepared and consumed fresh in households, as well as in restaurants. Among the poor it can represent as much as 90 % of total dietary energy intake (Chaudhry, 1968). Such heavy dependence on a single cereal protein source has resulted in widespread protein deficiency.

Flat breads are the most ancient of breads, although no records exist of their origin. Cereal gruel was probably baked into palatable flat breads in the Neolithic period (late Stone Age). The gruel was made into a dough, shaped into flat cakes and baked on hot stones or directly in a fireplace. The Egyptians spread knowledge of breadmaking and fermentations throughout the Mediterranean world by the end of the Neolithic period (about 1800 B.C.). Thus, by the Bronze and Iron Ages (1800 B.C.-1 A.D.) flat sourdough breads had become the staple European food. In the Middle Ages, loaves of bread were rounded or semicircular, flat and marked with a cross. This Christian symbol made it easier to break the loaf (Pomeranz and Shellenberger, 1971). The history of bread baking in Egypt and other North African countries, including baking techniques of the bedouins and nomads of the deserts, has been described by Wahren (1959, 1961, 1962).

Flat breads are currently consumed around the world. Until recently, few people had heard of flat breads, but restaurants with cultural roots in India, Pakistan, Greece, Mexico, Turkey and various Arab countries and growing recreational travel have changed that. The increasing popularity of flat breads has resulted in the success of a number of Middle Eastern bakery products such as pita bread, sold either fresh, chilled or frozen (Rashid, 1983).

Breads may be divided into three categories with respect to density: 1) those with high specific volume i.e. volume to weight ratio, such as Western white pan bread; 2) those with medium specific volume, such as French and rye breads; and 3) those with low specific volume, such as pita and lavosh bread and the flat breads of Northern Europe, the Middle East, India and Pakistan (Faridi, 1988). Flat breads are generally dense in texture being mostly crust with little crumb, and usually round but sometimes triangular or rectangular. Diameters vary from 5 - 10 cm, up to 70 cm and loaf thickness from paper-thin to 4 cm. The crust is thin and light, with brown or dark spots. The crumb is small in quantity and coarse and dense. Flat breads have a higher crust-to-crumb ratio than do conventional leavened wheat breads (Pomeranz, 1988).

Flat breads can also be divided into two principal groups on the basis of cross section: either single-layered or double-layered (Fig. 1.1). Double-layered breads are leavened with sourdough starters, or yeast. The most prominent examples are pita or Arabic breads and Egyptian baladi bread.

The single-layered breads can be further subdivided into leavened and unleavened breads. Leavened breads include tannouri, barbari, lavosh, Khobz al-daar (North African), and Scandinavian flat breads. Unleavened breads include traditional tortillas, chapaties, paratha and a number of European flat breads. Certain single-layered breads are prepared from a slurry of raw materials notably dosai, rogag and injera. European crepes and American pancakes can also be put in this category. Such products are prepared by pouring a slurry of raw materials on to a heated metal surface. Methods of preparation of flat breads differ in the manner of sheeting or in the final appearance of the end products. Five basic methods are used for the commercial production of leavened flat breads (Fig. 1.2) and many ingredients are essential (Fig. 1.3) (Qarooni et al., 1992).

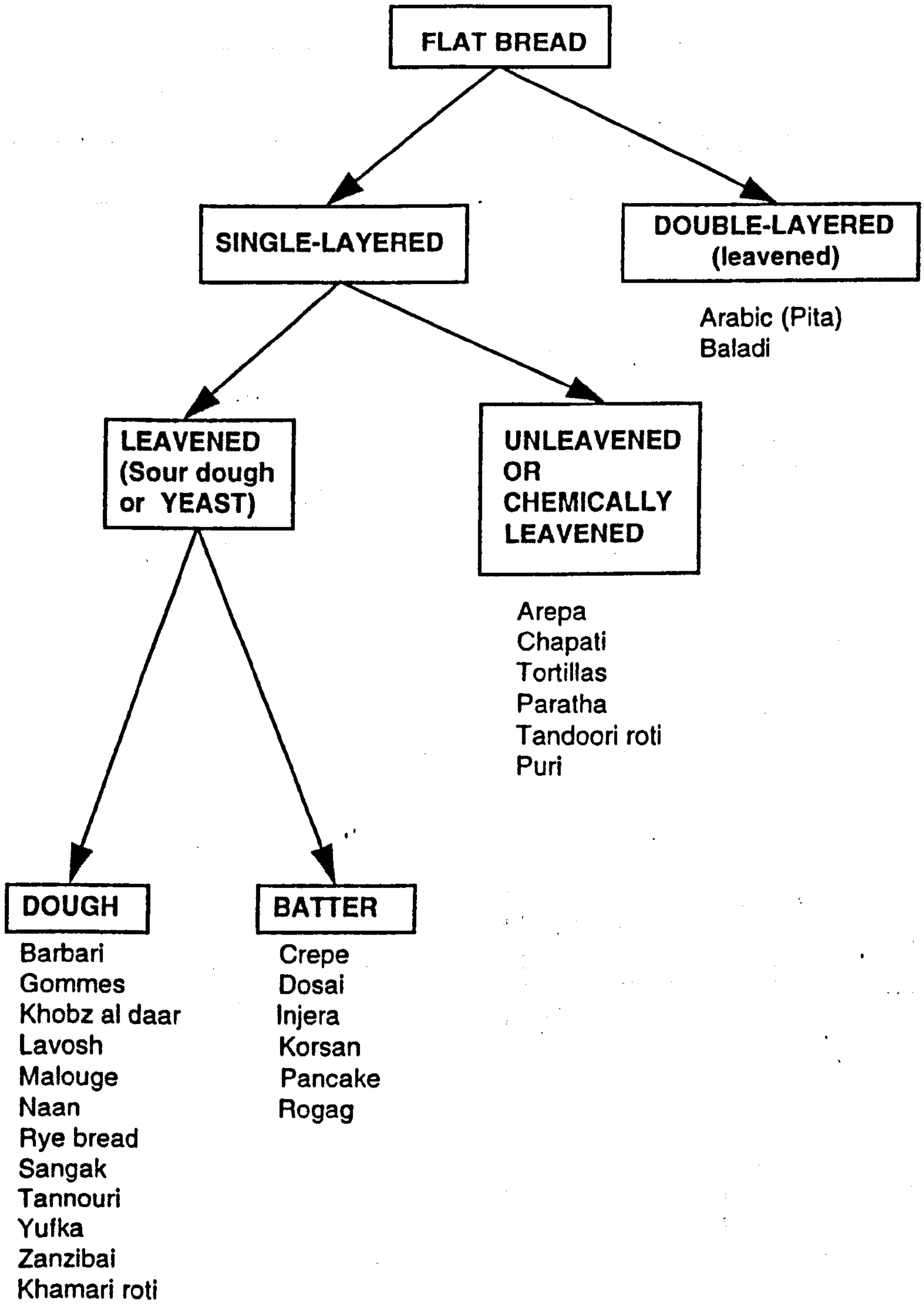

Fig. 1.1. Classification of flat breads.
Source: Qarooni _et al._, 1992.

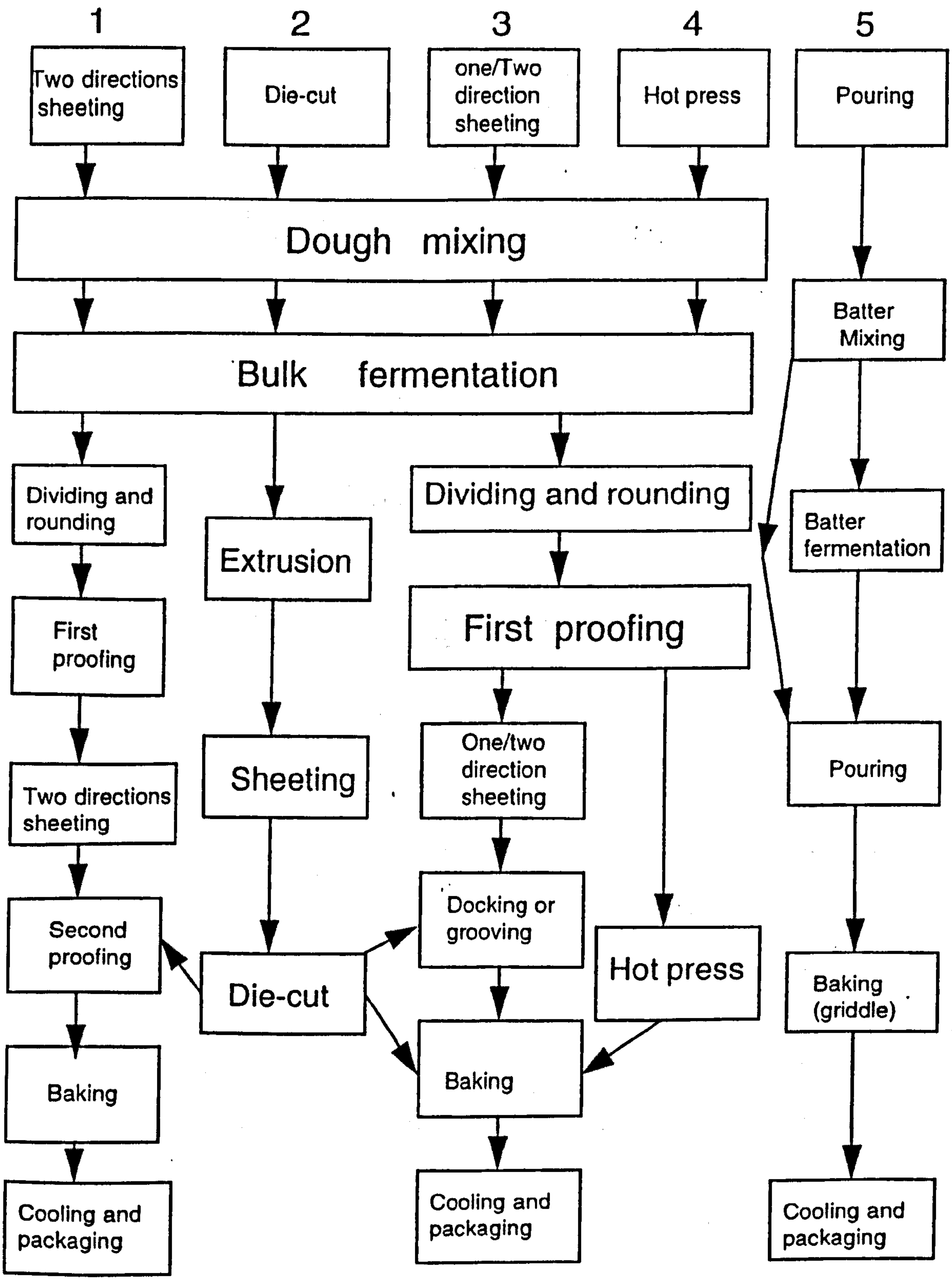

Fig. 1.2. Processing method of flat breads.
Source: Qarooni *et al.*, 1992

Essential:

Flour	Wheat, corn, rye, barley, sorghum, millet, or rice
Leavening agents	Yeast, sourdough, soda, baking powder(or without leavening agents)
Salt	Sodium chloride
Water	

Optional:

Sugar, shortening, milk, malt flour fruits, potato flour and flour from various legumes, meat, herbs, spices, sesame, poppy, and caraway seeds, emulsifiers, gums, reducing and oxidizing agents

Fig. 1.3. Ingredients of flat breads. Adapted from: Qarooni et al., 1992

1.1.1.a Unleavened breads: Production

Chapati: Flour is mixed with water and kneaded by hand to the desired consistency - a slack dough. Water absorption ranges from 65 to 75 %, occasionally being as high as 80 %. The dough is allowed to rest (30 min to 1 h), divided into suitable balls (from 50 g to more than 1 kg varying with region), and rounded. After a further rest for some minutes, it is then flattened and shaped either using a rolling pin or between the palms to yield a flat, round shape. Doughs are normally processed without resting and immediately transferred to a preheated hot plate or metal surface and turned three or four times during baking. The total baking time is generally 70 to 100 sec. However, the cooking time varies from 2 to 5 min depending on temperature, size, weight and thickness of the bread (Aziz and Bhatti, 1960; Chaudhri and Muller, 1976; Finney et al., 1980b).

Tandoori roti: Preparation of tandoori roti is similar to that of chapati, except that salt may be added at the time of kneading and dough balls are generally heavier. Roti is baked in a "tandoor", an oval-shaped and in-ground, oven. In this walls, plastered with clay are heated by burning wood or natural gas. The sheeted dough is placed on a cloth pad and pasted on to the heated walls. Depending on the heat in the clay, roties may be baked in 60 - 90 sec. The baked roti is recovered with a long iron, with an end formed into an L shape (Finney et al., 1980b).

Tandoori roties are generally prepared and sold at small bake shops that also operate as restaurants. More often than not, the roties are consumed at the point of manufacture. Fermented tandoori roties are the staple food of people in Baluchistan and frontier provinces of Pakistan. In these the dough is fermented by the natural microflora of the flour. Each day, a large chunk of dough is saved for use as a starter on the following day. The dough is prepared by the addition of starter, water, salt (1.5 - 2.0 %) and sodium bicarbonate to neutralize dough acidity and act as a leavening agent. The leavening action is, therefore, both biological and chemical.

Paratha: A unleavened bread made from whole wheat flour, shortening (44 %), salt (2.5 %) and water (65 %). The flour dough is mixed with a portion of shortening and sheeted like chapati and roti, then smeared on one side with shortening. The dough is folded, twisted, resheeted and cooked on a preheated hot plate. When both sides are partly cooked, bread is smeared on both sides with a further quantity of shortening and cooked to completion (4 - 5 min) (Finney et al., 1980b).

Puri: Dough is prepared, as for chapati, using patent white flour, sheeted using a small amount of shortening and gently lowered into preheated oil for 1 - 2 min. The cooking dough is flipped over once while being pressed gently with a slotted spatula, to help puffing.

1.1.1.b Leavened breads

Naan: Naan is a fermented flat bread prepared from flour, yeast (2 %), sugar (1 %), salt (2 %), water (35 %), yogurt (25 %) and shortening (6 %). The dough is mixed, immediately divided into balls, and allowed to ferment, while covered with wet cloth to avoid formation of a crust. It is then sheeted into thin, oblong or round flat pieces and baked at 315 °C for *ca.* 2 minutes (Rashid et al., 1982).

Khameri roti: A summer bread in which a sponge is formed from a part of whole wheat flour, salt, sugar and yogurt and then fermented overnight. A second portion of flour, soda and water are added on the following day. The baking procedure is similar to that of roti (Pomeranz, 1988).

Such breads are generally consumed at any time a day. Paratha and puri are liked at breakfast. Whole wheat flour is used for their production except that 2 to 5 % of the coarse bran may be sifted off before preparation. Naan and puri are often prepared using lower extraction flour, even 75 to 80 % (maida) (Finney et al., 1980b).

Several other products from wheat flour are popular in Pakistan. These include pan bread, buns, biscuits, cakes, pastries, patties, samosas, pies, rusks, noodles, vermicelli, sooji halwa, sohn halwa, khatai, and jalebi amongst other products.

When wheat is not available, other cereals may also be used to prepare chapaties. Murty and Subramanian (1982) reported that 70 % of sorghum grown in India was used to prepare roti. For production of chapati from sorghum, flour is usually mixed with warm water in increments and kneaded into a dough which has little cohesiveness. The roti is then rolled into a thin circle and baked. In some households, a portion of the flour may be cooked or soaked in water overnight to improve dough cohesiveness. Good sorghum roti should be creamy white, with a few slightly burnt spots, and have a soft texture and bland flavour.

Millet roti is traditionally prepared with stone-ground millet flour of 85 - 100 % extraction (Rashid, 1974; Desikacher, 1977). Wholemeal flour is mixed with water and sometimes salt to produce a dough of a suitable consistency. The dough is then rested under a moist cloth, moulded into a ball, rolled into a disc and baked on a ungreased preheated iron plate. Cooking time depends on temperature and the size of the roti. Sorghum and millet roti doughs do not trap gas efficiently and may tear more easily than wheat chapati doughs (Mason and Hoseney, 1980).

1.1.2 Supplementation of flat breads with flours

Many Third World countries in which flat breads are popular do not grow enough wheat to meet the demand for bread. Wheat is thus imported (Dendy et al., 1970; Tsen et al., 1974; Bond, 1975; El-Said and El-Farra, 1983) which places a strain on the economy of the country. Thus efforts have been made to utilise alternative native cereal grains to replace part of the wheat flour required for breadmaking. In general, flat breads are more tolerant of supplement addition than pan breads. Additives, such as potato flour, may modify the gluten matrix structure so that steam evolved during baking escapes readily through the surface of the loaf. This results in reduced loaf volumes. Certain supplements, however, may improve the quality of flat breads. Prabhavathi et al. (1976) reported improvements in the chapati-making quality of Indian wheats blended with durum wheat. Williams et al. (1988a) stated that Arabic breads made from durum wheat flour had a more desirable and softer texture. This improvement in quality could be due to the grain "hardness" in durum wheat. Milling causes more starch damage in durum flour than with softer wheats. A high level of damaged starch results in increased absorption of water and starch gelatinization yielding a softer crumb.

The suitability of blending wheat and triticale flour in production of chapati was studied by Rashid and Hawthorn (1974), Sharma et al. (1977) and Sekhon et al. (1980). A blend of triticale flour with wheat flour (1:3) gave similar chapati quality to wheat flour alone. Triticale varieties with higher protein contents (16 %) were most suitable for chapati making. When triticale was used alone, the bread was darken and somewhat tougher. Wheat flour could also be mixed with 15 - 20 % tapioca flour without adversely affecting chapati-making qualities such as sweetness and palatability. Bengal gram flour, however, gave a prominent flavour to the chapati even at low levels. Such flour blends did not lose chapati-making quality during storage for up to 30 days (Murty and Austin, 1963a, 1963b).

Morad et al. (1984) considered that substitution of wheat flour with up to 30 % ground sorghum produced acceptable Egyptian balady bread. Awadalla and Slump (1974) used 20 % whole or decorticated millet flour with 80 % Dutch wheat flour of 100, 80, or 70 % extractions in production of Egyptian flat breads. Baking quality was impaired, as judged by loaf volume and internal and external characteristics, but could be improved by addition of dough conditioners such as $KBrO_3$, fat and calcium stearoyl lactylate. Mustafa (1973) suggested blending of 10 % sorghum flour into wheat flour for preparation of Sudanese breads.

Elias et al. (1977) reported that adding 3 - 6 % potato flour to conventional balady dough yielded an acceptable loaf. Hamed et al. (1973a, 1973b) reported that addition of 10 - 15 parts of sweet-potato flour to 100 parts of high-extraction wheat flour (93 % extraction) produced good quality balady bread.

1.2 Role of chapaties and flat breads in nutrition

Protein-calorie malnutrition is endemic in the developing countries and exists in a spectrum of conditions from kwashiorkor, or straightforward protein deficiency, to marasmus or calorie and protein deficiency. Marasmus and marasmic kwashiorkor are far more prevalent than kwashiorkor (McLaren, 1969). In India, in only 10 % cases was protein deficiency a result of inadequate protein intake. Protein deficiency was found primarily to be due to insufficient intake of total energy (Sukhatme, 1970). Desai et al. (1970) also stressed the need for sufficient calories to obtain the maximal benefit from any nutritional supplements.

Flat breads are considered to be the staple food in North Africa, the Middle East, and South Asia (Irani, 1987). Protein malnutrition is a serious problem for people with a diet based on cereals or other starchy products (Finney et al., 1950; Chastain et al., 1975;

Carlson et al., 1981). In countries where rice, maize or wheat are the main foods there is a high prevalence of protein-calorie malnutrition syndromes among infant and preschool children. Women and children are most affected and diet can be improved by supplementation with cheap and nutritious foods such as soy and potato flour (Singh and Nath, 1989). Inexpensive high protein supplements in various foods and food products have considerable potential for improving diets. Potential protein supplements are extracted oilseed flours, food grade yeasts and fish meal. High consumption, acceptability and low price make bread an ideal vehicle for protein supplementation (Faridi and Finney, 1980).

Milner (1974) reported cereals supply 60 %, 50 % and 40 - 45 % dietary protein intake for Asia, Sub-Sahara Africa and Eastern Europe including USSR respectively.

Supplementing wheat flour with animal and vegetable protein is considered a promising strategy to alleviate protein deficiency. Data showing per capita protein consumption for Pakistan are given in Table 1.1 .

In Pakistan nutritional surveys and food balance sheets indicate varied protein deficiencies in vulnerable groups of the population, due especially to both low quality and quantity of proteins consumed. Cereals provide over 63 and 71 % of daily calories and protein intakes of an average person, respectively (Government of Pakistan, 1993). In certain areas, these components can form as much as 88 % of total caloric intakes (Khan et al., 1976).

Table 1.1: Contribution of various sources to daily protein supply in Pakistan

gram protein/day/person						
Animal origin	Cereals	Pulses & nuts	Vegetables	Total supply	Plant protein % of total	Cereal protein % of total
9.8	32.9	3.3	0.7	47.7	79	69

Source : FAO, 1966.

Aslam et al. (1982) reported Pakistanis consume primarily chapaties and roties with a curry made from pulses, accounting for 68.5 and 1.3 % of total energy intake from wheat and pulses, respectively and 75 and 3 % of the total protein intakes.

Energy deficiency widens a protein gap, and protein deficiency is conditioned by energy deficiency. Therefore, energy intake must be sufficient to ensure adequate protein supply (Hussain, 1973).

Protein in cereal grains is deficient in certain essential amino acids, primarily lysine in wheat flour although tryptophan, threonine and methionine are also low. Deficiencies in such amino acids reduce utilization of protein in the body and contribute to malnutrition. Lysine deficiency in wheat products is aggravated by losses arising from browning during baking. In conventional bread baking conditions over 10 % of lysine is destroyed (Saab et al., 1981).

Protein scores, based on the FAO 1973 Scoring Pattern indicated that lysine is the first limiting amino acid in all flours and breads, and threonine the second in wheat, rice, barley, millet, triticale and sorghum breads. Tryptophan was the second limiting amino acid in maize bread. Isoleucine is the third limiting amino acid in barley, millet and sorghum breads, valine in wheat and triticale breads; threonine and tryptophan in maize and rice breads, respectively.

To improve the nutrition of the people of Pakistan (especially those with low income and in rural populations), wheat flour should be fortified with suitable proteins relatively rich in such amino acids. Enrichment of the basic dough formula increases the overall nutritional status of the indigent population. Meat, milk, fish and poultry are expensive protein sources and beyond the reach of much of the populace. Therefore, it is important to identify cheap sources of high quality food proteins to compensate for deficiencies in wheat-based products like chapati.

1.2.1 Enrichment of chapati and flat breads

Amino acids from synthetic sources: Maleki and Djazayeri (1968) established that addition of 0.3 % L-lysine to wheat flour significantly improved the protein quality of bread. Supplementation of lysine-fortified flour with 0.62 % DL-threonine further improved protein quality, whereas methionine had no effect. Baking did not change the protein quality of bread supplemented with either lysine alone or lysine and threonine. Hussein et al. (1973) added sufficient DL-methionine and L-lysine to balady bread to supply the equivalent of 10 % daily protein. Protein efficiency ratio (PER) rose significantly with the amino acid supplementation, from 1.28 to 2.19 for the DL-methionine supplement and to 3.32 with L-lysine. Balady breads containing added 0.35 % lysine, 0.13 % DL-threonine and 0.7 % DL-methionine (flour basis) produced similar growth rates in rats to diets with 6.6 % fish protein concentrate (FPC) (Arafah et al., 1980). Hedayat et al. (1973) fortified Iranian village bread with L-lysine. Although studies with rats gave encouraging results, no significant improvements were observed in the nutritional status of a group of Iranian village children who received the bread for 210 days. Wheat grains fortified with 0.1 - 0.2 % lysine appear suitable for preparation of atta for chapaties, usually prepared in small batches (Graham et al., 1968).

Protein from animal sources: Ali et al. (1964) concluded that chapaties containing 2 % shark meat flour could satisfy the protein requirement of growing children and supplementation with 3 - 5 % was adequate for nursing mothers. Arabic and Indian breads were supplemented with fish protein concentrate (FPC) by Nikkila et al. (1976) giving significant improvements in nutritional value. Taste panel tests showed the acceptability of breads supplemented with 10 % FPC. High temperature required for the baking of Arabic bread (500 °C) or cooking puri in oil (190 °C) for 1 minute did not significantly reduce the protein value of FPC.

Arafah et al. (1980) conducted feeding trials with rats to study the effects of fortification of balady bread with both fish protein concentrate and green algae. Baking at 400 °C resulted in losses of 12 - 21 % of L-lysine in bread made with FPC to a protein efficiency ratio of 2.6 (mean value unsupplemented: 1.0). Diets of 6 % algae in composite bread flours did not improve growth rates in rats. Nutritive value of Arabic bread was also improved by supplementation with whey protein (Khalil and Hallab, 1975).

Protein from vegetable sources: Vegetable proteins in legumes and pulses have been used in reducing protein malnutrition. Production of edible pulses in Pakistan was 725.5 thousand metric tonnes during 1985 (Government of Pakistan, 1986) including a number of bean varieties (family Leguminaceae). The quality of pulse protein is superior to that of wheat, containing a higher proportion of lysine, threonine and tryptophan. Such crops are relatively deficient in sulphur-containing amino acids but rich sources of lysine and are natural and cheap sources of proteins for human nutrition (Aykroyd and Doughty, 1969) when diets lack animal protein (Rajalakshami, 1976).

In Pakistan, a number of pulses are consumed as whole or split grains in preparation of curries to be consumed with chapati or rice. Numerous sweets and snacks are also prepared from such pulses. Pulses are also good sources of carbohydrates, fat, vitamins (notably thiamine, riboflavin and niacin) and many minerals. Nutritional improvements of chapati supplemented with soy flour have been reported (Bhat, 1977). Imtiaz (1962) considered that chapati from whole wheat flours supplemented with 15 % medium-fat soybean flour and 10 % dried milk gave the best balanced diet. Lindel and Walker (1984) prepared chapati with various blends of cereal flours enriched with soy flour. In all cases, protein efficiency ratios were improved. Corn-soy chapati had a protein efficiency ratio 2.15, the highest ratio, compared to a value of 1.41 for corn alone. Shyamala and Kennedy (1962) reported protein efficiency ratio increased from 1.65 to 3.08 in chapati supplemented with 10 % defatted soy flour and to 3.07 in chapati with 10 % nonfat dried milk. Although such levels of soy supplement have been considered acceptable to consumers, Bass and Caul (1972) pointed out that taste panels can easily detect and characterize the flavour of soy in fortified chapati flours and breads.

Patel and Johnson (1975) and Patel et al. (1977) reported marked increases in contents of lysine, histidine, arginine, threonine and tyrosine in Moroccan flat breads supplemented with horsebean protein isolate. Up to 20% of horsebean isolate improved protein quality without changing aroma, flavour and texture quality. Hussein et al. (1974) supplemented balady bread with broad-bean protein. Finney et al. (1980a) reported that supplementing wheat flour with up to 20 % faba bean flour did not change sensory characteristics of bread. Shehata and Fryer (1970) determined that supplementing balady bread with 10 % chick-pea flour both increased nutritional value of bread and also organoleptic and physical properties. Dalby (1969) used cottonseed, chick-pea and dried yeast as protein supplements in bread. Chickpea has also been used in soups, salads and other main dishes (Ahmed et al., 1981). This seed contains about 17 - 26 % protein on dry weight basis, rich in lysine and other essential amino acids with the exception of methionine (Salwa et al., 1973). Gupta and Kawatra (1992) observed that supplementation of chapati flour with Bengal gram significantly improved calcium absorption. Acceptable balady bread was produced with 5 % supplementation of cottonseed flour by El-Sayed et al. (1978) and the effects of cottonseed flour on chapati quality were also examined by Archer (1970). Okezie and Dobo (1980) reported that up to 20 % defatted winged bean increased bread protein content by more than 78 %, and 15 % of winged bean flour produced acceptable bread (Blaise and Okezie, 1980).

1.2.2 Effect of baking on nutritional value of flat breads

There are conflicting reports on the influences of baking on protein quality. Eggum and Duggal (1977) reported a loss in net protein utilization value of chapati as a result of cooking, whereas Shyamala and Kennedy (1962) found the protein efficiency ratio of Indian chapati to increase during cooking. Khan and Eggum (1978) considered that incorporation of wheat, maize, millet, rice, barley, sorghum and triticale flour into unleavened Pakistani bread (roti) affected the nutritive quality slightly. Tsen et al. (1977) demonstrated that protein efficiency ratios of breads were significantly improved, and feed conversion ratios reduced, by replacing conventional oven baking with steaming. Baking lowered the protein efficiency ratio of five Iranian breads whereas the effects of fermentation were not significant. Effects of flour extraction rate, type and length of fermentation of dough (yeast-raised, sourdough and unleavened) and baking condition as relative bioavailabilities of magnesium (Faridi et al., 1983b), zinc (Faridi et al., 1983a) and iron (Ranhotra et al., 1981) were determined in studies with weanling rats. Bread did not improve the bioavailability of magnesium but improved the bioavailability of zinc and iron. El-Tinay et al. (1979) found no changes in threonine and lysine, and increases in tyrosine and methionine during fermentation of kisra. When the dough was fermented at pH 9.3, cooking had minor effects on the nutritional quality of kisra made with flours from various sorghum cultivars (Eggum et al., 1983). Tabekhia and Mohamed (1971) observed increases in riboflavin (14 %), nicotinic acid (13.6 %) and thiamine

(6.7 %) during the fermentation of balady dough. Bread was subsequently baked at 350 °C for 15 min, resulting in reductions in riboflavin (25.8 %), thiamine (24.8 %) and nicotinic acid (2.5 %). Toasting of the bread (120 - 130 °C) for 10 min after baking further reduced thiamine contents by 36 %, while riboflavin and nicotinic acid were not influenced.

Vaghefi and Delgosha (1975) studied fortification of Iranian sangak bread with vitamin A and found about 70 % of the added vitamin was recovered after baking. Eid and Bourisly (1986a, 1986b) fortified flour with thiamine, riboflavin, niacin and iron for the production of Iranian and Arabic breads. Losses caused by baking were minimal in enriched Arabic bread but pronounced in fortified Iranian bread. Maleki and Daghir (1967) reported significant losses of riboflavin in both white and brown Arabic breads, but negligible loss of niacin. Retention of riboflavin was higher in vitamin-rich samples than unenriched bread and added niacin retained completely. In Iranian village bread, studied by Hedayat et al. (1968), added niacin was stable but about 50 % of the added riboflavin and vitamin C and 80 % of vitamin A were destroyed. Enrichment of flour with iron can be a practical means to enhance intake of people in the Middle East. Nazar (1970) and Nazar and Hallab (1973) reported no adverse effects on dough characteristics and baking quality when up to 50 mg of iron was added per pound of flour, regardless of the iron salt used.

1.2.3 Advantages and disadvantages of leavened and unleavened breads

Faridi (1981) quoted several reasons for consumption of greater amounts of flat breads. Such products are good sources of dietary fibre because flours of high extraction rate were most often used. The formulation is simple and ingredients few, and such breads have lower caloric content on a dry basis than other breads. Thus would be perceived and desirable by people who are diet conscious. These bread are excellent food carriers either by incorporating foods such as meat or vegetables into the dough, or in the final product, food can be placed within the pocket-like pita bread or rolled with a single-layered flat bread such as chapati. Flat bread production offers certain advantages for the baking industry as ingredients are few, and costs and storage space reduced. Capital requirements are also relatively less than for pan bread. Moreover, there is less need for gluten strength and quality bread can be made both from medium soft and sometimes from durum wheat. As crust to crumb ratio is high, the bread tends to bake more uniformly, is more tasty, and is less crumbly than pan breads.

Flat bread contains high levels of fibre and phytate, which may reduce absorption of minerals within the intestine. Berlyne et al. (1973) noted osteomalacia in Israeli bedouins arising from intake of unleavened bread. Reinhold (1972) and Ter-Sarkissian et al. (1974) stated that daily phytate intake by adults is in the range from 2 to 5 g. Ismail-beigi et al. (1977) reported removal of phytate from village bread did not reduce the capacity to bind metal ions but concluded there were decreases in availability of dietary iron and zinc in whole wheat breads. Reinhold et al. (1976) found significant correlations between faecal dry matter and metal excretion. In a similar study in Pakistan, the diet of eight children with late rickets and two women with osteomalacia, was changed from chapati to bread made from white flour. After seven weeks levels of blood serum calcium, inorganic phosphorus and alkaline phosphorus had returned to normal (Ford et al., 1972). It is evident that if whole wheat unleavened flat breads supply more than 90 % of calories and protein, deficiencies in zinc, iron and calcium caused by fibre and phytate binding may result (Faridi, 1988). However, Dutta et al. (1980) concluded that high consumption of chapati may be an important factor in the prevention of folate deficiency in northern India and similar results were recorded in southern Iran by Russell et al. (1976).

Wheat bran has, however, proved effective in treatment of constipation and diverticular diseases. Whole wheat bread and larger particle size bran significantly increased human faecal weight (Wisker et al., 1985; Shetty and Kurpad, 1986). The cereal fibre (Leeds, 1985) retains water in the colon, forming a bulky, soft mass that is easily voided. This lowers pressure in the colon, lessens stress on the intestinal walls and alleviates problems such as diverticulitis, haemorrhoids (Stauffer, 1991), constipation and appendicitis (Ranhotra, 1981). Factors that increased effectiveness of wheat bran in increasing stool weight were coarse versus fine bran and raw versus cooked bran (Vahouny, 1985). Decreased intestinal transit time would be expected to decrease water absorption within the intestinal lumen, resulting in higher moisture in stools (Health and Welfare Canada, 1985; Anderson, 1985). Walker (1993) recorded the low occurrence of chronic bowl disease and diet-related cancers with high intakes of fibre in the daily diet. The role of dietary fibre in human nutrition and health has received much attention in recent years (Vohouny and Kritchevsky, 1982; Jenkins and Jenkins, 1984).

1.3 Wheat

Cereals have always been a staple food in the diet. The saving in energy and the increased productivity are great if cereals are consumed directly. The Sumerians, 4,000 years ago, accepted that "a given acreage of land used in the cultivation of wheat or barley filled stomachs, more quickly and more cheaply than the same land given over to livestock" (Tannahill, 1973).

Wheat, the most ancient of the cultivated cereals, is consumed throughout the world by about 5 billion people (FAO, 1985) and is a major source of nutrients. Wheat provides a larger amount of energy and protein than any other single cereal. Although primarily a source of carbohydrate, wheat also supplies protein, vitamins and minerals.

Annual world production of wheat exceeds that of any other grain, legume, or food crop (Harlan, 1976). In 1984, world production stood at 510 million (FAO, 1985), increased to 546 million metric tonnes in 1992 (Bureau of Census, 1993), to which Pakistan contributed 15.68 million in 1992 and expects to produce 16.4 million in 1993 (Government of Pakistan, 1993).

1.3.1 Wheat protein

The amount and type of protein is important for the functional uses of flour. Protein content is probably the most important factor in bread flour quality.

Wheat holds a special position among the cereal grains because of its ability to form dough, imparted by the gluten fraction. This gluten enables a leavened dough to rise by forming a structure in which minute cells retain carbon dioxide produced during the fermentation. The cohesive nature of gluten has peculiar extensible properties, in that it can be stretched and possesses a certain degree of recoil or spring (Kent, 1975). Bread is thus fundamentally foamed gluten (Atkins, 1971). In baking, the key properties of gluten are the high water absorption (two to three times its own weight) and ability to form viscoelastic films that contribute to dough handling properties and improved loaf characteristics (Bushuk, 1985).

Gluten is relatively easy to isolate in relatively pure form through being insoluble in water. Starch and soluble proteins can be removed from gluten by gently working a dough under a steady stream of water as used by Beccari in 1728 (Baily, 1941; Beach, 1961). The product contains (dry wt. basis) about 80 % protein and 8 % lipids the remainder being ash and carbohydrate (Simmonds and Wrigley, 1972; Frazier, 1983).

Wheat storage proteins are conventionally separated into four classes according to solubility (Osborne, 1907). Albumins are proteins soluble in water with a solubility not affected by reasonable salt concentrations. Globulins are soluble only in dilute salt solutions and are insoluble at higher salt concentrations. Prolamins are soluble in 70 % ethanol. Glutenins are only soluble in dilute acids and bases.

The Osborne extraction procedure (Osborne, 1924) has been refined in attempts to improve the definition of protein fractions (Mosse and Baudet, 1963; Bushuk, 1985). Byers et al. (1983) reviewed origin of variations, showing effects of temperature, solvent composition, flour particle size, sample to solvent ratio, the number of extractions with each solvent, the time of extraction and intensity of mixing. A range of solvents has been applied to the fractionation of gluten with varying degrees of success. These include increasing concentrations of urea (Pomeranz, 1965a, 1965b; Lee and MacRitchie, 1971), SDS (Graveland et al., 1979; Bottomley et al., 1982) and other detergents (Kobrehel, 1984).

The gluten complex is composed primarily of gliadin and glutenin proteins (Kasarda et al., 1976). The gliadin fraction consists mainly of monomeric proteins, associated by non-covalent hydrogen bonds and hydrophobic interactions, but also contains some aggregated (polymeric) protein, related structurally to certain glutenin subunits (Beitz et al., 1973). Glutenins appear to consist only of polypeptide subunits, aggregated into high molecular weight polymers by covalent disulphide bonds (Miflin et al., 1983). The insolubility of the glutenins is determined, to an extent, by their high molecular weight. Gliadins, with an average molecular weight of about 40,000, are extremely sticky when hydrated. Such proteins have little or no resistance to extension and appear to be responsible for the cohesiveness of doughs. The glutenin fraction appears to be a heterogenous group of proteins, varying in molecular weight from 100,000 to several million (Average three million). The glutenin fraction appears to provide resistance to extension in dough. Denaturing sodium dodecyl sulfate electrophoresis can be performed after disulfide bonds have been reduced with a reagent such as mercaptoethanol (Danno et al., 1974). Reduced glutenin migrates at a rate related to molecular weight estimated as <16,000 to about 133,000 (Hoseney, 1986).

The relative proportion of gluten increases as protein content increases. Amino acids characteristic of gluten thus increase with grain protein content (Mosse et al., 1985). Bell and Simmonds (1963) reported that as protein increased, the proportion of pyrophosphate soluble proteins decreased and gluten protein content increased. Tanaka and Bushuk (1972) and Orth and Bushuk (1972) have reported varietal variations in the various protein classes. Green and Kasarda (1971) suggested that gluten has a relatively large number of nonpolar side chains which impart hydrophobicity and contribute cohesive properties through hydrophobic bonding.

When fractionated by SDS gel electrophoresis, glutenin polymers separate into about 20 different subunits that fall into two distinct groups; high molecular weight (HMW) and low molecular weight (LMW) subunits (Huebner and Wall, 1976). Fullington et al. (1980) extracted total proteins from flour for quantification purposes using solutions containing SDS and mercaptoethanol. Fractionation was on the basis of the molecular weights of reduced, dissociated polypeptide chains by SDS-PAGE electrophoresis.

To understand dough characteristics the features of dough rheology such as dough strength, extensibility, peak time, and the nature of breakdown and stickiness must be related to the behaviour of proteins in flours.

Several studies (Belitz et al., 1987; Autran et al., 1987; MacRitchie et al., 1990, Shewry et al., 1992; Wrigley, 1993; Wrigley, 1994) have indicated the importance of the balance between the aggregating glutenin and monomeric gliadin polypeptides in rheology. The rheological characteristics of flour are varied between varieties. It is important to determine how the diversity of proteins influences the rheological and qualitative characteristics of flours for chapati production.

The variation in the total amount of glutenin has been related to breadmaking quality (Schofield and Booth, 1983). Moreover differences in the ratio of HMW and LMW subunits (Huebner and Bietz, 1985) and the quantities of groups of subunits in the glutenin fraction (Sutton et al., 1989) have also been related to variation in breadmaking quality. Various attempts have also been made to establish relationships between rheological properties of flour and content of HMW glutenin subunits (Branlard and Dardevet, 1985a; Manley et al.,1992; Gupta et al., 1992). This has demanded more studies of the influence of protein profiles on rheological properties of doughs generally using SDS-PAGE electrophoresis (Sathe et al., 1987).

1.3.2 Polysaccharides and other components

The human diet contains two broad classes of plant polysaccharide, conveniently referred to as starch and non-starch polysaccharides (FAO, 1984). Starch, the storage carbohydrate in cereals, is an α-linked glucan that forms the major source of carbohydrate. In addition to nutritional value, this polymer has important effects upon the physical properties of foods. The gelling of puddings, thickening of gravies and the setting of cakes and the body of bread are all strongly influenced by the properties of starches. In cereal grains, starch granules are embedded in a protein matrix.

Starch content of wheat ranges from 63 to 85.3 % (Cerning and Guilbot, 1974; Pomeranz and MacMaster, 1968) and is thought to be inversely related to protein content (Hopkins and Graham, 1935). Soft wheat varieties in general have a higher content of starch (69 %) than hard wheat varieties (64 %) (Miller, 1974). European wheats tend to have higher mean starch (62 - 75 %) and lower protein contents than American varieties (Cerning and Guilbot, 1974).

Starch granules consist of two main components: amylose, an essentially linear chain α-(1-4) glucose polymer containing up to 5000 glucose units and amylopectin, a branched structure of α-(1-4) glucose chains linked together by α-(1-6) branch points. Amylopectin molecules have been reported to contain up to 10,00,000 glucose units and 18 - 28 glucose units in each unit-chain. Amylose content of starches is from 25 - 27 % in common cereal genotypes but in 'waxy' types of barley, maize, rice and sorghum, the starch is composed almost entirely of amylopectin. Certain other genotypes may contain up to 40 % amylose. The properties of both starches and their components depend very markedly on genotype (Kent, 1983).

Starch granules are insoluble in cold water, but when heated in the presence of water, the polymers hydrate. The granule swells and bursts: a phenomenon called gelatinization. Amylose and amylopectin have close associations, possibly through hydrogen bonding and in gelatinization the association of starch polymers is disrupted by heating in aqueous solution or with other hydrogen-bond breaking solvents. The branched nature of amylopectin provides greater solubility and solution stability than with linear amylose. Separations of starch components have been based on ability to leach amylose from the granule. Aqueous solutions can utilise the complex-forming ability of amylose when amylopectin and amylose have been completely dispersed i.e gelatinization. Both amylose and amylopectin behaviours play important roles in the swelling of starch although swelling is primarily a property of the amylopectin (Pomeranz, 1988).

Since starch provides many of the textural characteristics of food, changes during food processing and preparation are important. Common food ingredients influencing starch behaviour are sugars, salt and lipids (Osman, 1975). The role of starch in baked products has been characterized as that of a "temperature-triggered water sink" (Hoseney et al., 1978). As temperature increases and starch starts to gelatinize, the polymeric components compete with other ingredients for available water. The extent of pasting depends on the availability of water to the starch granules. Derby et al. (1975) demonstrated that swelling of granules was controlled both by temperature and available water and presence of other ingredients. Sucrose was observed to delay gelatinization (D'Appolonia, 1972; Hoseney et al., 1977; Abboud and Hoseney, 1984). Differences in availability of water appeared to relate to starch behaviour. In bread crumb, the protein matrix is considered to hold the crumb together (Pomeranz and Meyer, 1984). Starch has also been found to play a major role in the structure of wafer sheets (Stevens, 1976). Yasunaga et al. (1968) compared hot pasting abilities of flours of 68 wheats of U.S. origin in breads using a Brabender Amylograph. In the absence of amylase activity, flours from soft and club wheats had poor pasting abilities and a marked seasonal effect was observed. Variation was also observed due to differences in protein and to an extent damaged starch (Meredith and Pomeranz, 1982). Alsberg (1927) considered starch contributes to breadmaking because it affects water absorption, dough consistency and viscosity, whereas Sandstedt (1961) suggested starch has the following functions in breadmaking: dilution of gluten to an optimum level, provision of sugars for fermentation, strong adhesion with gluten, the creation of flexibility during gelatinization in conditions of limited water, allowing the gas-cell film to stretch and absorb water from gluten by gelatinization. This causes the film to set and becoming rigid ensuring a bread structure permeable to gas so that the bread does not collapse on cooling.

It is generally accepted that bread from "strong" flours has better keeping quality than that from "weak" flours, indicating both quantity and quality of flour proteins are involved in bread staling. However, extensive research has shown that changes in the starch fraction are responsible for certain major changes in bread during staling. In retrogradation, an important property of starch gels crystalline regions in the gel are formed by aggregating amylose molecules. Amylose molecules have a strong tendency to associate through the formation of hydrogen bonds with adjacent amylose chains as solutions cool: this is manifested in the formation of precipitates (D'Appolonia et al., 1971; Dengate, 1984). In an earlier study, Schoch (1942) suggested that amylose was primarily responsible for gelation and may form a gel at concentrations as low as 1.5 %. Lindqvist (1979), in a study of cold gelation of wheat starch, proved that this occurs only if the amylose is first leached from the starch granules. Starch recrystallization and firming of the grain were accelerated by lowering storage temperature (4 °C) and at this temperature 60 % of the changes through retrogradation took place during the initial 12 hr (Jankowski and Rha, 1986).

Significant differences in mineral content by class of wheat have been found and related to environmental factors (Davis et al., 1984; Dikeman et al., 1982; Koivistoinen et al., 1974). Sandstrom (1987) reported that use of unrefined cereals can significantly increase dietary mineral intake in countries where cereals account for less than 50 % of total energy and protein intake. Generally wheat has mineral content of 1.8 %, distributed through the different parts of the grain (MacMaster et al., 1971). The bulk (60 %) of wheat minerals are found in the aleurone. Rate of grain extraction therefore has a great influence on the concentration of minerals in the flour. At 70 - 72 % extraction rate only 30 - 40 % of mineral content remains (Schroeder, 1971). The aleurone layer also contains high levels of thiamine and riboflavin (Somers et al., 1945) and 80 % of total kernel niacin (Heathcote et al., 1952).

Wheat has a relatively high content of thiamine and niacin compared to other cereal grains but is relatively low in riboflavin and devoid of vitamin A. This cereal also contains both pyridoxine and tocopherol (Hoseney, 1986). Variations in vitamin content in flour can relate to differences in varietal and geographic origins. The highest concentration of niacin, 740 mg kg^{-1}, was recorded in the aleurone tissue. The pyridoxine and pantothenic acid is also most abundant in this tissue, although a significant portion of these vitamins is in the embryo and scutulum.

1.3.3 Wheat classification

Four species in the genus *Triticum* are commercially recognized: *T.monococcum* (diploid), *T. turgidum* (tetraploid), *T. timopheevi* (tetraploid) and *T.aestivum* (hexaploid) . According to Feldman and Sears (1981), *T. monococcum* and *T. timopheevi* now enjoy very limited cultivation. Intensive cultivation and breeding programs have resulted in tens of thousands of commercial varieties of bread and durum wheats. This strain improvement process has led to both enhancements in yield and development of wheats with enhanced milling and flour-processing qualities of value to bakers and millers.

For commercial purposes, wheats are classified by properties other than botanical features. In practice, individual varieties differ in milling quality, dough characteristics and breadmaking quality. Factors used to differentiate wheats are hardness or softness of the grain, winter or spring, red or white colour and protein content i.e. strong or weak. Wheat quality properties are also influenced by genetic and environmental factors.

Commercial grades around the world can be compared with respect to physical and chemical properties so that the most suitable wheat for any use can be stipulated. Wheat can be closely matched to different uses according to grain hardness and protein content (Fig. 1.4). Durum wheats are preferred for pasta; soft wheats for biscuits, cakes and pastries, hard grained bread wheat for noodles, flat breads, pan bread and other products. Requirements also vary and much effort has been directed to determine the relationships between agronomy and suitability for individual products. Research into noodles, flat breads, chapati and other products has become important to wheat-exporting countries such as the United States, Canada and Australia, although domestic use in such countries is dominated by pan bread products (Orth and Shellenberger, 1988).

Various types of wheat are used for the preparation of flat bread (Williams and Blaschuk, 1980; Bakhshi et al., 1979). Austin (1980) attempted to classify Indian wheat varieties on the basis of suitability for various bakery products, including flat breads. In general, whiteness is desirable in breads and thus white are preferred over red wheats. In research to establish the suitability of U.S. and Australian wheats for the production of flat breads, Mousa et al. (1979) studied the influences of US wheat classes, flour extractions, and baking methods on the quality of balady bread. Faridi et al. (1981) and Faridi and Rubenthaler (1983b, 1984a) tested a number of U.S.-grown wheats for the production of Iranian and North African flat breads and Chinese steam bread, and found soft wheats to be very suitable. Finney et al. (1980b) suggested that soft white wheats produced in the Pacific Northwest are suitable for Egyptian, Moroccan, Iranian, Indian and Pakistani breads.

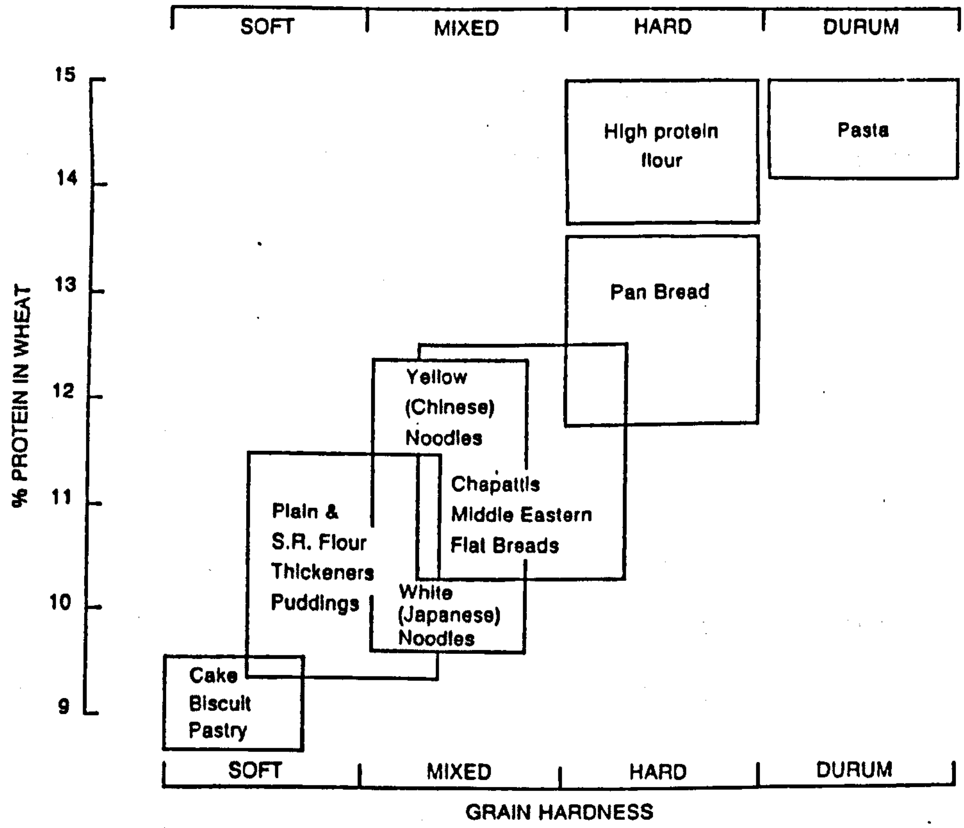

Fig. 1.4. Suitability of wheat for various products. Source : Moss, 1973

1.3.4 Extraction rate of flour

In general, flour with 80 % extraction rates are preferred for the production of flat breads, although 90 % extractions are also utilized in certain locations, notably in rural areas of the Middle East, India and Pakistan (Haq and Chaudhry, 1976; Shurpalekar et al., 1976; Majeed et al., 1987). In Pakistan, chapaties are prepared from whole atta, at 100 % extraction rate (Kent, 1983). Naan is prepared from flour of lower extraction, e.g., patent (Dutta et al., 1980) and 75 % extraction white flour (Chaudhri and Muller, 1976). In certain areas of Pakistan and Afghanistan, whole wheat naan is also consumed (Rashid, 1983). Protein content decreases from 14.2 % for 100 % extraction to 12.7 % for 66 % extraction flour, reflecting the removal of germ and aleurone as bran, both relatively rich in protein (Pedersen and Eggum, 1983). Chapati flour milled in the UK for the use of Asian immigrants is a granular fine wheatmeal of about 85 % extraction rate, made by blending with fine offal or bran (Kent, 1983) and 100 % extraction rate or brown flour. Qarooni et al. (1993) used 85 % extraction rate flour milled from US hard white winter, hard red winter and soft white wheats for the production of tannouri bread. Optimum quality in tannouri bread was produced from flour containing 11 - 13 % protein.

1.4 Quality in unleavened bread

Acceptability of bread can be related to a number of factors such as behaviour during preparation, rheological characteristics, and sensory qualities.

1.4.1 Technological studies

As the proofing time in flat breads production is short, baking procedures vary. Certain can be baked in regular ovens like pan breads, but others require higher temperatures in the range of 350 - 550 °C, for optimal quality. Certain flat breads like chapati are baked over a hot plate. High oven temperatures reduce baking time and lead to increases in moisture content and softness of the bread.

Rao et al. (1986b) studied the effect of baking temperature on chapati quality. Baking at a low temperature of 150 °C increased baking time and resulted in a chapati with undesirable grayish spots. The range 205 - 232 °C was considered optimal. Puffing time was found to be critical, as even a slight variation greatly influenced chapati quality, particularly with respect to colour and pliability. Faridi and Rubenthaler (1984b) reported that white and whole wheat breads baked at 480 °C for 90 sec, were significantly better in quality than those baked at 260 °C for 4-6 min. Longer baking times and the lower temperatures yield rough and dry crumbs. Breads baked at higher temperatures for less time had more desirable crusts. Quail et al. (1990) found that thinner doughs baked at higher temperatures for shorter times produced improvements in quality in Arabic bread. El-Samahy and Tsen (1981) reported that baking at increasing temperatures from 288 to 343 °C for 4 min reduced loaf weight and significantly darkened the top and bottom crust of balady breads. El-Samahy and Tsen (1983) measured heat penetration inside balady during baking. Internal temperature of the loaf increased linearly with baking time with the central portion reaching a maximum (99 °C) after 1.5 min in a 343 °C oven. Increasing dough weight from 125 to 225 g did not have a significant effect on heat penetration to the central portion.

1.4.2 Study of quality factors

Defining quality factors in a product can be a challenge. Sensory evaluation of foods and food products is one of the more important aspects of quality control. The two primary sensory characteristics of flat breads are considered to be colour and texture. These have been measured by a number of laboratory instruments, notably the Instron Universal Testing Machine (Ebeler and Walker, 1983). Kannur et al. (1973) quantified the colour of chapati using a colorimetric method. Texture has been investigated using a number of Instron tests and significant correlations between panel sensory scores, hardness and yield point measurement were reported for flat bread samples kept at ambient for several hours (Abrol, 1972; Rashid and Hawthorn, 1974). Faridi and Rubenthaler (1983b) evaluated textural properties of Tunisian and Moroccan breads using a Fudoh Rheometer fitted with a disc 2.5 cm in diameter and automatic-stop accessory adjusted to 3 mm penetration of bread crumb. Khan et al. (1972) used the precision penetrometer for determining the softness of chapati and Rao et al. (1986b) a Warner-Bratzler shear press and a device called a pliability tester to quantify textural changes in chapati during storage.

Mousa et al. (1979) used a sensory panel for measuring textural characteristics of Egyptian balady bread, scoring crust colour, crust character, crumb colour, grain and texture, flavour and taste and chewiness. Mann (1970) studied the shelf life of unleavened and leavened flat breads evaluating colour, aroma, texture, taste and overall acceptability. Chaudhry (1968) studied the suitability of US wheats for chapati making, evaluating quality on the basis of colour, flavour, texture and general acceptability. White club and Pakistani wheats were found to be superior to red wheats with colour being found the major factor lowering the grade of the samples. Dhingra et al. (1992) reported significant positive correlations between grain hardness and chapati texture. Amylose had a significant positive correlation with overall acceptability of chapati. Yasin et al. (1965) used a different set of criteria for chapati quality including stiffness, staleness, brittleness and pliability. Faridi and Rubenthaler (1984b) adopted a visual scoring system for evaluation of US pita bread (Table 1.2). Scoring depended on the following characteristics: complete separation into the upper and lower crusts of similar thickness; soft, white and moist crumb; light and shiny crust with brown spots; and pocket formation.

Table 1.2 : Scoring factors for pita bread quality

Factor	Score
Crust colour	10
Crumb colour	5
Upper to lower crumb ratio	20
Pocket formation	20
Crumb texture	10
Bread score(overall)	65

Source: Faridi and Rubenthaler (1984b)

The scoring system of Williams et al. (1988a) was based on dough-handling properties at the dividing and sheeting stage, crumb colour and texture, bite, flavour and regularity in the final product appearance. Majeed et al. (1987) reported evaluations of quality of roti prepared at a roti plant at 4, 12, 20, 28 and 36 hours intervals after production for appearance, breaking properties, sponginess, folding ability, softness and flavour.

Chapaties prepared from triticale flour were improved in quality by blending 60 % wheat flour in triticale flour. Further improvement was recorded by using a blend of 40 % wheat, 40 % triticale and 20 % bengal gram flour (Kent, 1983). Triticale chapaties were darker in colour and tougher than wheat chapati. Baking qualities were improved by addition of 75 % wheat to triticale (Akmal and Javed, 1987). Ullah et al. (1986) reported that acceptable chapaties with improved protein quality could be produced by blending triticale in the ratio of 1:1 or 1:3 with barley, rice and wheat atta. Flexibility and palatability of chapaties were satisfactory but were unacceptable in breads prepared from triticale alone.

Sidhu et al. (1990) reported that replacement of whole wheat flour with up to 25 % barley flour did not adversely affect chapati quality. Sood et al. (1992) described colour, appearance, texture and puffing of chapaties as good, but the flavour score was slightly reduced when up to 30 % hull-less barley flour was incorporated. Chewability of chapaties was acceptable when up to 40 % of barley flour was added in the blend. Anjum et al. (1991) prepared acceptable chapati with 2 % barley in wheat flour.

Shehata and Fryer (1970) determined that supplementation of balady bread with 10 % chick-pea flour improved both organoleptic and physical properties and bread with up to 20 %, protein quality improved without alteration of aroma, flavour and textural quality (Patel et al., 1977). Similar results were obtained for fortifying white Arabic bread (Hallab et al., 1974). However, Luzfernandez and Berry (1989) reported that although most sensory characteristics of fortified breads did not differ significantly, loaves fortified with 10 % germinated chickpea flour did not compare favourably with controls. Jain (1988) observed that sensory qualities such as appearance, texture, taste and flavour of chapati were not reduced by up to 20 % substitution of soy in wheat flour.

Malwinder et al. (1988) prepared chapati from wheat flour supplemented by musk melon seed flour at 0, 20, 25 and 30 % levels and reported from sensory evaluation tests, that up to 20 % gave acceptable chapati.

Knight and Hanson (1986) reported that the dried yeast protein could be successfully incorporated into spaghetti at a 15 % substitution for flour. When served with tomato sauce to a taste panel no difference was detected, although a good 'bite' was reported.

1.4.3 Rheological studies

Rheometry yields an evaluation of the important functional properties of flours. Viscosity, elasticity and plasticity are amongst the most important properties in raw materials, and relate to behaviour during processing and end product quality (Muller, 1973; Bloksma, 1990). Two recording mixers, the farinograph and the mixograph, have been used extensively to provide information on the behaviour of doughs during mixing and kneading. Rehman et al. (1988) determined the effect of various improving agents (potassium bromate, calcium peroxide and non-fat dry milk), added at various levels to flour of different extraction rates (60 % and 75 %), on physical dough characteristics. Flour at 75 % extraction exhibited more water absorption, greater dough stability and softening as compared with that at 60 % extraction. Addition of calcium peroxide and non-fat dried milk increased water absorption capacity at both extraction rates. Potassium bromate had no effect on absorption level (water absorption is the amount of water needed to centre the farinograph curve on the 500 BU (Brabender Units) line for a flour-water dough) (Mahmood et al., 1989), but dough development time, stability and softening were slightly decreased, notably in the 75 % extracted flour. Information derived from a typical farinogram is shown in Figure 1.5. Rheological characteristics of the composite flours of wheat and germinated chickpea flours were evaluated through Brabender farinograph and viscoamylograph by Luzfernandez and Berry (1989). Dough development (dough development or peak time is the time to the nearest half-minute from the first addition of the water to the development of the maximum consistency of the dough, maximum mobility, immediately before the first indication of weakening) and stability times decreased and amylograph peak viscosities increased for all fortified flours. Water absorption was decreased in chickpea (CP) and increased in germinated chickpea (GCP) flours, relative to the control, except for 10 % composite flours. Increases in water absorption were reported when wheat was supplemented with navy bean lupin flour (Campos and El-Dash, 1978; Sathe et al., 1981). On the other hand, Finney et al. (1980a) found decreases in water absorption, as quantified by the mixograph, when wheat was supplemented with germinated and ungerminated faba bean flours. This suggested that water absorption may be related to protein type rather than quantity (Deshpande et al., 1983). The results vary depending on the legume used. Okezie and Dobo (1980) reported that farinograph absorption, arrival time (time required for the top of the curve to reach the 500 line after the mixer has been started and the water added) and dough development time of winged bean composite flours gradually increased in relation to the proportion of defatted winged bean to wheat flour. When dry roasted navy and pinto bean cotyledon flours were blended into whole wheat flour, increases in water absorption, arrival time and dough development times were observed while stability decreased (Silaula et al., 1989).

Rao et al. (1987) measured the consistency of chapati dough using the Henry Simon Research Water Absorption Meter. This instrument was shown to give more sensitive resolution between consistencies of dough samples that the Brabender Farinograph or General Foods Texture-meter at differing levels of water absorption. Variation in flour particle size, protein and damaged starch contents were found to account for 88 % of the variability in dough consistency in several wheat flours.

Two load-extension instruments, the alveograph and extensograph, are used to determine the behaviour of dough and its gas retention capacity during fermentation, proofing and baking. Such instruments measure the resistance and extensibility of a uniformly moulded piece of dough, which relate to baking properties of the end product. Such information has been employed into classify wheats into different quality types with respect to usage

(Weipert, 1991; Locken et al., 1972).

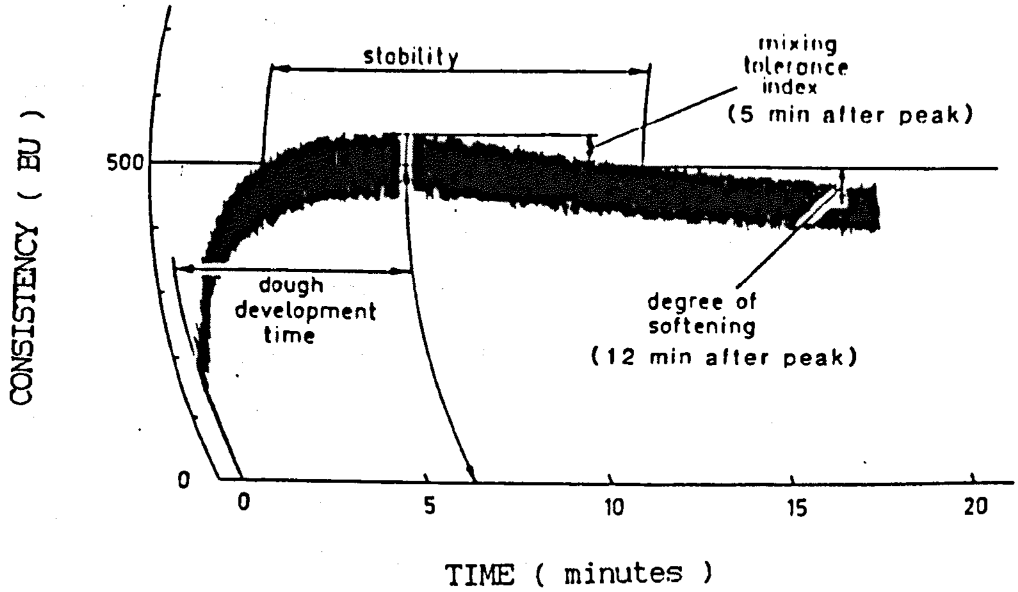

Fig. 1.5. Representative farinogram showing some common measured indices. Source: Pomeranz, 1988.

Qarooni et al. (1987b) tested 33 Australian wheat samples for suitability for the production of Arabic bread and reported that the best quality bread was made from white flour produced from hard wheat, of intermediate dough strength, with flour protein in the range of 10 - 12 % and starch damage 6 %. The suitability of hard wheat flours was attributed to high water absorption capacity. Qarooni et al. (1987a) adjusted the absorption of Arabic bread dough to a farinograph consistency of 850 BU. and concluded that if the dough consistency was above 850 BU., through addition of less water, dough sheeting quality was improved. The resulting loaves were dark in crust colour and tended to break during rolling and folding. The crumb was dense and became denser as water content was decreased. On the other hand, at high water contents, sticky doughs with inferior sheeting quality were produced. The resultant breads were asymmetric, light in crust colour and showed blistering of the surface. The crumb also had a woollier appearance, with large air cells, as water absorption increased. However, Faridi and Rubenthalar (1984b) observed the effect of various levels of water absorption (optimum for white 57 % and for whole wheat flour 62.5 % plus 10 %) on the physical quality of white and whole wheat pita breads. The quality of both breads improved at higher water absorptions, probably due to increased generation of steam during baking of bread that produced finer and more uniform crumbs and crusts. Unlike in Western pan breads, in which the optimum water absorption is 60 - 65 %, flat breads have optima ranging from very low (38 %), as in matzo, to very high (85 %), as in Iranian sangak (Faridi and Rubenthaler, 1983c).

Flour strength and protein content are important factors in production of flat breads. Traditionally, flat-bread production in North Africa, the Middle East, South Asia and Northern Europe was adapted to the strength of the local wheats, typically soft or durum. Therefore, use of high protein, strong US and Canadian wheats may cause problems in small scale production of flat breads. Balady dough, for example, must be overmixed to degrade the gluten network for optimum product quality. Thus protein quantity and quality are primary factors in measuring flour potential in relation to use for bread preparation. Increases in water absorption and loaf volume and decreases in mixing time are associated with higher flour protein contents (Sattar et al., 1986).

Ibrahim et al. (1983) examined the effect of flour particle size on the quality of Egyptian balady bread. Flour with the largest particle size had the lowest protein content and produced a loaf of relatively poor volume and appearance, whilst small particles produced bread of increased volume with good grain and texture and excellent colour.

Flat breads are to a large extent tolerant of sprout-damage in wheat that may result from late-season rainfalls. Flat breads made from flour milled from field-sprouted wheat were, in seven of nine test breads, judged to be equal to control breads (Finney et al., 1980b). Linko et al. (1984) prepared crisp flat breads by direct continuous extrusion cooking from mixtures of wheat and rye flours (1:1) of normal and high amylase activity (falling numbers 183 and 61, respectively) and obtained no significant differences between products obtained from flours with normal and high-amylase activities. Orth and Moss (1977) stated that with a reasonable protein content and a falling number (time in seconds for a viscometer to drop down in the gelatinized suspension is a measure for the α-amylase activity of flour and grain) over 120, chapati of adequate quality could be produced from a flour of 80 - 85 % extraction, typical of those used in India. However, variability in water absorption related to degree of sprout-damage may cause manufacturing difficulties. Production of Middle Eastern pocket bread was reported to be more difficult from flour with sprout-damage. The appearance of blisters, poor crumb colour, surface cracks, and also difficulties in rolling and folding were apparent to varying degrees. The samples with the highest protein content tended to be better than those of a lower protein but were still inferior to controls. Leelavathi and Haridas (1988) reported that germinated wheat flour gave lower yields of chapaties, due to decreases in water absorption in preparing doughs of optimum consistency. Chapaties prepared from germinated wheat flour were less pliable and had dark, charred colour spots. Eating quality was reduced due to harder texture, and bread had a sweetish taste. After storage, the germinated wheat chapati had improved eating quality and taste compared with the dried, more brittle control.

1.5 Yeast protein enhances flavour and nutrition

Yeast extracts (*Saccharomyces cerevisiae*) have been widely used in different aspects of food as both flavouring and flavour enhancers, condiments and for nutritional fortification. Flavour profiles are largely determined by yeast strain, autolysis conditions, equipment and processing. Yeast extracts are categorised as inactive yeast products. The term "yeast extracts" is sometimes used as a synonym for yeast autolysates (Reed and Peppler, 1973; Prescott and Dun, 1982).

According to the International Hydrolysed Protein Council (1977), autolysed yeast extracts are food ingredients used as natural food flavours composed primarily of:

1). Amino acids, peptides and polypeptides resulting from the enzymatic splitting of peptide bonds due to naturally occurring enzymes present in edible yeast.

2). The water soluble components of the yeast cell. Food grade salt may be added during processing (Prescott and Dun, 1982).

3). Maillard browning products from heat-induced reactions between sugars and amino acids.

Autolysed yeast with insoluble cell walls removed is vacuum-evaporated into a paste like, translucent, water-soluble product or spray-dried into powder termed autolysed yeast extract (Peppler, 1982; Przybyia, 1986).

Yeast extracts and autolysates are approved for use as flavourings and flavour enhancers by the Food and Drug Administration. Yeast products are used as condiments in the preparation of meat products: meat pie fillings, hot dogs, sausages and hams. This provides a means of not only enhancing flavour profiles but also of reducing the usage of more expensive meat extracts (Peppler, 1982). In powder form, such extracts are effective bases for preparing savoury flavours. Yeast products can also be used to increase the savoury nature of spices, onion, hams, cheese and to generate ranges of popular savoury flavourings (Lyall, 1970).

In bakery products, autolysed baker's yeast is a natural complement (Lyall, 1970). When added to an extruded snack formulation, especially those with cheesy flavour, flavour is changed or enhanced. A naturalness is also given to the product which strengthens the image of "wholesomeness" (Hough and Maddox, 1970). Soups, bouillons, sauces and gravies develop enhanced savoury characteristics following the addition of autolysates and extracts (Dziezak, 1987b). Yeast products serve also as ingredients of soups made for dietetic purposes, are of vegetable origin and therefore acceptable to varied ethnic groups (Binsted and Devey, 1970). Paste-concentrated yeast autolysates are used in Australia as a savoury bread spread (Reed and Peppler, 1973). Peppler (1982) suggested that by combining yeast extracts and autolysates with hydrolysed plant proteins it is possible to produce some truly unique flavour extracts, fortified with mono-sodium glutamate (MSG) and 5'-nucleotides to enhance other flavours.

Many other yeasts derivatives are available for functions other than flavour enhancement and nutritional fortifications. For example, yeast products impart unique texturising effects, serving as stabilizers and thickeners as well as obviating the need for addition of MSG (Dziezak, 1987a).

The protein in *S. cerevisiae* contains high levels of all the essential amino acids with the exception of methionine. Yeast contains protein (48 %), carbohydrates (36 %), ash (8 %), fat (1 %) and water (7 %). Yeasts are also excellent sources of the water soluble B-complex vitamins, including B1, B2 and nicotinic acid (Table 1.3)(Dziezak, 1987a).

Table 1.3: Comparison of vitamin B contents in dried yeast and other foods.

Food item	Vitamin (mg per 100 g)		
	B1	B2	Nicotinic acid
Dried Brewers yeast	18.4	3.68	53.4
Dried Torula yeast	15.0	-	-
Wholemeal Bread	0.3	0.1	2.6
Beef	0.1	0.2	-
Eggs	0.1	0.35	-
Cheese(Cheddar)	0.01	0.5	0.1
Milk(Liquid)	0.04	0.15	0.1
Tea(Dry)	-	0.9	-
Chicken	-	-	5.0
Kidney	-	-	7.0

- Data Not Available
Source: Dziezak, 1987a.

1.6 Consumer preference in relation to savoury flavour

Nutrition is not the primary factor that determines food consumption patterns. Other factors relate to choice and include cultural, gender, age, and quality parameters, preference and food price. Current information on the production, consumption and composition of food products indicates that the nutritional standard of about two-thirds of the world population is inadequate (Abbey, 1983).

Asian savoury products including snacks are widely available in the market. These are popular among ethnic communities and also UK consumers. Such products as pakora, samosa, shami kebab and Turkish kebab are widely available in the UK either from take-away retail and supermarket outlets. Variation in flavour preference can be related to various factors include flavour intensity, age, gender and socio-economic category of consumers. However, differences in flavour character have also been noted between countries. Spicy and Asian flavours have a longer history in Australia and New Zealand because of the ethnic mix and proximity to Asia (Slavin, 1990). Moreover, many consumers have preconceived ideas and perceptions of both products and flavours. Beliefs and prejudices may prevent consumers from eating nutritionally beneficial foods (Afors, 1992).

Almost three million people from ethnic groups in the UK provide both importers and retailers with an opportunity to develop new markets and products. Consumers may wish to eat frequently products that relate to their own culture (Henderson, 1992; Wrigley, 1993). Thus groups can be distinguished on the basis of food choice. There may be an impact on the majority cultural group through ethnic restaurants, speciality food shops and supermarket product development. To ensure success of new products, it is essential to understand the response of consumers. If not, nutritious foods will be rejected (Afors, 1992).

The soy sauce fermentation can be considered as a progenitor of savoury both flavours and the flavour industry. Other important developments include production of savoury flavouring ingredients such as acid hydrolysed vegetable protein from soybean, wheat and yeasts. Such processes generate compound flavourings and natural extracts (Ramsey, 1992). Investigations of autolysis of spent brewer's yeast in England and Germany in the current century led to the development of yeast extracts. Savoury flavours are still a growth area and yeast extracts have always been considered a versatile product (Maase, 1991). Various yeast extracts can be used as alternative natural flavour enhancers to monosodium glutamate and hydrolysed proteins. Applications include food products such as soups, sauces, gravies, canned meat, snacks, poultry and seafoods (Schoenberg, 1992; Labell, 1992; Anon., 1991a).

In 1908, monosodium glutamate (MSG) was reported to contribute palatability with an effect on the four basic tastes (sweet, sour, salty and bitter). The characteristic taste perceived from the presence of MSG was called *umami* (Ikeda, 1909). Subsequently other substances were recognised to have similar effects on taste in foods. Kodama (1913) described *umami* character in inosine-5-monophosphate (IMP). Kuninaka (1960) found a further *umami* compound, guanosine-5-monophosphate (GMP), with properties confirmed later by Kuchiba et al.(1991). These compounds are now widely added to enhance savoury flavours (Land, 1994).

Yeast extracts are commercially available as powders or pastes and are rich in both flavour peptides and amino acids. Such ingredients have been used extensively in food products (Duxbury, 1992). A major consideration is cost effectiveness in relation to other flavouring agents on the basis of equivalent flavour intensity (Reed and Nagodawithana, 1991). Moreover, there is no documentary evidence from either animals or humans indicating that yeast extracts cause reactions similar to those described for MSG. This implies that the glutamic acid in yeast extract is present predominantly in peptides and is thus incapable of eliciting adverse reactions (Nagodawithana, 1992). In addition to impart savouriness, yeast extracts provide nutrients not present in staple foods (Perkin, 1992). It has been forecast that the savoury flavour sector will reach 40 % of the total flavour market by 1995 and become increasingly fragmented in line with consumer trends (Anon, 1991b).

1.7 Product optimisation and relationship between data sets

1.7.1 Response surface

Response surface methodology (RSM) uses quantitative data to determine and simultaneously solve multivariate equations that specify the optimum product for a specified set of factors through mathematical models. Such models consider interactions among the test factors and can be used to determine how products change in relation to variation in factor levels (Giovanni, 1983). Moreover, RSM is more efficient than traditional experimental procedures because it decreases both the time and cost required to determine the optimum product. Through planned experiments, RSM aims to identify variable effects and seek conditions that optimise the response in question (Box and Draper, 1987). According to Henika (1982), RSM differs from the usual procedure in that it tests several variables at one time, using experimental designs to cut costs and measure several effects. Relationships between variables and responses are defined by using Taylor second-order equations. Henika (1972) stated that it is useful statistical tool for analysing experimental data from food products to optimise the physical properties of the food product using various levels of ingredients. Mead and Pike (1975) reviewed RSM in detail from a biometric viewpoint.

The principles and foundations of RSM were first developed by Box and Wilson (1951). Subsequently, the theory was modified and expanded into a powerful tool for empirical model development and optimisation (Davies, 1954; Box and Draper, 1987; Khuri and Cornell, 1987; Cochran and Cox, 1966; Khuri, 1992). Sidel and Stone (1983) have attempted to explain the application of RSM in a simple way and Myers et al. (1989) reviewed the progress of RSM in the general areas of experimental design and analysis and indicated how its role has been affected by other advances of applied statistics.

Response surface methodology has been effectively utilised in a variety of food products and process improvement efforts. Ylimaki et al. (1988, 1991) applied this method to the development of rice flour bread and Ling et al. (1977) studied the effects of nonfat dried milk fractions in bread making by using RSM. Henselman et al. (1974) prepared high protein bread using three protein sources at three levels using RSM to optimise both sensory responses and physical properties of the bread. Frye and Setser (1991) used RSM to optimise textural attributes of a cake with reduced calories by the use of bulking agents to replace sucrose. In an earlier study, Neville and Setser (1986) studied the texture optimisation of reduced-calorie cakes with an experimental plan based on the five variables at five levels and nine responses. Canola oil was successfully used in place of hydrogenated shortening in layer cakes together with appropriate levels of water and an emulsifier. Three variables at five levels and eight dependent variables were measured to optimise ingredients levels in canola cake formulations (Vaisey-Genser et al., 1987). Similar experimental designs were used to study the effects of three variables (fat, stabilizer and corn syrup solids) with three levels each on a overrun and firmness of whipped topping. The combination of variables selected by this procedure produced an optimum topping mixture on the basis of cost, availability and convenience (Min and Thomas, 1980). More recently RSM was used to optimise the formulation of Chinese wet noodles (Shelke et al., 1990).

A number of reports have described the use of RSM to optimise process conditions. Malcolmson et al. (1993) analysed the effects of peak drying temperature and blending of hard red spring farina with durum semolina on spaghetti quality. Ulgen and Ozilgen (1993) determined pH-temperature optimum for pasteurisation of citrus juice by response surface methodology. Lomothe et al. (1988) optimised saponification of cod liver oil without loss of polyunsaturated fatty acids studying four parameters. Xu and co-workers (1992) examined the effect of a number of processing variables, including protein and fat concentrations and pH, on gel properties and related the results to the rheological behaviour and structure of gel. Oh et al. (1985) applied RSM to study noodle quality as affected by water absorption, dough pH, mixing time, sheeting speed and reduction percentage in roll gap. Seven responses were investigated: colour and breaking stress of uncooked noodles, surface firmness, cutting stress, resistance to compression, cooked weight and cooking loss of noodles. Similar studies were conducted to evaluate the effect of temperature, calcium hydroxide concentration and cooking time on physicochemical properties of amaranth flour for production of tortillas (Vargas-Lopez et al., 1990). Batistuti et al. (1991) also successfully used RSM to optimise the production of a snack food from chickpeas by extrusion. The independent variables (process temperature at 125 - 137 °C; feed moisture contents at 13 - 27 %) were studied at five levels in the extrusion of defatted chickpea flour.

Response surface technique has also been used in studies of protein denaturation (Nielsen et al., 1973), bacterial growth (Schroder and Busta, 1973), soybean extruded products (Agvilera and Kosikowski, 1976), whipping properties of an ultrafiltered soybean product (Lah et al., 1980), rheological properties of flours (Skeggs, 1985), optimisation of boneless ham yields (Motycka et al., 1984), response transformation (Draper, 1985), processing conditions improvement (Malundo et al., 1992; Floros et al., 1992), casein production (Fichtali et al., 1990), enzymatic hydrolysis of canola meal (Ma and Doraikul, 1986) and optimisation of pimiento pepper lye peeling processes (Floros and Chinnan, 1987).

1.7.2 Partial least square regression

Multivariate data analysis has increasingly been used in food research during the last decade (Martens and Harries, 1983; Powers, 1984a, 1984b; Gacula and Singh, 1984; Martens et al., 1983c). Partial least squares regression is a comparatively new approach to multivariate data analysis, originally developed by Wold (1982).

Two-block predictive PLS regression has proven beneficial both for calibration (Marten and Jensen, 1983;

Wold et al., 1983a) and for general data interpretation (Wold et al., 1983b, 1983c, 1984). Until recently, it has been more popular among chemometricians than among statisticians (Naes and Martens, 1985).

This approach has been successfully used in the calibration of fluorescence spectra of botanical components in the wheat flour (Jensen et al., 1982; Jensen and Martens, 1983). It can be a useful tool for determining the extent to which sensory attributes capture the information contained in dissimilarity data and for developing predictive models of acceptability (Popper et al., 1987). Williams et al. (1988) applied PLS to relate composition of a range of samples to flavour. Martens (1986) studied the sensory attributes of pea samples blanched and frozen and analysed within one month following harvesting. Peas were selected to represent relevant variation with respect to maturity levels and cultivars. The PLS1 regression indicated that sensory analysis of frozen green peas can to some degree predict average consumer response; about 73 % of variation in consumer preference could be explained using a profiling panel with bilinear modelling. Fjeldsenden et al. (1981) explored the relationships between analytical sensory and compositional data of swedes from different seasons and growth sites. The results confirmed that PLS2 could be useful for obtaining a total picture of different relationships between multiple Y and X variables. A weak relationship between sensory and compositional variables was found as only about 20 % of the total sensory variation was predicted by chemical variables alone. In other studies, PLS was used to explore how combinations of compositional and physical measurements improve prediction of sensory quality in cauliflowers (Fjeldsenden et al., 1981; Martens et al., 1983a, b). This was found to be described by combinations of dry matter, sugars and acids. An important factor is that PLS works well with small experimental sets that have fewer samples than variables (Piggott, 1990).

1.8 Aim of experimental work

In Pakistan, it has been reported that cereals provide 70 and 76 % of average daily calories and protein intakes respectively (Government of Pakistan, 1970). Wheat contributes roughly half of all the calories and protein in the Pakistani diet and is consumed predominantly in the form of unleavened flat bread (chapati, roti) throughout the country by all socio-economic groups.

Although wheat is a cheap source of energy and protein it does not contain sufficient of the essential amino acids for a balanced diet. The result is low levels of nutrition limiting health and child development. Such situations exist in villages and among the urban poor who can not make up cereal proteins deficiencies from other affordable protein sources. It has been established previously that protein malnutrition is prevalent in those countries where the staple diet is from cereal grains. Considering the deficiencies in wheat, several workers (Juneja et al., 1980; El-Minyawi and Zabik, 1981; Elgedaily et al., 1982 ; Repetsky and Klein, 1982; Nmorka and Okezie, 1983; Ballester et al., 1984 and Luzfernandez and Berry, 1989) have made efforts to prepare composite flours to produce nutritionally balanced cereal products. Such efforts have mainly been on the improvement of leavened breads.

Yeast extracts have been very widely used in various products of food as flavouring and flavouring enhancers, condiments and protein fortification ingredients but not often in cereal products. Their potential for this study is obvious.

Therefore, the main aim of this study was to optimise the appeal and acceptability of a range of chapati breads produced from composite flours with addition of appropriate yeast extracts and in parallel to improve the nutritional value of product. The main objectives of this investigation can be summarised thus:

(a). Determination of preference of consumers for savoury flavours through surveys.

(b). Study of the factors in flours that determine acceptability.

(c). Formulation of chapati flour using British wheat varieties.

(d). Production and optimisation of savoury-flavoured composite flours for chapati production using response surface methodology.

(e). Physicochemical analyses of flours and products.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Wheat and commercial chapati flour samples

Samples of eight British wheat varieties were obtained from Plant Breeding International, Maris Lane, Trumpington Cambridge CB2 2LQ, England. Four samples of commercial chapati flour were supplied by Finnedon Mills Limited, Rixon Road, Finnedon Industrial Estate, Wellingborough, Northants NN8 4BI, England.

Tempering of wheat: Wheat samples were cleaned to remove extraneous materials, washed, dried and conditioned by adding sufficient water to achieve a moisture content of 14 % (AACC, 1983). Wheat was tempered in batches of 3 kg in air-tight bottles adding the required quantity of water. Grain was mixed for 5 min and kept for 24 h before milling.

Milling of wheat and preparation of chapati flour: Milling was carried out in a Laboratory Mill Quadrumat Junior (Mod-NR279002), Brabender OHG, Duisburg, Germany. Bran and shorts were pulverised through a Cyclotec 1093 Sample Mill (Tecator AB, Box 70, S-263 01, Hoganas, Sweden) equipped with a 1 mm mesh sieve and mixed into white flour to prepare a wholemeal blend. For chapaties, flours of two wheat varieties were blended. Flours of individual varieties and blends were stored in closed polyethylene bags at ambient temperature prior to analyses.

2.1.2 Chemical analysis of flour and product

Reagents and chemicals: All chemicals were procured as analytical grade from commercial suppliers.

Determination of moisture content: Moisture contents of wheat, flours and products were determined as described in AACC (1983). Samples (2 g) of well-mixed material, in triplicate, were weighed into aluminium dishes (55 mm diameter) provided with lids. These were air-dried at 130 ± 0.5 °C for 1 h, cooled in a desiccator and weighed immediately after attaining ambient temperature. Moisture contents were calculated as weight loss through heating.

Determination of ash content: Samples (3 g) were weighed in crucibles, heated to 550 °C then cooled in a desiccator prior to use. Samples were carbonized until no further fumes were observed. Crucibles were then placed in a muffle furnace (550 °C) and incinerated until a light gray ash was obtained (4 h), cooled in a desiccator for 50 min and weighed. Triplicate determinations were made.

Protein determination : Total nitrogen was determined by the Kjeldahl method (46-12, AACC, 1983) using the Kjeltec system, (Tecator: 1002 distilling unit and 1007 digestion unit, Tecator AB. Box 70, S-263 01 Hoganas, Sweden).

Triplicate samples (200 mg) were digested with 5 ml sulphuric acid in a tube for 25 min. Catalysts (2 g potassium sulphate and 0.1 g of cupric selenite) were used. 0.20 M (for yeast extract); 0.05 M, (for flour and product) hydrochloric acid solutions were used for titration of ammonia released. Protein conversion factors for wholemeal flour, extraction rate flours and product were N X 5.83, N X 5.7 and N X 6.25, respectively.

Determination of damaged starch: Damaged starch and maltose were determined as described by AACC (1983). Damage was calculated as the amount of starch (g) hydrolysed by α-amylase per 100 g of flour on the basis of 14 % moisture.

Reagents: (a). Acetate buffer: Sodium acetate trihydrate 0.68 % (w/v) and 0.3 % (v/v) glacial acetic acid (pH 4.6 - 4.8).

(b). Sulphuric acid solution 1.835 M.

(c). Sodium tungstate solution 10.7 % (w/v).

(d). Alkaline ferricyanide solution, 0.1 M: 33 g pure dry $K_3Fe(CN)_6$ and 44 g anhydrous $NaCO_3$ were dissolved in water and diluted to 1 l.

(e). Acetic acid-salt solution: 70 g KCl and 40 g $ZnSO_4.7H_2O$ were dissolved in 20 % (v/v) glacial acetic acid.

(f). Soluble starch-KI solution: 1 g soluble starch was suspended in a small quantity of cold water and poured slowly into boiling water with constant stirring. The gelatinised starch was cooled and 25 g KI added. The mixture was diluted to 50 ml with water to which 1 drop of 40 % (v/v) NaOH solution was added.

(g). Thiosulphate solution 0.1 M contained 0.38 % (w/v) borax.

Standardization of sodium thiosulphate with potassium iodate: 0.15 g pure dry potassium iodate was dissolved in 25 ml of cold distilled water and 2 g KI and 5 ml 1 M sulphuric acid was added. Iodine liberated was titrated with thiosulphate solution, until the colour changed to a pale yellow. Distilled water (200 ml) and 2 ml starch solution were added and titrated further until the colour changed from blue to colourless. Triplicate titrations were performed.

$$1 \text{ ml M } Na_2\ S_2\ O_3 = 0.03567 \text{ g } KIO_3$$

(h). α-Amylase preparation: Fungal amylase from Aspergillus oryzae (No A0273, Sigma Chemical Co., Fancy Road, Poole Dorset, BH17 7NH, England) were obtained at 50-200 Sigma units mg-1 protein.

Procedure: Reagent (a) was maintained at 30°C. Flour (1 g; 14 % moisture basis) was weighed into 125 ml conical flask and enzyme (0.05 g) added. Reagent (a) (45 ml) was added to form a uniform suspension which was incubated for 15 min. The flask was removed from the water and 3 ml of reagent (b) and 2 ml of reagent (c) added with mixing. After a 2 min rest, the mixture was filtered (Whatman paper No. 31), discarding the first 10 drops of filtrate. To 5 ml of filtrate in a test tube, 10 ml of ferricyanide reagent (d) were added. This solution was mixed and heated in water (100 °C) for exactly 20 min. The test tube was cooled under running water and the contents poured into a 125 ml flask. The tube was rinsed with 25 ml acetic acid-salt solution and solutions pooled. After mixing, 1 ml of soluble starch-KI solution was added and titrated with 0.1 M thiosulphate until the blue colour had completely disappeared.

Determination of blank: A control was prepared to examine changes in the ferricyanide reagent, arising from reducing impurities in reagents. Acetate buffer (a) (45 ml), 3 ml sulphuric acid solution (b) and 2 ml sodium tungstate (c) were mixed in a flask and to an aliquot (5 ml) was added 10 ml of ferricyanide solution (d). In controls, 10 ml thiosulphate solution was needed to discharge the blue starch-iodine colour.

Calculations: Subtraction of ml thiosulphate required for sample analysis from ml thiosulphate used in blank yielded ml ferricyanide reduced.

Reducing sugars were quantified as mg maltose/10 g (AACC, 1983).

$$\% \text{ damaged starch} = \frac{1.64}{100} \times 5 \times (\text{maltose content})$$

2.1.3 Analysis of yeast extract

Three samples of yeast extract were supplied by Quest International (Bromborough Port, Wirral, Merseyside L62 4SU, England).

The HPLC method of Nguyen and Sporns (1984) was selected because it enabled the simultaneous quantification of flavour potentiators and salt (as chloride) in yeast extracts.

Apparatus and reagents: High performance liquid chromatography was performed using a LKB 2150 HPLC pump (LKB-Produkter AB. Box 305, S-16126 Bromma, Sweden) with a manual injection module fitted with 20 µl loop. Effluent was monitored at 254 nm using a Varian UV 50 and a refractive index detector. Both detectors were attached to a Trio computing Integrator (Trivector International Limited, Sandy, Bedfordshire SG19 1RB, England). Chromatography was performed using Partisil SAX (25 cm x 4.6 mm) with a 10 cm x 4.6 mm silica gel precolumn.

The mobile phase was 0.017 M potassium dihydrogen phosphate adjusted to pH 4.0 with 0.017 M phosphoric acid. Standards were monosodium glutamate (MSG)(purity 99-100%), disodium inosine 5'monophosphate (IMP) and disodium guanosine 5'monophosphate (GMP) (Sigma grade, purity 99.9 %, Sigma Chem. Co., Fancy Road Poole Dorset, BH17 7NH, England), NaCl (Aristar grade, BDH Ltd, Broom Road Poole, BH12 4NN, England) were dissolved in HPLC-grade water and dilutions were analysed to produce linear regression equations. These were used to quantify flavour potentiators and salts in the yeast extracts. Triplicate samples of each yeast extract were analysed and variation between samples was within 5 % of mean values.

Procedure: Yeast extract (2 g) was added to 30 ml of phosphate buffer and the mixture stirred for 15 min. Acetone (40 ml) was added and stirring continued for a further 5 min. The mixture was then filtered (Whatman No. 1) and washed with 20 ml of 50 % aqueous acetone. The beaker and insoluble residue was rinsed (6 X 20 ml) with 50 % aqueous acetone. The filtrate was transferred to a round bottom flask, evaporated to *ca*. 10 ml and then diluted with water to 50 ml. This solution was filtered through 0.45 μm Millipore filter prior to injection into the HPLC. The mobile phase flow rate was maintained at 1 ml min^{-1}. Each chromatogram was completed in approximately 35 min. Peaks were identified from the chromatogram by comparison of retention times with standards and quantified using integration of peak areas. Mixtures of standards and experimental samples were injected alternately. Injection of blank (water) was run after every determination.

Determination of sodium chloride in yeast extracts: Samples were ashed as described previously and the residue transferred from the crucible into a 200 ml conical flask with 50 ml water. To each flask was added 1 ml of potassium chromate (5 %) as indicator and the contents were titrated with 0.1 M $AgNO_3$. At the end point there was a colour change from yellow to a darker orange-yellow. In a second conical flask 1 ml of potassium chromate in 50 ml of water containing 0.5 g calcium carbonate was taken as a reference for comparison of colour.

Determination of protein with microbiuret method (Itzhak and Gill, 1964): This method was used for protein estimation in yeast extract as the Kjeldahl method gave over estimates of values due to presence of the nucleotide nitrogen. A standard curve of 100 - 2000 µg bovine serum albumin was found to be linear and appropriate dilutions of samples were performed so that values fell within the linear range.

Reagents

a. 0.21 % (w/v) $CuSO_4.5H_2O$ in 30 % (w/v) NaOH

b. 30 % (w/v) NaOH

Procedure: The following samples were prepared.

1. 2 ml H_2O, 1 ml reagent a.
2. 2 ml protein solution, 1 ml reagent a.
3. 2 ml H_2O, 1 ml reagent b.
4. 2 ml protein solution, 1 ml reagent b.

The absorbance of each sample at 310nm was determined on Beckman DU 7500 spectrophotometer (Beckman Instruments Inc. 2500 Harber Blvd. Fullerton CA 92634-311, USA) using distilled water as the reference. The formula (2-1)-(4-3) was used to estimate the protein concentration.

2.2 Production of chapati

A dough was prepared by mixing flour (100 g) with 60 - 70 % of water (6 - 7 % more water than water absorption from the farinograph), kneaded by hand, then placed in a bowl, sprinkled with water, covered and allowed to rest at ambient temperature for 30 min. This dough was divided into two 85 g portions, each of which was moulded into a smooth, round ball. Each ball was rolled into a smooth disc 17.5 cm in diameter, on a flour-dusted wooden board using a rolling pin. This wooden board had four strips of wood each 3.5 mm thick and 17.5 cm long, fixed to form a rectangle with 17.5 cm sides. This maintained uniformity in diameter and thickness in the finished chapati. The dough was quickly transferred to a cast iron griddle pre-heated to 245 °C over an electric cooker. After 30 sec the chapati was turned over and pressed with a soft cloth to spread the steam uniformly internally. This action also assisted in puffing the chapati. After a further 30 sec, it was turned for a second time and 30 sec later it was removed from the heat. The total baking time was 90 sec. The chapati was left to cool and packed in individual polyethylene bag at 32 °C. This process is summarised in Fig. 2.1.

Physical properties of dough such as stickiness, resistance to sheeting and shrinkage after sheeting were

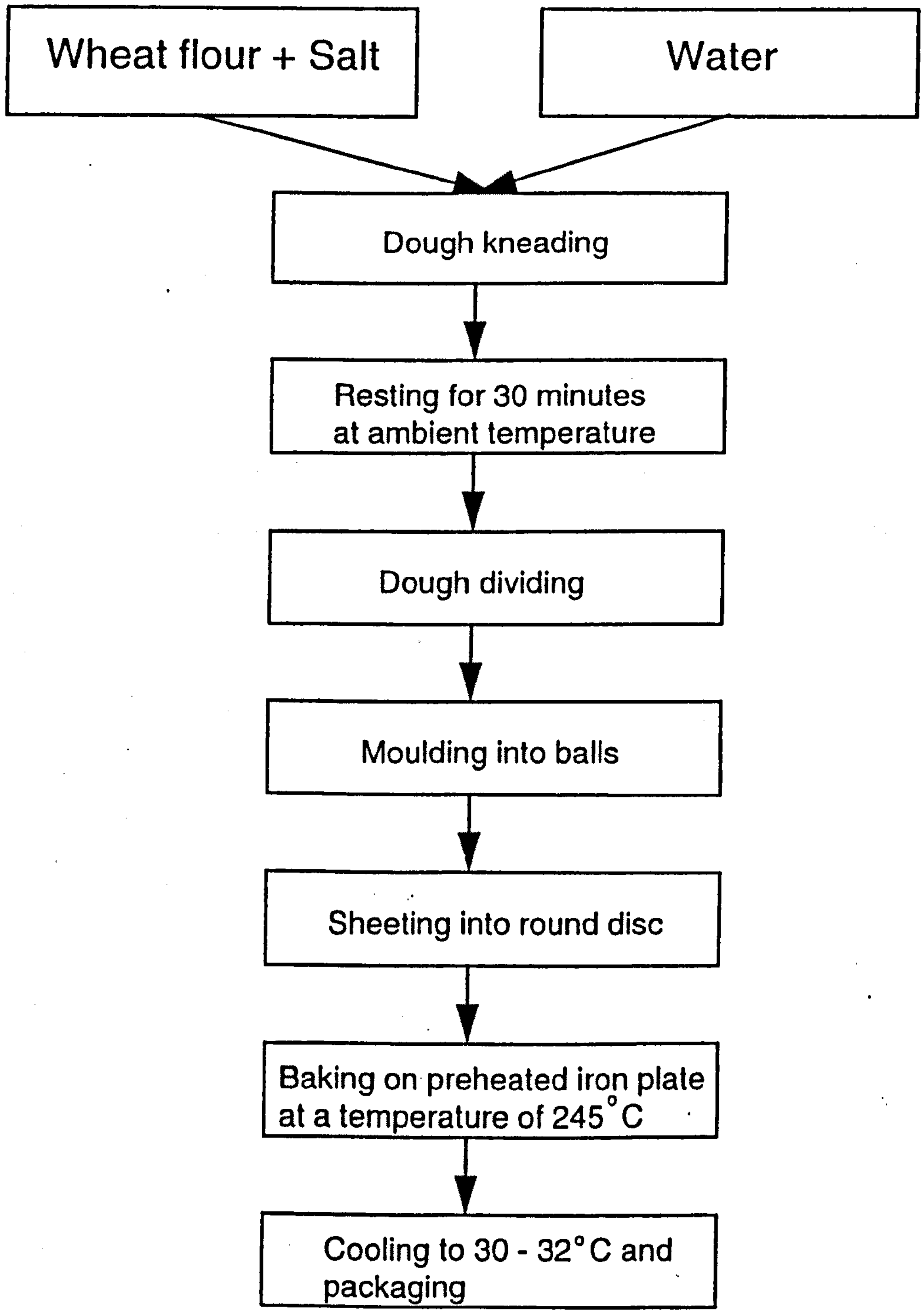

Fig. 2.1. Production of experimental chapati

assessed by visual judgement during chapati preparation. Intensity of each character was marked as low (X), medium (XX) and High (XXX).

2.3 Assessment of texture of chapati

2.3.1 Sensory evaluation

Sensory evaluation was carried out by assessing textural characteristics of chapaties on a scale from 1 to 10, where 1 = softer and 10 = harder, 5 was considered the mean texture after 2.5, 10 and 24 h of storage (Brennan, 1988). Texture was defined as the experience derived from sensation of the skin after ingestion of the product. It related to summation of hardness, softness, ability to fold and susceptibility to tear of chapati. A piece of chapati which could be folded into scoop shape without tearing skin and chewed easily was considered as soft (Majeed *et al*., 1987). A panel of 12 Pakistani students and wives, all users of the product, was used: 6 men and 6 women aged from 30 to 45 years. A batch of 4 samples was prepared using two griddles at a time. Reverse order was applied for second replication. Samples of chapati, packed individually in coded polyethylene bags along with score sheets (contained assessment times) were distributed to assessors at their homes. Each bag contained 1/2 a chapati. Assessors were briefed individually. Experiments were performed in duplicate. A sample score sheet is given in Appendix 1.

2.3.2 Instrumental measurement of texture

Texture (toughness) of chapati was estimated at three intervals (2.5, 10, 24 h) of storage utilising a Instron Universal Testing machine (Ebeler and Walker, 1983)(Instron Ltd, Corporate Headquarters, 2500 Washington Street, Canton, Mass, 02021, USA). A cutter of 22 mm diameter was used to cut two circular pieces of chapati at random. Samples were stacked in duplicate under the probe (20 mm dia). Chart speed, cross head speed and depth of application of compression force were 30 cm min^{-1}, 1 cm min^{-1} and 2 mm, respectively. More the crosshead goes into pieces of chapati less tough they are.

2.3.3 Sensory evaluation of flavour in chapaties

Same panel (texture) was used for evaluation of acceptability in savoury chapaties. The products were presented to the panel used for the evaluation of texture. In preliminary studies, to determine the appropriate salt level, 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 % of NaCl (dry weight basis of flour) was added to the flour. In a similar set of experiments, chapaties containing varying concentrations of yeast extracts with different flavours, but similar quantities of sodium chloride, were prepared. This strategy was used to select acceptable concentrations of yeast extracts for chapati production. A third series of experiments used response surface methodology for final study of acceptability in chapaties. Each experiment was performed in duplicate. A typical score sheet is given in appendix 2. Taste was evaluated as a hedonic scale 1 - 9; 1 = extreme dislike, 9 = extreme like. Each sample was given a separate random code number and presented to assessors at their homes.

2.4 Determination of the falling number

This method determines the α-amylase activity in flour samples and is based on the degradation of a gelatinised starch paste by α-amylase. The time in seconds for a "viscometer " to drop down in the gelatinised suspension yields an estimate of enzyme activity in both grain and flour.

Preparation of sample: Flour (100 g) was sifted through a sieve with 180μ aperture sieve mounted on a shaker in a horizontal plane for five min.

Determination: Determination of falling number was carried using a falling number apparatus (Falling Number AB, Norrlandsgatan 16, Stockholm C, Sweden). Flour (7 g) was placed in a viscometer-tube and 25 ml of distilled water (20 °C) was added. The tube was fitted with a rubber stopper and shaken vigorously 30 times to obtain a uniform suspension. The stopper was then removed and flour was pushed back into the liquid using the stirrer. The tube with stirrer was placed into a boiling water bath. As the tube touched the bottom of the rack, a stopwatch was started and the tube secured. After five seconds from the immersion of the tube, stirring of the suspension was started by hand at two turns per second. After a total of 59 seconds, the stirrer was lifted to the uppermost position, held for 0.5 sec in this position and then released after exactly sixty sec from starting the stopwatch. When the stirrer had dropped by its own weight until the lower edge of the upper stop touched the stopper, the watch was stopped. The total time in seconds from the immersion of the tube into the water bath until the viscometer-stirrer had dropped through the gelatinised suspension, was taken as the Falling Number value.

2.5 Determination of flour granularity by the Simon laboratory sifter

Each nest of 3 sieves was cleaned with a small brush and the sieves were then refitted with the 8N at the top and the 12N at the base (ensuring that the rubber ball cleaners move freely between each cover).

Flour (30 g) was placed with a ball cleaner on the 8N sieve. The lid was clamped on and the nest of sieves fixed firmly to a sifter. This was activated for 5 min, then stopped and the nest of sieves tapped to dislodge flour adhering to the underside of covers. Stock on each sieve and in the receiver was weighed after brushing on to a piece of glazed paper. If it was found that more than 0.5 g of flour had been lost, the measurement was repeated.

The results obtained were expressed as a percentage of tails and throughs according to the sieve specifications (Table 2.1).

Table 2.1: Sieve specifications.

Sieve	Aperture size (Microns)	Particle size range of tails
8N	180	over 180 microns
10N	130	Between 130 and 180 microns
12N	110	Between 110 and 130 microns

Size of particles in the receiver (throughs) is less than 110 microns.

2.6 Determination of grade colour of flour (Kent-Jones and Martin Colour Grader)

The grade colour value relates to bran content, extraction rate and efficiency of the milling process (Table 2.2). To confine values within the scale (maximum 18), 50 % patent flour (6.2 grade colour value) was mixed into wholemeal flour before determining the colour value of chapati flours.

Procedure: A smooth paste was prepared by mixing 30 g of flour and 50 ml of distilled water. This was poured into a clean sample cell. It was important to ensure that the paste was free from bubbles during filling. The front surface of the cell was cleaned with a soft cloth and clamped firmly into the upright position and the light spot was adjusted to the zero position. The cell holder was lowered carefully and the grade figure recorded. The cell holder was lifted to the upper position, and the sample cell removed. Triplicate determinations were made and the average calculated of the readings (overall variation should not be more than 0.2 of a grade unit).

Table 2.2 : Colour grade figures.

Type of flour	Grade Figure Range
Top patent flours / Special cake flour	-1.0 to 2.0
Straight - run flours (72 % extraction)	2.0 to 4.5
High extraction low grade flours (80 % extraction)	5.0 to 7.5
85 % extraction flour	8.0 to 12.5

2.7 Rheological tests of flour

2.7.a Farinograph method

Farinograph tests were performed using a 300 g Brabender farinograph bowl (Brabender OHG D-4100 Duisburg, Kulturstrabe 51-55, Germany) with the constant flour method (AACC, 1983). Water absorption, dough development time, dough stability and tolerance index were measured. Absorption values were based on dough consistency at the 500 BU line.

Normal working temperature of the farinograph was 30°C. Flour (300 g at 14 % moisture basis) was placed in the bowl and the machine started. After 1 min, water was added from a burette from the right corner of the bowl until the pen reached the 500 BU line position. Addition of water was then stopped and checked after some minutes the position of curve to be centered the 500 BU line. The experiment was repeated thrice to make sure that the curve was centered on the 500 BU line and burette reading recorded. For the final titration, water was added within 25 sec after starting the machine and the machine allowed to run for 20 min.

Interpretation of farinogram (see Fig. 1.5)

Dough development time: the time from the first addition of the water to the development of the dough's maximum consistency. The value has also been referred to as "peak time".

Stability: the difference in time to the nearest half-minute, between the time when the curve first intercepts the 500 BU line (Arrival time) and the when the curve leaves the 500 BU line (departure time).

Tolerance Index: is the difference in Brabender units from the top of the curve at the peak to the top of the curve measured 5 min after the peak.

2.7.b Extensography

An extensograph measures the force needed to stretch a piece of dough (**resistance to stretching**) and time required to stretch the dough to the breaking point (**extensibility**) (AACC, 1983).

Thermostat (set at 30 °C) and water circulation pump were Switched on one hour prior to conduct the experiments to attain the temperature of extensograph to 30 °C. Water absorption of the sample was ascertained to give dough consistency of 500 BU with farinograph method (2.7a).

Flour (300 g) was decanted into the bowl and the farinograph switched on. NaCl (6 g) was dissolved in 135 ml of distilled water (30°C) and poured into the flour. Sufficient water was added from the burette (1.5 - 2 % less than the predetermined water absorption) to give a consistency of 500 BU after one min of mixing. The mixer was then stopped and the dough allowed to rest for five min with the bowl covered. The farinograph was started again and run for two min. A small quantity of water was added, during mixing, at 0.5 min intervals to maintain consistency at 500 BU (Brabender Units). The dough was removed from the bowl, cut into two portions of 100 g, rolled into ball on the homogenizer (20 turns) and moulded into a cylinder shape by passing through the moulder. The moulded pieces were then clamped into dough holders and permitted to rest in the incubation cabinet of the extensograph for 30 min (curve 1). The dough holder was placed on the extensometer arm and the dough stretched to record the extensibility and resistance to extension on the graph of the machine. A duplicate test was made on the second piece of dough. The dough pieces were remoulded, allowed to relax for a further period of 60 min and the process repeated (curve 11).

Interpretation of extensogram

A. Resistance to extension: measured as the height of the Extensogram in Extensograph Units after 50 mm stretching, this indicates the force opposing to stretching of the dough.

B. Extensibility: measured as the length of the Extensogram base in mm indicating stretchability of dough.

C. The ratio A:B: It indicates the behaviour of a dough, its stability and boldness.

2.8 SDS-PAGE of cereal proteins

One-dimensional SDS-PAGE was used to fractionate and quantifying proteins of wheat flour. The method, based on the discontinuous buffer system (Laemmli, 1970) has been modified by Payne et al. (1979, 1980a). Disulphide bonds of proteins were broken using 2-mercaptoethanol, and SDS to disrupt noncovalent bonds. The SDS is absorbed on to the surface of the protein components to mask differences in the surface charge of the proteins. This ensures a fractionation principally on the basis of differences in molecular weight.

Gradient SDS-PAGE gels enable a much larger range of protein sizes to be fractionated than gels of uniform pore dimension. Resulting protein bands are sharper and polypeptides of similar molecular weight are more likely to resolve.

Electrophoresis was performed using vertical gel apparatus (GE - 2/4 LS) with 140 X 175 mm glass plates, LKB 2197 power supply and a LKB 2219 Multipump II thermostatic circulator (Pharmacia LKB Biotechnology Division, Midsummer Boulevard, Central Milton Keynes, Bucks. MK9 3HP, UK.). The composition of the polyacrylamide solution (Table 2.3) was sufficient for running two gels.

2.8.1 Gel mixture preparation

Solutions of 10 % and 20 % gel were degassed for 20 min. Activators were added before pouring the mixtures into gel casting chambers. Similarly, stacking gel solutions were mixed, degassed and activators were added before pouring on top of the resolving gel.

2.8.2 Moulding of polyacrylamide gel

Gradient gels 170 X 130 X 1.5 mm thick were moulded in a gel slab casting apparatus (GSC-2, Pharmacia LKB Ltd). Glass plates were cleaned thoroughly by immersing in 2 % (v/v) Decon solution overnight, washing with tap water followed by distilled water and 70 % (v/v) ethanol, and air dried. The 1.5 mm thick spacers (130 mm in length) were fixed between the two edges of plates and sealed with yellow tape (Cat. no: 19-1257-01, Pharmacia LKB Biotechnology). The plates were placed in an oven at 60 °C for 30 min and cooled. Gel cassettes were then placed in the casting apparatus. A peristaltic pump (Sr.90843, Watson - Marlow Ltd, Falmouth, Cornwall TR11 4RU, England) was fixed between a gradient mixer chamber (LKB GM-1, Pharmacia) and the gel casting apparatus. Methanol (45 ml, 20 %) was poured into chamber B, tilted slightly and the pump started. When the methanol level reached the tube, the pump was stopped: a 10 % acrylamide solution was poured into chamber A and a 20 % solution to B. A mixer was inserted into chamber A and the barrier between the two chambers removed. The contents were immediately pumped into the gel casting apparatus followed by 45 ml 50 % glycerol-

Table 2.3: SDS polyacrylamide gradient (10 - 20 %) gel solutions for separation of wheat protein.

	10 %	20 %
Resolving gel		
35 % Acrylamide (ml)	11.13	22.26
2 % Bisacrylamide (ml)	5.20	10.40
3 M TRIS, pH 8.8 (ml)	5.00	5.00
Distilled water (ml)	18.10	1.96
Sucrose (g)	-	6.00
Activators		
10 % SDS (µl)	400	400
TEMED (µl)	26	26
10 % Ammonium persulphate (µl) (freshly prepared)	150	150
Stacking gel		
35 % Acrylamide (ml)	2.23	
2 % Bisacrylamide (ml)	1.04	
1 M TRIS, pH 6.8 (ml)	2.5	
Distilled water (ml)	13.8	
Activators		
10 % SDS (µl)	200	
TEMED (µl)	20	
10 % Ammonium persulphate (µl) (freshly prepared)	200	
Reservoir buffer		
Buffer, pH 8.3 contained 0.192 M Glycine, 0.025 M TRIS and 0.1 % (w/v) SDS.		
Glycine (g)	57.625	
TRIS (g)	12.10	
SDS (g)	4.0	
Made up to 4 l with distilled water		

bromophenol blue solution. The gel was allowed to polymerise for 1 hr (Fig. 2.2).

The slab casting apparatus was dismantled. Yellow tape was applied on the bottom of plates containing gel, and the stacking gel solution added to the top of each gel with a slot former (14-teeth) inserted into stacking gel solution. This gel was allowed to stand for 1 h to complete polymerisation.

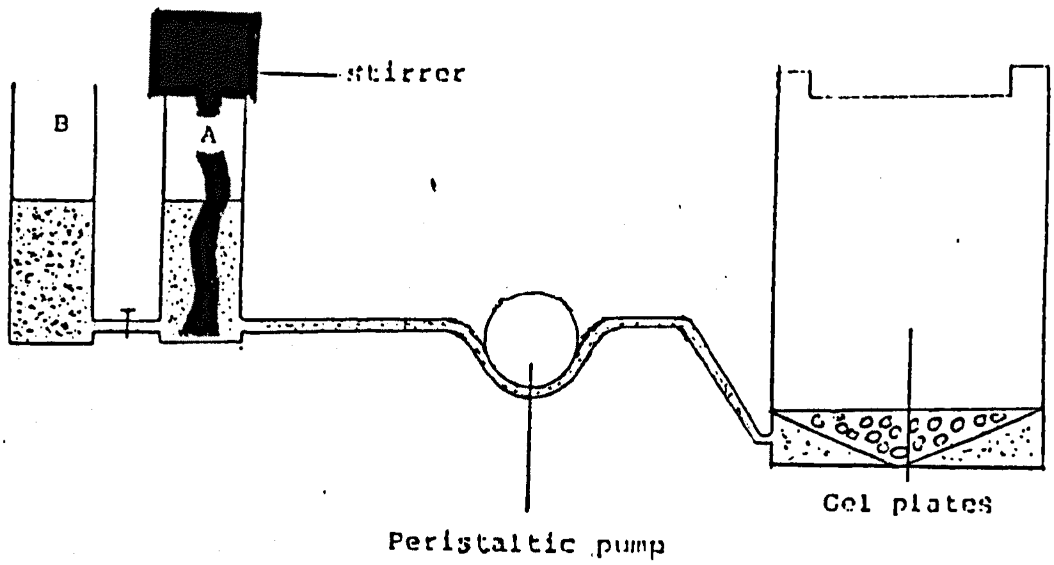

Fig 2.2: Diagram of apparatus for casting gradient gels

2.8.3 Preparation of protein extracts

The extraction procedure used in this study was similar to that described by Ng and Bushuk (1987). Extracts were prepared by suspending 40 mg of flours (dry basis) in 1 ml of loading buffer solution containing 2 % (w/v) SDS, 10 % (v/v) glycerol, 5 % (w/v) 2-mercaptoethanol, 0.063 M TRIS/HCl (pH 6.8) and 0.01 % (w/v) Pyronin Y (tracking dye). Protein extraction was carried out in 1.5 ml microfuge tubes for 2 h with occasional shaking, heated for 2.5 minutes and cooled. The suspension was centrifuged at 13000 rpm for 10 minutes in a microfuge and an aliquot of the clear top layer was used as the protein extract. Extract (10 - 25 μl), containing the reduced proteins was loaded using a long needle syringe (Cat.no. 2010 - 150, LKB Pharmacia Biotechnology) on to the stacking gel so that a thin layer was formed at the bottom of the well. End slots were loaded with dye as protein bands there were skewed. A primary standard reference protein mixture (3 mg) contained: Cytochrome c (MW = 12,300), Myoglobin (17,200), Carbon anhydrase (bovine erythrocyte)(30,000), Ovalbumin (45,000), Bovine serum albumin (66,250) and Ovotransferrin (78,000). The dried protein was dissolved in 1 ml of the buffer mixture and 5 - 25 μl were loaded on to each gel.

2.8.4 Electrophoresis

Yellow tape was removed from the bottom of plates and 0.5 % agarose solution used to fill the base. The gel was fixed in the electrophoresis unit, the comb removed and the wells rinsed with buffer. The upper and lower reservoirs were filled with electrophoresis buffer. The lower reservoir was cooled by coolant passing through a glass tube heat exchanger. A thermostated circulating water bath was adjusted to 18 °C and gel was pre-electrophoresed at a current of 15 *mA* for 30 min to equilibrate. After rinsing of the wells, samples were applied, overlaid with buffer and run at a current of 15 *mA* for 1 h without circulation of buffer until the proteins had completely entered the stacking gel. The buffer circulation pump was switched on and electrophoresis performed at 20 *mA* (430 *V*, 100 *W*) for 22 h, at which point the tracking dye, Pyronin Y, had emerged from the gel.

2.8.5 Staining of gel

The gel was removed from the glass plates and immersed in a fixative (300 ml) containing 5 % (v/v) isopropyl alcohol and 10 % (v/v) acetic acid for 2 h at 37 °C and 22 h at ambient temperature. The fixing solution was drained off and the gel washed with water. Proteins were stained using a solution containing 0.1 % (w/v) PAGE-83 dye, 25 % (v/v) methanol and 10 % (v/v) acetic acid for 1 h at 37 °C and 23 hr at ambient temperature. The background was destained by immersing the gel in a solution (250 ml) containing 25 % (v/v) methanol and 8 % (v/v) acetic acid for 24 h and further destained with 250 ml solution for 24 h. The gel was stored in preserving solution (7 % acetic acid) prior to quantification of protein and photography.

Quantification: The degree of dye binding by each band of protein was measured by scanning densitometry of gel (LKB Pharmacia, Ultroscan XL Bromma Enhanced Laser Densitometer). The total absorption by the dye in each band (proportional to the area of the peak in the scan profile) was automatically calculated by the integral integrator (Gelscan XL version 2.1 software). Relative peak area (% under each band) was used for quantifications.

2.9 Lyophilisation of chapaties

Chapaties were grated into small bits with a hand grater and frozen at -20 °C. Lyophilisation was performed with Freeze-drier (Edwards, model E 12) at -40 °C condenser temperature and 20 - 25°C tray temperature under vacuum for 48 h until drying was complete.

2.10 Methodology of survey

For the Glasgow survey, data were collected through personal interviews and questionnaires distributed and collected using a quota sampling technique (Chisnall, 1986), to approximately represent age and sex variation in the population. Questionnaires were filled from 7 parts of the city and 20 respondents were selected from each part. The median of stay of people in Glasgow was 3 years.

For the Pakistan survey, data were collected through postal questionnaires sent to 225 individuals living in different areas of the country. In order to get better response, more weighting was given to education of respondents, 115 questionnaires were sent to employees of University of Agriculture, Faisalabad. 50 and 60 questionnaires were sent to respondents from rural areas and other parts of the country, respectively. Questionnaires were translated from English to Urdu with a consideration of cultural interpretation.

Questionnaires were composed of structured questions, divided into three sections. The first related to socioeconomic questions on age, sex, profession, education, marital status, number of children in the household, and consumption patterns. The second elicited information on savoury flavour preferences from both adults and dependent children. Information regarding children's preferences were collected from their parents. Respondents were asked to indicate preference on a five-point rating scale from "least preferred" (1) to "most preferred" (5). The final section of the questionnaire was designed to determine the importance of quality factors in chapati. Consumers were asked to rate each quality character on a five-point rating scale from 1 (not important) to 5 (very important). The design of the questionnaire is given in Appendix 3. Information regarding profession of respondents were used to segregate upper (business men), middle (surgeons, engineers and university teachers) and low income groups (agriculturists, retired individuals, clerks, school teachers, labourers and students).

2.11 Statistical analysis

Sensory data were analysed by analysis of variance

(Randomised Complete Block Design). A general linear model was used for survey data analysis (Minitab Version 8.2, Minitab Inc., 3081 Enterprise Drive, State College, PA 16801-3008, USA). Tukey's test was applied to compare sample means (Steel and Torrie, 1980).

Relationships between rheological characteristics and protein composition of flours were analysed by Partial Least square regression (PLS2) (Unscrambler V 3.1, Camo A/S Jarlevien N-7041, Trondheim, Norway (1990)). 2-vector loadings plots were drawn using cross validation method to explore relationships between variables of two data sets. Variables situated far from the origin were correlated positively and those present on opposite sides of origin were considered as correlated negatively.

Response surface methodology was applied to analyse the data generated in the optimisation phase (Table 2.4). Data were analysed using the multiple regression procedure. It was possible to fit second-order polynomial equations which contained linear, quadratic and interaction terms for two variables (Gacula and Singh, 1984). Two dimensional contour plots for quality parameters were generated to determine the effects of the variables on the selected response.

The equation for the response surface was:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{12} AB$$

Table 2.4 : Design Matrix for two Factors with Blocks for fitting Second-Order Response model.

	Coded levels	
Block	A	B
Block 1	-1	-1
	1	-1
	-1	1
	1	1
	0	0
	0	0
	0	0
Block 2	0	0
	-2	0
	2	0
	0	-2
	0	2
	0	0
	0	0

Models used in the optimising phase of study are as follows:

Model 1: Yeast extract composite chapaties

	-2	-1	0	1	2
YEP L % (A) =	0.5	1.0	1.5	2.0	2.5
NaCl added % (B)=	0.0	0.2	0.4	0.6	0.8

Response (Y)= 1. Taste   11. Texture hardness

a. Instrumental measurements

Model 2: Yeast extract composite chapaties

	-2	-1	0	1	2
YEP 77 % (A) =	1.0	1.5	2.0	2.5	3.0
NaCl added % (B)=	0.0	0.2	0.4	0.6	0.8

Response (Y)= 1. Taste   11. Texture hardness

a. Instrumental measurements

Composite flour for chapati production: In preliminary studies, composite flours were prepared by blending flours from single wheat varieties; Fresco and Galahad in ratios- 20:80, 30:70, 35:65, 40:60 and 80:20; Mercia and Galahad- 20:80, 30:70, 40:60, 50:50 and 60:40. Formulations for optimisation phase are given in Table 2.5.

Table 2.5: Formulations for British wheat composite flour chapaties (based on Table 2.4).

Fresco +	Galahad	Mercia +	Galahad
%	%	%	%
25	75	38	62
40	60	50	50
20	80	30	70
33	67	42	58
30	70	40	60
13	87	25	75
42	58	50	50
38	62	50	50
25	75	33	67

Response (Y)= Texture hardness

a. Sensory evaluation

b. Instrumental measurement

Data of these combinations were analysed by the multiple regression technique using Minitab and quadratic curves of fitted and raw data were drawn. N.line and R.line were denoted as normal line (raw values) and regression line (fitted values), respectively.

3. FOOD PREFERENCE SURVEYS

3.1 Savoury flavours preference and unleavened flat bread traits.

The objective of these studies was to ascertain preferences for savoury flavours and determine the principal quality characteristics in chapati. Members of the Pakistani community in Glasgow were compared with consumers in Pakistan. In both these surveys, the underlying objective was to identify suitable flavour products that could be used to enhance the nutritional value of chapati.

3.2 Survey.1: Glasgow

3.2.1 Results

Limitations of study: Respondents were reluctant to complete questionnaires relating to a broad range of savoury flavours. Moreover, it was difficult for respondents to understand the basis of quality characters for products in the absence of prototypes. Structured questionnaires left limited possibility for respondents to describe in depth individual opinions.

Response rate: Initially 140 subjects were selected. Ten surveys could not be conducted due to lack of interest by the consumers. Four questionnaires were not returned. Thus 126 questionnaires were received as completed. The dietary section of six questionnaires was incomplete or contained obviously erroneous data. A further five questionnaires did not contained adequate answers to questions regarding origin, sex or marital status. A total of 115 questionnaires was therefore analysed, representing an effective response rate of 82 %.

Demographic characteristics: Demographic characteristics such as age, sex, marital status and children in a family are shown in Table 3.1.

Consumption pattern: Consumption patterns of respondents are shown in Table 3.2. Most males stayed away from home in connection with employment and consumed foods available at the workplace in the middle of the day.

Table 3.1: Demographic characteristics of consumers surveyed in Glasgow.

Variables	Description
Origin	Pakistani
Sex	
Male	67 %
Female	33 %
Age	
Less than 25 years	14 %
26 - 40 years	72 %
41 - 60 years	13 %
More than 60 years	1 %
Residence in Glasgow	
Minimum	1 week
Maximum	38 years
Median	3 years
Marital status	
Married	86 %
Unmarried	13 %
Widow	1 %
No of children	
Minimum	1
Maximum	7
Median	3

Table 3.2: Consumption pattern for chapati of respondents of Glasgow.

Variables	Description
Chapati prepared/bought	
Bought	7 %
Prepared from wheat flour	70 %
Both	23 %
No of times chapati consumed in a day	
Three	34 %
Two	39 %
One	27 %
Flavour preference	
Savoury	97 %
Sweet	3 %

Adult preference regarding savoury flavours (Fig. 3.1): It was evident (Table 3.2) that 97 % of respondents preferred a range of savoury flavours, significantly different from each other (P<0.05) (Table 3.3).

Children preference regarding savoury flavours: For children (Fig. 3.1), mean scores ranged from 2.12 (cheese) to 3.93 (chicken). The order of preference was largely similar for adults and children but ratings differed significantly (P<0.05) (Table 3.3) .

Quality in chapati: Consumers showed interest in the improvement of chapati quality, scores of quality traits ranged from 3.41 to 4.33. Fig. 3.2 shows ranking of quality factors for chapaties. Mean scores differed significantly at 5 % (Table 3.3).

Table 3.3: Analyses of variance for Glasgow survey

Source	F Value	P Value
Adults v Children preference	17.84	0.00
Savoury flavours	68.51	0.00
Q. attributes	80.05	0.00
Male v Female preference	0.00	1.00
Savoury flavours	45.40	0.00
Male v Female preference	0.25	0.62
Q. attributes	39.74	0.00
Age groups preference	2.67	0.05
Savoury flavours	27.37	0.00
Age groups preference	3.25	0.04
Q.attributes	33.13	0.00

Q = Quality

Male v female preference: From male-female preference data, differences were found in savoury flavours ranking between the sex groups (Fig. 3.3). Rankings of flavours were in general similar. However, chicken and meat flavours were ranked highest by both groups.

Other differences pertaining to chapati quality characteristics and price consciousness of the product were also examined (Fig. 3.4). Male respondents were more conscious of nutritional value than female. However both groups gave high rankings for overall taste and desirable flavour in chapati. The overall difference in preference between the sexes was found to be non-significant (Table 3.3).

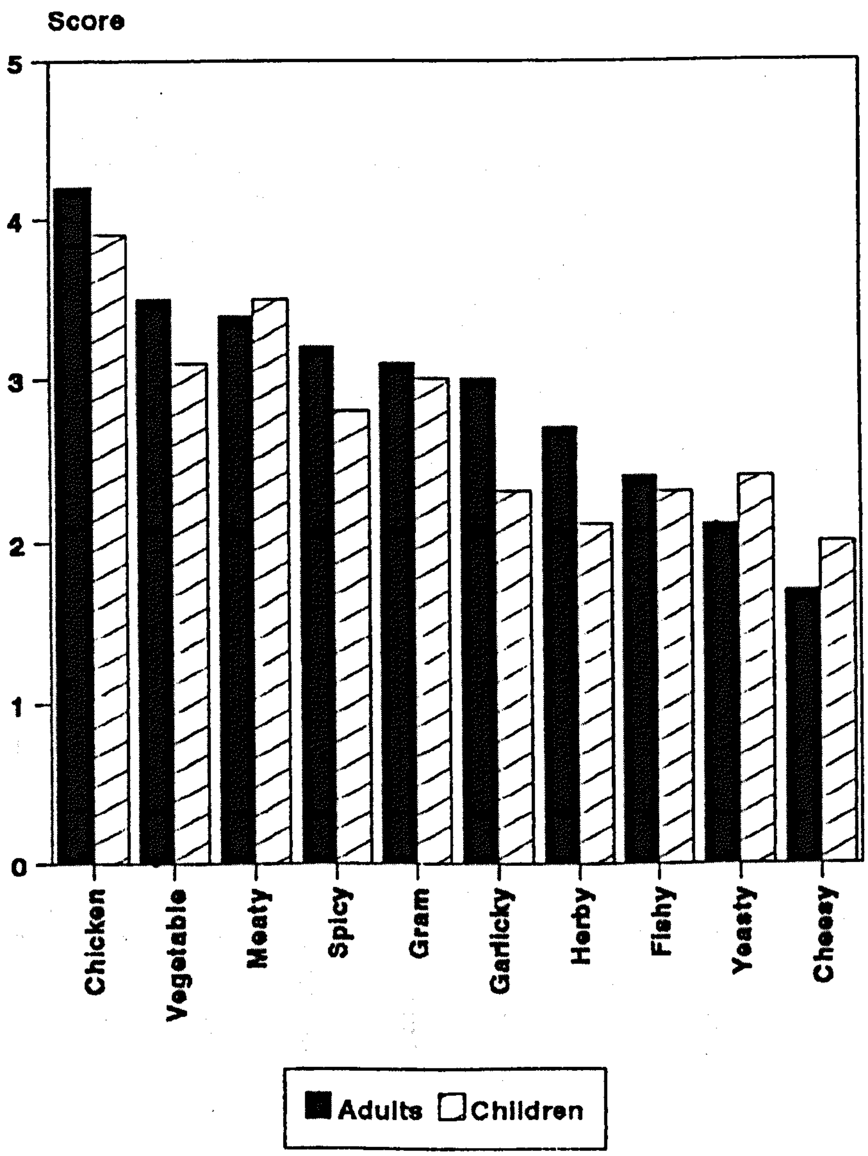

Fig. 3.1 : Adults and children preference regarding savoury flavours (Glasgow).

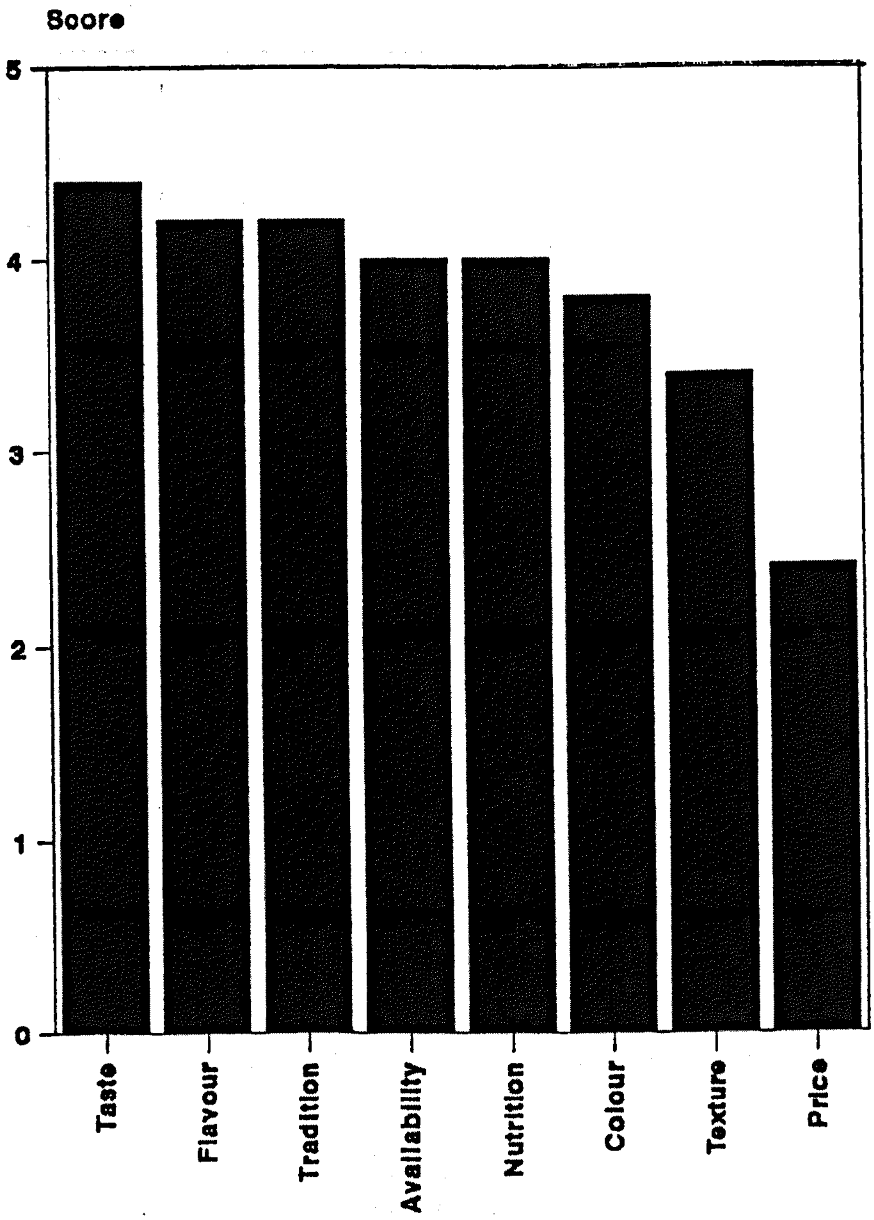

Fig. 3.2: Consumer importance in relation to quality characteristics of chapati (Glasgow).

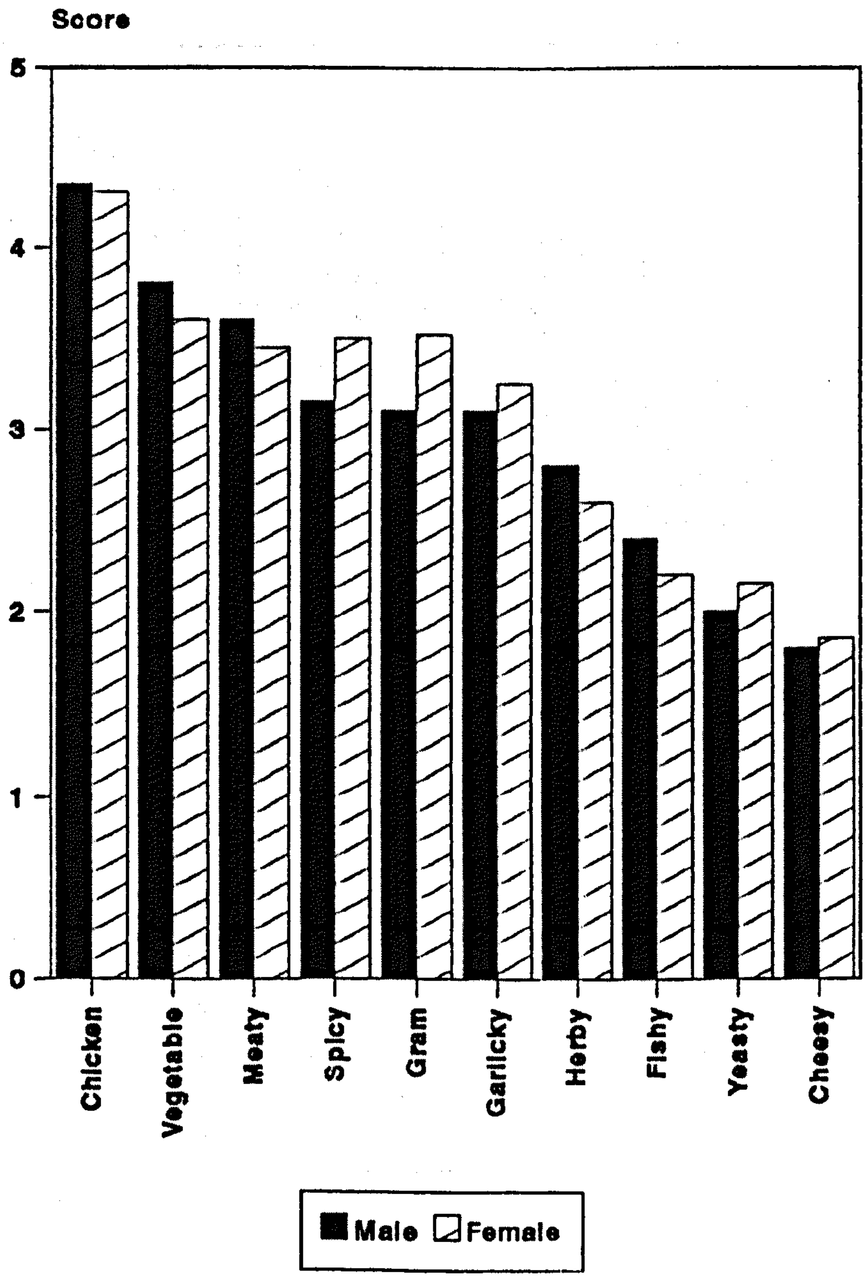

Fig. 3.3: Male V female preference regarding savoury flavours (Glasgow).

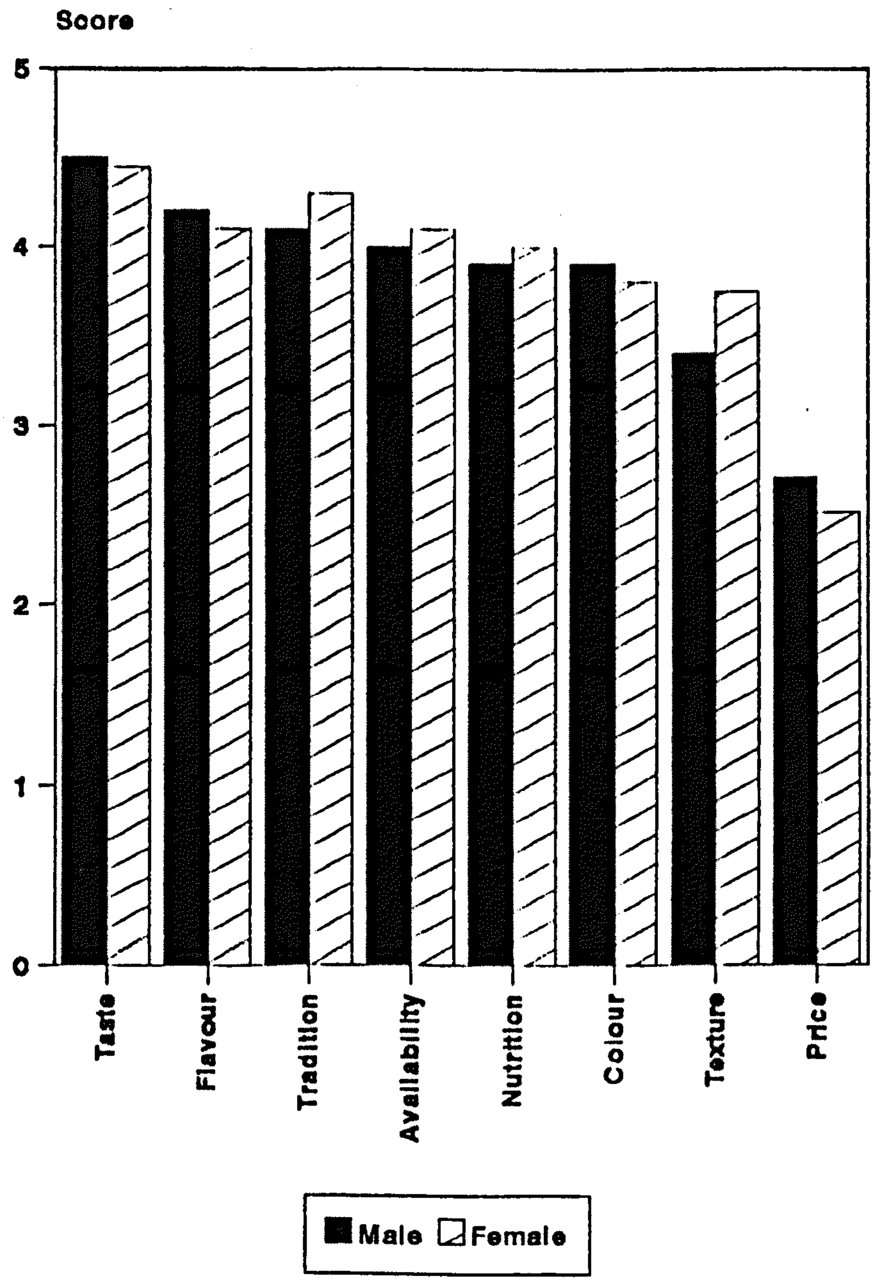

Fig. 3.4: Male V female ranking regarding importance of quality attributes of chapati (Glasgow).

Effect of age on preference: Chicken flavour was equally favoured by all age groups (Fig 3.5). Younger adults preferred "gram" flavoured products such as pakora and preference for these increased with age. However, differences of opinion in the selection of flavours like spicy, vegetables, meaty, garlicky and herby were observed (Table 3.3).

In selection of quality characteristics of chapati, young respondents ranked overall taste and flavour more highly than other factors (Fig. 3.6). Analysis of variance (Table 3.3) showed significant effects of age on preference ($P<0.05$) of respondents.

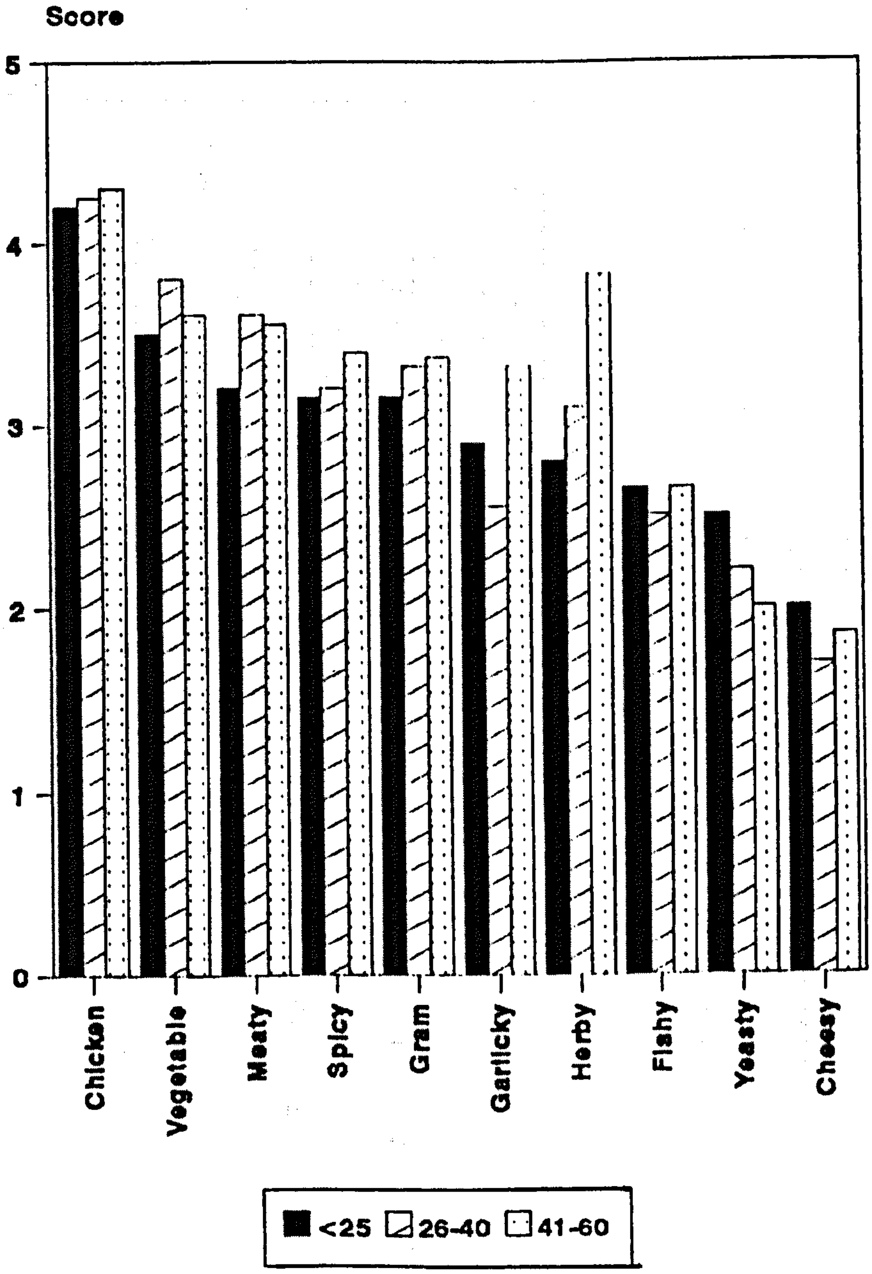

Fig. 3.5: Effect of age on preference of savoury flavours (Glasgow).

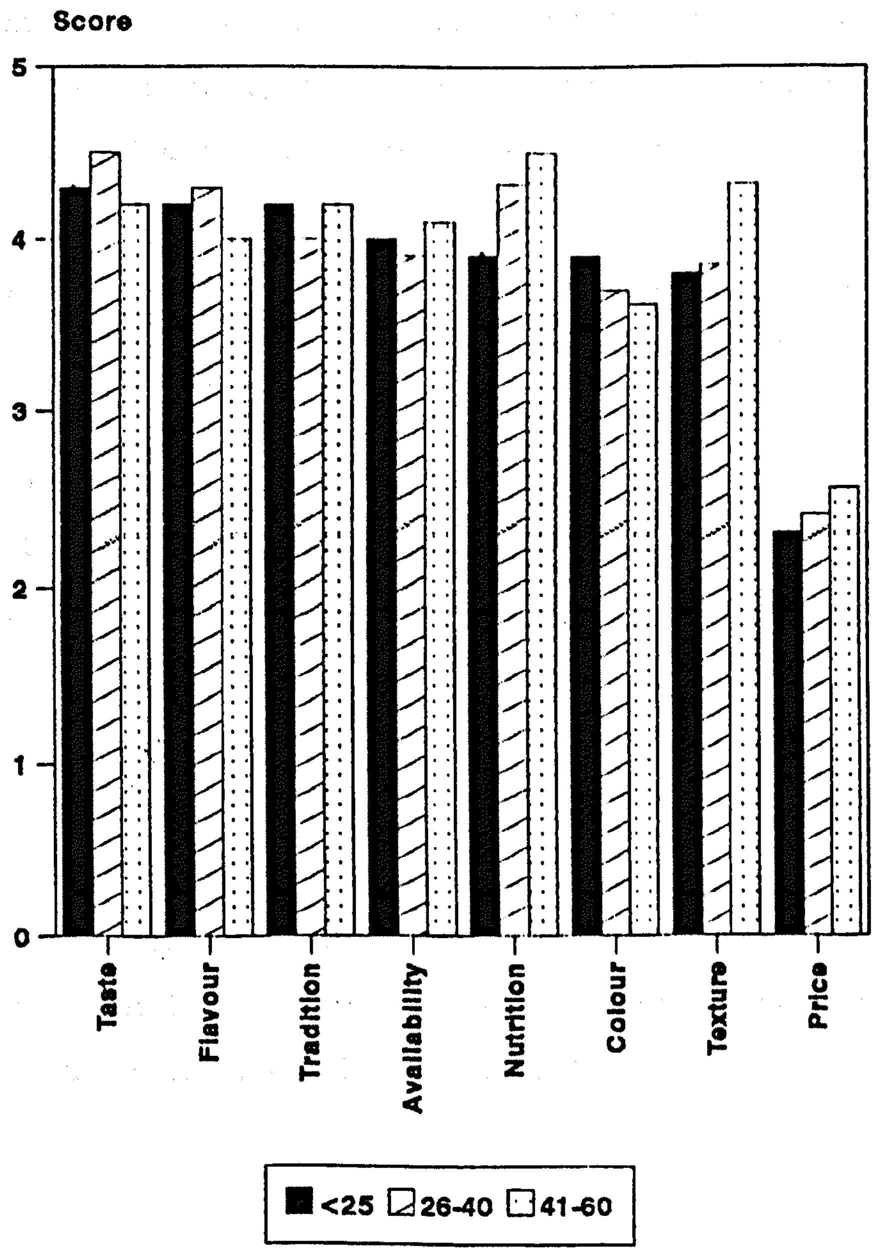

Fig. 3.6: Effect of age on importance of quality characters of chapati (Glasgow).

3.3 Survey. 2: Pakistan

3.3.1 Results

Response rate: A total of 140 replies from 225, were received from which 126 questionnaires were considered suitable for analysis. This represented a total response of 62 % and effective response rate of 56 %.

Demographic characteristics: The majority of respondents (88 %) belonged to urban areas in various parts of Pakistan. Of the respondents 92 % were male: the majority (75 %) of respondents were in the age group 26 - 40 year old, the most populous segment of Pakistani society(Table 3.4).

Consumption pattern: Almost 69 % of respondents prepared chapaties at home (Table 3.5) and 21 % used either ready prepared chapaties or purchased at point of consumption. Of the respondents 50 % only procured wheat grain as raw material while 36 % purchased commercial chapati flour. Consumption of wheat flour was 200 to 325 g day^{-1}.

Table 3.4: Demographic characteristics: Pakistan.

variable	Description
Origin	Pakistan
Urban area	88 %
Rural area	12 %
Sex	
Male	92 %
Female	08 %
Age	
Less than 25 years	04 %
26 - 44 years	75 %
41 - 60 years	18 %
More than 60 years	03 %
Marital status	
Married	77 %
Unmarried	21.4 %
Widow	1.6 %
Children (number)	
Minimum	01
Maximum	10
Median	5

Table 3.5: Consumption pattern of chapati by sample population in Pakistan.

Variable	Description
Chapati	
Bought	10 %
Prepared	69 %
Both	21 %
Flour	
Bought	36 %
Prepared from wheat	50 %
Both	14 %
Daily consumption of wheat flour in the form of chapati	
Maximum	325 g/person
Minimum	200 g/person
Frequency for consumption of chapati	
Three times per day	62 %
Two times per day	33 %
One times per day	05 %
Flavour preference	
Savoury	74 %
Sweet	17 %
Both	09 %

Occupation and education: In this study, 50 % respondents had postgraduate qualification followed by bachelor, secondary, primary and intermediate education (Fig. 3.7). The largest proportion of respondents was engaged in teaching followed by clerical jobs and business. The residue consisted of others: engineering, agriculture, unskilled workers, pensioners, students and doctors (Fig. 3.8).

Adult preference regarding savoury flavours: The majority of people ranked savoury flavour highest (Table 3.4). Comparison of mean scores of savoury flavours (Fig. 3.9) revealed that meaty and chicken were ranked highest. Cheesy flavour was rejected indicating disinterest in milk products (LSD 0.4). Analysis of variance (Table 3.6) showed significant difference among savoury flavours ($P<0.05$).

Preference of children for savoury flavours (Fig. 3.9): Ranking by children was similar to that for adults (Table 3.6). There were small differences but these were non significant. Chicken flavour was ranked highest followed by meat. No significant difference was observed between adults and children preference (Table 3.6).

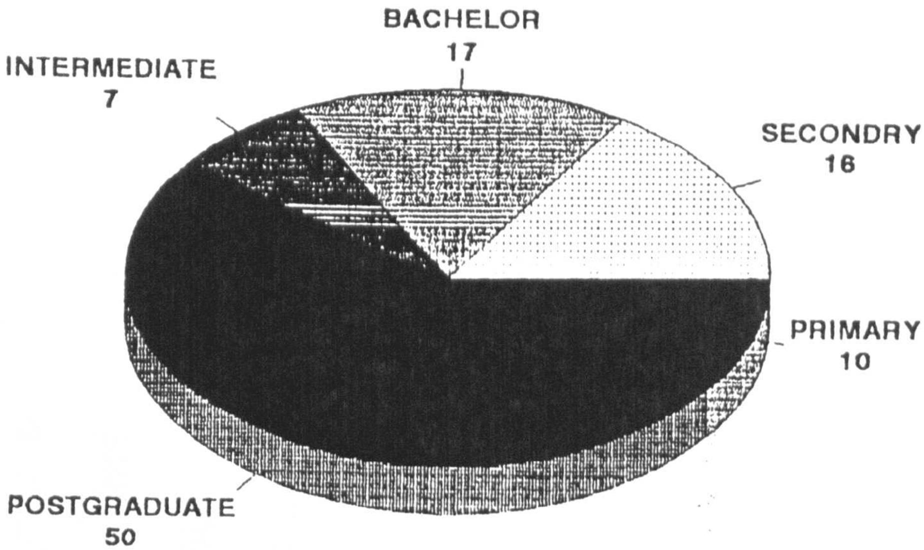

Fig. 3.7: Education level of respondents of Pakistan.

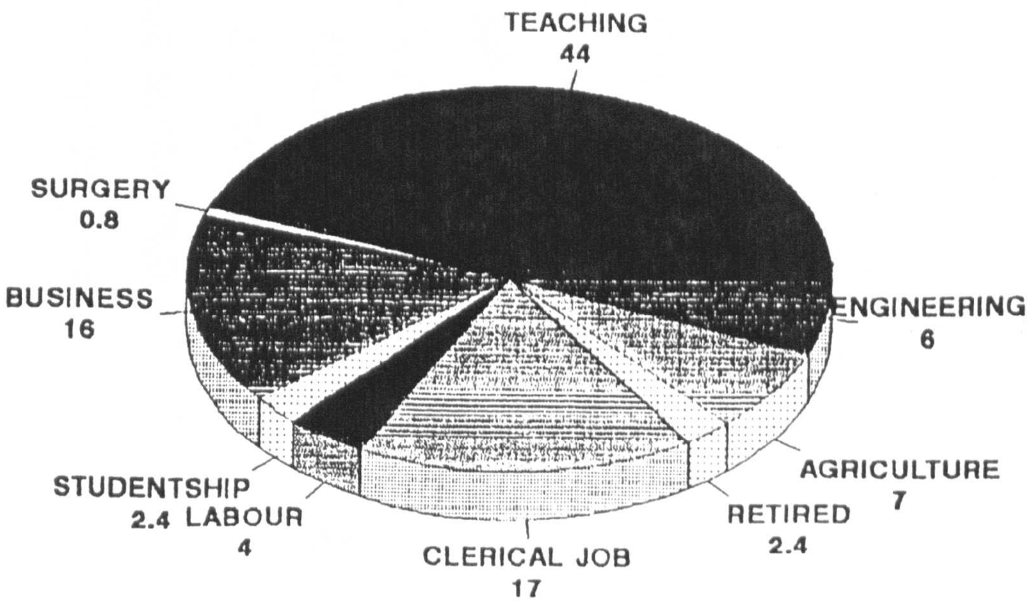

Fig. 3.8: Occupation of respondents of Pakistan.

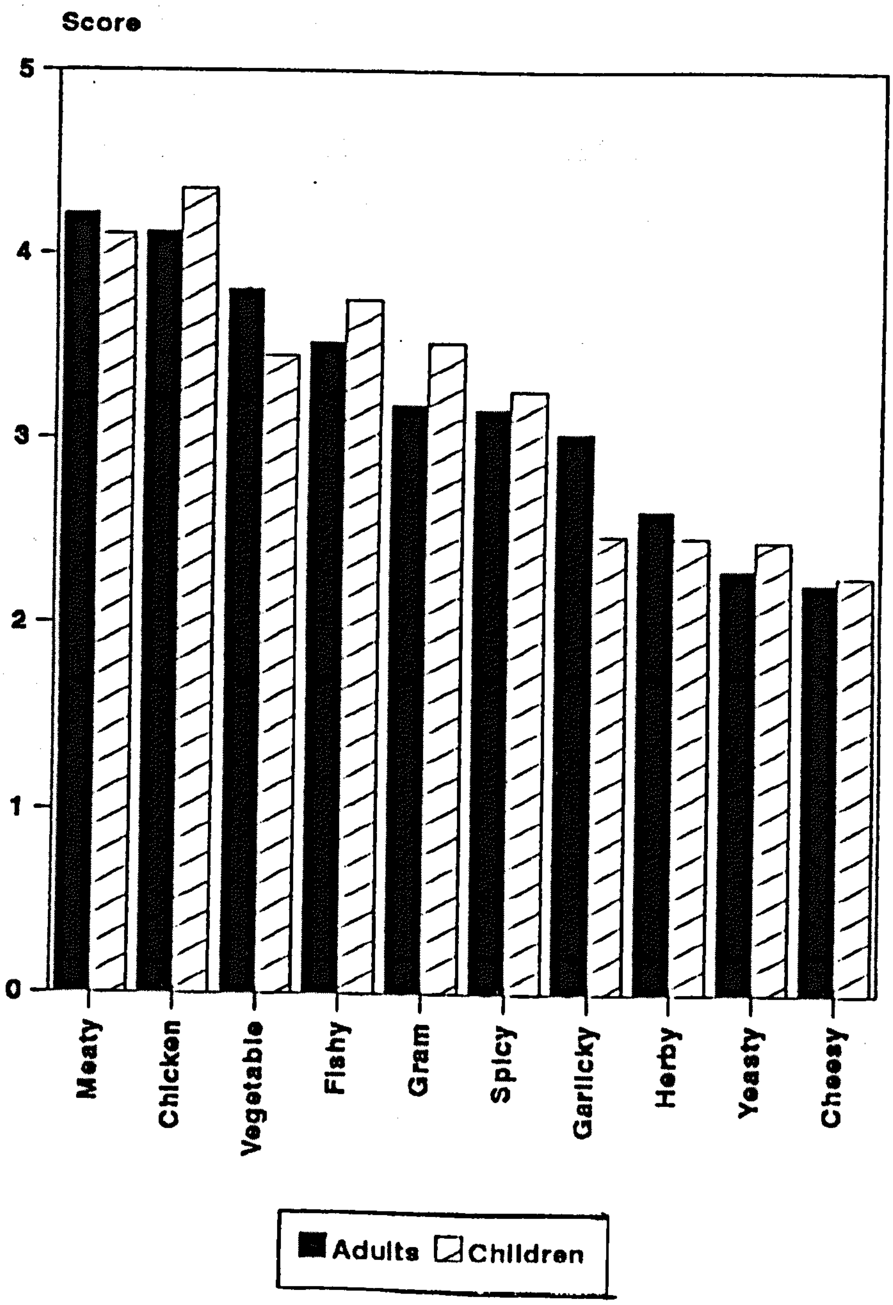

Fig. 3.9: Adults and children preference regarding savoury flavours (Pakistan).

Quality factors in chapati: Nutritional quality obtained a mean score 4.5, the highest recorded (Fig 3.10).
As previously, respondents gave the lowest importance to price. Quality attributes were found to be differ significantly ($P<0.05$) to each other.

Male v female selection: No significant differences were found to exist in the selection of savoury flavours between the two sexes (Table 3.6 & Fig.3.11). Relative difference in quality characteristics and price consciousness were also investigated (Fig. 3.12). Both sexes gave equal ranking to nutritional value. However differences in selection of colour, availability and price of product were non-significant at 5 % level. Tasty, flavoured and soft texture products were favoured by both groups.

Table 3.6: Analyses of variance for Pakistan survey

Source	F Value	P Value
Adults v Children preference	0.02	0.88
Savoury flavours	87.03	0.00
Q. attributes	74.38	0.00
Sexes preference	0.27	0.61
Savoury flavours	13.44	0.00
Sexes preference	2.72	0.10
Q.attributes	11.14	0.00
Age groups preference	5.63	0.00
Savoury flavours	18.74	0.00
Age groups preference	3.66	0.03
Q.attributes	8.75	0.00
Income groups preference	7.82	0.00
Savoury flavours	43.20	0.00
Income groups preference	0.94	0.47
Q.attributes	33.00	0.00

Q = Quality

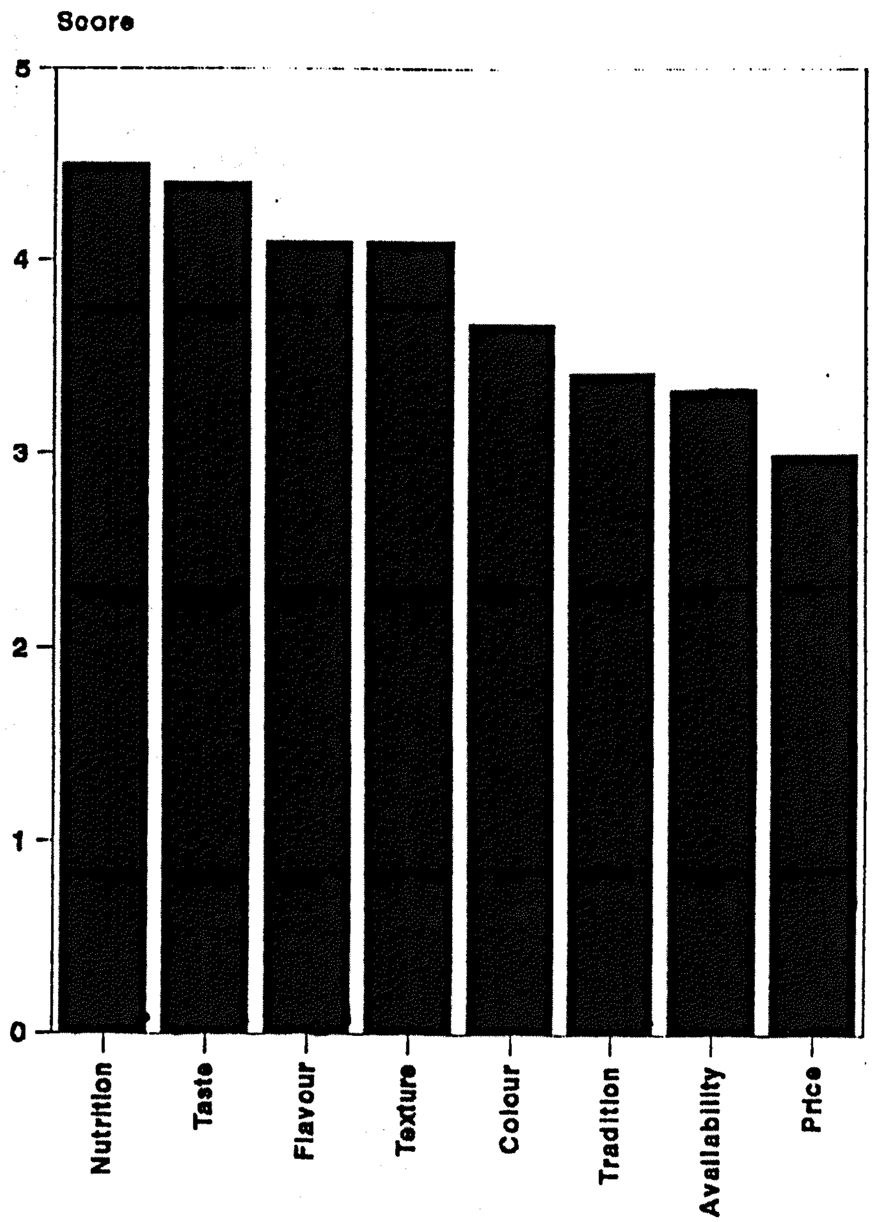

Fig. 3.10: Consumer importance regarding quality of product (Pakistan).

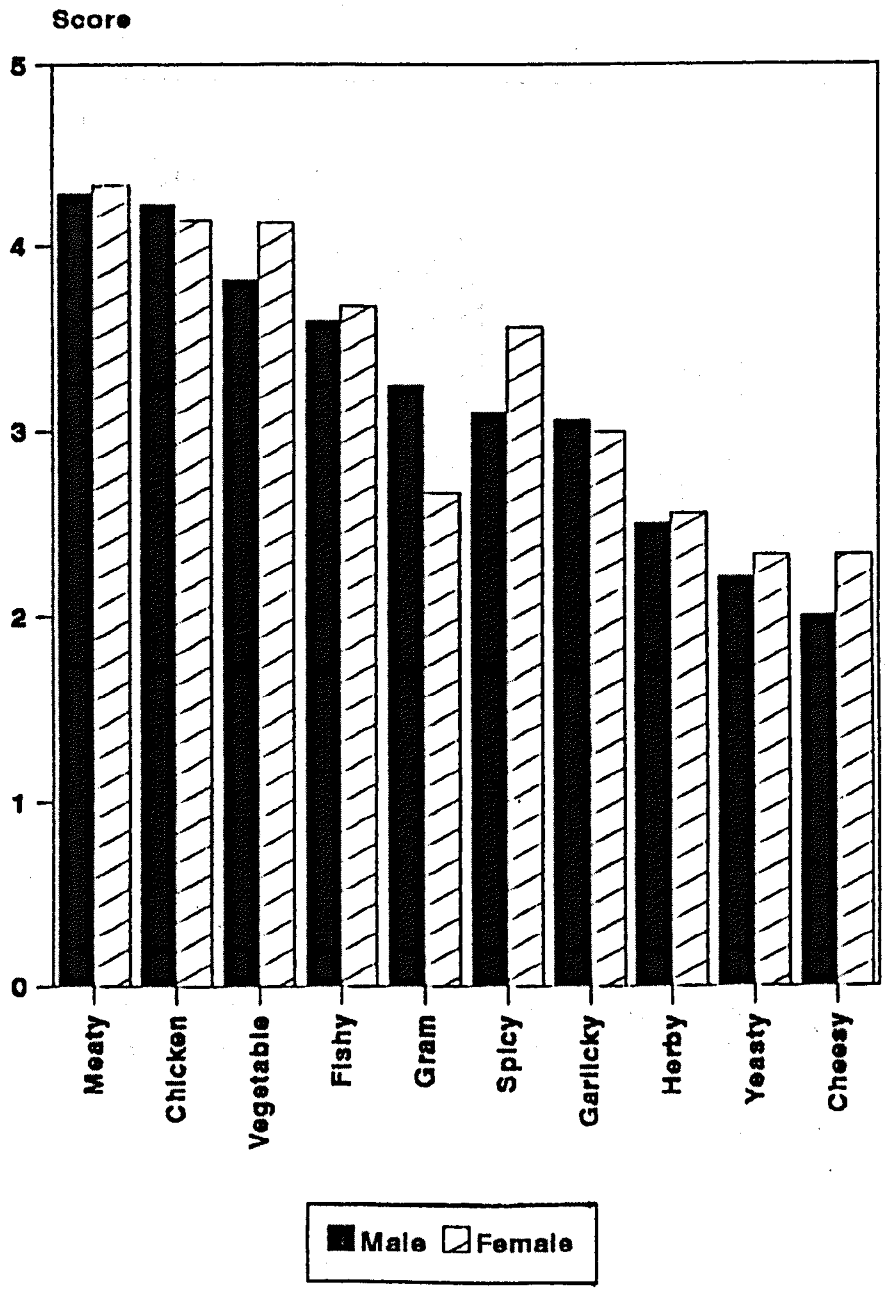

Fig. 3.11: Male V female preference regarding savoury flavours (Pakistan).

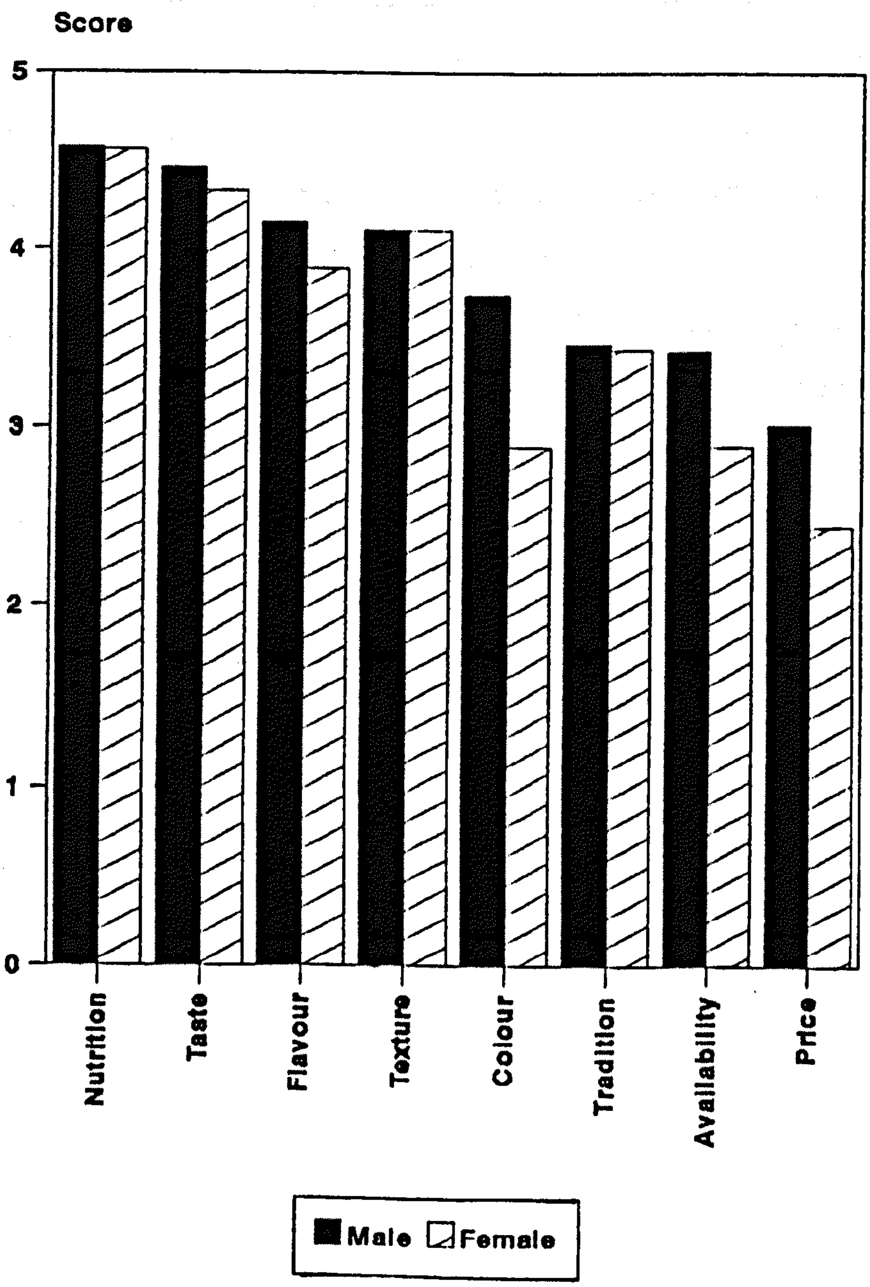

Fig. 3.12: Male V female importance regarding quality of product (Pakistan).

Effect of age on consumer preference for savoury flavours: "Meaty" and "chicken" flavours had higher score for preference from groups >60 years, followed by 41 - 60, and under 25 - 40 years (Fig. 3.13). Flavoured products such as pakora were more popular among the older (>60) people than younger. People >40 year were more likely to use garlic in cuisines. However, significant (P<0.05) differences of opinion in the selection of savoury flavours were observed (Table 3.6).

Similar trends were observed in the selection of quality traits of chapati (Fig. 3.14). Nutrition was an equally important factor to all age groups and price was considered least important by all groups.

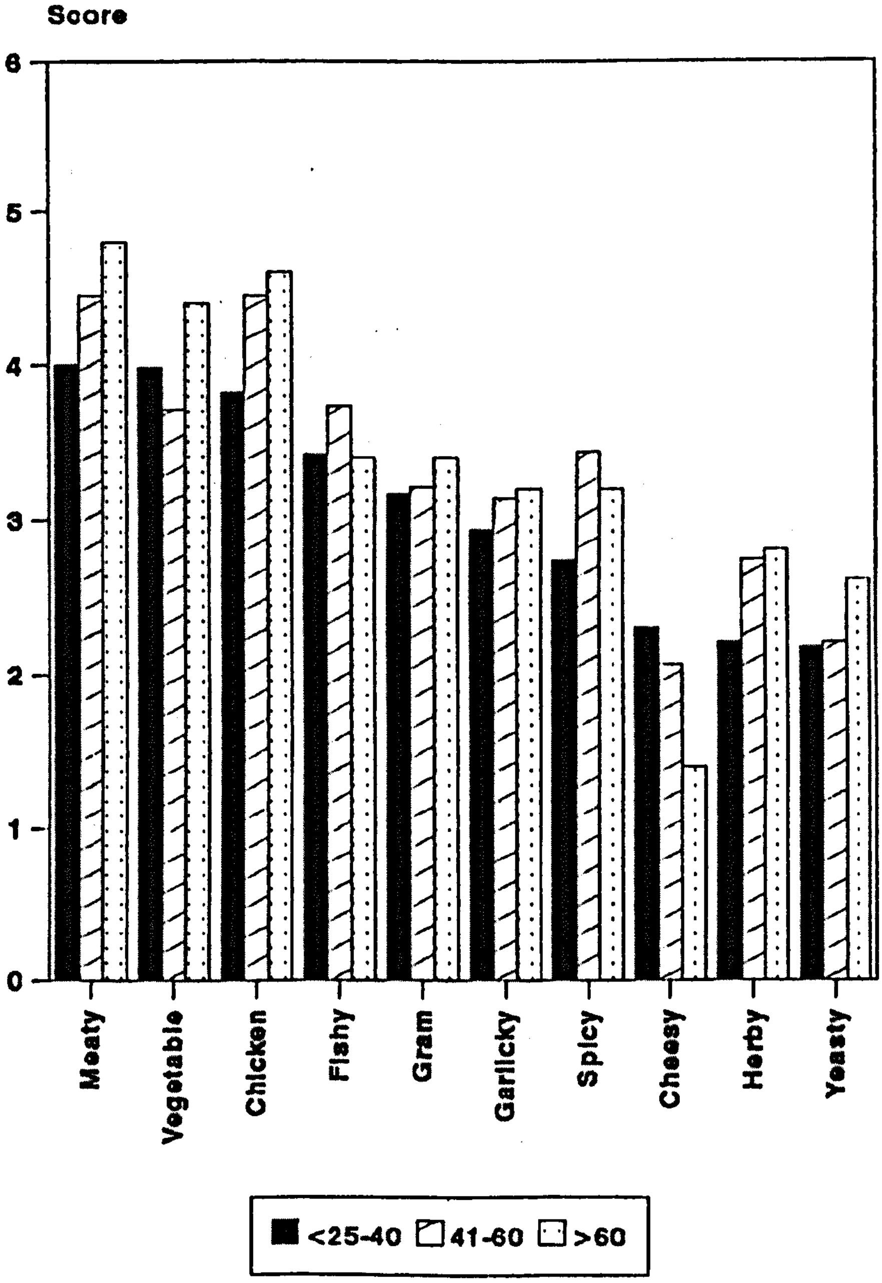

Fig. 3.13: Consumer preference (age effect) regarding savoury flavours (Pakistan).

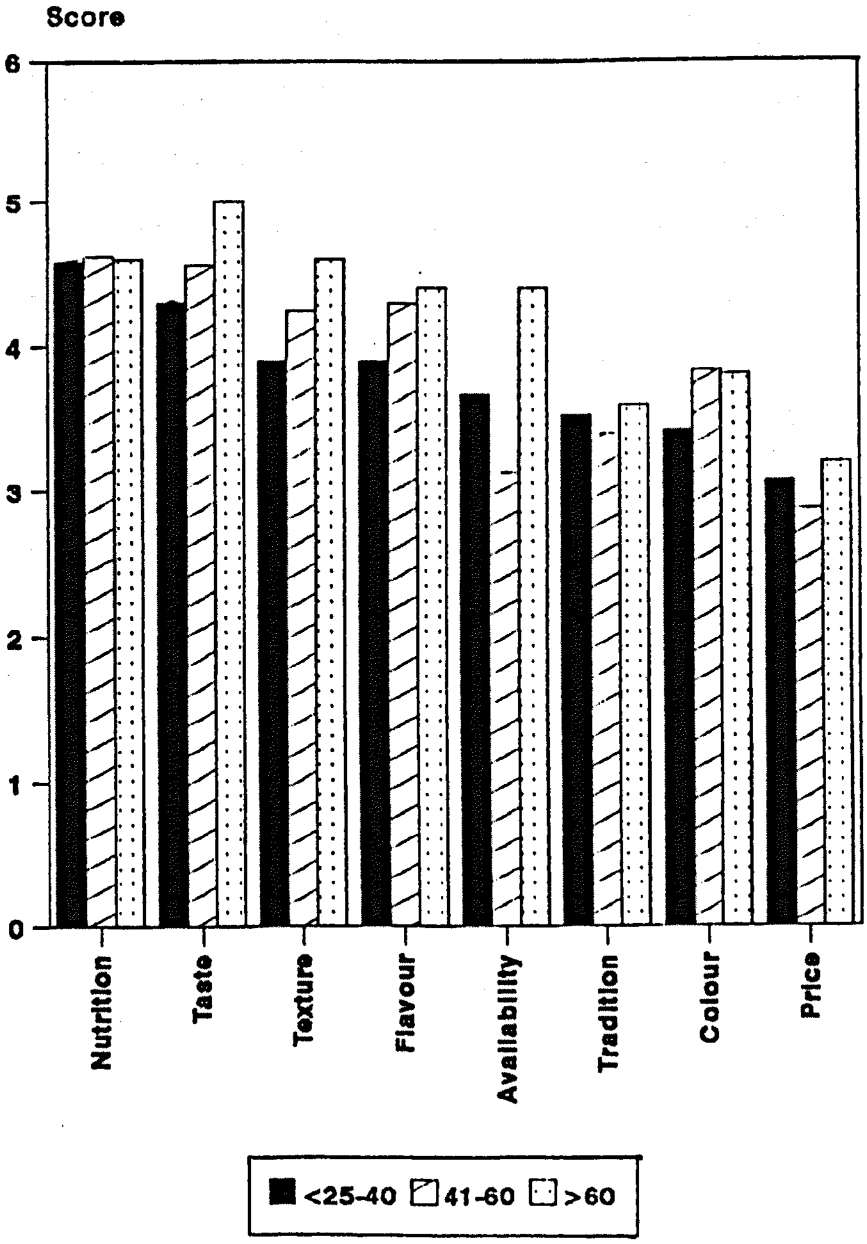

Fig. 3.14: Consumer importance (age effect) regarding quality of product (Pakistan).

Effect of income on preference: Upper and middle income groups showed a preference for "chicken" flavour, ranked less highly by low income groups (Fig.3.15). Fish was given higher ranking by middle than upper group, but "spicy" flavour had a higher preference for high followed by middle incomes. Garlic was used more by middle than by upper income groups whereas vegetable was preferred by upper and followed by middle incomes. Significant difference was observed between preference of groups ($P<0.05$) in relation to savoury flavours.

Nutrition was equally important for all groups but upper income group gave higher ratings for taste, flavour, colour and texture of the product whereas low income group was more sensitive to product availability at low price (Fig. 3.16). Differences between groups were non-significant (Table 3.6).

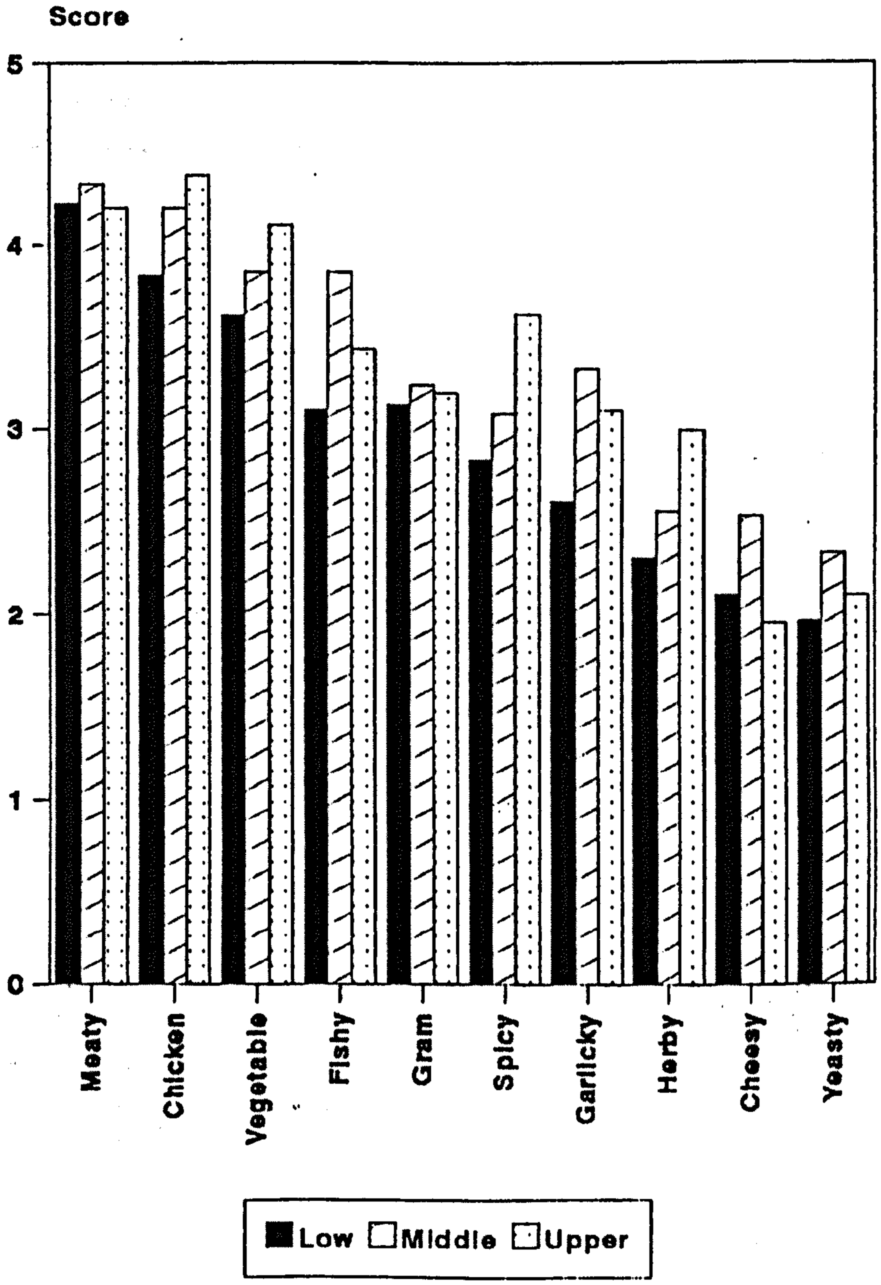

Fig. 3.15: Effect of income on consumer preference of savoury flavours (Pakistan).

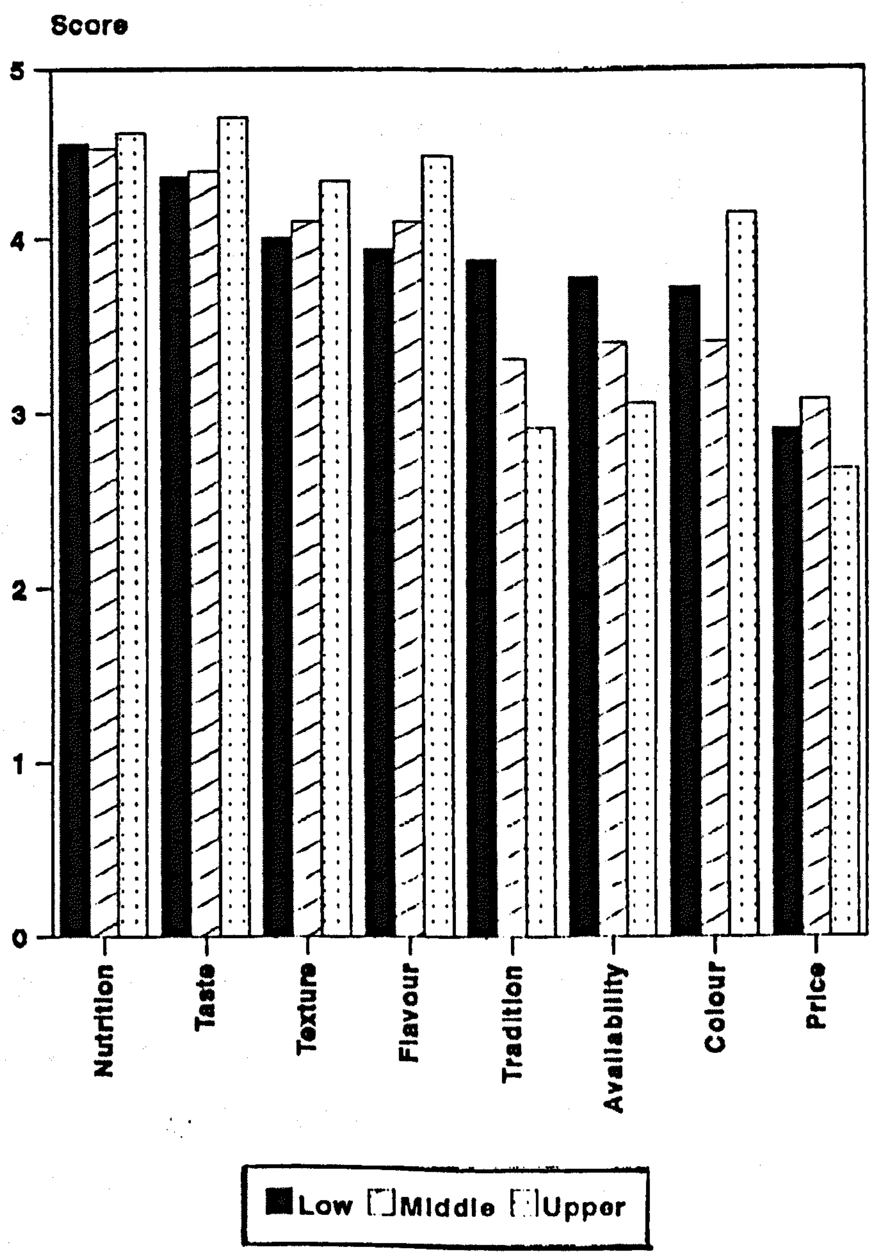

Fig. 3.16: Effect of income on consumer importance for chapati quality factors (Pakistan).

3.4 Discussion

These two surveys suggested that there was an interest in improving the nutritional value of chapaties by addition of suitable yeast extracts. This could be achieved if imparting savouriness did not reduce its quality characteristics; enhanced consumer appeal depends on acceptance by consumers, otherwise the most nutritious food is of no value to the malnourished.

The quality characters of chapati were found to be significantly different ($P<0.05$) in both studies. In both surveys, nutritional value was ranked the highest. Supplementing wheat flour with protein has a potential for low income people of Pakistan for economic alleviation of protein deficiency. The protein quality of a vegetarian diet can be improved by properly combining protein from different sources. A supplemented flour for chapati production could readily find a role in the diet of the target population without changing food habits (Bass and Caul, 1972; Milner, 1974).

Availability and price were found to be of lesser importance to those population samples than core beliefs, values and customs passed to individuals from parents and grand-parents. These are more important influences on purchase and consumption behaviour.

Texture is also an important quality factor used by consumers to assess acceptability. This has gained new importance for consumers because attitudes toward texture are shaped by physiological and psychological factors, socially and culturally learned expectations, sex, socio-economic group and eating occasion (Szczesniak, 1990). Changes in texture also alter flavour perception and sometimes, appearance (Szczesniak and Kahn, 1971).

Demographic characteristics are most often used as the basis for market segmentation of consumers. Such factors are generally easy to identify and quantify. Furthermore, they can often be associated either singly, or in composite, with usage of specific products. The typical Pakistani consumer eats chapaties three times a day. This unleavened bread was found to be a staple food both for Pakistani consumers living in Pakistan and in the UK. Per capita daily consumption of wheat in the form of chapaties by people of Pakistan was 342 g (Govt of Pakistan, 1993) and similar results were obtained by Khan and Eggum (1978). Carlson and Kipps (1983) concluded that habits are related to family, cultural and religious values and rituals.

Consumers were willing to a accept wide range of savoury flavours, but with significantly different hedonic ratings ($P<0.05$). Chapaties are normally consumed with savoury cuisines, a habit retained in the new domicile (UK). Savoury cuisines in Pakistan comprise meat, fish, eggs, vegetables and pulses in solid and semisolid forms. Chicken and meaty flavours obtained higher acceptability ranking. Similar flavours could be achieved by inclusion of yeast extracts (Best, 1992; Maase, 1991; Nagodawithana,

1992) at low cost, resulting in economical products. Being of vegetable origin, yeast extracts are acceptable to consumers of varied ethnic groups (Binsted and Devey, 1970) and are natural bakery products (Lyall, 1970; Peppler, 1982). Connotations of naturalness also strengthens product image (Hough and Maddox, 1970). Cheese flavour received the lowest ranking exhibiting the lack of interest of the Pakistani consumers in milk products. Rennin is used in the production of cheese, which is unlawful in the opinion of people of certain religious beliefs (Naele et al., 1993). Other influences on eating habits include cultural factors.

Children preference in relation to savoury flavours were found to be significantly ($P<0.05$) different from their parents in the Glasgow unlike to those living in Pakistan. This might be due to the effects of environment, changing family lifestyle and exposure to different foods. Schultz, (1974) reported many interacting factors that affect attitude and acceptance of food product such as geographical area, family lifestyle, ethnic groups, peers and teachers.

Gender is thought to account for differences in psychological factors between sexes (Douglas, 1977; Hamburg and Lunde, 1986). Male-female data revealed no significant difference in the preference for savoury flavours between the consumer groups. Chicken and meaty flavours were found to be popular. Although male consumers gave more weighting to nutritional value, taste and flavour was rated highly by both groups. Baker (1990) concluded that consumer pressures are currently toward foods offering more desirable flavour and textural features.

Since product needs often vary in relation to age, marketers have also found this to be a useful variable in defining market segments. Many products may obtain a place in the market by concentrating on consumers in a specific age range. People between the ages 41 and 60 and >60 years considered use of garlic important in their cuisine. The role of garlic in Asian dishes may relate to a perceived medicinal value in the reducing of blood cholesterol level. This is thought to minimize the chances of heart attack.

Age had a significant effect on the preference of savoury flavours and quality traits in the products. Younger people are considered more sensitive to taste and smell than older and were less price conscious (Frye et al., 1990). This does not mean that high prices for new products will be acceptable. Thus, the need to improve the quality of a food product is often tempered by the need to keep prices at competitive levels whilst still generating reasonable profits (Baker et al., 1988). Concern for physical fitness and health-related factors have contributed to a shift from "tasty" for preference for soft texture and a nutritious diet in the older age group. It was extrapolated that children had a higher preference for yeast-leavened foods such as white and pita breads and hot dog rolls than adults.

Level of education is rising in parallel with income in Pakistan. Composition of the work force has changed as the level of education has risen. Social classes vary in terms of values, product preference and buying habits: richer people have greater choice in foods than poor.

The main reasons of success of snacks and fast foods can be related to flavour, texture and appearance of the product in addition to ease of preparation and convenience (Guadagni and Venstrom, 1972; Baker, 1990). Preference within savoury flavours was significantly ($P<0.05$) affected by income but the quality characters of chapati were equally important for all income groups of consumer in Pakistan ($P>0.05$). Thus, changes in consumer preferences are generating an interest in new flavouring systems and methods of processing for ethnic foods (Yaylayan 1991). This market is expected to expand 25 % in the next 5 years (Mintel, 1989). The conclusion of the surveys was that consumers favour a cheaper product with good taste, appealing flavour, a soft texture and high nutritional value.

4. SUITABILITY OF WHEAT VARIETAL FLOURS FOR PRODUCTION OF CHAPATIES

4.1 Quality factors in commercial chapati flours for production of chapaties

4.1.1 Objective

These studies were conducted to choose reference chapati flour out of samples of commercial chapati flours by determining compositional, sensory, physical and rheological characteristics.

4.1.2 Results

Flour samples were analysed for moisture, protein content, ash and damaged starch (Table 4.1).

Protein contents were found to have significant ($P<0.01$) correlations with water absorption ($r = 0.98$), peak time ($r = 0.99$), R/E at 30 min ($r = 0.97$) and at 60 min ($r = 0.99$) periods of rest but was less significant ($r = 0.94$, $P = 0.05$) with dough development time.

Highest ash content (1.52 %) was observed in Mehrani brown and lowest (0.72 %) in Mehrani white. Ash had a significant ($P<0.05$) correlation ($r = 0.99$) with flour colour.

Damaged starch ranged between 6.97 % and 10.66 %. There were significant correlations ($r = -0.92$, $P<0.05$) with particle size (130 - 180 μm) of flour, DD ($r = -0.98$), extensibility (at 60 min $r=0.96$) and R/E (at 60 min $r = -0.99$). Also, strong correlations were observed with water absorption (r = -0.94), dough stability (r = -0.93), tolerance index (r = -0.84), resistance to extension (at 60 min r = -0.93), extensibility (at 30 min r = 0.94) and R/E ratio (at 30 min r = -0.92).

Table 4.1: Chemical analysis of commercial chapati flours.

Flour	Moisture %	Protein % (d.b.)	Ash content % (d.b.)	Damaged starch % (d.b.)
Mehrani White	14.00	12.43	0.72	10.33
Mehrani Medium	14.10	12.46	0.96	10.66
Mehrani Wholemeal	13.80	13.69	1.30	7.38
Mehrani Brown	14.20	14.10	1.52	7.00
F.A. Bird Brown	14.35	11.64	1.45	6.97
S.d.	0.08	0.03	0.06	0.04

d.b. = dry basis
S.d. = Standard deviation (pooled)

Commercial chapati flour samples were analysed for particle size, falling number and grade colour value (Table 4.2) .

Falling numbers had positive correlations with protein content (r = 0.73), water absorption (r = 0.70), dough development (r = 0.79), dough stability (r = 0.86), R/E at 30 min (r = 0.63) and at 60 min (r = 0.66) resting periods of dough. There was also a significant correlation (r = -0.91, $P<0.05$) with tolerance index of dough.

Grade colour values for flours ranged from 10.43 to

>18.00, in relation to bran content. The Mehrani brown and F.A. Bird had the highest colour values (>18.00) while Mehrani white had the lowest (10.43).

Table 4.2: Physical properties of commercial chapati flours.

Flour	Ps >180 µm %	Ps 130-180 µm %	Ps 110-130 µm %	Ps <110 µm %	Falling numbers	Grade colour
Mehrani White	6.03	11.77	23.00	59.10	545	10.43
Mehrani Medium	12.33	9.53	11.90	66.37	508	12.90
Mehrani Wholemeal	16.20	17.77	12.83	52.80	570	17.43
Mehrani Brown	18.93	15.03	9.03	56.96	531	>18.0
F.A. Bird Brown	15.20	17.57	17.57	49.66	365	>18.0
S.d.	0.30	0.20	0.07	0.12	6.29	-

Ps = Particle size S.d. = Standard deviation (pooled)

Rheological studies on commercial chapati flours were conducted using Brabender Farinograph and Brabender Extensograph.

Representative farinograms obtained from commercial chapati flours are shown in Fig. 4.1 and 4.2. The data obtained from such studies have been tabulated in Table 4.3.

Water absorption showed significant ($P<0.05$) correlations with dough development ($r = 0.99$), dough stability ($r = 0.94$), resistance to extension (R) at 60 min (r = 0.93), R/E at 30 (r = 0.93) and R/E at 60 min (r = 0.98) of resting periods of dough. Dough stability showed significant correlations with resistance to extension (r = 0.90) and R/E (r = 0.92) after 60 min resting.

Table 4.3: Farinographic parameters of commercial chapati flours.

Flour	Water absorption (%)	Dough development (min)	Dough stability (min)	Tolerance index (BU)
Mehrani White	57.30	5.25	9.25	55
Mehrani Medium	57.90	5.50	9.25	40
Mehrani Wholemeal	59.00	7.00	16.50	35
Mehrani Brown	59.20	7.00	14.00	45
F.A. Bird Brown	56.80	4.25	4.00	85

BU = Brabender Unit

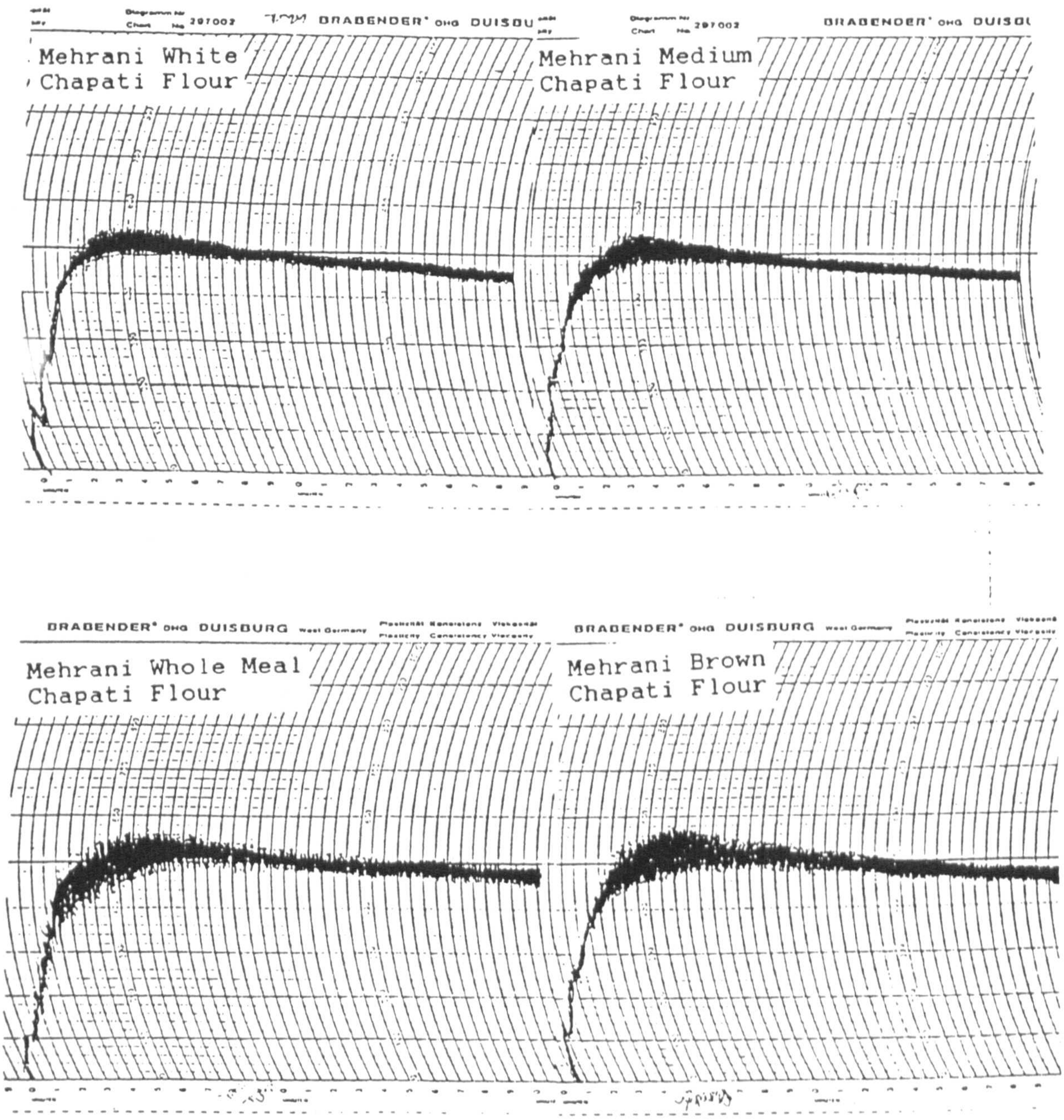

Fig. 4.1: Farinograms of Mehrani chapati flours.

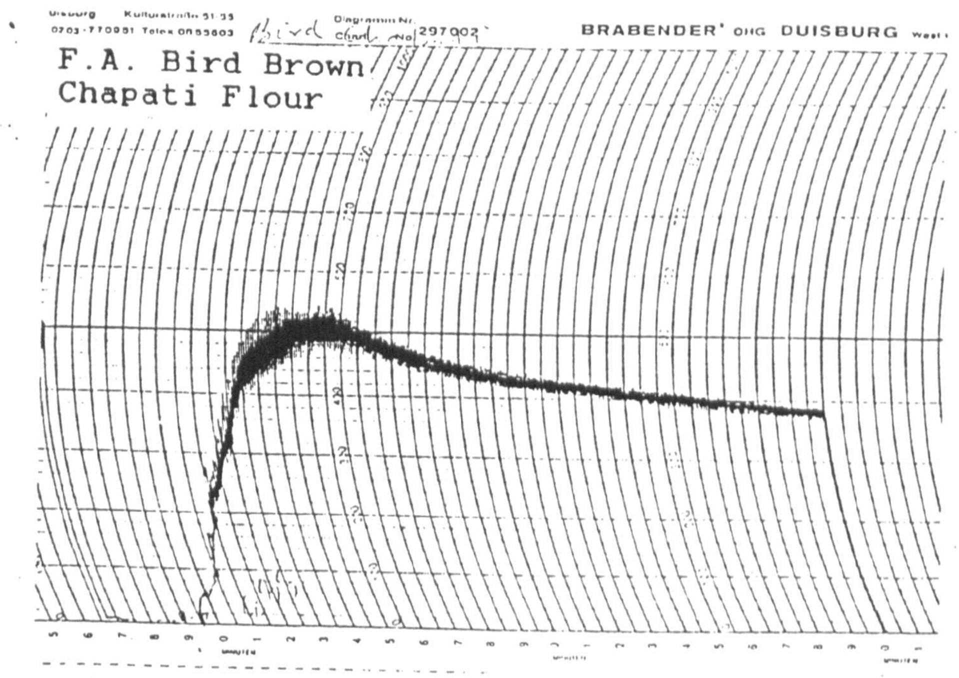

Fig. 4.2: Farinogram of F.A. Bird Brown chapati flour.

Representative extensograms from various chapati flours are depicted in Fig. 4.3 and 4.5. The results are summarised in Table 4.4.

Resistance to extension (R) at 60 min had shown significant correlations ($P<0.05$) with R/E at 30 and 60 min ($r = 0.92$, $r = 0.90$, respectively) and R at 30 min showed significant correlations with R/E at 30 min ($r = 0.89$) and R/E at 60 min ($r = 0.80$) of resting time of dough.

Table 4.4: Extensographic characteristics of commercial chapati flours.

Flour	Res. (EU) 30 min	Res. (EU) 60 min	Ext. (mm) 30 min	Ext. (mm) 60 min	Res/ Ext 30 min	Res/ Ext 60 min
M. White	173	173	132	140	1.31	1.23
M. Medium	155	165	121	128	1.28	1.28
M. Whole-meal	158	210	79	87	2.00	2.40
M. Brown	255	253	90	91	2.80	2.78
F.A. Bird Brown	60	60	77	77	0.78	0.78

M = Mehrani   Res. = Resistance
Ext. = Extensibility
EU = Extensograph unit

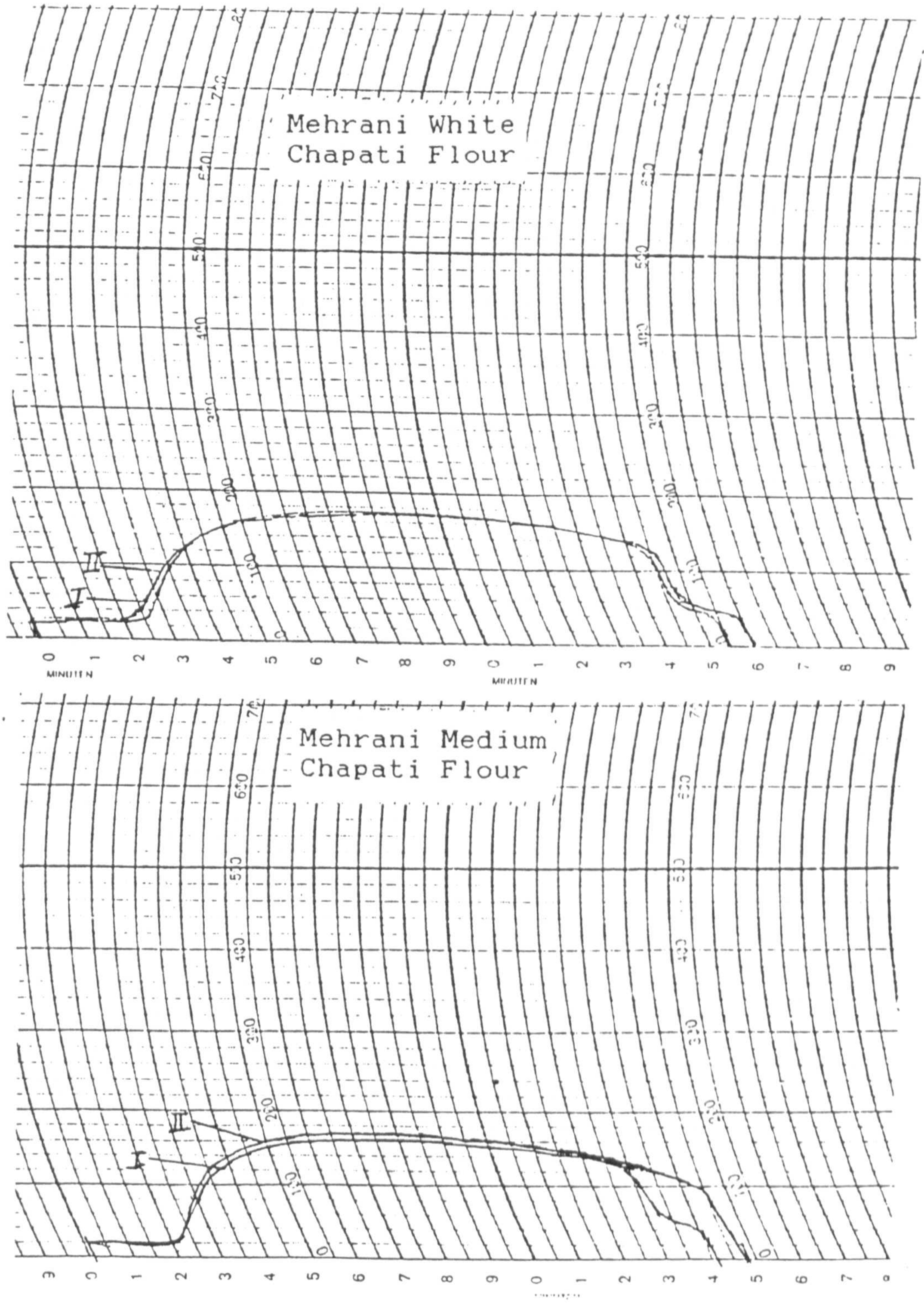

Fig. 4.3: Extensograms of Mehrani chapati flours.

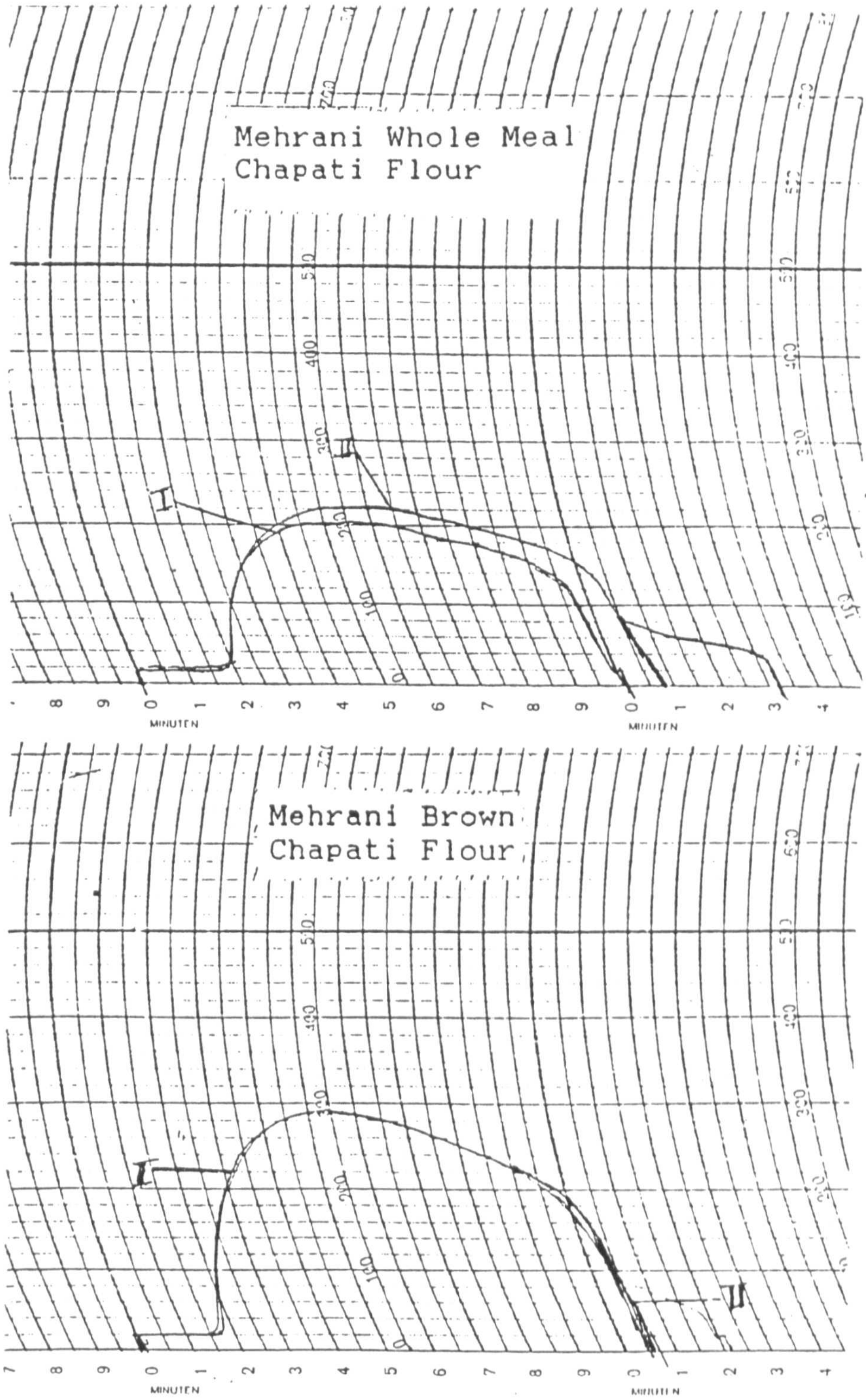

Fig. 4.4: Extensograms of Mehrani chapati flours.

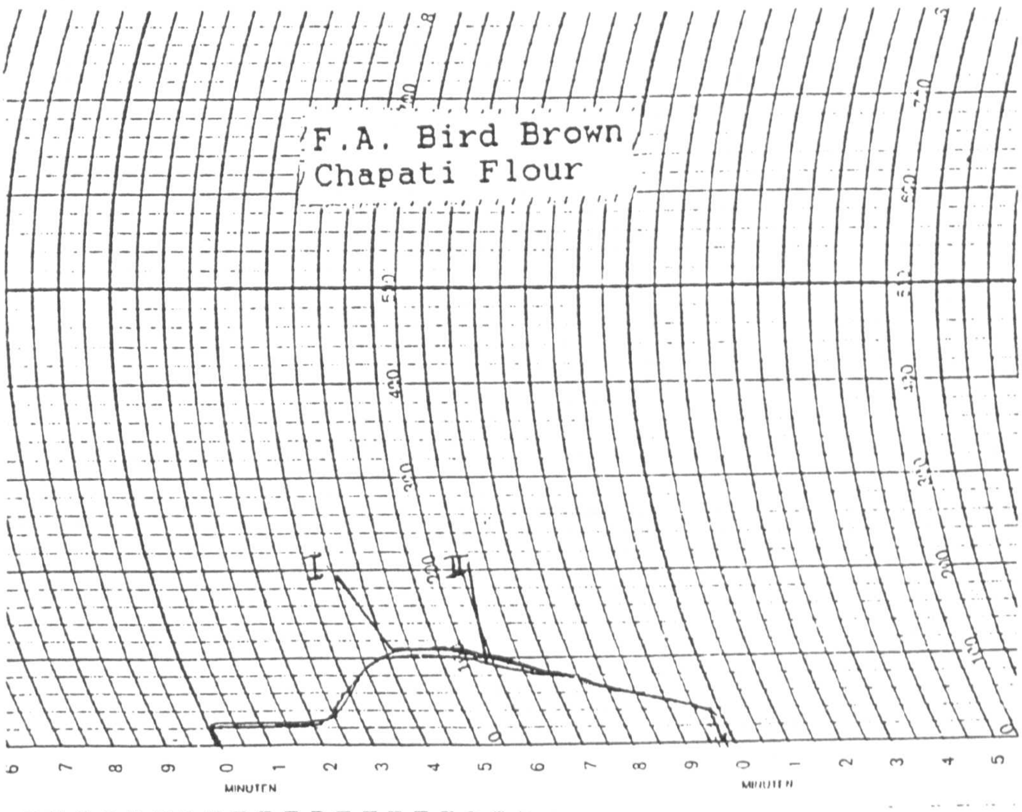

Fig. 4.5: Extensogram of F.A. Bird brown Chapati flour.

4.1.3: Sensory analysis of chapaties made from commercial chapati flours

Texture of chapaties was evaluated (2.3.1) by a panel of 12 judges at three intervals of storage. Analysis of variance (Table 4.5) of different flours showed a significant difference between samples ($P<0.05$). Tukey's test was used to determine which samples were significantly different. The flours of Mehrani brown and Mehrani wholemeal did not differ significantly, but were significantly different from Mehrani medium and Mehrani white. Storage intervals affected the quality of chapati significantly. Judges 5 and 6 were also significantly different from the others. When the scores of judges were examined, it was evident that although judges 5 and 6 scored low, their scores were consistent with the means for the panel: 3 h interval received the lowest scores and 24 h received the highest scores.

Table 4.5: Analysis of variance of effect of storage on sensory texture of chapati using a scale from 1 (softer) to 10 (hard).

Source	F. Value	P. Value
Assessor	37.00	0.00
Sample	33.98	0.00
Storage interval	264.97	0.00

Assessor means

8	7	4	11	12	2
6.5a	6.4a	5.7a	5.6a	5.5a	5.5a

1	3	10	9	6	5
5.3ab	4.9bc	4.4bc	4.0bc	3.2c	3.0c

Sample means

M. Brown	M. Wholemeal	M. Medium	M. White
5.5a	5.5a	4.5b	4.4b

Interval means

24 h	12 h	3 h
6.5a	4.9b	3.5c

Note : Any two values not followed by the same letter are significantly different at the 5 % level of probability.

Compression tests were performed with the Instron universal testing machine to determine hardness of texture of chapaties made from commercial chapati flours after 3, 12 and 24 h of storage intervals (Table 4.6). Storage intervals affected the softness of chapati significantly ($P<0.05$).

The highest values were observed for Mehrani brown flour while the lowest values in Mehrani wholemeal after 3 and 12 h; medium was recorded after 24 h.

Table 4.6: Effect of storage on physical texture of chapati determined with Instron machine (Full scale= 10Kg).

Flour	3 h	12 h	24 h
Mehrani White	3.2f	4.6e	7.2ba
Mehrani Medium	3.0f	5.2d	6.4c
Mehrani Wholemeal	3.0f	5.1d	7.7a
Mehrani Brown	4.4e	5.6d	8.4a
S.d.	0.2	0.2	0.3

S.d. = Standard deviation (pooled)

Note: Any two values not followed by the same letter are significantly different at the 5 % level of probability.

4.1.3 Discussion

For assessment of dough, water absorption, dough development, dough stability and tolerance index were selected as parameters most representative of rheological properties of wheat flour dough. Water absorption is claimed to be an important characteristic for wheat and composite flour (Sollars and Robenthaler, 1975).

The shape of the farinogram was also used to characterize flours. Various indices have been defined: dough development time or peak time being the most important. This increased with increasing water absorption and protein contents of the flour. Markley et al. (1939) noted a correlation coefficient of 0.88 between the peak time and crude protein content in 89 wheat samples, a positive relationship between them. Similar results were obtained by Haridas et al. (1989). The extensogram curves indicated clearly from the varying form of the curves, that the flours differed markedly in extensibility of doughs and other characteristics. Changes in extensibility influence sheeting behaviour of dough after resting periods. Flours with a low R/E ratio values (<1)tend to become slack and flow excessively in rolling. High R/E values (>2.5) indicate that resistance to stretching compared to extensibility was excessive, generating doughs that tend to tighten up during resting periods. These become difficult to sheet.

Texture softness/hardness of chapati made from these flours was evaluated by sensory analysis as well as instrumental techniques after 3, 12 and 24 h storage. It was evident (Table 4.5) from analysis of variance that Mehrani white and Mehrani medium flours (low extraction) were significantly different ($P>0.05$) from Mehrani brown (containing more bran than wholemeal as indicated from grade colour value and ash content) and Mehrani wholemeal. F.A. Bird Brown chapati flour dough did not perform well in test and was considered unfit for chapati production. Farinograph and extensograph curves indicated a weak, inelastic and highly-flowing dough. Flours with such dough characteristics are generally obtained from flours prepared from damaged wheat, high in α-amylase, and soft wheats. The dough showed no improvement after resting for 60 min (curve 11). Among Mehrani chapati flours, Mehrani Wholemeal showed good performance in relation to farinographic indices such as high water absorption, long dough development and stability times and low tolerance index. It had a moderate resistance to extension ratio, and showed improvement in dough extensibility after a 60 min resting period. The dough was mostly soft, but it was elastic and although not fit for leavened bread, was very suitable for the production of chapati. Mehrani Wholemeal flour had high protein, low damaged starch, uniform particle size values, and was lighter in colour than other flours. Mean sensory texture score showed no significant ($P<0.05$) differences between Mehrani wholemeal and Mehrani brown flours. Softness in chapati texture was highly correlated with flour colour and consequently bran content; this may be due to increase in water absorption (Navickis and Nelsen, 1992). Mehrani flours are blends of soft and hard wheat varieties. Sekhon et al. (1980) obtained a softer texture in chapaties by blending wheat and triticale flours in 3:1 ratio. Similar improvements in leavened bread-making properties were attained by Unrau and Jenkins (1965) and Rao et al. (1978) by blending triticale and wheat flours.

It should be noted that sensory chapati texture had significant ($P<0.05$) correlations with protein content, damaged starch, water absorption, dough development, dough stability, particle size (130 - 180 µm), Grade colour, extensibility and R/E ratio of extensograms. The strength of flour, and correlations between flour tests and chapati softness, suggest that these tests are more suitable for the description of flour quality for the production of chapati. Results are well supported by previous findings (Weipert, 1992; Swarnjeet et al., 1982; Quail et al., 1991).

Chapaties are usually produced from flour of high extraction rate i.e. wholemeal flours (Chaudhry, 1968; Douglas, 1981; Finney et al., 1980a; Kent, 1975; Qarooni et al., 1992 and Sawaranjeet at al., 1982). This practice increases the water absorption of flours and improves dough sheeting quality. Improvements in sheeting quality have significant effects on softness and shelf life of the final product. Low extraction white flours are used for the preparation of flat breads such as Arabic bread (65 % extraction rate) (Nazar and Hallab, 1973), Balady from 72 to 87.5 % extraction rate (ER) (Dalby, 1969), Naan from patent flour (Dutta et al., 1980) and Barbary from 78 % ER (Faridi et al., 1982). High contents of bran in the wholemeal flours have been also correlated with nutritional value (Faridi, 1981; Qarooni et al., 1992; Newman et al., 1992). Chapaties prepared from wholemeal flour were found to be smooth, soft and pliable in sensory terms. Similar results were reported by Yamazaki and Greenwood (1981).

4.2 Wheat cultivars and flour specification.

4.2.1 Objective

The goal of these experiments was to determine the suitability of British wheat cultivars for chapati production.

4.2.2 Results

Samples of British wheat varieties were analysed for moisture, protein and damaged starch contents (Table 4.7).

The moisture content of wheat grain ranged from 9.50 to 11.50 %. Moisture contents of grain were adjusted by tempering prior to milling.

Protein had significant correlations ($P<0.05$) with water absorption ($r = 0.91$) and also ($P<0.05$) with extensibility ($r = 0.78$) of dough at 60 min and FN ($r = 0.72$). However, there were also some correlations with extensibility of dough at 30 min ($r = 0.69$), dough stability ($r = -0.54$), tolerance index ($r = 0.57$) and particle size (130 - 180 μm) ($r = 0.45$).

Table 4.7: Chemical analysis of wheat and flour.

Variety	Description	m.c. % Grain	m.c. % Adjusted	m.c. % Flour	P.C. % (d.b.)	DS % (d.b.)
Torfrida	Hard	10.52	15.50	14.20	12.80	13.06
Pastiche	Hard	10.20	15.50	14.10	14.81	10.79
Mercia	Hard	9.50	15.50	13.70	11.11	10.19
Fresco	Hard	10.35	15.50	14.00	13.48	9.92
Avalon	Hard	11.50	15.50	13.90	14.43	9.51
Hereward	Hard	11.50	15.50	13.95	13.00	11.75
Riband	Soft	10.90	14.50	13.40	12.24	8.28
Galahad	soft	10.50	14.50	13.50	14.81	8.87
S.d.		0.04	0.11	0.07	0.08	0.06

DS = Damaged Starch m.c. = moisture content
d.b.= dry basis P.C. = Protein content
S.d. = Standard deviation (Pooled)

Flour samples were analysed for particle size, falling number and Grade colour values (Table 4.8). Galahad and Riband had the highest content of the coarser particle size (>180 µm) and Fresco, Hereward and Avalon the lowest, reflecting the grain hardness. Galahad and Riband produced very fine powdery flour, which adhered to a sieve during sifting and resulted in a low yield of the undersieve fraction, reflecting softness. Generally, the hard-milling wheats, as predicted, gave flours with good flow properties, and soft-milling wheats produced flours with poor flow properties.

The average falling number (FN) value for flours was 472 reflecting the dry conditions during grain development. The highest value (545) was obtained for Galahad and the lowest (379) for Mercia. FN had shown strong correlation with extensibility (at 30 min r = 0.44, 60 min r = 0.60) and R/E ratio (at 30 min r = -0.41, 60 min r = -0.44) of dough.

Table 4.8: Physical properties of flours of wheat varieties.

Cultivars	Ps >180 µm	Ps 130 - 180µm	Ps 110 - 130µm	Ps <110 µm	FN	G.C.
Torfrida	23.98	22.97	10.47	42.58	405	17.10
Pastiche	22.17	24.27	13.56	40.00	521	15.83
Mercia	24.50	18.60	10.96	45.94	379	14.10
Fresco	21.96	23.14	14.02	40.88	532	16.05
Avalon	19.47	22.68	13.70	43.87	445	16.63
Hereward	21.59	19.90	12.28	46.22	474	16.80
Riband	25.60	15.60	9.00	48.80	477	15.00
Galahad	27.55	17.80	12.16	41.90	545	15.35
S.d (pooled)	0.10	0.05	0.07	0.21	6.0	0.07

Ps = Particle size FN = Falling Number
G.C. = Grade Colour S.d. = Standard deviation

Farinograph mixing characteristics (Table 4.9) provided useful information on the baking performance of conventionally mixed doughs. Representative farinograms obtained with varietal flours are shown in Fig. 4.6 and 4.7.

Water absorption had shown strong correlation with dough development (r = -0.50), dough stability (r = -0.63), tolerance index (r = 0.61), particle size 130-180 µm (r = 0.43), FN (r = 0.60), extensibility after 30 and 60 min (r = 0.70, r = 0.77*).

The dough development time showed strong correlations with dough stability (DSt) (r = 0.66), tolerance index (r = -0.65), and resistance to extension (R) after 30 and 60 min (r = 0.58, r = 0.50 respectively). The DSt of dough was strongly correlated with TI (r = -0.85, P<0.05), R after 30 and 60 min (r=0.86, r=0.87, P<0.001) and R/E after 30 and 60 min (r = 0.89, r = 0.85, P<0.001). Tolerance index had negative correlation with R after 30 and 60 min (r = -0.60, r = -0.58, respectively) and R/E ratio after 30 and 60 min (r = -0.59, r = -0.54, respectively)..

Table 4.9: Farinographic parameters of British wheat cultivars.

Cultivars	Water absorption (%)	Dough development (min)	Dough Stability (min)	Tolerance index (BU)
Torfrida	61.90	5.00	>17.50	45
Pastiche	68.50	6.50	6.50	70
Mercia	54.60	10.00	>17.50	10
Fresco	60.20	14.50	>17.50	30
Avalon	64.50	5.50	6.00	70
Hereward	61.50	6.00	8.00	40
Riband	60.90	5.75	4.00	100
Galahad	64.60	4.00	3.25	120

BU= Brabender Unit

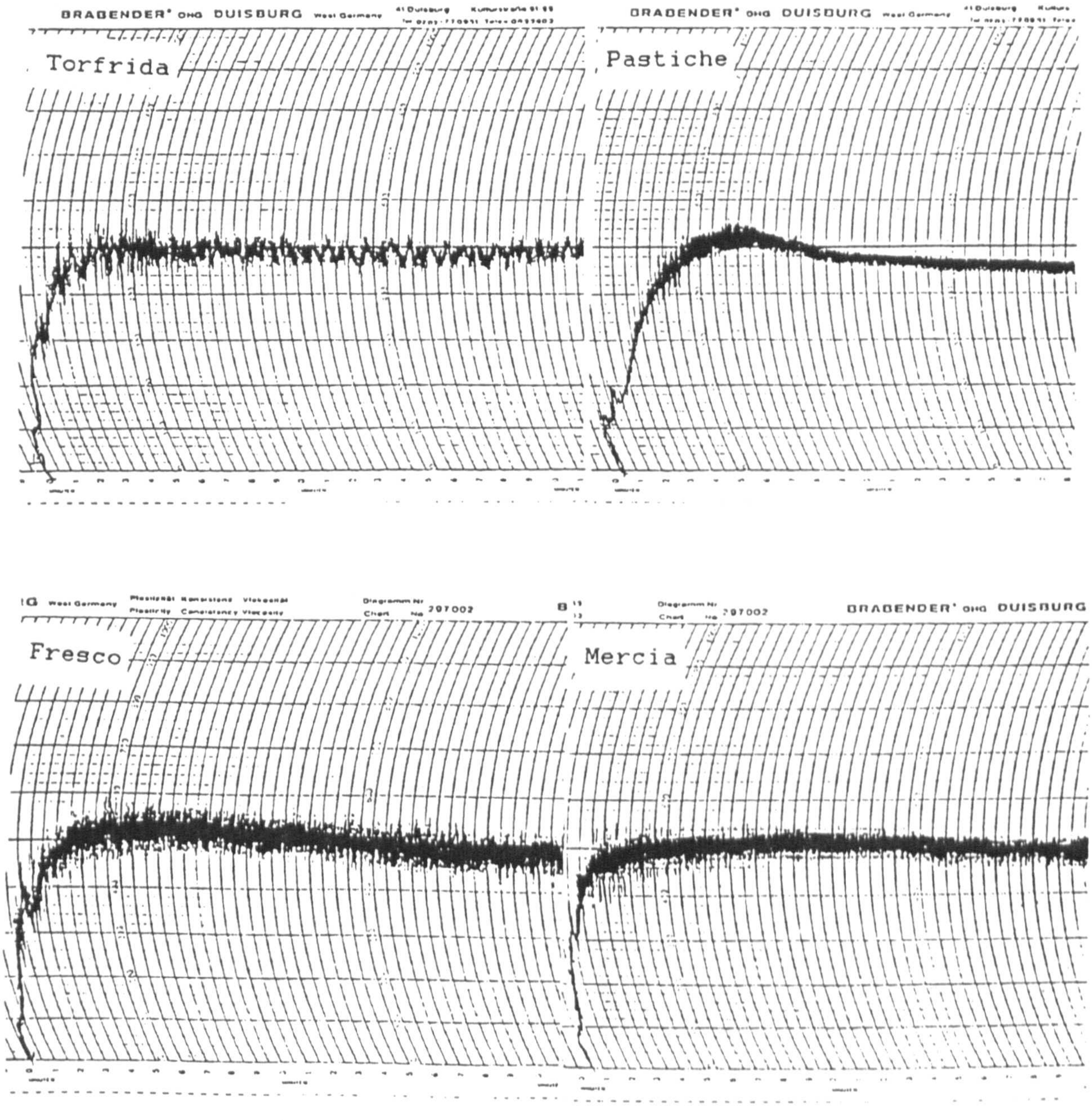

Fig. 4.6: Farinograms of wheat cultivars.

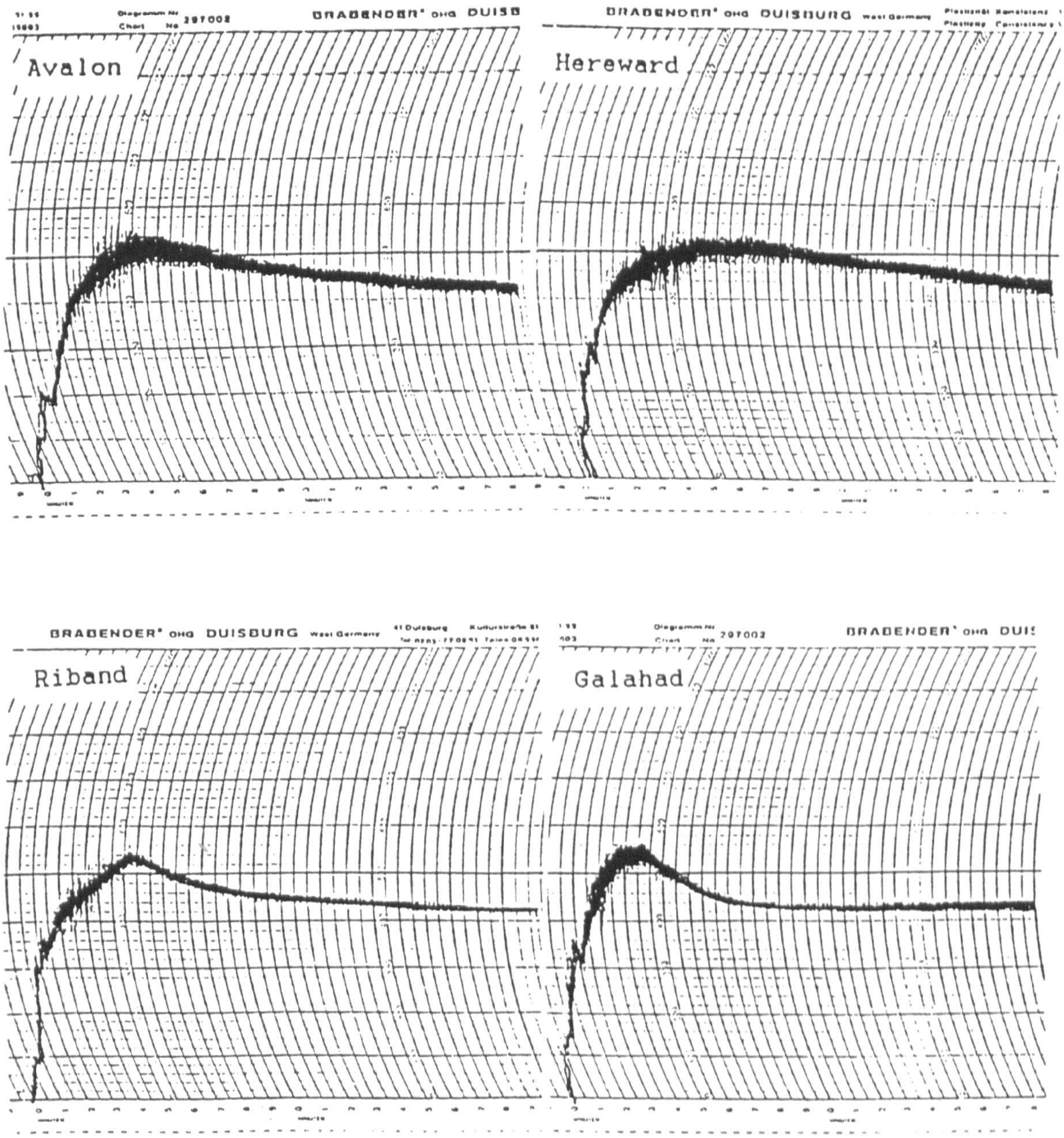

Fig. 4.7: Farinograms of wheat cultivars.

Representative extensograms are shown in Fig 4.8 and 4.9. The Fresco flour showed the strongest curve in terms of resistance to extension (565 EU) at 30 min and the Torfrida flour (780 EU) at 60 min resting (Table 4.10).

Correlations of flour characteristics revealed that resistance to extension had shown highly significant correlation ($P<0.01$) with R/E after 30 min ($r = 0.95$, $r = 0.96$) and 60 min ($r = 0.98$, $r = 0.96$) of resting time. Extensibility at both the periods had shown negative correlation with resistance to extension (E30, R30 $r = -0.43$, R60 $r = -0.48$ and E60, R30 $r = -0.51$, R60 $r = -0.58$) and with R/E ratio (E30 with R/E 30 and R/E 60, E60 with R/E 30 and R/E 60, $r = -0.63$, $r = -0.62$, $r = -0.73$*, $r = -0.72$*, respectively) of dough.

Table 4.10: Extensographic characteristics of wheat cultivars.

cultivars	R (EU) 30 min	R (EU) 60 min	E (mm) 30 min	E (mm) 60 min	R/E 30 min	R/E 60 min
Torfrida	545	780	56	55	9.70	14.18
Pastiche	123	155	113	118	1.10	1.31
Mercia	230	305	51	55	4.50	5.54
Fresco	565	680	84	85	6.72	8.00
Avalon	125	143	124	115	1.01	1.24
Hereward	160	205	101	101	1.58	2.03
Riband	90	100	80	82	1.12	1.22
Galahad	70	95	77	91	0.91	1.04

Ability of each dough to sheet was also determined

(Table 4.11). Dough from Torfrida had a low sheeting ability and insufficient extensibility. It had excessive flow and no cohesiveness. The farinogram (Fig 4.6) indicated a lack of consistency and smoothness during mixing. Mercia showed the extensogram of a typical short dough: high in resistance to sheeting as it possessed insufficient extensibility and contracted after sheeting (Table 4.11). The dough of Pastiche had adequate elasticity and sheeting ability but contracted after rolling. It was too sticky to handle. Doughs from Avalon and Hereward were soft but highly elastic, in contrast to Riband and Galahad doughs which were proven to be weak, inelastic and highly "flowy" doughs. The curve of dough of Fresco showed strong behaviour, extensible and elastic. The dough was less sticky and had better sheeting quality but contracted after rolling.

Table 4.11: Behaviour of doughs of British wheat cultivars during processing.

Cultivars	Handling (Stickiness)	Resistance to sheeting	Shrinkage after sheeting
Torfrida	X	X	X
Fresco	X	XXX	XXX
Mercia	X	XXX	XXX
Pastiche	XXX	XX	XXX
Hereward	XXX	XX	XXX
Avalon	XXX	XX	XXX
Riband	XXX	X	X
Galahad	XXX	X	X

Low = X Moderate = XX High = XXX

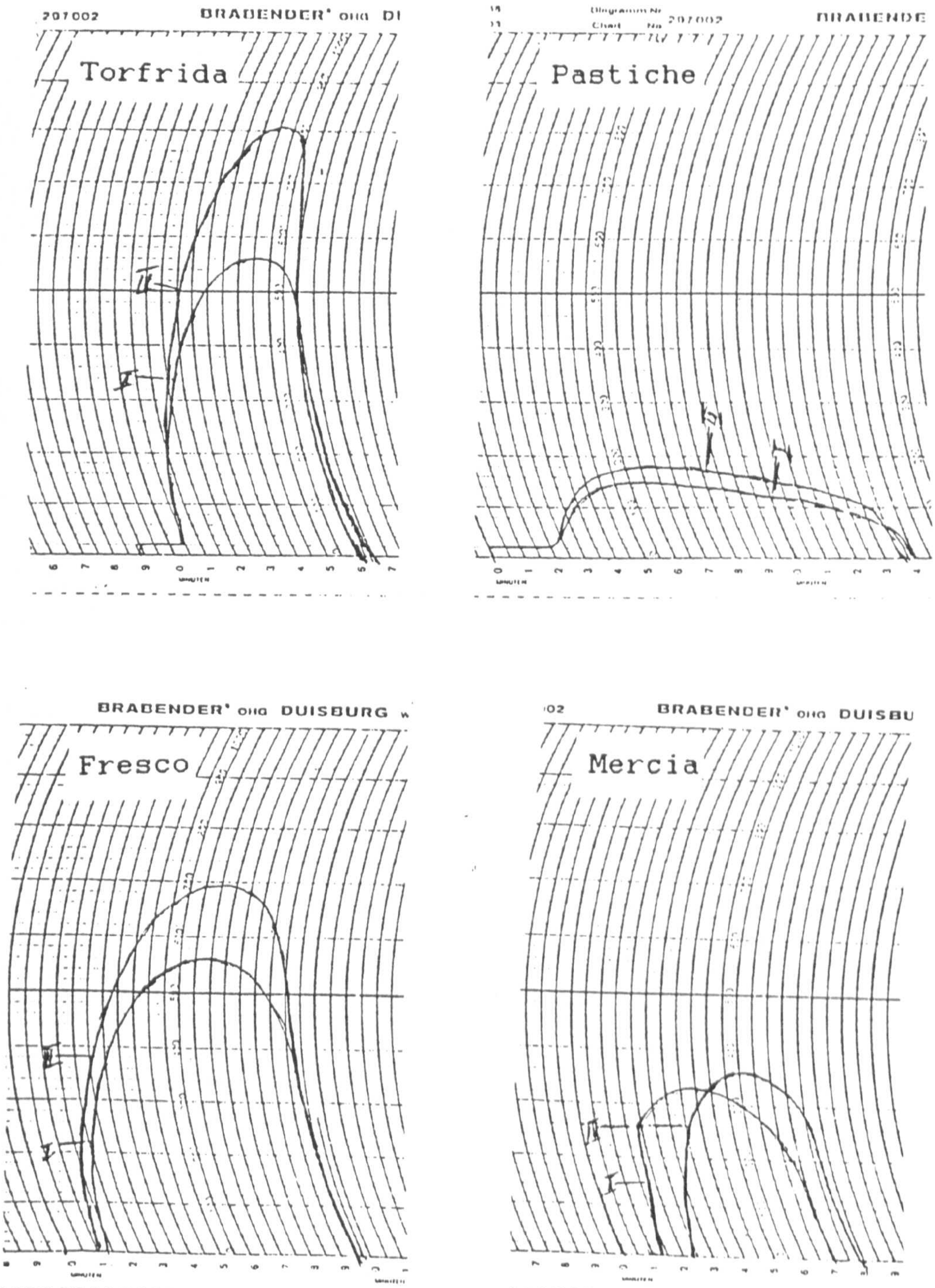

Fig. 4.8: Extensograms of wheat cultivars.

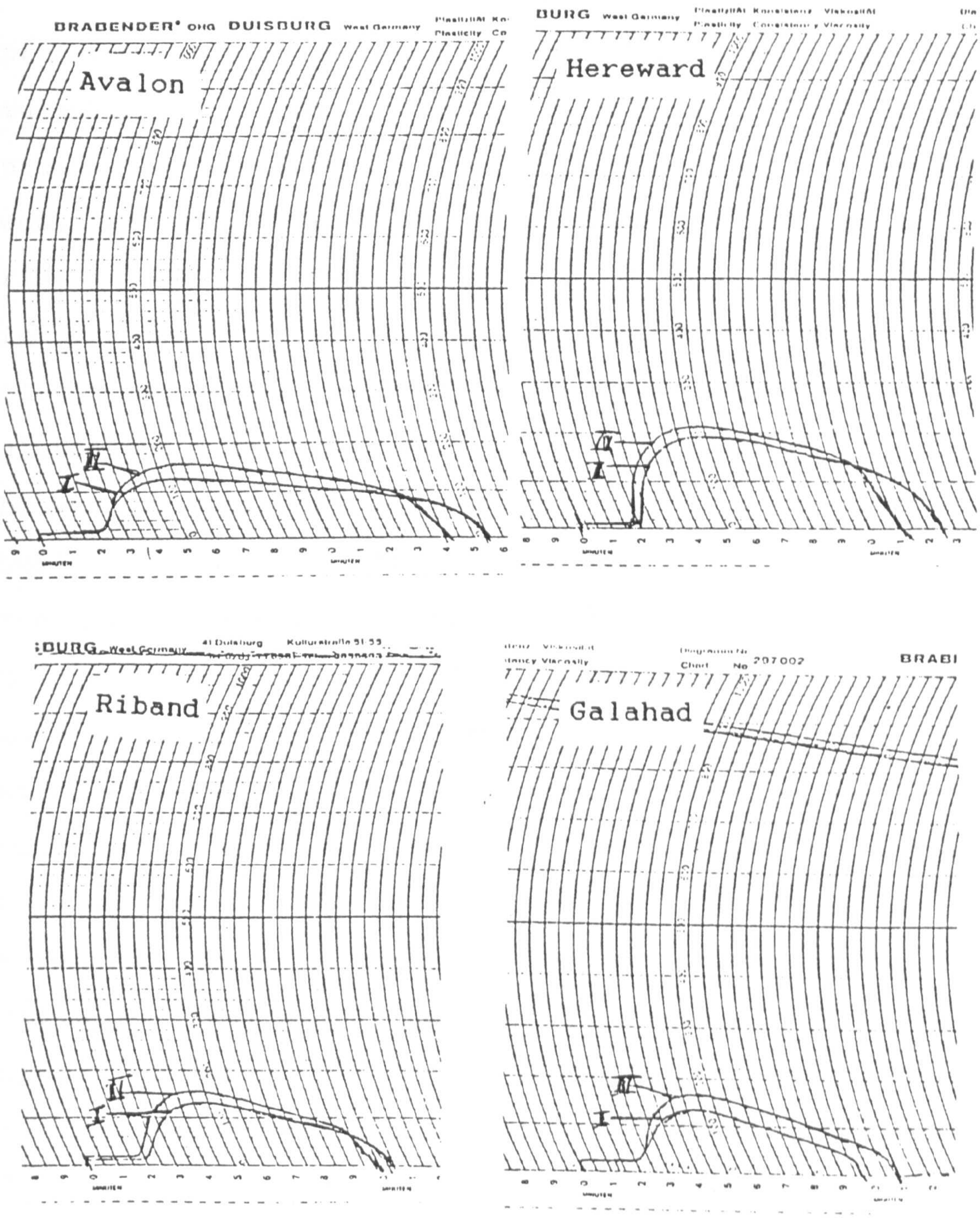

Fig. 4.9: Extensograms of wheat cultivars.

4.2.3 Discussion

The results of this study suggested that flours prepared from the single wheat cultivars tested were not suitable for chapati production. Six of the varieties produced dough with high viscoelastic properties which would produce leavened bread, and the remaining two gave rise to weak, extensible doughs which might be suitable for biscuits. The varieties possessing highly viscoelastic gluten are strong while those with the more extensible gluten are referred to as weak wheats. A proper balance between viscosity and elasticity is needed to obtain good quality chapati. When the elastic component predominated, the dough behaved as short and stiff as in the case of Mercia (Table 4.10, 4.11). Galahad and Riband were lacking in elasticity: this resulted in nonresilient, slack and sticky doughs. Both were found to be poor in sheeting quality, an essential feature of chapati. Different terms have been applied by various groups of investigators in referring to properties of dough, sometimes termed as viscoelastic or simply dough properties or "rheological optima" (Schafer, 1972). Mehrani wholemeal chapati flour, a commercial chapati flour which is a blend of flours from different cultivars, had the best rheological optima suited for the production of good quality chapati. Good rheological optima should be achieved by blending wheats of opposing characteristics in appropriate proportions. According to Bolling (1981), wheat and flour classification based on physical dough properties (rheological optima) has gained importance in Germany over the last 15 years. However, blending effects using different varieties of wheat have also been reported from European countries other than Germany (Fitchett and Frazier, 1988).

Cultivars used in this study were proven to differ in their rheological optima, and were individually unsuitable for chapati making. Riband and Galahad could not be used due to being too weak in rheological optima, whereas other had strong to extra strong rheological optima. Amongst them, the doughs of Fresco and Mercia behaved as the strongest but such doughs had poor sheeting ability in showing resistance to sheeting and contraction after rolling into chapaties. The doughs from Avalon, Hereward and Pastiche had more or less similar properties. Similar results have been discussed by Moss (1980).

Qarooni et al. (1988) reported that superior quality flat bread could be produced from hard grain wheat associated with high damaged starch and high water absorption. In this study, damaged starch ranged between 8.28 - 13.06 %. The proportion of damaged starch was influenced by wheat grain hardness. Torfrida, considered hard, had the highest content (13.06 %) of damaged starch whereas Galahad (8.87 %) and Riband (8.28 %), both soft, contained least. Damaged starch and particle size (130 - 180 µm) were found to be positively correlated ($r = 0.57$). Pomeranz et al. (1984) reported a similar correlation (n

= 12, r = 0.78). The falling numbers of all wheat varieties were greater than recommended (Perten, 1984) (250) for adequate supply of the fermentable sugars to yeast throughout the fermentation period for optimal baking results in leavened bread. Haridas et al. (1989) reported that 14.1 - 16.5 % damaged starch was considered optimal for chapati. Galahad and Riband had similar amount of protein to flours analysed by Serna-Saldivar et al. (1988).

This study suggested that among hard-grained cultivars, either Fresco or Mercia could be blended with the soft-grained variety Galahad for preparation of flours suitable for chapati production.

4.3 Blending of British wheat cultivars for preparation of chapati flour

4.3.1 Objective

The objective of these studies was to determine the maximal levels of hard wheat cultivars (Mercia/Fresco) in composite flours based upon a soft wheat cultivar (Galahad) for chapati production.

4.3.2 Results

The water absorption capacity of composite flours decreased with increasing amounts of Fresco and Mercia. Significant ($P<0.01$) correlations (Mercia $r = -0.99$; Fresco $r = -0.95$) existed between water absorption and varietal flour level. Highest water absorptions (64.0 %, 63.2 %) were observed with flours containing 30 % Fresco and 20 % Mercia and lowest values (59.8 %, 60.8 %) with 60 % Mercia and 80 % Fresco, respectively.

In extensographic studies there were gradual increases in peak time with increase in Mercia and Fresco. The average peak times were 6.7 min and 9.5 min for Mercia + Galahad and Fresco + Galahad composites. However, the peak time ranged between 5.25 and 8.25 min for Mercia + Galahad and 6.0 and 14.0 in composite flour of Fresco + Galahad. The highest peak times (8.25, 14.0 min) were recorded for composite flours containing 60 % Mercia and

80 % Fresco. Significant correlation (r = 0.99) existed between peak time and level of varietal flours in both the cases.

Similar trends were noted for dough stability. The highest value of 16.25 min was noted with flour containing 60 % Mercia and >17.5 min for 80 % Fresco. The lowest values (4.5 and 6.0 min) were obtained with composite flours containing 20 % Mercia and 20 % Fresco, respectively. Significant (P<0.01) correlations (Mercia r = 0.91; Fresco r = 0.88) were observed between dough development time and level of varietal flours.

The tolerance index values of composite flours were ranged between 95 and 45 BU, and 80 and 35 BU for Mercia + Galahad and Fresco + Galahad, respectively. Significant correlation (P<0.01, Mercia r = -0.99; Fresco r = -0.89) existed between tolerance index and replacement of Galahad with either Fresco or Mercia in composites.

Composite flours containing 60 % Mercia and 80 % Fresco yielded highest values for R/E ratio (resistance to extension / extensibility) (2.5 at 30 min, 3.60 at 60 min and 5.10 at 30 min, 7.50 at 60 min, respectively). Composites of Mercia and Fresco (20 %) had the lowest values (1.30 at 30 min, 1.6 at 60 min and 1.70 at 30 min, 2.00 % at 60 min, respectively). There was a general increase noted with increases in the contents of both hard wheat cultivars (Fresco / Galahad). In both cases, dough strength increased during resting time. Significant correlations were observed between R/E values at 30 min (r

= 0.98) and at 60 min (Mercia r = 0.99; Fresco r = 0.98).

Table 4.12: Rheological Characteristics of composite flours.

Flour		WA	DD	DSt	TI	R/E	R/E
Merc (%)	Gala (%)	(%)	(min)	(min)	(BU)	30 min	60 min
20	80	63.2	5.25	4.50	95	1.30	1.60
30	70	62.5	6.00	5.50	80	1.60	2.50
40	60	61.7	6.50	7.25	65	1.75	2.50
50	50	60.8	7.50	14.25	55	2.30	3.00
60	40	59.8	8.25	16.25	45	2.50	3.60
Fres (%)	Gala (%)						
20	80	63.5	6.00	6.00	80	1.70	2.00
30	70	64.0	7.00	11.00	60	1.65	2.23
35	65	63.8	7.50	16.00	55	2.10	2.60
40	60	62.2	8.00	16.00	55	2.60	3.20
60	40	61.3	10.00	>17.5	35	4.20	5.40
80	20	60.8	14.00	>17.5	35	5.10	7.50

Merc = Mercia
Gala = Galahad
Fres = Fresco

4.3.3 Discussion

On the basis of the rheological characteristics, a maximum of 60 % of Fresco or Mercia could be blended with Galahad. Beyond this level, farinograms indicated dough would become stronger but it was predicted chapati-making qualities would deteriorate. Composite flour containing 40 % Mercia was observed to be similar in dough development to Mehrani Wholemeal chapati flour (MWCF), but lower in dough stability. Flour containing 60 % Mercia flour was near to commercial wholemeal with respect to dough stability. Increased R/E values also reflected deterioration in chapati preparation qualities (Table 4.12). Similar results were noted with flour containing 40 and 60 % Fresco where dough development, dough stability and tolerance index were observed to be similar to Mehrani Wholemeal chapati flour, but R/E values were higher reflecting a deterioration in quality.

Blends of strong and weak wheat flours at optimum levels produced enhancements in sheeting quality and in chapati quality (MWCF) compared with those from a single variety. Moreover superior crust colour was produced presumably due to high levels of damaged starch arising from the hard wheat in the blend (Qarooni et al., 1992). The blend effects measured as a gain in sheeting ability of composite flour compared with the sheeting ability associated with the individual flours, was the result of optimising viscoelastic properties (Weipert, 1992).

4.4 Optimisation of flours for chapati production

4.4.1 Objective

The objective of these studies was to optimise chapati flours prepared by blending of Fresco and Galahad, and Mercia and Galahad.

4.4.2 Results

Analysis of variance for texture of chapati evaluated through sensory and instrumental measured techniques are presented in a Table 4.16. Significance of R^2 values, coefficient of variation (CV) and model significance were used to judge the adequacy of fit of models. Analysis of variance of each response variable and for full regression was calculated (Table 4.13).. High R^2 values indicated strong dependence of response measured. The significance of regression coefficient within each model (Table 4.14, 4.15) showed the direction of the effect of each independent variable in the blends. Coefficient of variables in linear and quadratic terms showed significant effects on hardness of chapaties, indicated adequacy of models. The predicted models developed for sensory evaluation at the three storage intervals were considered adequate and in both cases possessed no significant lack of fit and satisfactory levels of R^2, CV and model of significance ($P<0.05$). The R^2 values for 2.5, 10, and 24 h

Table 4.13: Sensory and Instron analyses of chapaties prepared from composite flours (Fresco + Galahad; Mercia + Galahad).

Flour		Sensory score			Instron unit		
Fres + %	Gala %	2.5 h	10 h	24 h	2.5 h	10 h	24 h
13	87	2.60	6.95	8.70	37	42	57
20	80	2.20	6.30	8.20	33	37	53
25	75	2.20	5.80	8.00	29	35	51
30	70	1.80	4.85	6.50	24	30	45
33	67	1.60	4.50	6.30	20	28	42
38	62	1.80	5.80	6.40	24	30	45
40	60	2.10	6.10	6.90	26	32	47
42	58	2.20	6.75	7.20	32	39	52
S.d.		0.04	0.03	0.04	3.60	5.88	3.66
Merc + %	Gala %						
25	75	2.90	5.80	8.40	25	40	60
30	70	2.80	5.60	8.20	24	37	57
33	67	2.70	5.50	8.30	23	35	53
38	62	2.50	5.20	7.00	22	33	50
40	60	2.00	4.80	6.40	20	28	47
42	58	1.80	4.60	6.20	19	27	45
50	50	1.60	4.40	6.00	17	24	41
S.d.		0.06	0.04	0.04	2.72	3.62	3.26

Fres = Fresco   Gala = Galahad
Merc = Mercia   S.d. = Standard deviation (pooled)

Table 4.14: Best predicted coefficients, St dev. and optimum level of Fresco, affecting chapati quality parameter (texture at different intervals) obtained by regression test.

Variable	Coefficient	St. dev.	Optimum response (Fresco %)
	FRESCO + GALAHAD		
Sensory analysis			
2.5 h			
bo	4.28 **	0.61	32
b1	-0.155*	0.046	
b2	0.00246*	0.00082	
10 h			
bo	12.20**	1.69	29
b1	-0.484*	0.130	
b2	0.0083*	0.00228	
24 h			
bo	12.20**	1.72	37
b1	-0.30*	0.132	
b2	0.00405	0.00231	
Instron measurement			
2.5 h			
bo	67.0**	10.89	32
b1	-2.69*	0.84	
b2	0.0423*	0.015	
10 h			
bo	60.3**	9.71	31
b1	-2.50*	0.744	
b2	0.401*	0.013	
24 h			
bo	82.6***	9.97	32
b1	-2.28*	0.764	
b2	0.0353*	0.0135	

* = Significant (P<0.05)
** = Significant (P<0.01)
***= Highly significant (P<0.001)
St dev = Standard deviation

Table 4.15: Best predicted coefficients, st dev. and optimum level of Mercia, affecting chapati quality parameter (texture at different intervals) obtained by regression test.

Variable	Coefficient	St dev	Optimum response (Mercia %)
	MERCIA + GALAHAD		
Sensory analysis			
2.5 h			
bo	4.52***	0.344	50
b1	-0.0595**	0.0091	
10 h			
bo	7.44***	0.278	50
b1	-0.0627***	0.0074	
24 h			
bo	11.6***	0.810	50
b1	-0.119*	0.0215	
Instron measurement			
2.5 h			
bo	34.0***	1.128	50
b1	-0.340	0.030	
10 h			
bo	57.4***	2.833	50
b1	-0.692***	0.075	
24 h			
bo	80.00***	1.930	50
b1	-0.803	0.0513	

** = Significant (P<0.01)
*** = Highly significant (P<0.001)

intervals were good for Fresco blends (0.66, 0.64, 0.67, respectively) and very good for Mercia blends (0.87, 0.92, 0.83, respectively). The models developed for objective assessment at the three intervals had also adequate R^2 values for Fresco blends (0.65, 0.64, 0.64, respectively) and for Mercia blends (0.96, 0.93, 0.98, respectively) and models of significance. The CV values ranged from 6.2 - 11.89 % for Fresco and 2.1 - 8.0 % for Mercia blends.

Sensory evaluation of chapati prepared with blends of Fresco and Galahad: The plots for texture of chapati at the intervals of 2.5, 10 and 24 h are depicted in Figures 4.10 - 4.12. The regions of optimum softness (Lowest score) were ranged from 29 to 37 % replacement of Galahad with Fresco. The area of least good response was defined by high levels of Galahad or high levels of Fresco. N.line and R.line in the plots denote normal line (raw data values) and regression line (fitted values), respectively.

Instron Measurements: The plots at 2.5, 10 and 24 h are shown in Figures 4.13 - 4.15. Best response regions were found with 68 % Galahad or 32 % Fresco at 2.5 and 24 h, and 69 % Galahad and 31 % Fresco replacement at 10, whereas worst response regions were found with high level of Galahad or high level of Fresco in blends.

Table 4.16: Analysis of variances, CV and R-seq for evaluation of models for quality parameter (Texture) of chapati at different intervals.

Interval	F	P	CV %	R^2
	FRESCO + GALAHAD			
Sensory				
2.5 h	7.73	0.030	10.26	0.66
10 h	7.10	0.035	8.78	0.64
24 h	8.13	0.027	7.20	0.67
Instrumental				
2.5 h	7.56	0.031	11.89	0.65
10 h	7.21	0.034	8.72	0.64
24 h	7.10	0.035	6.20	0.64
	MERCIA + GALAHAD			
Sensory				
2.5 h	42.54	0.001	8.00	0.87
10 h	71.69	0.000	2.93	0.92
24 h	30.40	0.003	6.00	0.83
Instrumental				
2.5 h	129.22	0.000	2.90	0.96
10 h	84.52	0.000	4.10	0.93
24 h	245.45	0.000	2.10	0.98

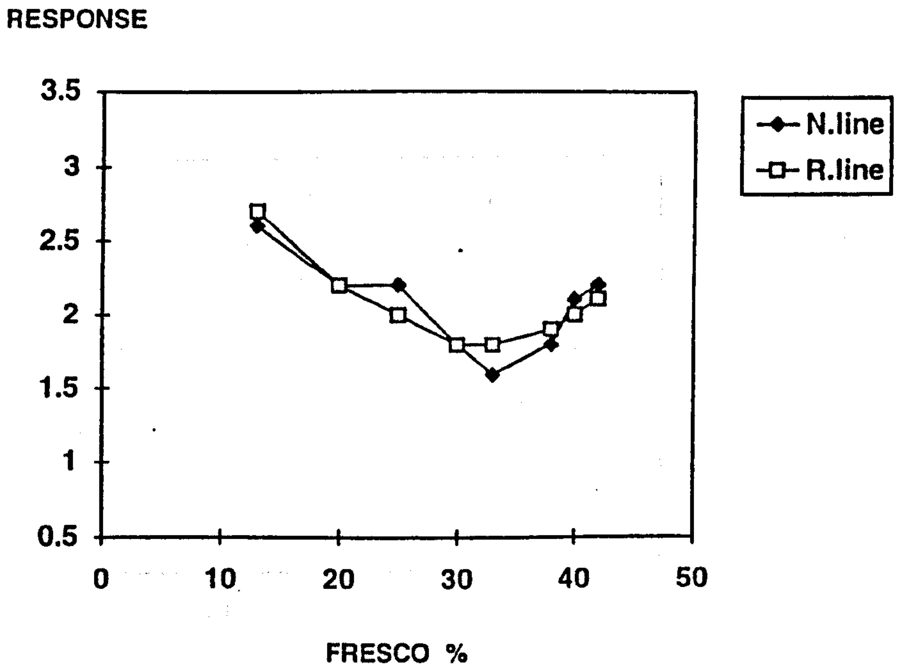

Fig. 4.10: Sensory evaluation of texture of chapaties after 2.5 h of preparation.

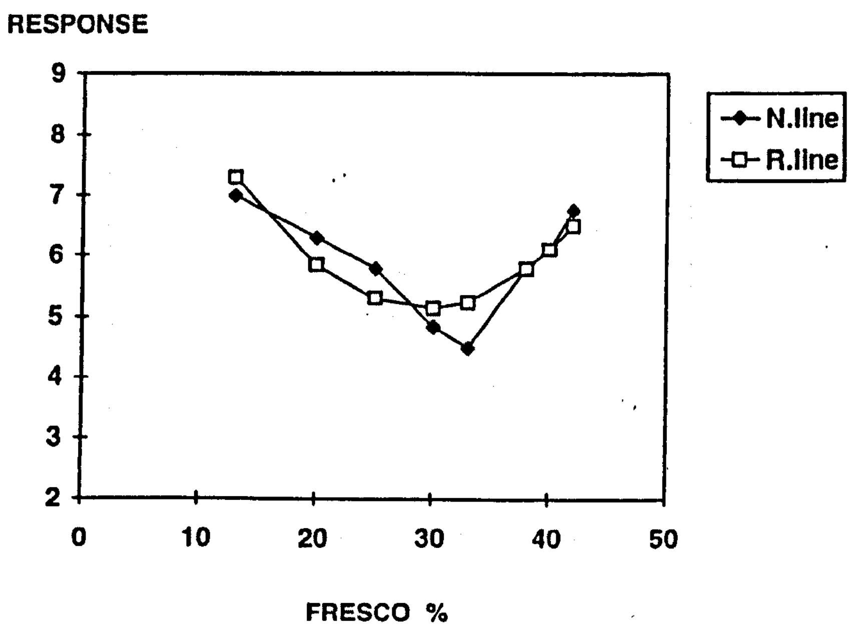

Fig. 4.11: Sensory evaluation of texture of chapaties after 10 h of preparation.

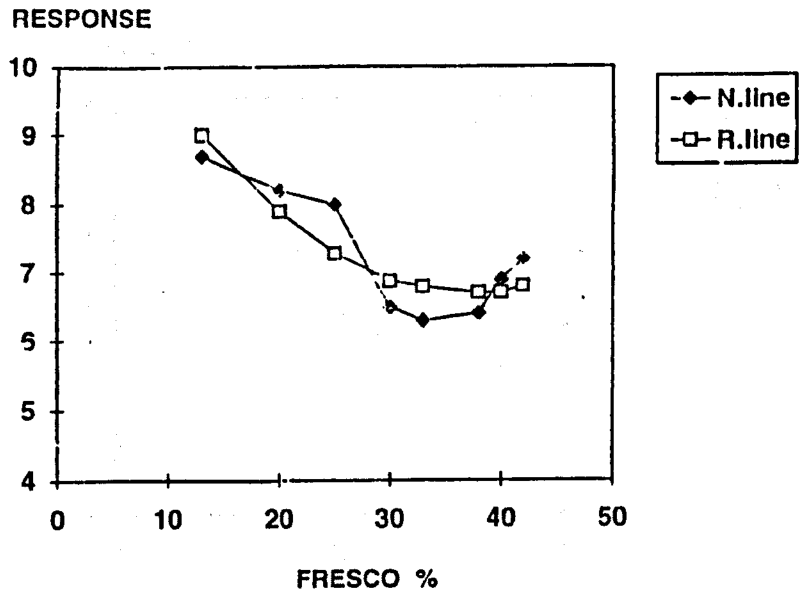

Fig. 4.12: Sensory evaluation of texture of chapaties after 24 h of preparation.

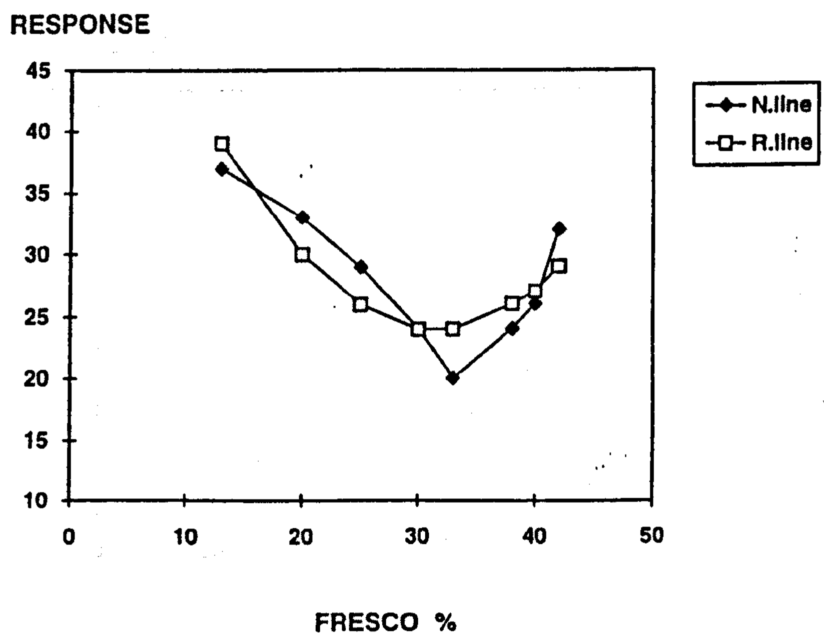

Fig. 4.13: Hardness in chapaties measured with Instron after 2.5 h of preparation.

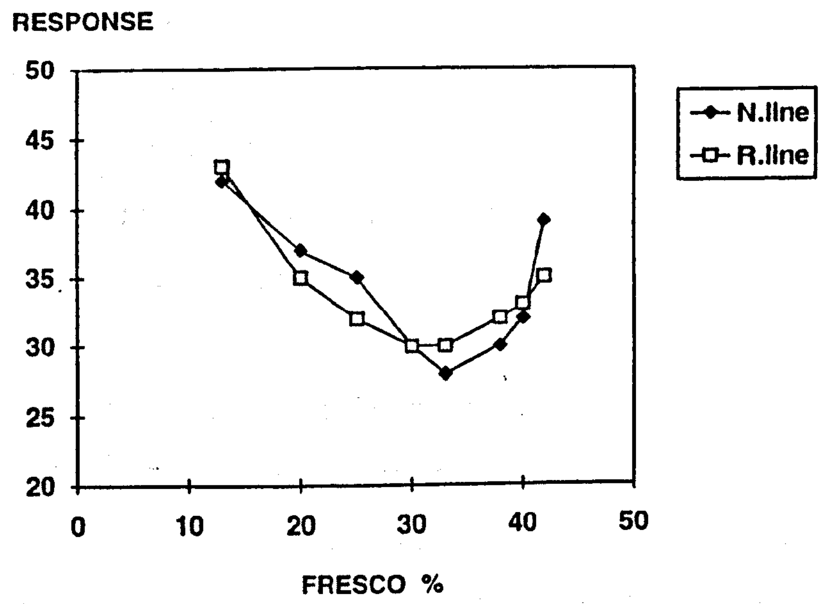

Fig. 4.14: Hardness in chapaties measured with Instron after 10 h of preparation.

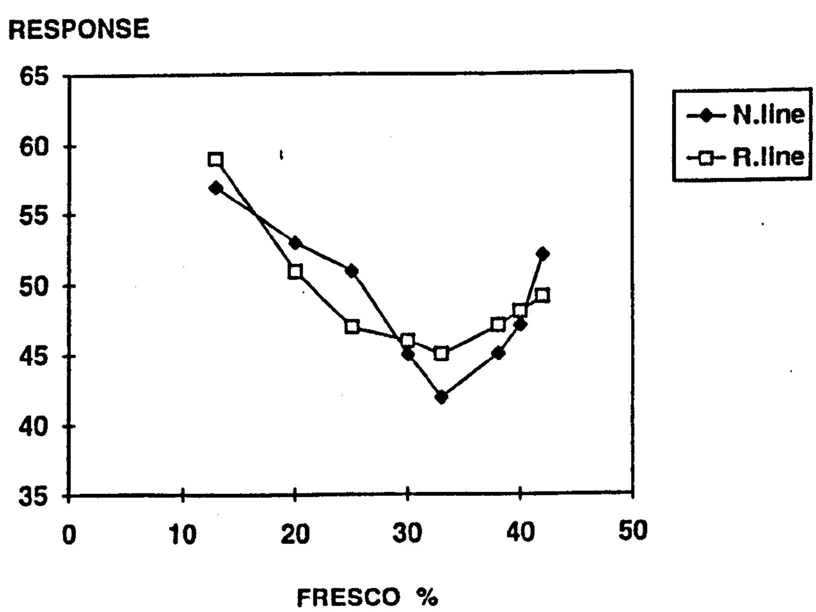

Fig. 4.15: Hardness in chapaties measured with Instron after 24 h of preparation.

Sensory analysis of chapati prepared with blends of Mercia and Galahad: The plots for texture of chapati at different intervals (2.5, 10 and 24 h) are shown in the Figures 4.16 - 4.18. The best response area was found with 50 % Mercia in blends. The worst response area was found for high levels of Galahad. In all the plots a similar trend towards softness of chapati was recorded.

Instron measurements: The plots of instron assessments are shown in Figures 4.19 - 4.21. The best response region was found with 50 % Mercia in Galahad blends. Worst response area was found with a high level of Galahad or Mercia in blends.

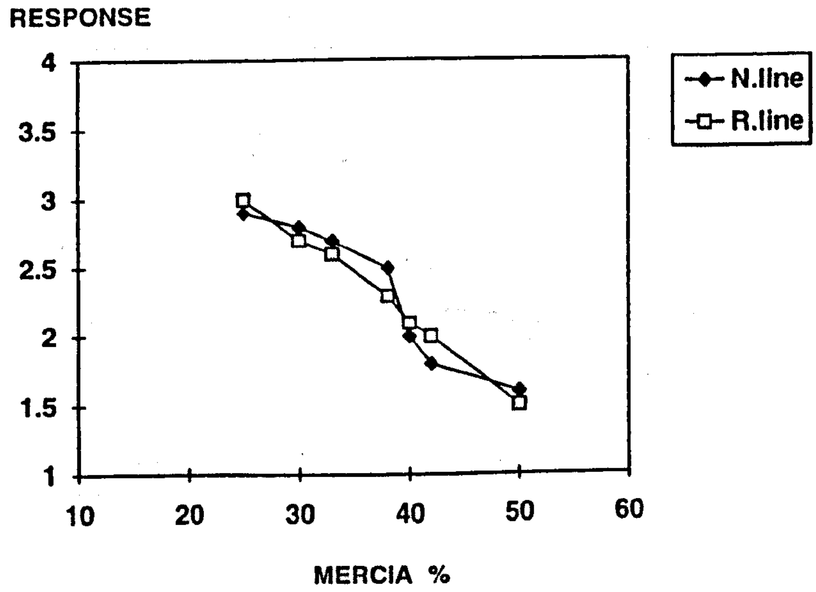

Fig. 4.16: Sensory evaluation of texture of chapaties after 2.5 h of preparation.

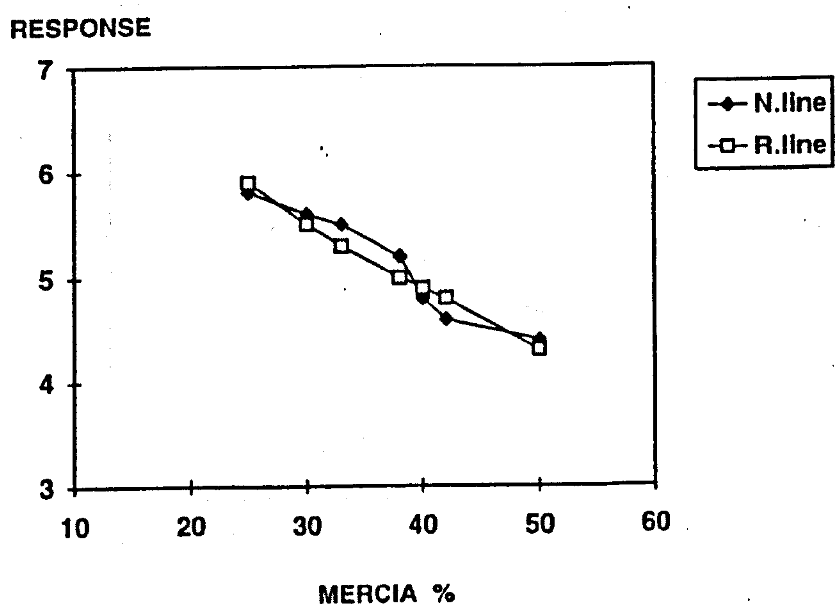

Fig. 4.17: Sensory evaluation of texture of chapaties after 10 h of preparation.

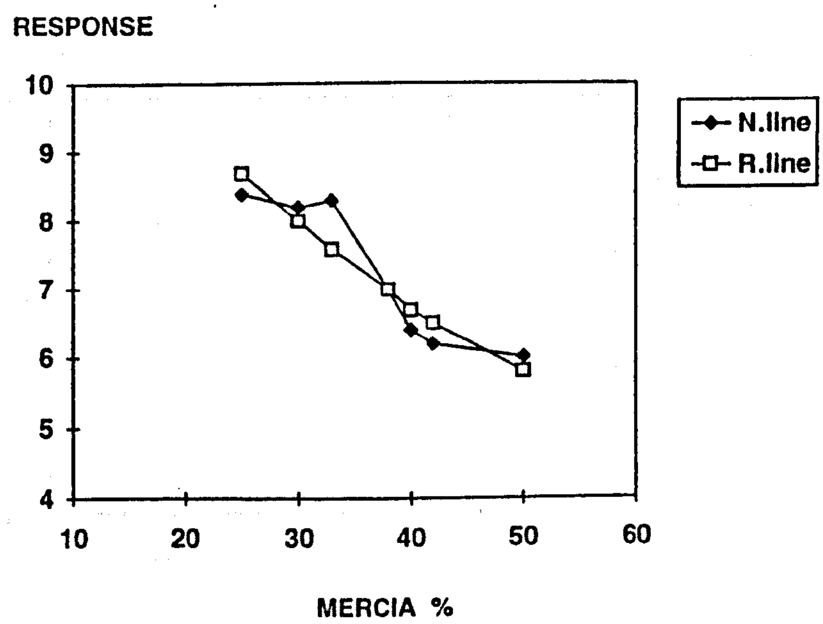

Fig. 4.18: Sensory evaluation of texture of chapaties after 24 h of preparation.

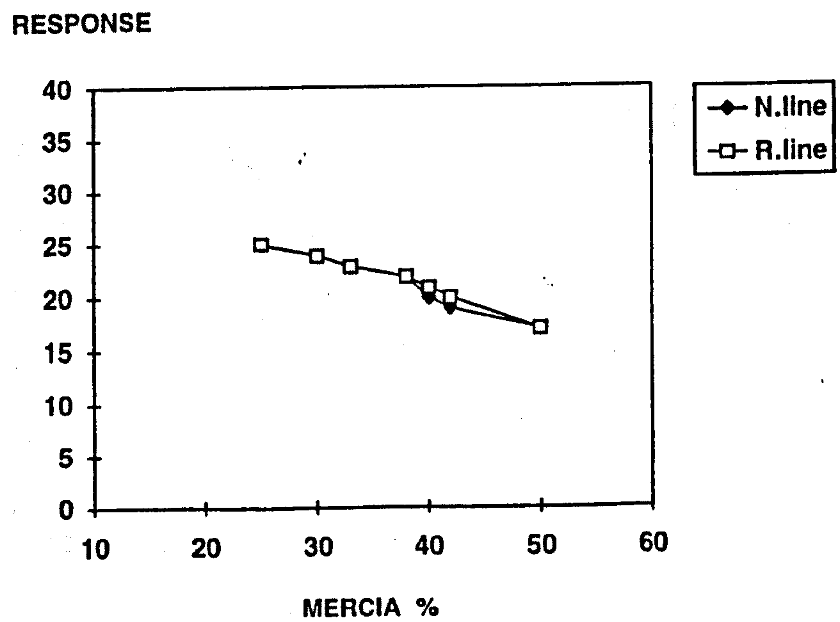

Fig. 4.19: Hardness in chapaties measured with Instron after 2.5 h of preparation.

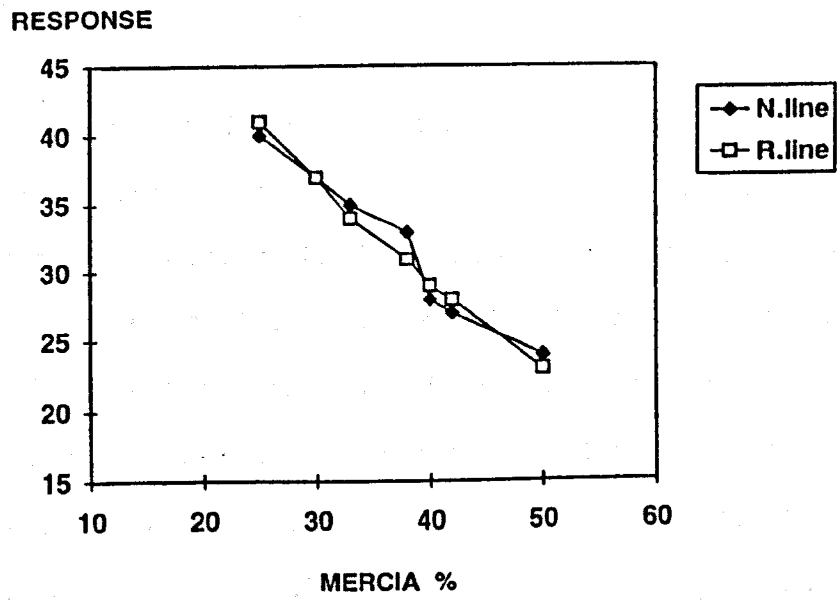

Fig. 4.20: Hardness in chapaties measured with Instron after 10 h of preparation.

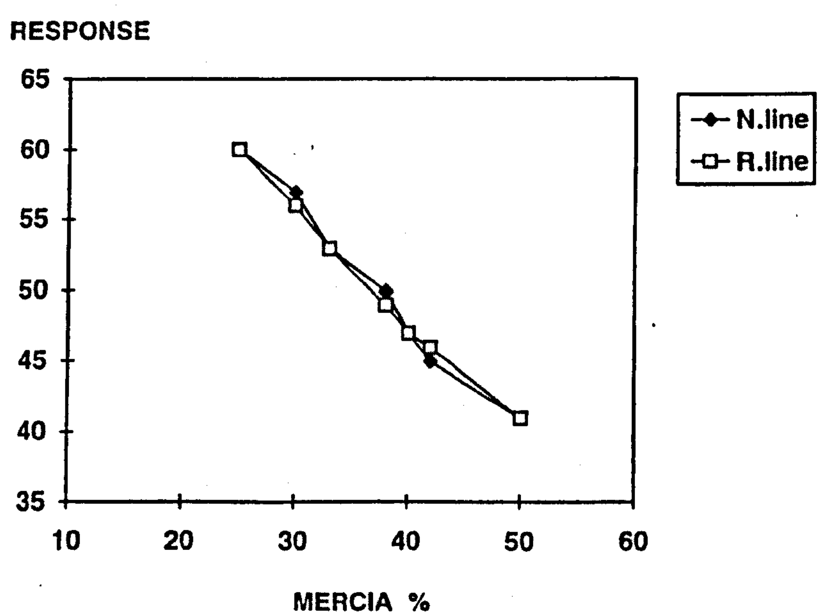

Fig. 4.21: Hardness in chapaties measured with Instron after 24 h of preparation.

Physicochemical characteristics of blended flours: Physicochemical characteristics of blended flours are summarised in Table 4.17.

Rheological characteristics of blended flours: Representative farinograms and extensograms from blended flours are shown in Figures 4.22 - 4.29. The data obtained is tabulated in Table 4.18. Blended flours containing Fresco yielded average values of 6.81 min for dough development and 69 BU for tolerance index. Dough stability ranged from 4.5 to >16.0 min. Blends of Mercia in flour showed values 6.10 min, 7.71 min and 74 BU for dough development, dough stability and tolerance index, respectively, and had reduced quality over Fresco blends.

Table 4.17: Physicochemical characteristics of blended flours.

Flour		Flour protein (%)	DS (%)	FN	GC	Ps <180 µm (%)	Ps 130 - 180 µm (%)
Fres	+ Gala						
13	87	14.63	9.0	543	15.44	26.82	18.48
20	80	14.54	9.1	543	15.49	26.96	18.87
25	75	14.47	9.1	542	15.52	26.14	19.13
30	70	14.40	9.2	541	15.55	25.87	19.40
33	67	14.36	9.2	541	15.58	25.69	19.56
38	62	14.63	9.2	540	15.62	25.40	19.82
40	60	14.27	9.3	539	15.63	25.31	19.94
42	58	14.24	9.3	539	15.64	25.18	20.00
S.d. (pooled)		0.04	0.05	3.78	0.13	0.04	0.05
Merc +	Gala						
25	75	13.88	9.20	504	15.00	26.79	18.00
30	70	13.70	9.27	495	14.98	26.63	18.34
33	67	13.54	9.36	493	14.93	26.51	18.10
38	62	13.40	9.37	482	14.52	26.28	18.11
40	60	13.30	9.44	479	14.85	26.33	18.12
42	58	13.25	9.42	475	14.82	26.27	18.13
50	50	12.96	9.54	462	14.73	26.33	18.00
S.d. (pooled)		0.06	0.05	5.11	0.04	0.05	0.05

S.d. = Standard deviation
Fres = Fresco
Merc = Mercia
Gala = Galahad

DS = Damaged Starch
FN = Falling Number
Ps = Particle size
GC = Grade Colour

Table 4.18: Rheological characteristics of blended flours.

Flour		WA	DD	DSt	TI	R/E 30	R/E 60
Fres	Gala	%	Min	Min	BU	min	min
13	87	64.2	5.50	4.50	85	0.89	1.17
20	80	63.6	6.00	6.00	80	1.33	1.96
25	75	63.5	6.25	8.25	75	1.50	2.00
30	70	63.6	7.00	14.00	62	1.65	2.23
33	67	63.3	7.00	15.00	60	1.84	2.34
38	62	63.2	7.25	16.00	60	2.52	3.15
40	60	62.8	8.00	16.00	55	2.24	3.19
42	58	62.8	7.50	>16.0	50	2.65	3.28
Average		63.4	6.81	-	69	1.82	2.42
Merc	Gala						
25	75	63.0	5.25	4.50	95	1.00	1.53
30	70	62.5	6.00	5.25	80	1.36	1.90
33	67	62.2	5.50	5.50	90	1.40	2.25
38	62	61.9	5.50	6.50	75	1.60	2.40
40	60	61.7	6.50	8.25	62	1.70	2.44
42	58	61.5	6.50	9.50	60	1.74	2.36
50	50	60.8	7.50	14.50	55	2.24	2.92
Average		61.9	6.10	7.71	74	1.58	2.26

WA = Water Absorption
DD = Dough Development
TI = Tolerance Index
BU = Brabender Unit
DSt = Dough Stability

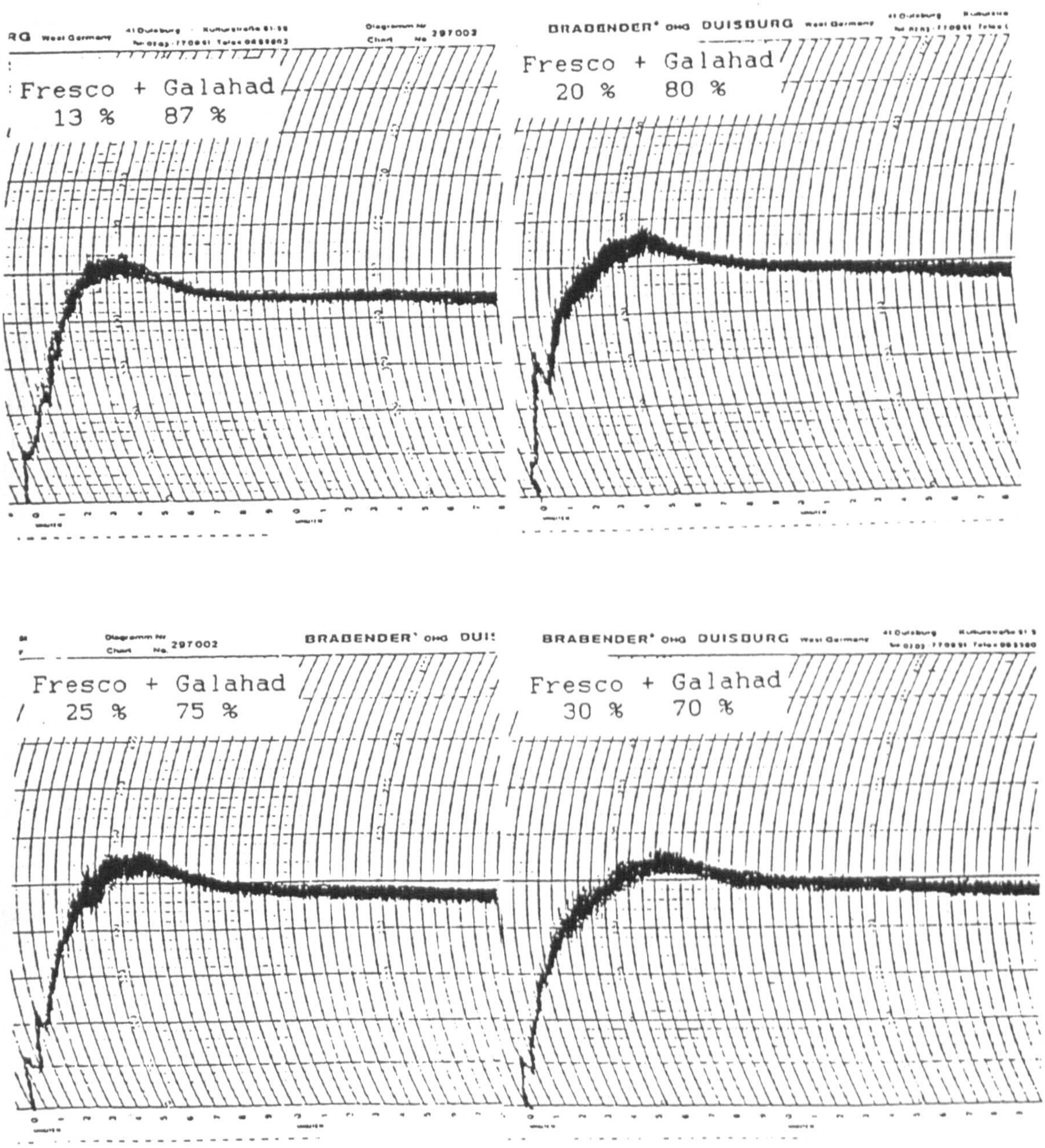

Fig. 4.22: Farinograms of blended flours.

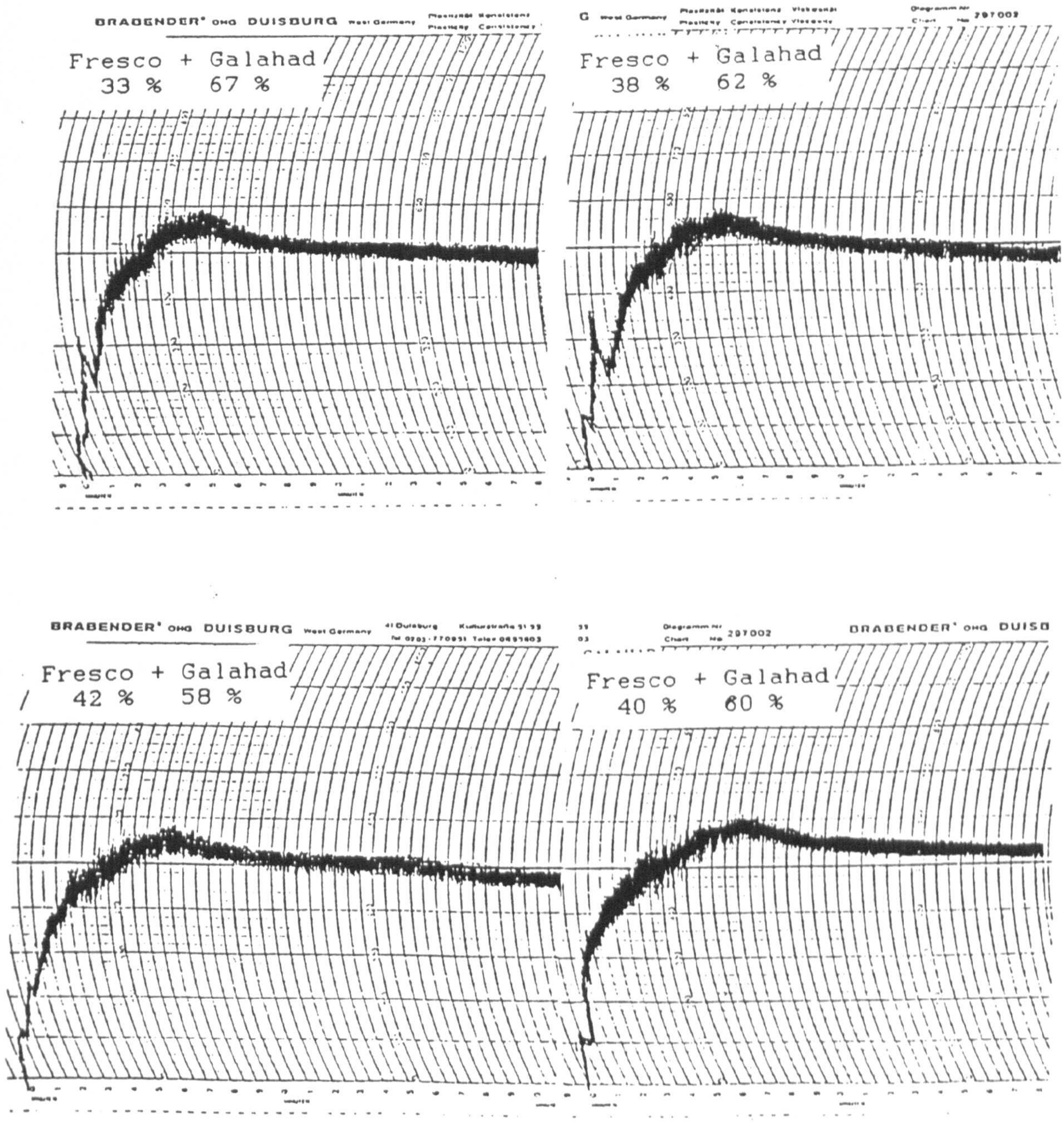

Fig. 4.23: Farinograms of blended flours.

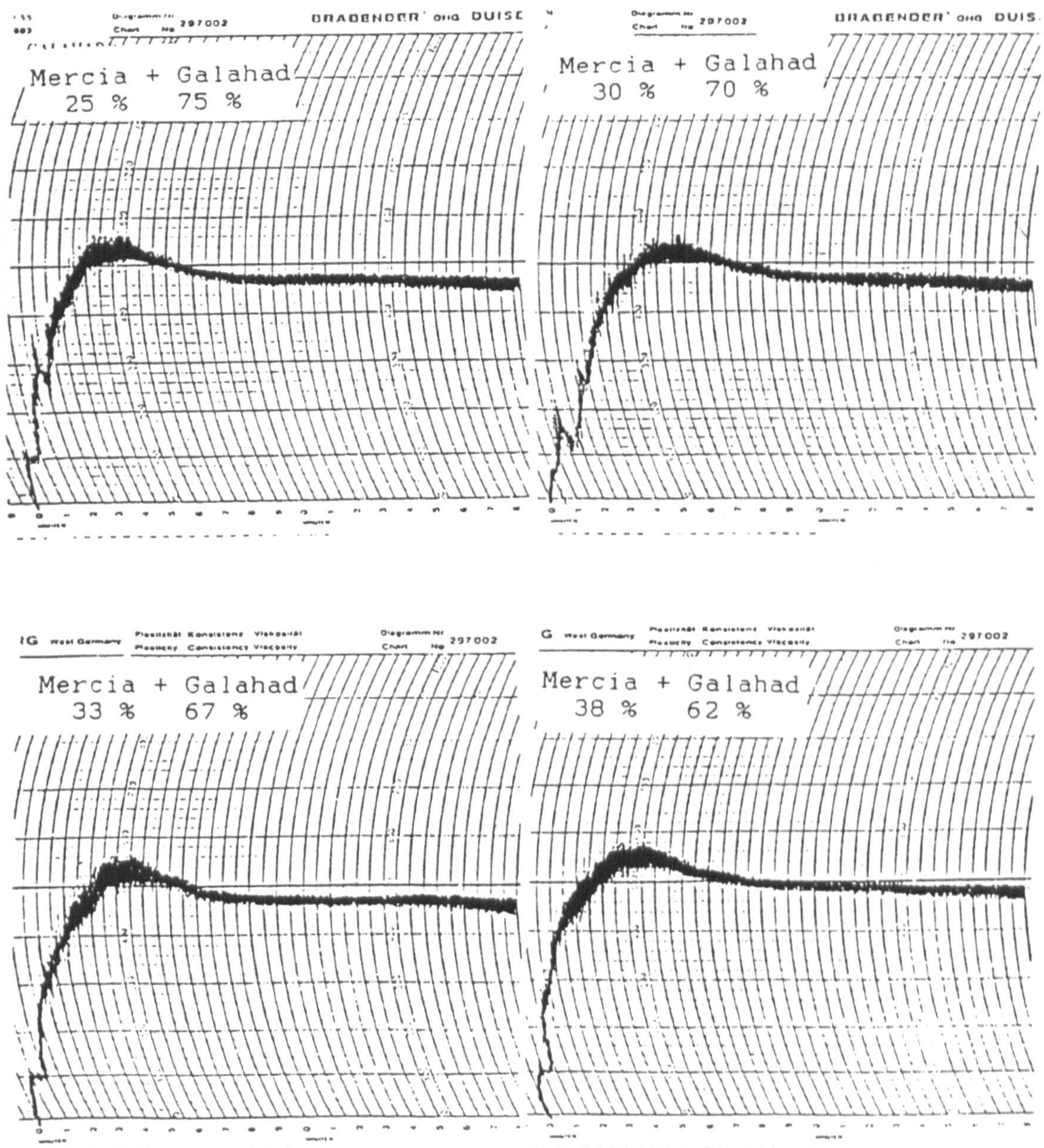

Fig. 4.24: Farinograms of blended flours.

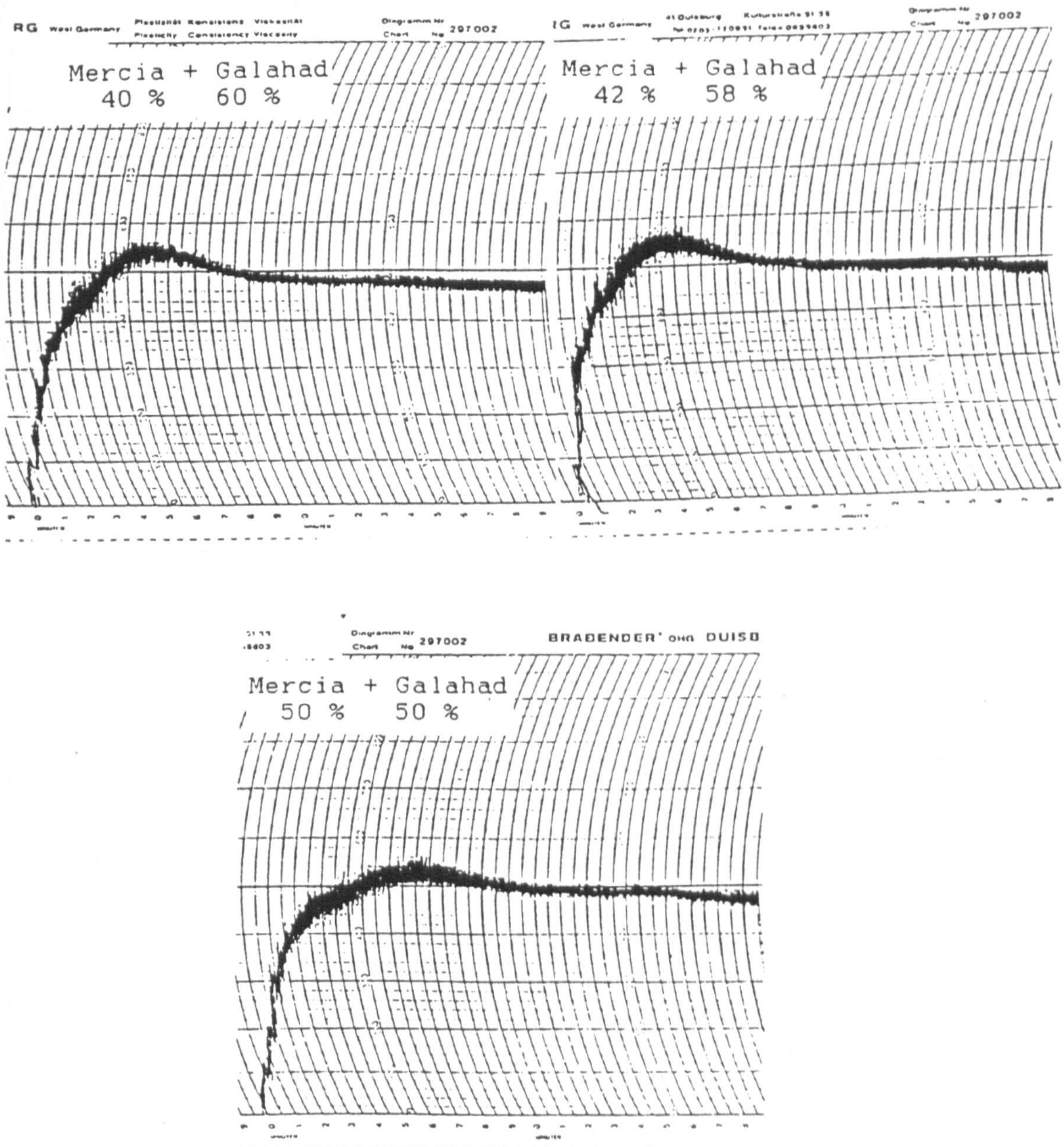

Fig. 4.25: Farinograms of blended flours.

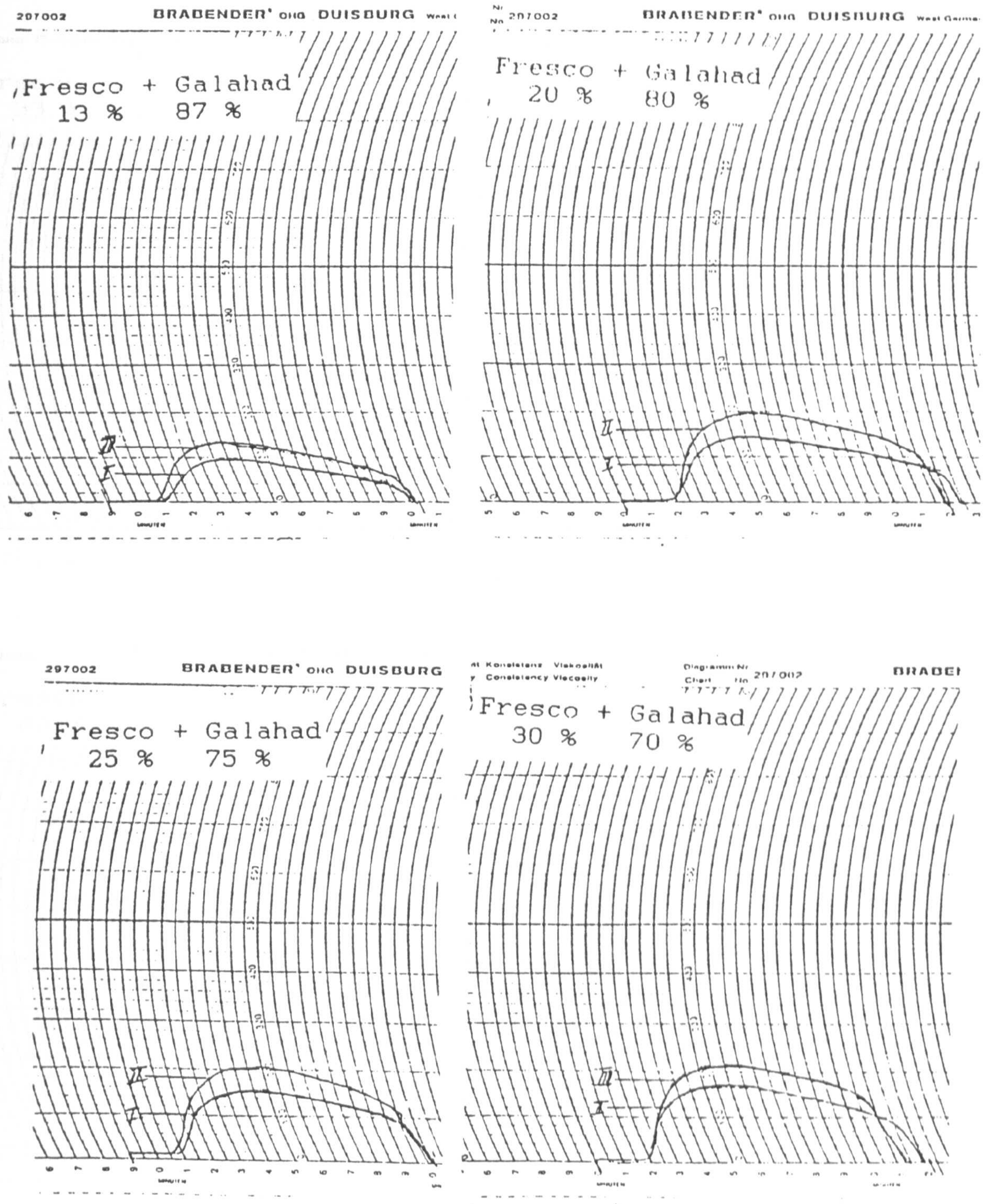

Fig. 4.26: Extensograms of blended flours.

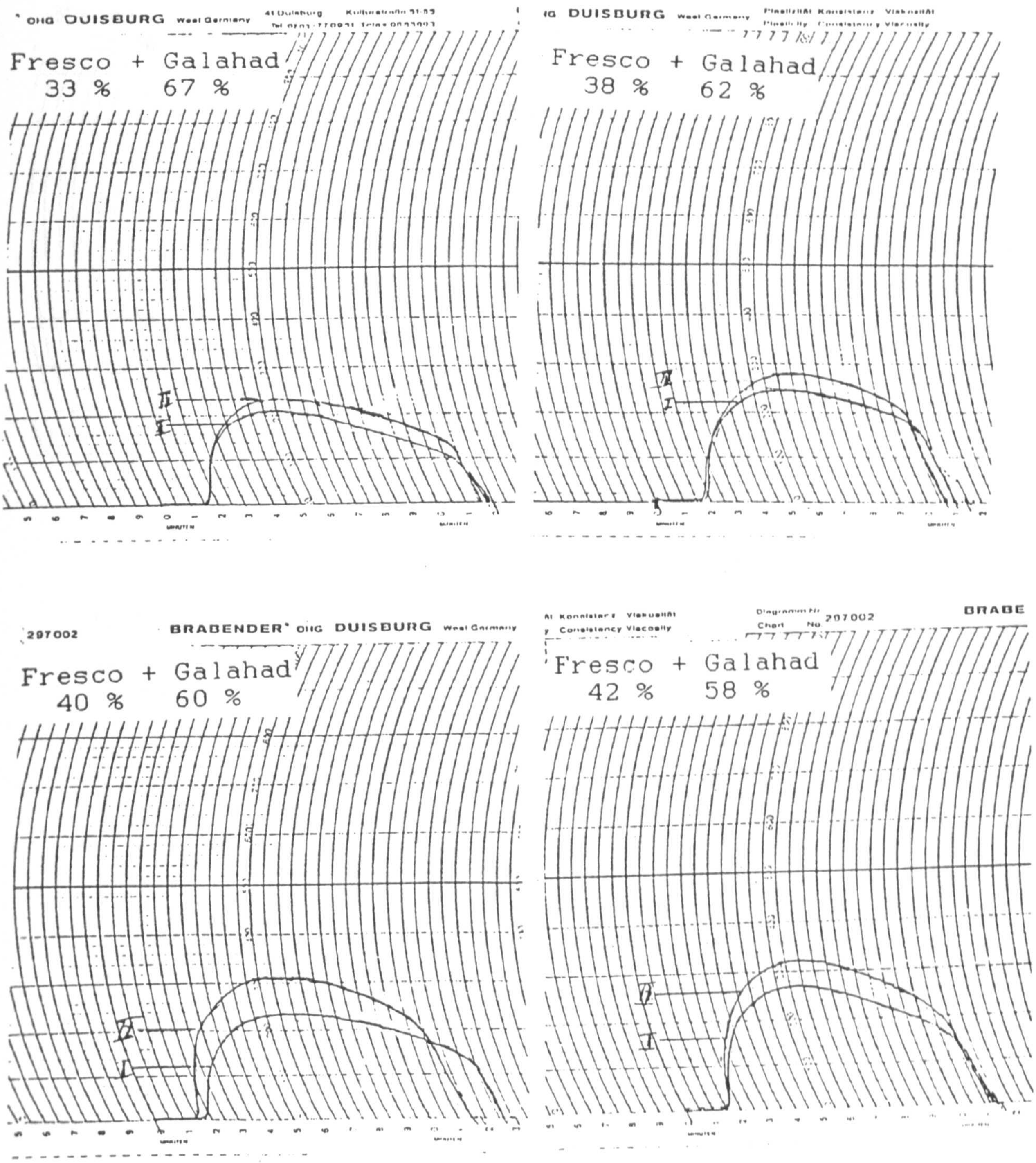

Fig. 4.27: Extensograms of blended flours.

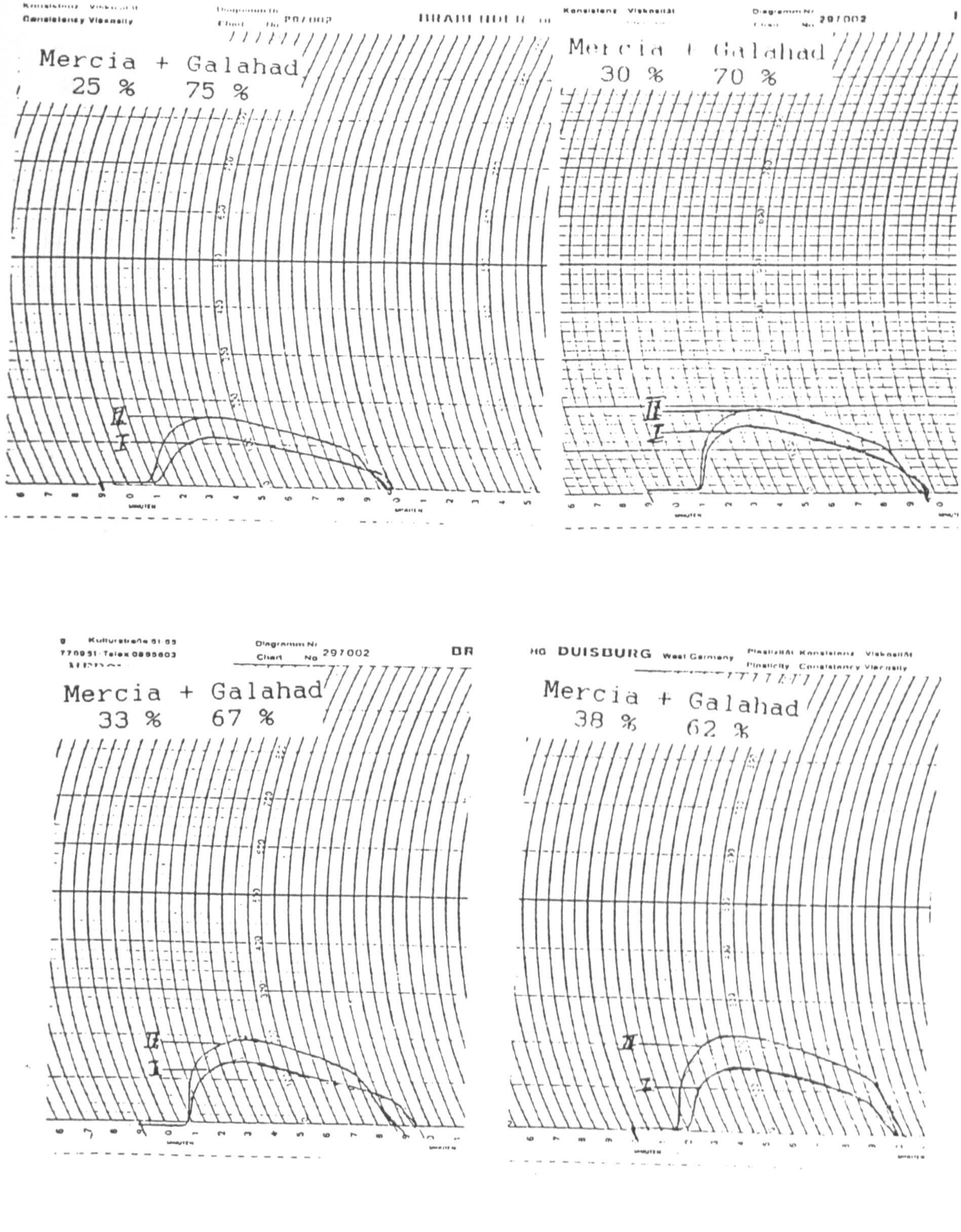

Fig. 4.28: Extensograms of blended flours.

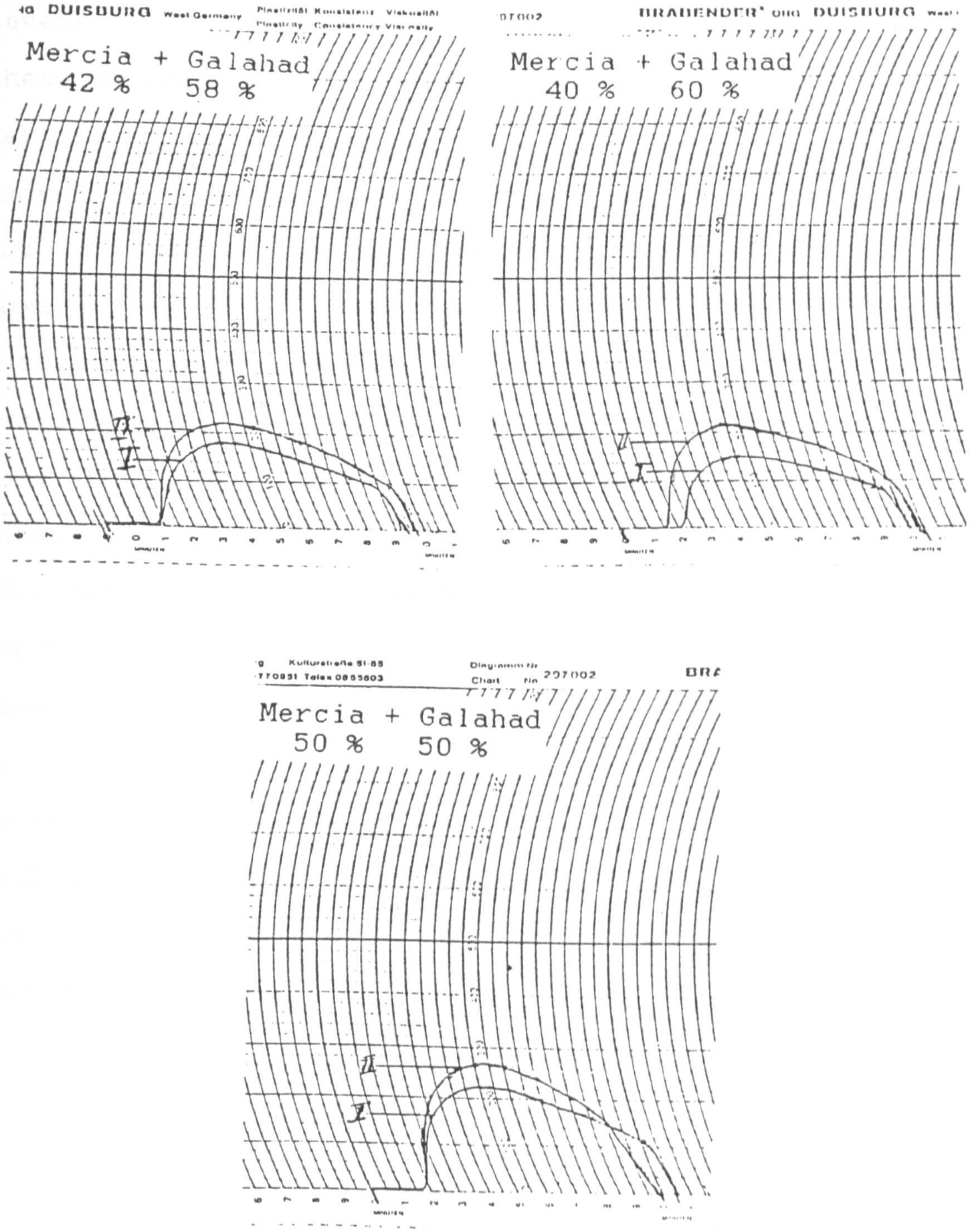

Fig. 4.29: Extensograms of blended flours.

Physical characteristics of doughs prepared from blended flours: Stickiness in the doughs increased in relation to levels of Galahad; sheeting ability and resistance to sheeting improved with increases in contents of Fresco and Mercia. Shrinkage after sheeting of dough into a disc, an undesirable character for chapati dough was increased as more of Fresco and Mercia were incorporated in blends. However, moderate or low physical characteristics of dough such as low to moderate stickiness, low to moderate resistance to sheeting and low shrinkage after sheeting were suitable (Table 4.19) for making of chapati. Intensity of each character was assessed as either low (X), moderate (XX) and high (XXX) based on the previous experience of the baker (researcher's wife) and researcher. The blend containing 33 % Fresco and 67 % Galahad was observed to be moderate in stickiness, low in resistance to sheeting and shrinkage, after sheeting reflecting good sheeting ability. Blends having 50 % Mercia and 50 % Galahad had similar properties except that they were reduced in stickiness.

Table 4.19: Physical properties of dough during processing.

Flour		Handling (Stickiness)	Resistance to Sheeting	Shrinkage after Sheeting
Fresco+Galahad				
13	87	XXX	X	X
20	80	XXX	X	X
25	75	XXX	X	X
30	70	XX	X	X
33	67	XX	X	X
38	62	X	XX	XX
40	60	X	XXX	XXX
42	58	X	XXX	XXX
Mercia+Galahad				
25	75	XXX	X	X
30	70	XXX	X	X
33	67	XXX	X	X
38	62	XX	X	X
40	60	XX	X	X
42	58	X	X	X
50	50	X	XX	XX

Low (poor): X Moderate : XX High : XXX (good)

4.4.3 Discussion

The R^2 values were found to be all sufficiently high to enable predictions of values of responses to be made with confidence (P = 0.05). Values of R^2 ranging between 0.58 and 0.81 were considered as good to excellent (Shelke et al., 1990; Tseo et al., 1983). Malcolmson et al. (1993) and Frye and Setser (1991) stated that R^2 below 0.61 should be regarded as poor and less predictive.

Results in this study indicated that with the decreases in content of hard grain cultivars in blends, the textural hardness of chapaties decreased. However >35 % of Fresco or >50 % Mercia adversely affected physical preparation quality. This might be due to over strength and shortness of doughs, previously reported limiting factors among the hard wheats for the production of quality flat breads (Quail et al., 1991).

Haridas (1982) reported that chapaties considered quite chewy had been made with flour of high protein content and had a higher tearing resistance value. Hard wheat flours with high protein content and gluten strength are generally used for leavened bread making. Hard durum wheat is best for pasta; and soft white wheat with low protein content and weak gluten is used for pastry production (Hoseney et al., 1978). Thus, blending of hard and soft wheats with appropriate proportion could balance the properties required to obtain the optimum product.

Chapaties lost softness gradually during storage

(Table 4.13). Changes in firmness have been reported to be due to changes in starch, protein, lipid and water content from aging (D'Appolonia and Morad, 1981). Such changes increase brittleness and decrease tearing resistance in chapaties (Rao et al., 1986a). With leavened bread (Maga, 1975), chemical changes unrelated to moisture may also take place during storage.

The results of sensory and Instron evaluation were highly correlated at 2.5 h (r = 0.90), 10 h (r = 0.95) and 24 h (r = 0.96) intervals as reported previously for other breads (Kramer, 1972). Further, it has been proved that the Instron could be used with confidence as a predictor of sensory score as correlation coefficient ranged between 0.90 and 1.0 (Bourne, 1982). This has been previously related to consumer acceptance (Baker et al., 1988). The plots of sensory and Instron data permitted the identification of blends where all predicted characteristics met or exceeded the commercial chapati flour (MWCF) (Fig.4.10 - 4.21). The blends of Fresco and Galahad (33 + 67) and Mercia and Galahad (50 + 50) yielded high values for water absorption and produced soft, tender and pliable chapaties regarded as desirable (Kandhari, 1975; Douglas, 1981). Austin and Ram (1971) stated that the texture of chapati remained soft and pliable for several hours prepared from blended flour, regarded as a most desirable quality character from the standpoint of consumer acceptance (Yamazaki and Greenwood, 1981).

Farinograph data indicates properties of the doughs only during mixing. For further characterisation, extensograph should be used. Table 4.17 summarises the parameters measured by both instruments. Dough water content at a 500-BU farinograph consistency ranged between 62.8 and 64.2 % for Fresco + Galahad, and 60.8 and 63.0 % for Mercia + Galahad blends. Dough development increased in relation to content of Fresco and Mercia in blends. Dough development times, dough stabilities and tolerance indices of selected blends were found to be similar to those from the reference sample of commercial chapati flour. The results agreed with previous findings (Luzfernandez and Berry, 1989).

Ratio R/E was also used for comparing the properties of flours. The fundamental properties responsible for resistance to deformation are elasticity and viscosity of the dough: these influence the shape of extensograms (Navicks and Nelsen, 1992). Increase in ratios was more pronounced at higher levels of replacement of Fresco and Mercia and lower levels of Galahad in blends and vice versa. Strong wheats are regarded as usually having a high protein content, characterised by a high water absorption, a long development time, more stability, small mixing tolerance index and in load-extension tests, by large resistance and a large extensibility (Table 4.9 - 4.10). Flours lacking in these properties are referred to as weak (Pomeranz, 1988). The rheological optimum, suggesting dough with the best physical properties for chapati making, was achieved by blending strong and weak wheats with opposing characters as has been reported previously (Schafer, 1972; Bolling, 1981; Fitchett and Frazier, 1988).

In this study, physical properties of dough observed during preparation of chapati had relevance with rheological characteristics of selected blends (Table 4.19). The dough of Fresco blend (33 + 67) had moderate stickiness, low resistance to sheeting and low shrinkage after sheeting. A Mercia blend (50 + 50) dough had low stickiness, moderate resistance to sheeting and shrinkage after sheeting. The gluten content of chapati flour should be low and the protein matrix should be very extensible to allow the necessary stretching of the dough into the pancake shape without shrinkage back (Douglas, 1981). Good sheeting quality has been reported to have a significant effect on the quality and shelf life of the product (Qarooni et al., 1992).

Physicochemical properties of blends were similar to those of the commercial chapati flours (Tables 4.1 - 4.2). The values were considered to be moderate and blends were suited for chapati preparation (Adsule and Lawande, 1986; Williams et al., 1988; Hanslas, 1986; Qarooni et al., 1992). Quail et al (1991) further demonstrated that damaged starch in the range of 6.0 - 9.0 % gave optimal results for flat bread production. Flours of low falling numbers showing high amylase activities due to sprouted grain are unsuitable for chapati production (Luckow et al., 1990; Rao et al., 1986a).

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5. RELATIONSHIP BETWEEN PROTEIN COMPOSITION AND FLOUR QUALITY PARAMETERS

5.1 Objective

Quantification of total proteins of British wheat cultivars from SDS-PAGE with scanning densitometry to determine relationships with rheological characteristics of flour related to chapati production.

5.1.1 Results and discussion

Protein extractability

The average protein extractability was 70 % (range 63 - 74 %). The total protein content was affected by the relative amounts of flour proteins (Table 5.1), in agreement with the findings of Danno and Hoseney (1982). Their finding was that 72 % of total nitrogen was extracted from flour by mixing whereas Danno (1981) reported 77 % of nitrogen solubilised into a clear solution by gentle mixing of flour with aqueous 0.5 % sodium dodecyl sulphate.

The average protein extractability without sonication has been reported as 72.1 % (range 60.6 to 78.1 %) (Singh et al., 1990b). This was found to increase to 100 %, with a range of 90.4 to 109.8 % with the application of sonication. Khan et al. (1994) reported 95 - 100 % protein solubility for sonicated and dithiothreitol (DTT) treated samples of hard spring wheat. It is evident from Table 5.1 that protein from lower-strength gluten was more easily extracted than those from the higher-strength (better rheological properties; Table 4.9 and 4.10) e.g. Fresco and Mercia. These results are consistent with previous findings (Orth and Bushuk, 1972; Dachkevitch and Autran, 1989) and form the basis for residue protein test used by some plant breeders for early quality evaluation in wheat varieties. Singh et al., (1990a) obtained 64.5 % and 79.4 % extraction of total protein from strong and weak varieties, respectively, using a simple mixing method.

Molecular pattern of proteins

Molecular weights (MWs) of storage protein patterns of 8 British cultivars and commercial chapati flour (Mehrani wholemeal flour) were determined using a primary standard reference (Fig. 5.1). Similar volumes of protein extract were loaded for each sample to the gel, and thus differences in the intensity of bands between samples reflect differences in protein content.

Typical densitometer scans of the electrophoretic pattern of each variety are shown in Fig. 5.2 - 5.6.

The patterns were divided into five groups of bands, A1 - A5. Assignment of relative molecular weight ranges corresponding to each area was made on the basis of previous studies (Cole et al., 1981; Fullington et al., 1983, 1987; MacRitchie et al., 1991). The high molecular weight (HMW) glutenin subunits are clearly differentiated into group A1, $\geq 80 \times 10^3$ (80 KD); A2 group is mainly w-

Table 5.1: Distribution of protein among five molecular weight fractions (Percent of total area (A1 - A5).

Variety	Protein % (d.b.)	Protein extracted %	Relative area (%)					
			A1	A2	A3	A4	A5	RT
Torfrida	12.80	70	7.00	17.60	18.50	32.35	25.10	0.34
Pastiche	14.81	74	6.75	18.75	21.50	31.45	19.10	0.39
Mercia	11.11	63	5.90	19.80	22.15	31.70	20.40	0.39
Fresco	13.48	70	7.70	18.25	21.75	34.80	17.90	0.42
Avalon	14.43	69	8.35	15.40	17.90	35.65	22.70	0.36
Hereward	13.00	72	6.95	17.40	19.90	31.45	23.00	0.36
Riband	12.24	65	6.25	18.65	17.70	34.45	20.70	0.32
Galahad	14.80	74	6.00	20.65	18.30	34.60	20.65	0.32
Mehrani wholemeal flour	13.69	71	7.10	21.35	21.00	29.60	20.35	0.39
S.d.	0.07	2.95	2.72	3.40	4.14	3.65	2.10	0.03

Average of two replicates   S.d. = Standard deviation (pooled)

RT (Ratio): $\frac{A1+A3}{A2+A4+A5}$

gliadin, 80 - 51 KD; A3 mainly low-molecular-weight (LMW) glutenin subunits from 50 - 41 KD (Tao and Kasarda, 1989). Certain higher molecular weight gliadins have mobilities in the A3 range (Kasarda et al., 1987). Group A4 corresponds mainly to α- and ß-gliadins but certain LMW appear in A4 (Tao and Kasarda, 1989). They range from 40 - 28 KD. Area A5 corresponded to LMW albumins and globulins, ≤ 28 KD. In groups A3, A4 and A5 different protein entities overlap somewhat. For protein molecular weights near 38 KD and 12 KD there was apparent aggregation of 2-3 bands resulting in an unexpectedly high value. This may relate to somewhat heavy loading of samples. Varietal differences can be noted in the patterns (Table 5.1). Quantifications performed by the densitometer are percentages of total area, and are also related to the amount of dye bound by each polypeptide.

A1, A2 and A3

The proportions of proteins corresponding to areas A1 and A2 were higher in those varieties with high protein contents. Varieties with stronger rheological behaviour (Table 4.9 and 4.10) contained a higher proportion of protein corresponded to area A3. The proteins of area A1 - A3 were mainly composed of glutenin components (Beitz and Wall, 1972, Cole et al., 1981), although gliadins also contribute somewhat less than half the area A3 (Kasarda et al, 1976), and w-gliadin should contribute slightly to A2 (Charbonnier, 1974).

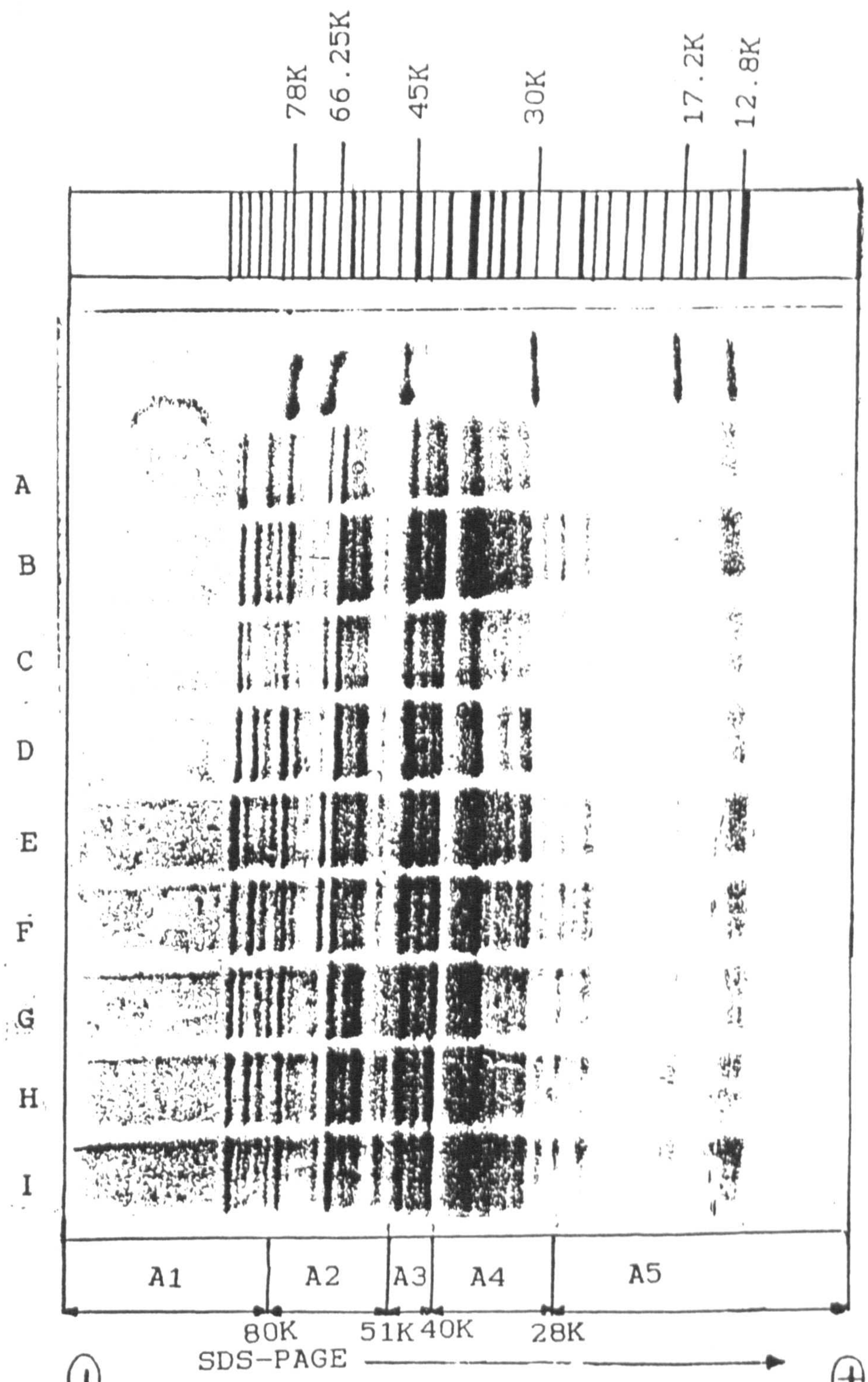


Fig. 5.1: Sodium dodecyl sulphate - polyacrylamide gel electrophoresis of the flours A. Torfrida; B. Pastiche; C. Mercia; D. Fresco; E. Avalon; F. Hereward; G. Riband; H. Galahad; I. Mehrani wholemeal chapati flour.

A4-Gliadins

The area A4 relates largely to gliadin proteins, the proportions of which were increased significantly with increased protein content for three varieties but not for Riband and Pastiche. These results are as reported previously; Johnson and Mattern (1977) observed that alcohol-water-soluble proteins (largely gliadins) increased with increased protein content. Dexter and Matsuo (1977) found a significant increase in the proportion of alcohol-water-soluble protein for one durum wheat but not for a second, as total protein increased. Tanaka and Bushuk (1972) found no significant increase in alcohol-water-soluble proteins, however, for two spring wheat varieties. The results of this study support an increase in the proportion of gliadin with increase in protein content as the gliadins are the dominant fractions in the wheat endosperm.

Area A5

Area A5 contains low molecular weight albumins and globulins (Fullington et al., 1980; Cole et al., 1981). The area under A5 was less than that under A4 in the present study (Table 5.1), as reported previously by MacRitchie (1989). The values, ranged between 17.9 - 25.1 %, for A5 agree reasonably well with albumin-globulin contents of 13.6 - 21.4 % estimated by Pence et al. (1954) from wheat samples ranging from 7.0 - 14.2 % total protein contents. Generally, the proportions of these

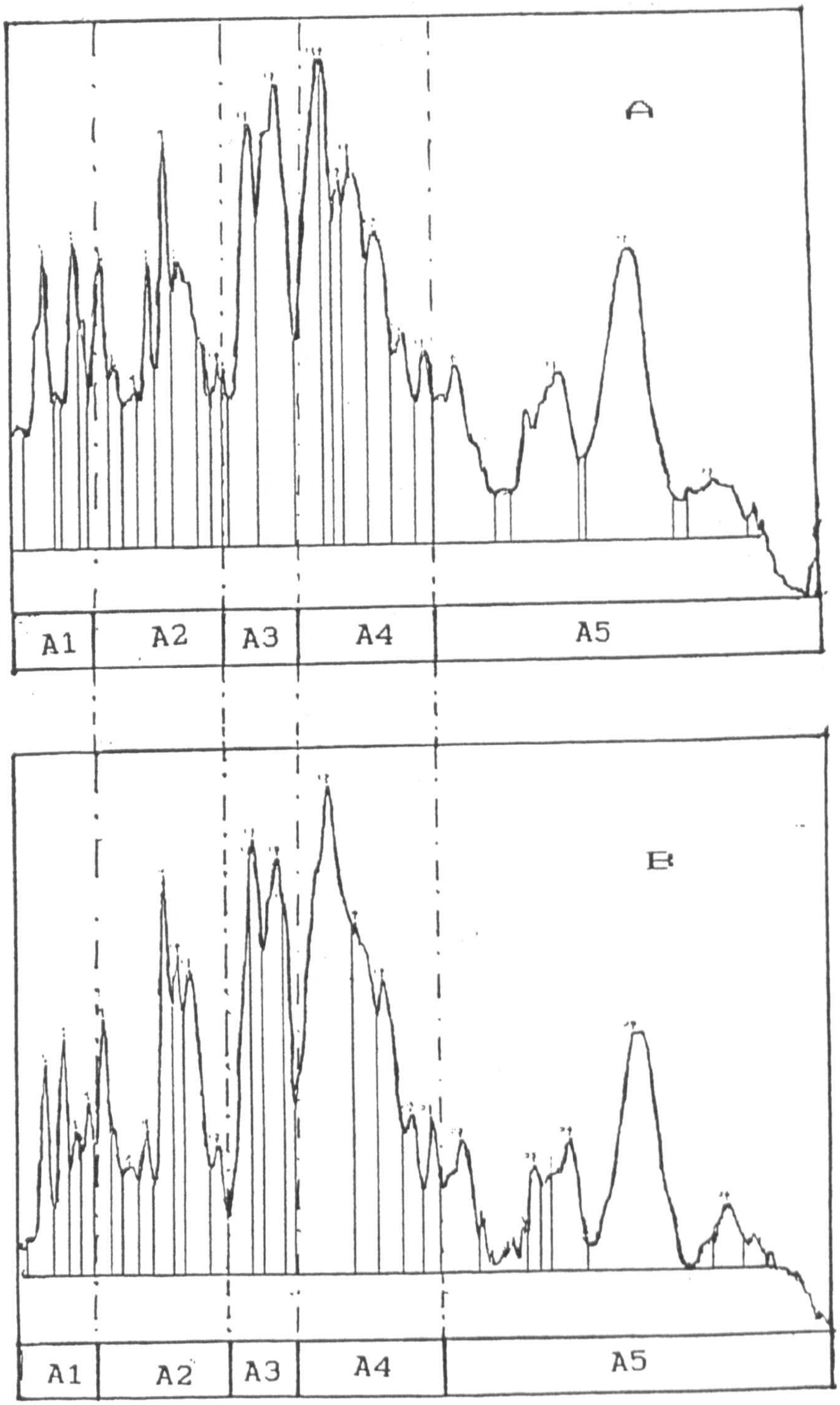


Fig. 5.2: Densitometer scans of sodium dodecyl sulphate -polyacrylamide gel electrophoresis patterns of flours A. Torfrida; B. Pastiche.

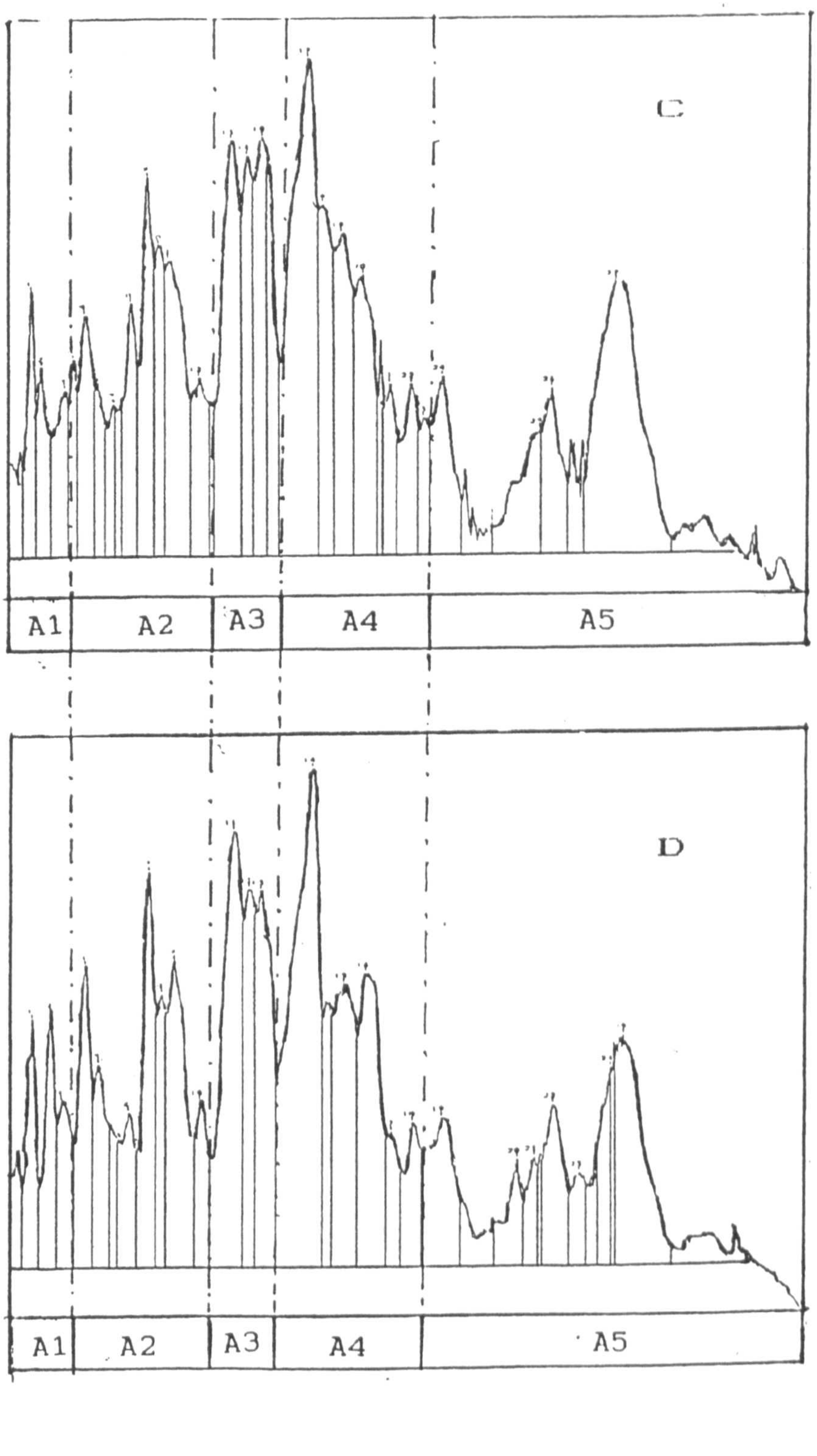
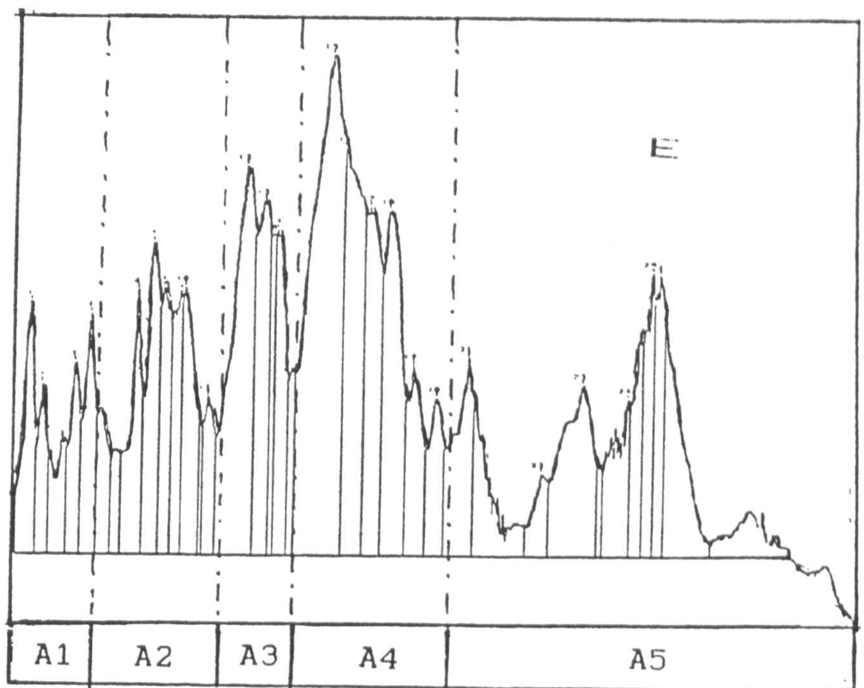


Fig. 5.3: Densitometer scans of sodium dodecyl sulphate -polyacrylamide gel electrophoresis patterns of flours C. Mercia; D. Fresco.

proteins decreased with increase in protein content except with Fresco (17.9 %). This however, contained higher proportion of the protein (13.48 %) than Mercia (11.11 %) or Riband (12.24). Dexter and Matsuo (1977) also reported that albumins and globulins decreased in proportion as total protein content increased. Tanaka and Bushuk (1972) observed a decrease for only one of two varieties whereas Soliman et al. (1980) found no decrease in the quantity of salt soluble proteins when total protein content of wheat was raised through chromosome addition. Decreases in proportion of albumins and globulins with increase in total protein were also reported by Fullington et al.,(1983) and MacRitchie et al.,(1991).

The ratio between glutenins (A1+A3), gliadins, albumins and globulins (A2+A4+A5) was highest in Fresco (0.42) followed by Mercia and Pastiche (0.39). These varieties are strong with respect to peak time (Table 4.9 and 4.10). As this ratio increased, strength of dough also improved. Galahad and Riband flours were found to have poor rheological properties and also the lowest ratios as reported by MacRitchie (1989). The dough strength and baking potential of flour may be manipulated by varying the ratio of polymeric to monomeric proteins.



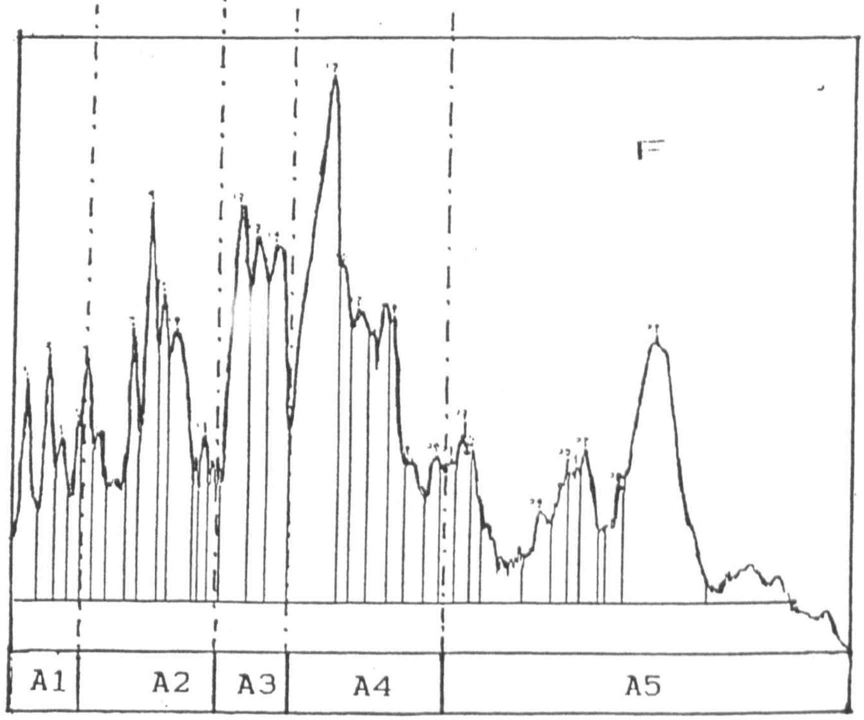


Fig. 5.4: Densitometer scans of sodium dodecyl sulphate -polyacrylamide gel electrophoresis patterns of flours E. Avalon; F. Hereward.

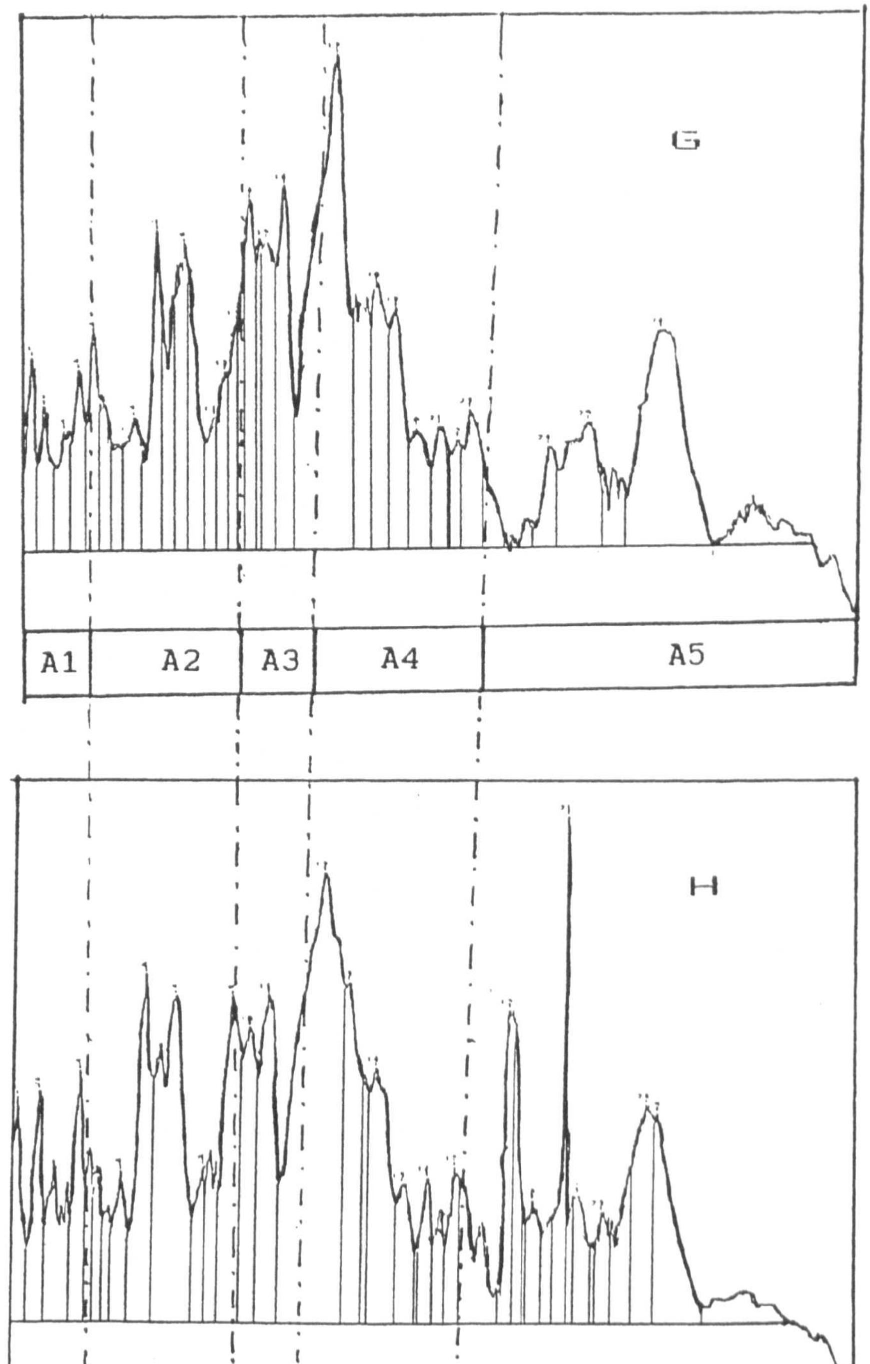


Fig. 5.5: Densitometer scans of sodium dodecyl sulphate -polyacrylamide gel electrophoresis patterns of flours G. Riband; H. Galahad.

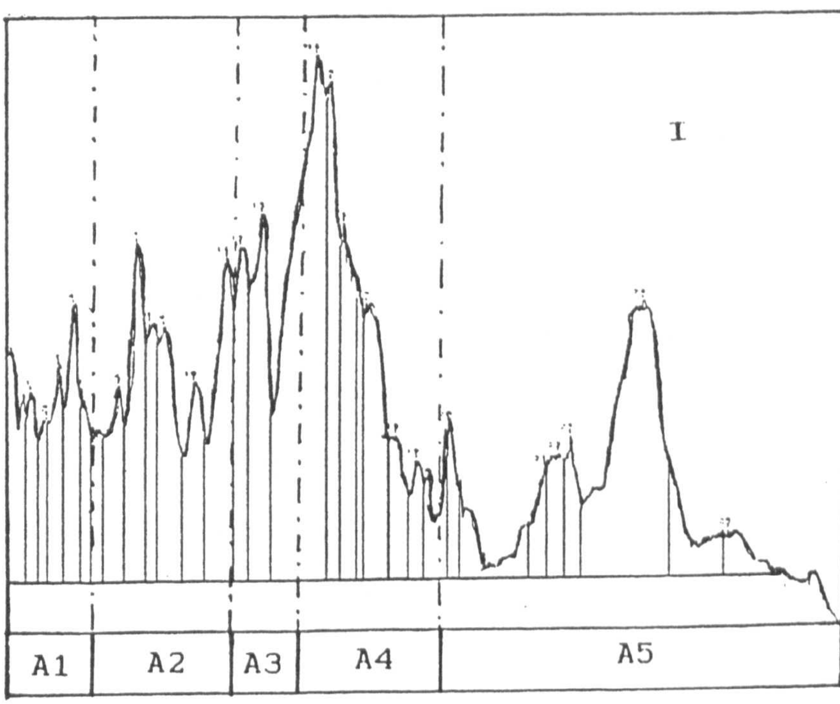


Fig. 5.6: Densitometer scans of sodium dodecyl sulphate -polyacrylamide gel electrophoresis patterns of flours I. Mehrani wholemeal chapati flour.

Association between protein composition and quality attributes

Table 5.1 shows the average values for protein in the glutenin, gliadin, albumins and globulin fractions and Tables 4.3, 4.4, 4.9 and 4.10 provide information about five rheological criteria. Application of Partial Least Squares regression (PLS2) provided information on relationships. A two dimensional loadings plot was generated to elucidate relationships between these variables (Fig. 5.7). Variables situated together far from the origin are positively correlated. If present on opposite sides of origin, they are considered as negatively correlated. Dough development time was significantly correlated ($r = 0.78$, $P<0.05$) with glutenin content (A1+A3) and with ratio of A1+A3 / A2+A4+A5 ($r= 0.81$, $P<0.01$). Dough development also showed a significant strong correlation ($r = 0.74$, $P<0.05$) with content of low molecular weights (LMW) glutenin (A3). Dough development was correlated negatively ($r= -0.80$, $P<0.01$) with group A2+A4+A5 (gliadins, albumins and globulins, respectively). This indicated that these protein fractions had adverse effects on the strength of flour. Fresco and Mercia had higher values of dough development than weaker flours, consistent with the findings of MacRitchie (1984). Peak development time decreased as the proportion of glutenin decreased. Dough stability showed significant negative correlation ($r = -0.66$, $P<0.05$) with gliadins (A2+A4), reflecting weakening of flours with increases in the gliadins content. Tolerance index was correlated significantly with gliadin content (r = 0.73, P<0.05) and with GL+G+A (r = 0.75, P<0.05) indicating weakening in strength of flour as the proportion of these proteins increased. Both weak varieties (Riband and Galahad) showed higher percentages of gliadin proteins (55.3 and 55.0 %, respectively) than those generating strong flours.

The SDS-PAGE profiles of protein fractions from the cultivars Riband and Galahad showed similarities in HMW-G composition. This was correlated with poor performance in dough development, dough stability and tolerance index as reported by MacRitchie et al. (1991). In an earlier study, MacRitchie (1987) reported that an increase in the gliadin fraction decreased mixing requirements, whilst an increase in glutenins induced large increase in dough development time. MacRitchie (1989) also demonstrated that dough strength may be manipulated by varying the ratio of polymeric to monomeric proteins (i.e glutenins to gliadin, albumins and globulins). The gliadins contribute to dough extensibility, whereas the glutenins confer strength and elasticity to dough (MacRitchie, 1984).

Although extensibility was correlated with w-gliadin content (A2), this correlation was seen to be both negative and weak (r = -0.53). A weak positive correlation (r = 0.60) was also observed with HMW glutenin content (A1). The positive correlation with HMW glutenins and negative correlation with

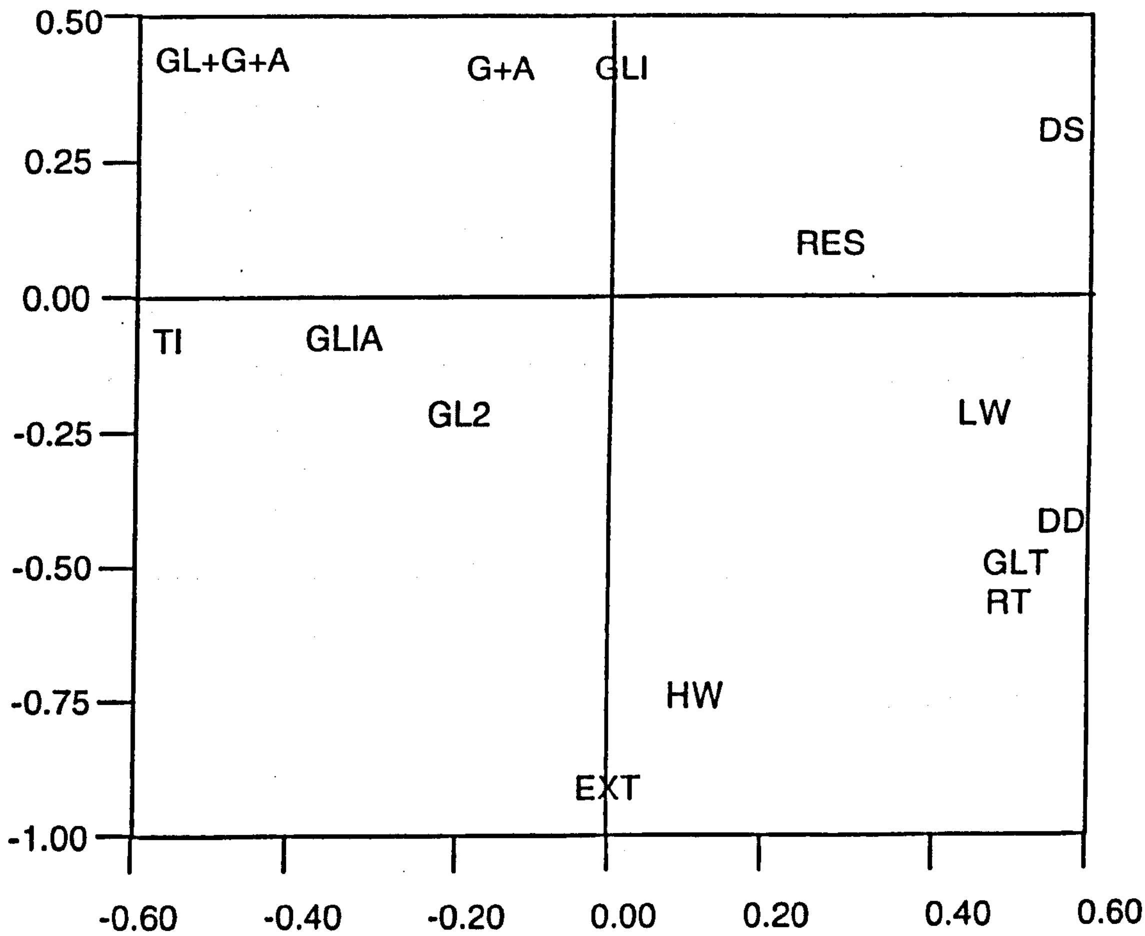


Fig. 5.7 : Two dimensional loadings plot (factor <1,2> <1,2>) illustrating relationships between Rheological characteristics: Dough development time (DD), Dough stability (DS), Tolerance Index (TI), Resistance to extension (Res), Extensibility (EXT) and protein fractions: High molecular weight glutenins (HW), w-gliadins (GLI), Low molecular weight glutenins (LW), α+ßgliadins (GL2), Globulins and Albumins (G+A), Glutenins (GLT), Gliadins (GLIA), Gliadins+Globulins+ Albumins (GL+G+A), Ratio of HW+LW/ GL+G+A (RT).

gliadin are possibly due to variable overlap of components between protein fractions, depending upon the isolation procedure used (Beitz and Wall, 1980; Chakraborty and Khan, 1988). However, Guthrie (1896) has reported that increased gliadin contents produce a weak, sticky and inelastic gluten and flours in which glutenin predominates yield strong, tough, elastic, non-adhesive gluten. Although resistance to extension (RES) showed weak negative correlations with gliadin contents (A2+A4) and α+ß gliadins content (A4), it was correlated significantly (r= 0.75, P<0.05) with Dough stability (DS). These results are in close agreement with those of Singh et al. (1990b), who reported that extensograph dough resistance and farinograph dough development were positively correlated with glutenin content (r=0.84, 0.89, P=0.001).

Correlation between rheological tests and storage proteins bands

After separation and quantification of storage proteins of 8 wheat cultivars and a commercial chapati flour, relationships between polypeptides and rheological data were sought. 23 - 29 protein bands (Fig. 5.1) were quantified by densitometer analysis of SDS-PAGE gels. It is evident from Fig. 5.8 that not all of the bands were positively correlated with each parameter. Moreover, certain had opposite physical influences. This related to the balance between extensibility and resistance to extensibility (r= -0.59). Tolerance index and resistance to extension were also inversely related (r= -0.54). Polypeptide band 12 showed two significant correlations but with different signs; the correlation between polypeptide B12 and TI was negative (r= -0.66, P<0.05) and that with resistance to extension was positive (r= 0.67, P<0.05). Therefore, it appeared that content of this polypeptide was related to strength, as increases in resistance to extension and decrease in tolerance index are indicative of strength of dough. Dough development was also correlated significantly (r=0.67, P<0.05) with polypeptide B12. Tolerance index was positively correlated with polypeptide B9 and negatively with polypeptide B7 (r=-0.77). Dough stability had an inverse relationship with tolerance index but highly significant positive correlation with polypeptide B7 (r= 0.83, P<0.01). Resistance to extension was negatively correlated with polypeptide B9 but positively with polypeptide B7. Dough stability was correlated positively with polypeptide B5. It appeared from these associations that polypeptides B5, B7 and B9 were positively correlated with flour strength. Polypeptide B10 was positively correlated with extensibility (r= 0.68, P<0.05).

Thus, five polypeptides out of 35 observed as bands were clearly correlated with rheological quality parameters. Of these bands 3 related to w-gliadin polypeptides and 2 to HMW glutenin. Polypeptides B9, B10 and B12 corresponded to MWs 70, 66.2 and 59 KD of w-gliadin. Correlations between the quantity of certain gliadin components and rheological data would require further study before relationships can be established. In particular, it is known that gliadins have a favourable influence upon the dough extensibility (Preston et al., 1975). Finney (1971) reported that extensibility was the major quality characteristic influenced by gliadin content. Extensibility was as expected negatively linked to resistance to extension but, to a certain degree, extensibility contributed to the character of the dough strength (Branlard and Dardevet, 1985a).

Comparison of results obtained in this study with those of previous authors is difficult due to use of different electrophoresis methods (Sozinov and Properelya, 1974; Wrigley et al., 1981). Moreover, information on correlations between storage protein polypeptides and rheological tests is limited.

Comparison of the results obtained here with those of Payne et al. 1987 suggested that polypeptide bands 5 and 7 may be related to HMW glutenin subunits 7 and 10 respectively, known to have favourable influences on dough strength. Branlard and Dardevet (1985b) reported that High molecular weight glutenin subunits 2*, 5, 7, 9, and 10 were related to strength and tenacity of doughs.

The study suggests that increase in the proportions of gliadins, albumins and globulins have adverse but glutenins and Polypeptides 5 and 7 have favourable effects on the strength of dough.

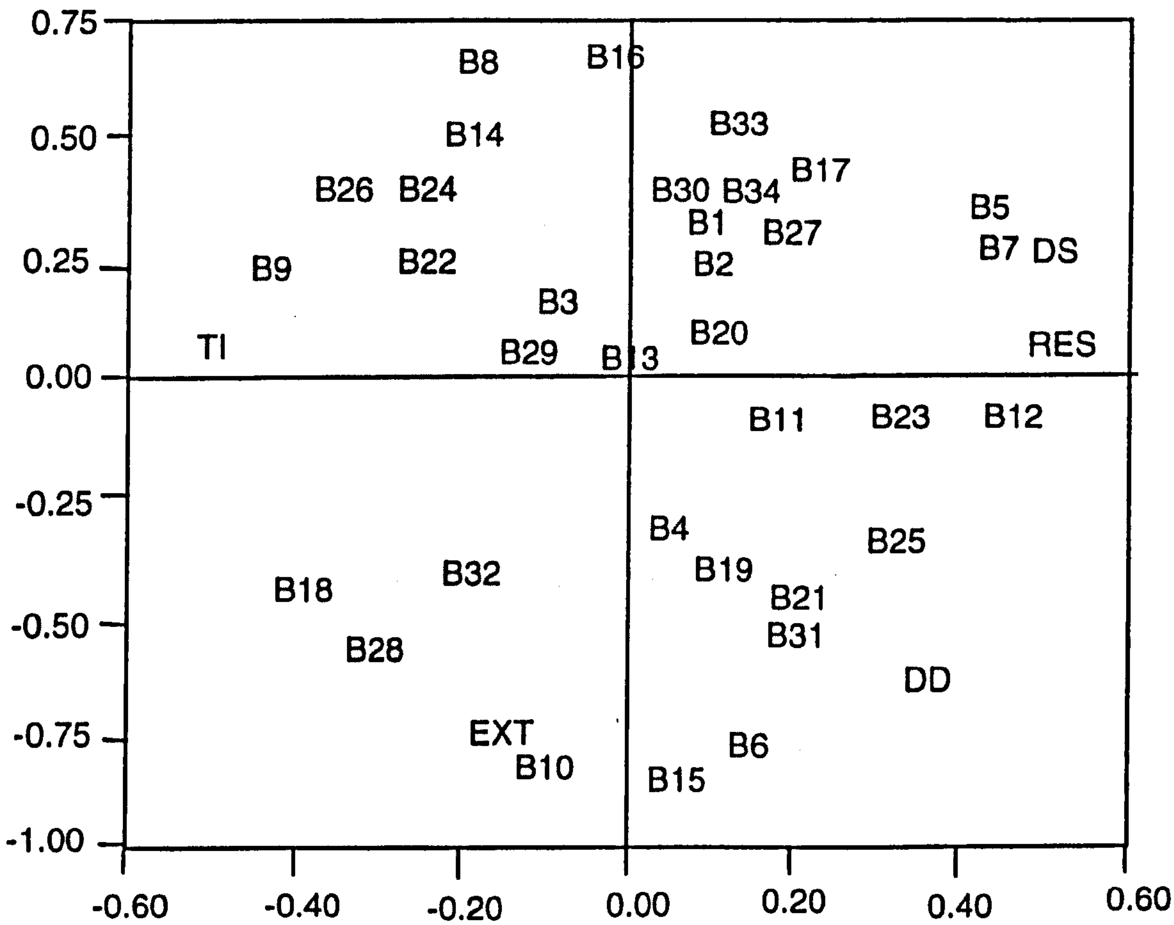


Fig. 5.8: Two dimensional loading plot (factor <1,2> <1,2>) illustrating relationships between Rheological characteristics: Dough development time (DD), Dough stability (DS), Tolerance index (TI), Resistance to extension (RES), Extensibility (EXT) and storage protein polypeptides.

6. ACHIEVING CHAPATI OF ENHANCED NUTRITIONAL VALUE

6.1 Chemical composition of yeast extract

Yeast extracts (2.1.2, 2.1.3) were analysed for moisture, total solids, protein, nitrogen, ash content, and sodium chloride (Table 6.1). The total solids were determined by difference.

The highest moisture content (5.87 %) was noted in case of Std. AYP and the lowest (3.00 %) was observed in YEP 77. However, all samples were free flowing dried powders which could be mixed easily in flour samples. Protein contents ranged between 22 to 37 % determined by the method of Itzhak and Gill (1964). The nitrogen content, from Kjeldahl method, ranged between 6.62 and 7.28 %. Crude protein contents derived from Kjeldahl determination (N X 6.25) were higher than with Itzhak and Gill method. Such differences may relate to the nitrogen derived from nucleotides (Yang et al., 1977). Therefore, a factor of 6.25 was used to convert Kjeldahl nitrogen values to crude protein content which may not be accurate. Dziezak (1987a) reported that Brewer's dried yeast contained 48 % good quality protein with an excellent profile of essential amino acids except for methionine. Protein contents were markedly lower in the present study. Yeast extracts also contained appreciable amounts of ash ranging from 13.5 to 44.4 %, primarily influenced by the level of sodium chloride.

6.2 Chromatographic analysis of yeast extracts

Inosine 5′ monophosphate, guanosine 5′ monophosphate, monosodium glutamate and sodium chloride were quantified in yeast extracts by HPLC (Table 6.1). Typical chromatograms are shown in Fig. 6.1.

Measurement of peak areas gave a linear response for all compounds quantified (Table 6.2). The retention times of IMP, GMP, MSG and Sodium Chloride were found to be 17.8, 29.8, 6.8 and 12.9 min, respectively. Small variations in retention times of compounds were related to differences in concentrations. These results are similar to those reported by Nguyen and Sporns (1984) and Law (1991).

Table 6.1: Chemical composition and flavouring potentiators in yeast extracts.

Parameter	YEP L	YEP 77	Std AYP	S.d.
Moisture content (%)	5.00	3.00	5.87	0.09
Total Solids (%)	95.00	97.00	94.13	0.09
Protein content* db(%)	31.00	37.00	22.00	0.20
Nitrogen content**db (%)	6.62	6.78	7.28	0.07
Ash content db (%)	43.00	44.40	13.48	0.09
Sodium Chloride*** (%)	38.94	39.63	8.58	0.13
IMP (%)	0.026	4.48	0.48	0.05
GMP (%)	0.00	1.72	0.00	0.06
MSG (%)	5.52	0.00	0.00	0.07
Sodium Chloride**** (%)	39.87	43.48	9.53	0.08

db = dry basis — Itzhak and Gill method*
IMP= Inosine 5' monophosphate — Kjeldahl method**
GMP= Guanosine 5' monophosphate — Silver nitrate method***
MSG= Monosodium glutamate — HPLC method****

Table 6.2: Linearity of peak area measurement.

Compound	Conc. Range Tested	Corr. Coeff.	Linear regression equation
IMP	1 - 0.0625 mg ml^{-1}	0.999	$Y = 0.022X10^{-6}\ X + 0.2$
GMP	1 - 0.0625 mg ml^{-1}	0.997	$Y = 0.03X10^{-6}\ X + 0.11$
MSG	20 - 0.5 mg ml^{-1}	0.993	$Y = -\ 0.48X10^{-5}\ X + 1.85$
Chloride	20 - 0.5 mg ml^{-1}	0.996	$Y = -0.69X10^{-5}\ X + 0.99$

Fig. 6.1: HPLC chromatogram from RI (Lower) and UV (Upper) detectors of 1. Glutamic acid (1 mg/ml, retention time 6.83 min); 2. Chloride (1.5 mg/ml, retention time 12.93 min); 3. IMP (0.5 mg/ml, retention time 17.77 min); and 4. GMP (1 mg/ml, retention time 29.77 min) at flow rate of 1 ml/min with 0.017M pH 4.0 phosphate buffer.

6.3 Sensory analysis of chapati containing NaCl and yeast extract

Acceptability of chapaties varying in NaCl content: The objective of this part of the study was to establish a NaCl content in chapati which would be acceptable to target consumers. Chapaties were prepared by adding 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 % NaCl (dry weight basis) into flour. Acceptability of the product was evaluated on a 9 point hedonic scale using a panel of twelve, all users of the product. Analysis of variance (Table 6.3) showed significant differences among the samples. The differences between chapaties containing 0.5, 1.0 and 1.5 % NaCl were found to be non-significant but all of these were significantly different from samples containing 3.0, 3.5 and 4.0 % salt. Chapaties containing 1.5 % NaCl scored highest followed by those with 1.0 % NaCl. It was concluded that optimum salt content was in the range from 1.0 - 1.5 %. As chapaties are mostly consumed with savoury foods, 1 % NaCl was chosen for subsequent studies.

Table 6.3: Analysis of variance of chapaties containing salt (NaCl).

Source	F Value	P Value
Assessor	1.35	0.22
Treatment	58.43	0.00

Treatment Means

1.5 %	1 %	0.5 %	2 %	2.5 %
6.54a	6.25a	5.92ab	5.00b	4.16bc

3 %	3.5 %	4 %
3.29cd	2.54d	2.00d

Note : Any two values not followed by the same letter are significantly different at the 5 % level.

Acceptability of chapaties containing various levels of yeast extract; YEP L: The objective was to establish the effect of a yeast extract at varying concentrations on acceptability of chapaties. Chapaties were prepared by partial replacement of flour with 0.5 (B), 1.0 (C), 1.5 (D), 2.0 (E), 2.5 % (F) of yeast extract YEP L and evaluated for acceptability on a 9 point hedonic scale by the panel of 12 assessors used in optimising NaCl content. Concentration of NaCl was maintained at 1 % by adding table salt. Analysis of variance (Table 6.4) indicated significant differences existed among the samples. The 1.5 % yeast extract chapati (sample D) obtained the highest mean score, significantly different from samples with <1.5 % and >1.5 % yeast extract. Samples with <1.5 % yeast extract (A, B and C) were not significantly different from each other but differed significantly from those with >1.5 % yeast extract (E and F). The highest yeast extract content (2.5 %) F was scored lowest. The chapati containing 1.5 % yeast extract (D) was highly acceptable to the panel due to imparting a savouriness to the product not present in chapati containing only NaCL (A). According to the assessors, chapaties containing 1.5 % yeast extract had flavour notes varying from "lamb" to "chicken" meat. This may be due to presence of MSG in the YEP L yeast extract, as Hall (1978) suggested that MSG is responsible for perception of meatiness in flavours.

Table 6.4: ANOVA of acceptability of chapaties containing YEP L.

Source	F Value	P Value
Assessor	1.40	0.20
Sample	34.10	0.00

Sample Means

D	C	A	B
7.17a	5.54b	5.53b	5.50b

E	F
4.24c	4.10c

A : 0 %
B : 0.5 %
C : 1.0 %
D : 1.5 %
E : 2.0 %
F : 2.5 %

Note : Any two values not followed by the same letter are significantly different at 5 % level.

Acceptability of chapaties containing various levels of yeast extract; YEP 77: Analysis of variance (Table 6.5) of chapaties having 0.5 (B), 1.0 (C), 1.5 (D), 2.0 (E) and 2.5 % (F) of YEP 77 showed that there was a significant difference among samples. Sample A containing 1 % salt only was significantly different from samples with >1 % yeast extract but not from those containing 0.5 % yeast extract (B). Increase in acceptability was observed with increase in level of yeast extract, and chapaties >2 % yeast extract were rated highest by all panelists. This suggested that YEP 77 could be incorporated into chapati at up to 2.5 % (weight basis of flour) in order to enhance savouriness and acceptability.

Table 6.5: ANOVA of acceptability of chapaties containing YEP 77 (yeast extract).

Source	F Value	P Value
Assessor	1.75	0.08
Sample	45.48	0.00

Sample Means

F	E	D	C
7.50a	7.49a	6.41b	6.14b

B	A
5.87bc	5.52c

A : 0 %
B : 0.5 %
C : 1.0 %
D : 1.5 %
E : 2.0 %
F : 2.5 %

Note : Any two values not followed by the same letter are significantly different at 5 % level.

Acceptability of chapaties containing various levels of yeast extract; Std. AYP: AYP was used at concentrations between 0.5 and 2.5 % in chapaties (Table 6.6). Concentrations of 0.5 (B) and 1 % (C) were not significantly different from each other but were significantly different from 0 % and >1 % yeast extract (D - F). Increase in yeast powder beyond 1 % was reported to impart bitterness to chapaties with no distinct flavour note appearing as a result of the yeast powder. This material was not readily soluble. Autolysates are the entire contents of the lysed cell, including the water soluble components, the solubilized proteins and cell wall (Trivedi, 1986), whereas autolysed yeast extracts are the concentrated product obtained following the removal of insolubles and bitter tasting compounds (FDA, 1986; Dziezak, 1987a; Schmidt, 1987). This yeast product was not studied further.

Table 6.6: ANOVA of acceptability of chapaties having Std. AYP.

Source	F Value	P Value
Assessor	1.61	0.21
Sample	111.99	0.00

Sample Means

A	B	C	D
8.25a	7.71b	7.25b	5.83c

E	F
4.88d	4.88d

A : 0 %
B : 0.5 %
C : 1.0 %
D : 1.5 %
E : 2.0 %
F : 2.5 %

Note : Any two values not followed by the same letter are significantly different at 5 % level.

6.4 Optimization of acceptability of chapaties

Response surface methodology (RSM) was used to optimise a flour supplemented with yeast extract for chapati production. Levels of salt and yeast extract were determined by a randomize mixture design on the basis of the preliminary studies (Table 6.3 - 6.6). Yeast extract ranged from 1.0 - 3.0 % for YEP 77 and 0.5 - 2.5 % for YEP L in increments of 0.5 %. Chapaties were evaluated for acceptability of taste on a 9 point hedonic scale by the panel of assessors (Table 6.7). Best-fitting models were determined by regression procedure and used as predictors of the treatment factors and to estimate properties of independent variables. The signs of the regression coefficients within each equation show the direction of the effect of each independent variable, the squares and the interaction (Table 6.8). Considering such information together with the F values (Table 6.9) provided insight. The significant F values provided guidelines for further model building. Increasing yeast extract (A) had evidently the most important single influence. Both cases indicated that acceptability of taste improved with increasing yeast extract content.

The R^2 values for the best-fitting models were high for taste (0.96, 0.89) for YEP L and YEP 77, respectively. Satisfactory levels of R^2, CV % and model significance indicated good fit of the models with no significant lack of fit. Such results are consistent with previous results

Table 6.7: Sensory analysis for acceptability of taste and texture hardness measured with instron machine of yeast extract supplemented chapaties.

Yeast extract %	NaCl (added) %	Sensory Taste	Instron 2.5 h	 10 h	 24 h
			YEP L		
1.0	0.2	3.78	27	33	42
2.0	0.2	5.97	25	31	40
1.0	0.6	4.66	30	37	48
2.0	0.6	6.20	27	29	39
1.5	0.4	8.13	26	30	38
0.5	0.4	2.77	30	37	46
2.5	0.4	4.12	29	32	40
1.5	0.0	2.69	26	30	39
1.5	0.8	3.40	29	33	43
S.d.		0.05	1.51	2.53	4.17
			YEP 77		
1.5	0.2	6.94	25	38	46
2.5	0.2	7.42	24	33	49
1.5	0.6	5.33	27	36	51
2.5	0.6	4.55	23	31	42
2.0	0.4	8.27	22	29	39
1.0	0.4	4.80	26	37	50
3.0	0.4	4.88	23	31	43
2.0	0.0	7.94	22	29	48
2.0	0.8	5.35	24	26	44
S.d.		0.04	1.63	2.24	3.53

S.d. = Standard deviation

Table 6.8: Models for selected attributes used in Response Surface Optimization Phase.

<u>Taste</u>

YEP L

$$Y = 7.94^{**} + 0.54A^{**} + 0.21B - 1.25AA^{**} - 1.35BB^{**} - 0.16AB$$

YEP 77

$$Y = 7.91^{**} - 0.01A - 0.81B^{**} - 0.98AA^{**} - 0.53BB^{**} - 0.32AB$$

<u>Texture</u>

YEP L

2.5 h
$$Y = 26.00^{**} - 0.58A^{*} + 0.92B^{**} + 0.88AA^{**} + 0.38BB^{*} - 0.25AB$$

10 h
$$Y = 29.90^{**} - 1.67A^{**} + 0.67B^{*} + 1.51AA^{**} + 0.76BB^{**} - 1.50AB^{*}$$

24 h
$$Y = 38.80^{**} - 1.92A^{**} + 1.08B^{*} + 1.56AA^{**} + 1.06BB^{**} - 1.75AB^{*}$$

YEP 77

2.5 h
$$Y = 22.70^{**} - 0.92A^{*} + 0.42B + 0.88AA^{**} + 0.51BB^{*} - 0.75AB$$

10 h
$$Y = 30.40^{**} - 1.83A^{*} - 0.83B + 2.01AA^{**} + 0.38BB + 0.00AB$$

24 h
$$Y = 41.60^{**} - 1.71A^{*} - 0.71B + 2.02AA^{**} + 1.83BB^{**} - 2.87AB^{*}$$

Code for ingredients: A = Yeast extract, B = NaCl
* = Significant ($P<0.05$)
** = Highly significant

Table 6.9: Analysis of variance for evaluation of models for quality parameters of optimally prepared chapaties.

Source	F	P	CV	R^2
		Taste		
YEP L				
Model	54.40	0.000	7.65	0.96
YEP 77				
Model	18.26	0.001	7.51	0.89
		Texture		
YEP L				
2.5 h Model	11.76	0.002	2.55	0.83
10 h Model	16.51	0.001	3.15	0.88
24 h Model	12.52	0.002	3.20	0.84
YEP 77				
2.5 h Model	6.06	0.016	3.96	0.70
10 h Model	4.70	0.031	7.2	0.63
24 h Model	8.60	0.006	4.30	0.78

(Shelke *et al.*, 1990).

To analyse the combined effects of the two independent variables, yeast extract and salt, on the dependent responses of chapati acceptability, two dimensional contour plots were generated for each of the fitted models.

The contour plot for taste of chapaties supplemented with YEP L is shown in Fig. 6.2A. The region of optimum response was central, with 1.5 % of yeast extract and 0.4 % of salt. The area of minimum response was defined by high levels of both yeast extract and salt.

The contour plot for taste of chapaties prepared by partial replacement with YEP 77 is presented in Fig. 6.2B. The region of optimized response was defined by yeast extract 2.0 % and salt 0.3 %. Minimal response was found with high levels of yeast extract and salt. These studies indicated that the optimum level of yeast extract acceptable to consumers to impart savouriness to chapaties ranged from 1.5 - 2.0 % (containing 40 % of NaCl). Previously, Dziezak (1987a) reported addition of 2.0 to 4.0 % yeast extract in formulation of baking powder biscuits. In contrast, Knight and Hanson (1986) used 15 % of yeast protein to enrich spaghetti. The chapati containing YEP L was found to be ranked higher than those with YEP 77. A possible explanation is the presence of the chicken note in the product which was ranked highest (Chapter 3). The YEP L also contained an appreciable amount of MSG contributing meaty character to chapaties.

Johnson (1983) considered the glutamate content as enhancing savoury character. Moreover, this extract also contained IMP which increased the taste intensity of the mixture of MSG and IMP, possibly due to a synergistic effect (Yamaguchi, 1979; Kawamura, 1990). Also similar effects of MSG were discussed by Maase (1991) and Schoenberg (1992).

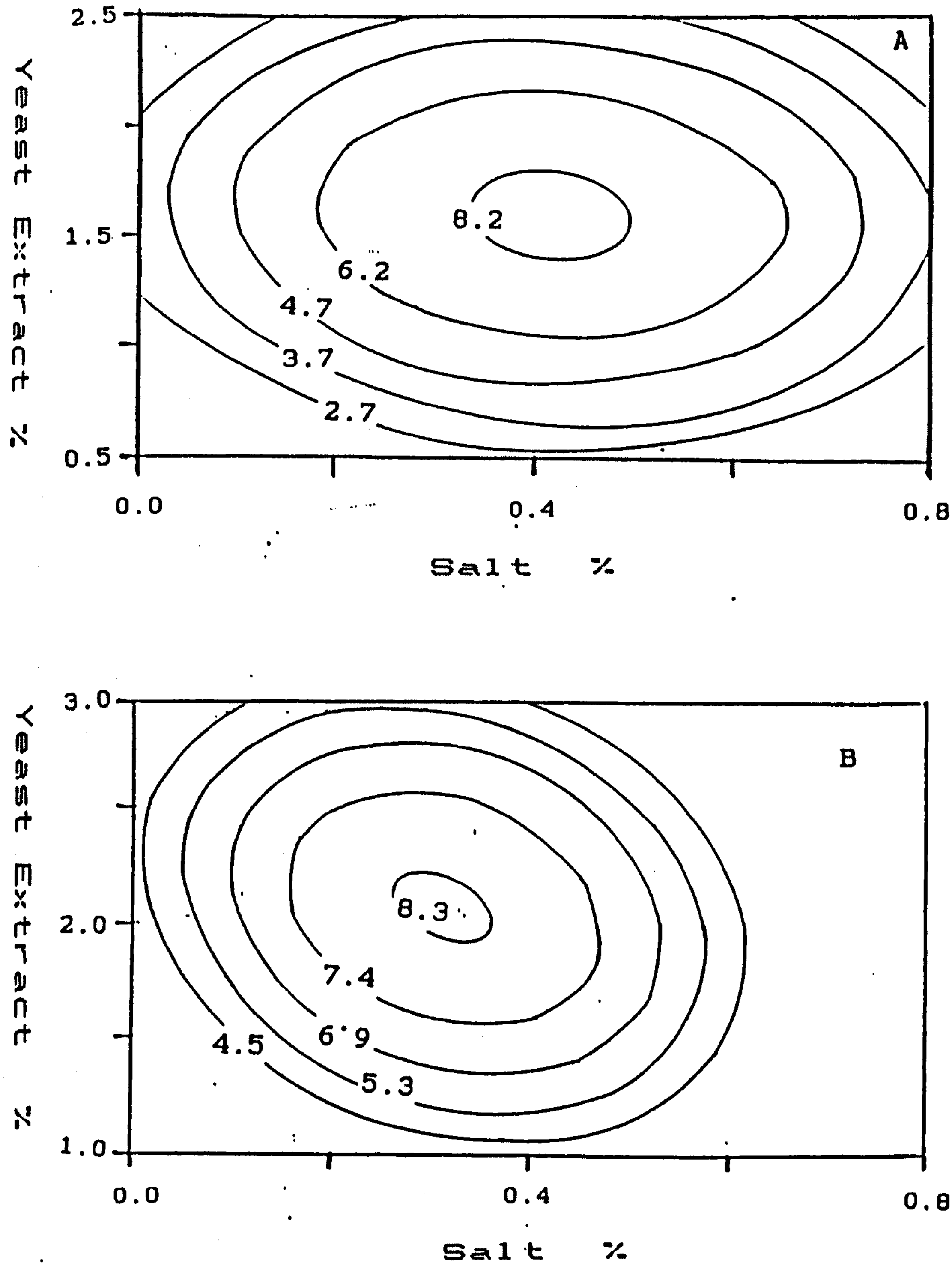


Fig. 6.2: Contour plots for taste in chapaties containing (A) YEP L (B) YEP 77.

The effects of yeast extract on textural hardness in chapati at different intervals of storage (2.5, 10 and 24 h) were studied using Instron machine (Table 6.7). The models developed for instrumental assessment for three intervals of storage also had adequate R^2, CV and significance. The value of R^2 ranged from 0.63 - 0.88. Values ranging from 0.52 to 0.90 have been considered good to excellent (Frye and Setser, 1991) but generally <0.6 is considered poor and less predictive, suggesting the models could not predict adequately (Malcolmson et al., 1993). Henika (1982) considered that models that explained 85 % of variance were very good. In the present study, the coefficients of multiple determination for all model equations show that they accounted for above 63 % of variability (Table 6.9); R^2 values were found to be sufficient to enable prediction of responses to be made with confidence ($P<0.05$).

Contour plots generated for hardness responses to chapaties containing YEP L are shown in Figures 6.3A, 6.3B and 6.3C. Regions of optimum response were found with 1.5, 1.75 and 1.75 % yeast extract, and 0.25, 0.4, 0.4 % NaCl at 2.5, 10 and 24 h, respectively.

Contour plots for chapaties using flour supplemented with YEP 77 are presented in Figures 6.4A, 6.4B and 6.4C. Areas of optimum responses were defined by 1.75 to 2.5 % yeast extract, and 0.4 to 0.5 % NaCl.

From the results obtained, it can be deduced that texture quality does not decrease if as much as 2.5 %

yeast extract is incorporated into chapaties. These results are not unexpected, as yeast protein has been reported to impart freshness and delay the onset of staling in bakery products (Knight and Hanson, 1986).

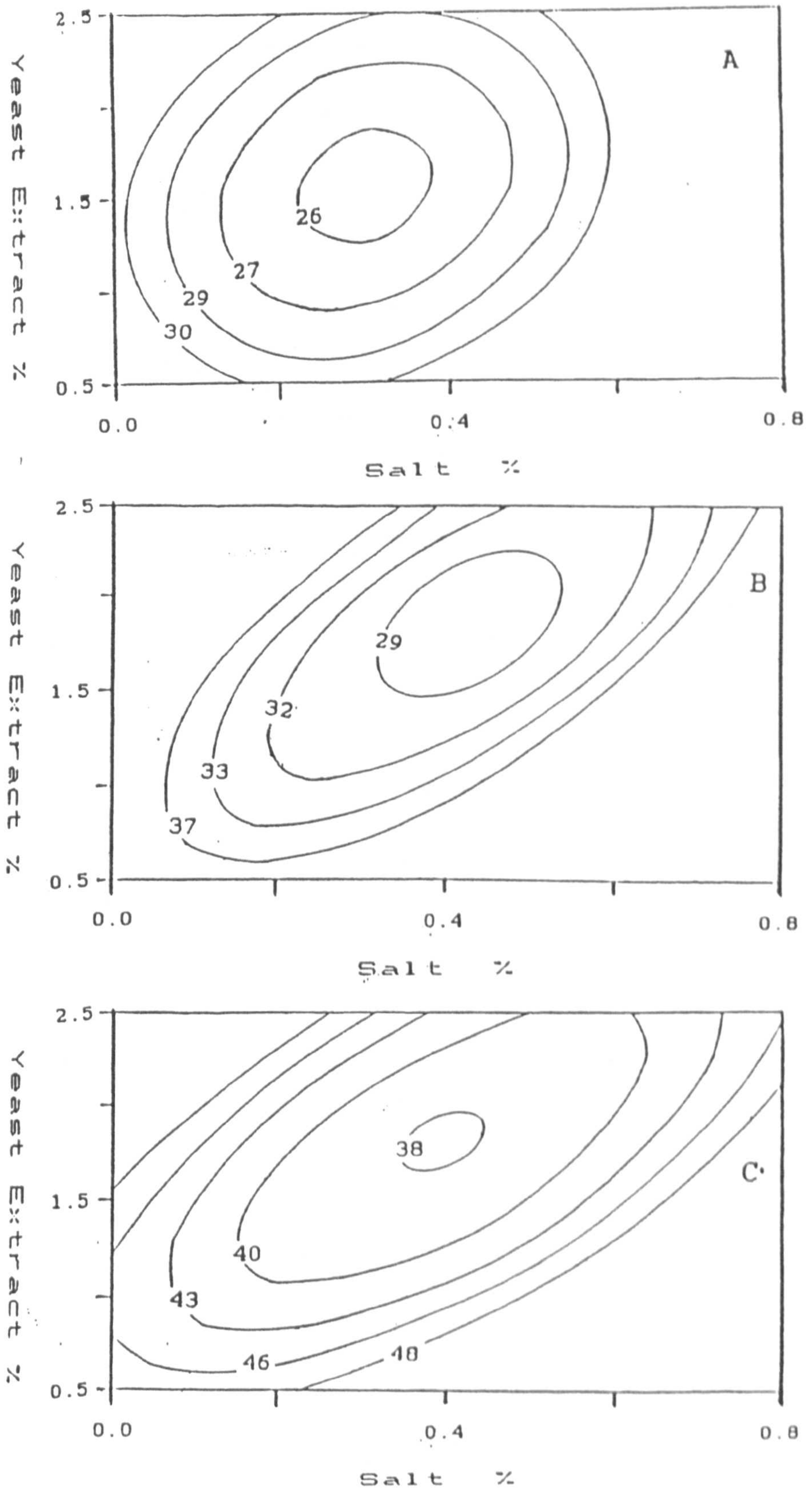


Fig. 6.3: Contour plots for hardness in chapaties containing YEP L assessed at (A) 2.5 h (B) 10 h (C) 24 h storage intervals.

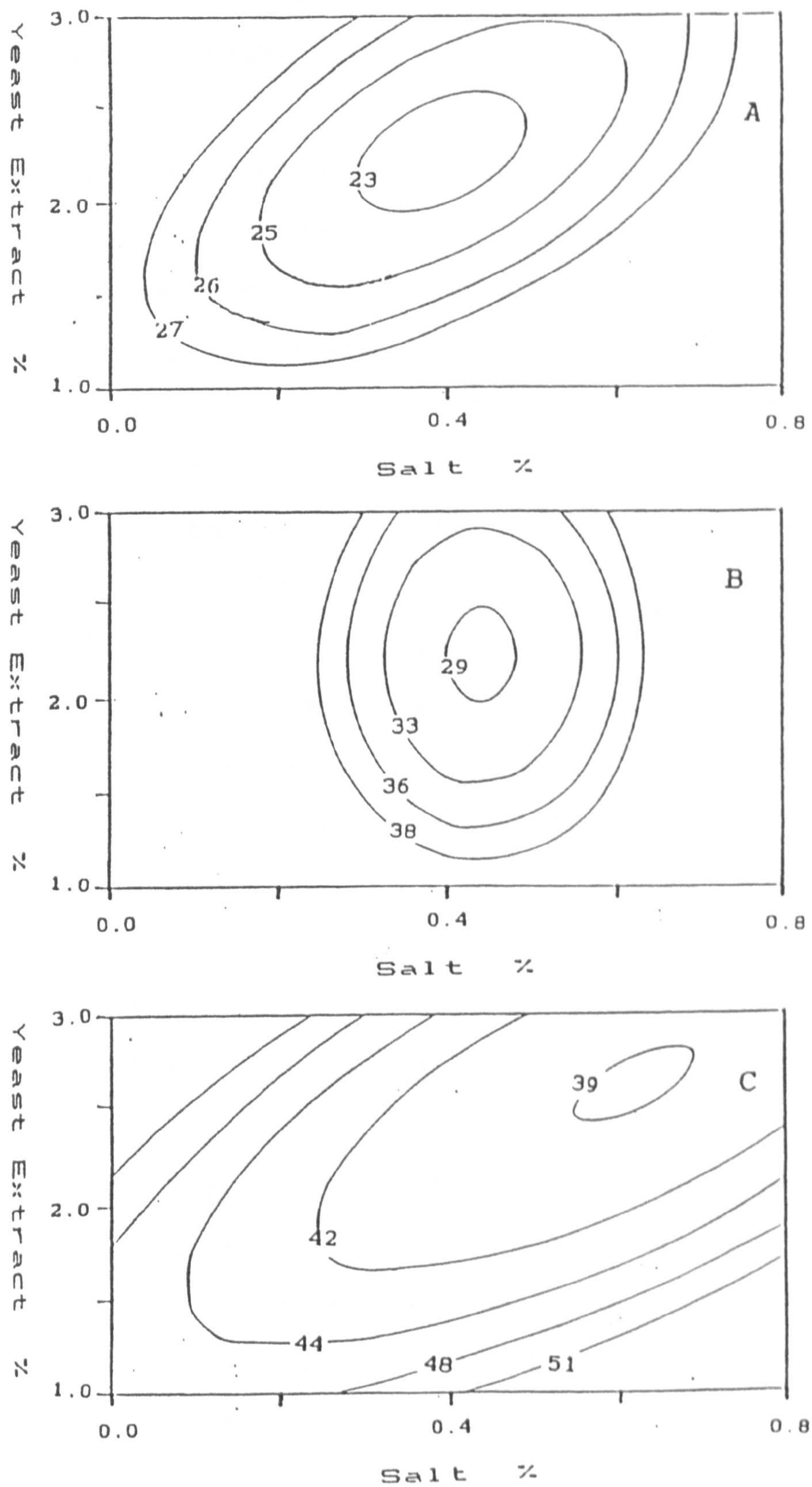


Fig. 6.4: Contour plots for hardness in chapaties containing YEP 77 assessed at (A) 2.5 h (B) 10 h (C) 24 h storage intervals.

6.5 Effect of salt and yeast extracts on rheological properties of dough.

The effects of varying concentrations of NaCl, MSG and yeast extract on the rheological properties of chapati dough was examined using a farinograph (Table 6.10). Representative farinograms are shown in Figures 6.5 - 6.6.

The effect of addition of 0.5 % NaCl to flour was to decrease water absorption by 2.1 %. The effect of subsequent increments was less marked and related to salt concentrations: at 1 % NaCl, 1.8 %; at 1.5 % NaCl, 1.4 %. Monosodium glutamate at 0.5 % decreased water absorption by 1.9 % and at 1.5 %, the decrease was 3 %. Addition of 1.5 % YEP L had more effect (4.90 %) than YEP 77 (2.4 %), salt and MSG. This might be due to the decrease in amount of "bound" water which was confirmed by Hlynka, (1962). The farinographic dough consistency decreased with addition of salt into flour. Similar effects have been reported by Bennett and Coppock (1953) and Moore and Herman (1942).

Rheological behaviour of the dough was markedly altered by the addition of 1.5 % of sodium chloride, MSG, YEP L and YEP 77 with effects on peak time, dough stability, tolerance index and extensibility. Improvement in dough properties presumably resulted from increasing hydrophic interactions between gluten proteins (He et al., 1992).

Table 6.9: Effect of Partial replacement with salt, MSG and yeast extract on rheological properties of dough.

Treatment	WA %	DD Min	DSt Min	TI BU	Ext mm	FN
Control (Unsalted)	57.6	7.0	18.0	30	18	513
Salt 0.5 %	56.4	8.5	17.5	35	23	-
Salt 1.0 %	55.3	9.0	18.0	20	26	-
Salt 1.5 %	54.5	8.75	18.0	20	26	630
MSG 0.5 %	56.7	7.75	16.5	40	17	-
MSG 1.5 %	55.0	9.75	17.0	30	22	668
YEP L 1.5 %	54.8	7.5	15.0	70	27	580
YEP 77 0.5 %	56.4	7.25	14.5	25	25	-
YEP 77 1.5 %	55.0	8.0	14.5	70	26	545

WA = Water Absorption
BU = Brabender Unit
TI = Tolerance Index
Ext= Extensibility
DD = Dough Development
DSt= Dough Stability
FN = Falling Number

Peak time is a measure of the time required for a dough to reach its maximum consistency and in the present study ranged from 7.0 to 9.75 min. Addition of 1.5 % MSG, 1.0 and 1.5 % salt increased peak times whereas yeast extracts had only a limited effect.

Dough stability is an indication of the tolerance to mixing and values in this study ranged from 14.5 to 18.0 min. The yeast extract YEP L, YEP 77 and MSG reduced the stability, whereas salt had no effect.

The tolerance index values of variously treated doughs ranged from 70 - 25 BU. NaCl reduced the tolerance index (20 BU) at 1.5 % whereas MSG and yeast extract at high concentrations yielded increases. With increased contents of salt, yeast extract and MSG in doughs, extensibility was found to increase, in agreement with the findings of Fisher _et al_. (1949), also indicated in stress relaxation experiments (Grogg and Melms, 1956, 1958; Hlynka 1954). Yeast extracts influenced dough rheology by strengthening the gluten and enhancing the dough machinability. Yeast extract has electrolytes with an effect on rheological properties based on gluten protein aggregation. Bennett and Ewart (1965) and Galal _et al_. (1978) suggested ions may enhance protein association or dissociation. When sodium chloride is present, association between proteins becomes dominant strengthening the dough and reducing water absorption, ionic, hydrophobic and hydrogen bonds are involved (Bernardin, 1978; Salovaara, 1982).

Thus, no essential technological problems were met when as much as 3 % of yeast extract was used. Difference in the rheological behaviour of variously treated doughs were not critical in relation to baking performance as none of the doughs was exceptionally sticky.

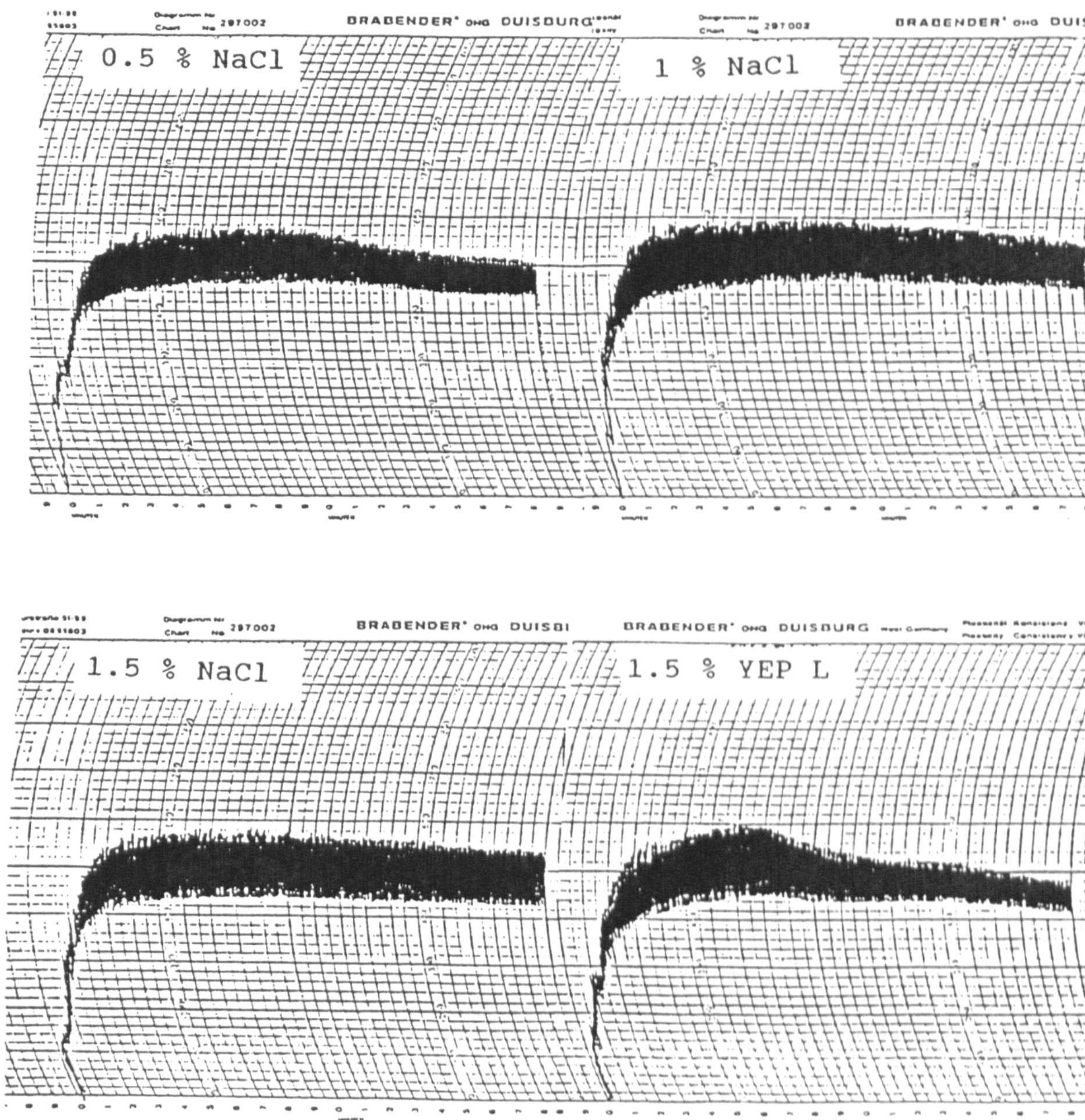


Fig. 6.5: Farinograms of doughs containing NaCl and Yeast extract.

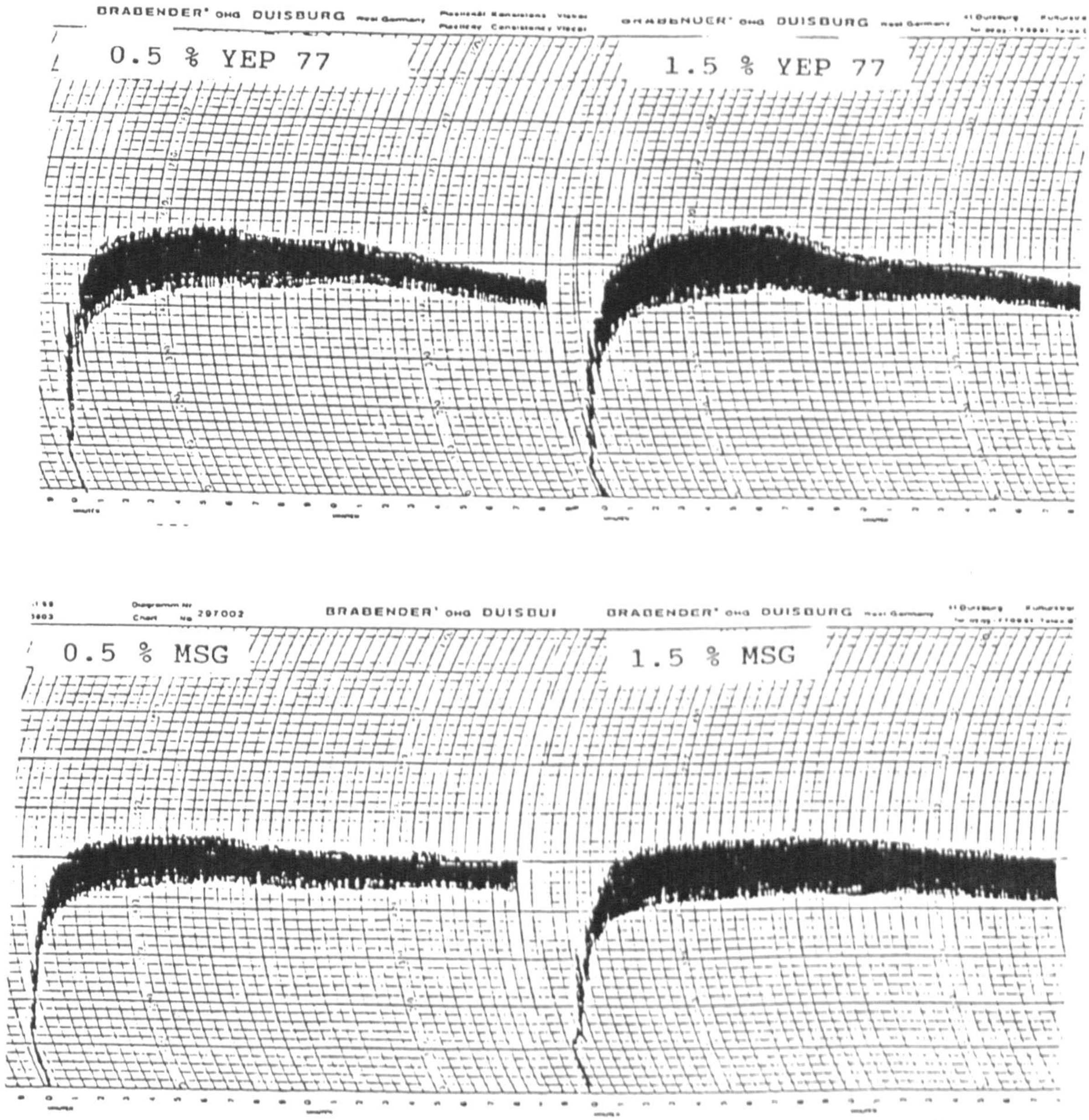


Fig. 6.6: Farinograms of doughs containing MSG and yeast extracts.

6.6: Nutritional impact of yeast extract on chapaties

The samples of chapati were freeze-dried and analysed for ash and protein contents (Table 6.10).

Table 6.10: Chemical analysis of chapaties.

Product	Moisture %	Ash % (db)	Protein % (db)
Control	38.0	1.57	13.10
YEP L (1.5 %)	36.0	2.44	13.98
YEP 77 (2 %)	37.6	2.70	14.43
S.d. (pooled)	0.10	0.06	0.07

db = dry basis S.d. = Standard deviation

Moisture content of fresh chapati ranged from 36 to 38 %. Chapaties containing yeast extracts retained less moisture than controls possibly through the lower initial water content of dough. The ash content of yeast-containing samples was found to be increased due to higher salt contents. The crude protein contents derived from Kjeldahl determination (N X 5.83) of YEP L (1.5 %), YEP 77 (2.0 %) and control samples resulted in 0.88 - 1.33 % increases of good quality protein in the supplemented chapaties. The protein quality of bread is a function of lysine content, which is fairly high in yeast extract (Dziezak, 1987a).

7. DISCUSSION

7.1 The nature of the problem

Wheat in the form of chapati is the major source of protein and energy in the Pakistani diet. The recommended dietary allowance (RDA) was estimated as 2550 calories and approximately 63 g protein per day per capita in 1991-92 (Govt of Pakistan, 1993). Chapaties are consumed throughout the country by all socio-economic groups and contribute as much as 90 % of the total intake (Chaudhry, 1968) to people living in villages, who account for 80 % of total population.

Soft to medium hard wheats are considered best for the production of desirable tender chapaties. Those produced from hard wheat are often tough, hard and difficult to chew (Nagao, 1981). Austin and Ram (1971) showed that variation in wheat properties gives marked differences between chapaties.

In Pakistan, wheat supplied to mills is uncategorised by class, grade or origin. About half of the mills grind atta as wholemeal; the remainder produce atta of about 85-90 % extraction, bran and maida (fines). Essentially all of Pakistani wheat is milled to flour and consumed as chapati or roti, and most is milled in small chaki mills (stone grinding mills) from wheat purchased directly or grown by consumers. Wheat procured by Government agencies is milled in roller mills of the type used in the USA and Europe. The Government allocates wheat to provincial Governments which control distribution to the roller-mills for processing. Atta is sold at a regulated price through Utility Stores and on the open market in both urban and rural areas. Flour mills in the United Kingdom produce chapati flour of various extraction rates including Brown, Wholemeal, Medium and white chapati flour using blends of wheat varieties.

As wheat proteins are deficient in certain amino acids and have low levels of certain others, consumption of chapati as sole protein source may lead to protein-malnutrition. Such a situation exists in the villages and among the poorest class as consumers cannot make up the deficiencies in wheat protein from other sources. Moreover, lysine deficiencies in wheat protein are aggravated by losses arising during baking of chapati (Saab et al., 1981; Arafah et al., 1980). Vitamin deficiency is also prevalent; Crowley (1972) reported that riboflavin deficiency existed in 100 % of the families of Pakistan as all consumed less than the recommended daily intake, irrespective of income.

In the past, attempts have been made to supplement nutritional quality of chapaties with synthetic amino acids, fish protein concentrate and legumes. However, the success of such strategies appears to be limited by several factors including high cost, low availability and convenience, and poor product image (reduced colour and taste score) (Bass and Caul, 1972; Ali et al., 1964). A further problem is low protein digestibility and flatulence. To alleviate protein and vitamin deficiencies in people of Pakistan, especially those with low income and in rural populations, wheat flour should ideally be fortified with cheap sources of high quality proteins and vitamins that maintain a desirable good product image.

Savoury dishes are popular amongst Pakistani people since their cuisine is based upon meat, vegetables and pulses, consumed with chapaties. In the developed world yeast extracts are used widely in snacks, soups, gravy, meat products and many other food products to impart a range of savoury flavours. Yeast extracts were used in the present study to enhance nutritional quality of chapati and to impart savoury flavour. Supplementation of wheat flour with yeast extract would be considered a promising strategy to reduce protein deficiency. Important factors include good product image, cheapness, transport and stablility, low moisture content and good quality protein. Moreover it should be possible to enhance acceptability of chapati.

7.2 Creating new savoury products for Pakistan

Generally chapaties are prepared by a female member of the family at home, but they are also sold ready prepared at restaurants and baking shops. Chapaties are usually eaten three times a day with curry and pickle.

In 1974, the Government of Pakistan installed 16 automated roti plants in seven big cities to provide yeast leavened rotis at a subsidised rate taking into account such features as cost, convenience, hygiene and energy costs. Although such leavened roti was cheaper it was not rated highly by consumers, possibly due to problems of taste, texture and shelf life. Other factors limiting appeal include insufficient retailing facilities, tradition, antigovernment slogans, lack of advertisement and illiteracy. At that time 15 % wheat was milled and distributed to market by the government through roller mills; the rest was procured and ground on small stone mills by consumers (Crowley, 1972). The dependency on such cheap wheat might be another major reason of failure. Currently, wheat price in the open market is high and the major part of Pakistani wheat is purchased by government agencies. Food department is responsible for supply of wheat to roller mills and distribution of flour to shops that charge a fair price.

Savoury products including samosa, shami kebab, shek kebab, pakora, chat, dahi barra and qeema (minced meat) naan are sold in small shops. Typical ingredients include wheat flour and gram flour, vegetables, meat, spices, fermented milk, red chili, herbs, and table salt. Although such products are relatively expensive, they are popular and relished by consumers due to their savoury taste. In the present study, liking for ten types of savoury flavours was evaluated through survey studies. Flavours were significantly different from each other, and chicken and meaty flavours were rated highest by all age and income groups. Cheesy flavour was ranked lowest reflecting its rejection by consumers with religious beliefs limiting appeal of dairy products (Naele et al., 1993). Chicken and lamb are expensive sources of protein in Pakistan and are not affordable by low income groups. Savoury chapati supplemented with chicken flavour yeast extract can have taste similar to natural flavour. Yeast extract is a cheap and rich source of protein and vitamins, and supplementations with such products should have little effect on price of chapati, but give high nutritional benefits. Savoury chapati could be a new product with attractive and desirable flavour but similar properties to the traditional chapati.

Two delivery routes can be implemented for provision of yeast extract supplementation to consumers. Yeast extracts can be added at predetermined levels during flour production in roller mills by millers. It is believed that flour in the fair price shops is purchased largely by low and middle income groups. Wheat sold in the open market is generally regarded as being of better quality and is considerably more expensive, affordable only by the upper class. As flour produced in roller mills is consumed by the major segment of the population, as a staple food and this is controlled largely by the government agencies, such an approach appears to be a reasonable way for supplementation. Consumers of this flour would be expected to be those in greatest need of supplementary nutrients. Alternatively, yeast extract flavoured chapati can be produced on roti plants and sold in the open market through retail shops at prices affordable by consumers.

7.3 Optimisation of chapati flour

In Pakistan, specifications for wheat procurement are based on FAQ system (Fair Average Quality), which specifies impurities including dust, stone and straw. There is no consideration of class, grade and variety. Consequently, wheat supplied to roller mills is uncategorised by class, grade or origin. In such mills, lack of analytical facilities makes quality control of flour impractical. The flours distributed to market are thus of variable quality. Such problems are exacerbated by many factors including the attitudes of plant breeders who pay more attention to yield and ignore quality factors. Lack of education in merchants, farmers and millers, however, also contributes.

Quality, in the broadest sense, is conformance to predefined requirements. Suppliers should seek to satisfy the customer by conformance with such requirements. Flour quality can be related to the ability of the flour to produce a uniform end product of characters agreed by supplier and consumer. Generally, flour strength is synonymous with flour quality. Presence and absence of strength factors may relate to suitability of a flour for a specific use. This may be associated with both protein quality and quantity. High strength flours are considered particularly suited for production of leavened breads. Low strength flours are recommended for production of cakes and biscuits. Medium strength flours have proven suitable for production of chapati. There can be a problem with excessive strength when it comes to the sheeting process of production. Blending of high and low strength wheats is required to produce a suitable flour for production of chapati.

The ranges in compositional and functional factors for the optimal flour for chapati production are summarised in Table 7.1. Flours with properties outside these defined ranges are unlikely to be suited to chapati production. The correlation matrix for flour characteristics indicates that protein content, damaged starch, water absorption, dough development time, dough stability, tolerance index, particle size (130 - 180 µm), grade colour, extensibility, R/E ratio and baking score for texture of chapati are all correlated significantly. These correlations suggest that such quantitations are suitable for description of the chapati making qualities of flour (Swarnjeet, et al., 1982). Chapaties are usually prepared from flour of high extraction rates (90 - 100 %). There are a number of reasons, such as, for example, the correlation in softness in chapati texture with increasing bran content due to increases in water absorption (Navickis and Nelsen, 1992). Chapaties produced from optimal flours were smooth, soft and pliable, desirable characteristics in consumer acceptance.

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soluble and insoluble proteins. The molecular structures and weights of these proteins have been studied carefully in attempts to clarify relationships between flour quality and composition. Research to identify and measure quality factors of wheat proteins has continued for many years and significant progress made in developing tests that can be used to evaluate various characteristics of flour. Little progress has been made to determine the relationships between quality characteristics and protein composition through statistical techniques. Such approaches may be acceptable and cost effective for breeding purposes.

Autran et al. (1986a, 1986b) used principal component analysis on data from nine quality tests examining 112 durum wheat and breeding lines. Gluten firmness and elasticity and detergent (SDS) sedimentation were strongly associated, and typically variety-dependent. Such factors were independent of protein content. Simple correlations have been calculated between the proportion of each of the high molecular weight (HMW) glutenin subunits and strength, tenacity, swelling and extensibility of flour, measured with Chopin Alveograph (Branlard and Dardevet, 1985b). Strength and tenacity were correlated positively with bands 5 and 10 and negatively with bands 1 and 12. Subunit 9 was correlated positively with swelling and strength. Subunits 1 and 2* were correlated positively with swelling and strength. This suggests that gliadin and HMW glutenin subunits interact in determining the rheological characteristics of the dough. Branlard and

Dardevet (1985a) established relationships between protein contents and breadmaking quality in wheat with simple correlation methods. Multiple regressions were used to examine relationships between the functionality tests and gliadin bands. Highly significant relationships were obtained between resistance to dough extension and specific gliadins and between resistance and HMW glutenin (Campbell et al., 1987). Linear regression analysis has also been applied to correlate relative areas of the major gliadin peaks with quality of flour (Huebner and Beitz, 1987).

The characterization and fractionation of cereal proteins may be among the most difficult problems in biochemical analysis due to protein heterogeneity, variation in solubility characteristics and tendencies of both covalent and noncovalent polypeptides to aggregation. Types of proteins or their relative proportions may directly influence functional properties of bread. For example MW native glutenin relates closely to wheat bread making quality (Huebner and Wall, 1976), as does the presence of specific high-MW glutenin subunits (Payne et al., 1980b). Understanding relationships between quality and groups of wheat polypeptides provides useful information relating to rheological characteristics of wheat. It can make it possible for breeders to concentrate on specific proteins as markers of quality. Genetical approaches may also use such information to link functionality to loci on specific chromosomes. Although this field of study is in its early development, a detailed knowledge of cereal proteins and their properties can lead to methods of value in identification, classification and quality control. According to Cressey et al. (1987), selection of cultivars for dough resistance can be performed with protein markers.

In the present study, Partial Least Square (PLS) regression was used to explore relationships between protein composition and rheological characteristics of wheat flours. PLS proved to be an efficient multivariate technique, not only finding relationships but also taking into account the degree of variation in these parameters which can be represented graphically. It can also help to rank parameters and clarify their relative influence on samples and extent of differences between samples. More recently, multivariate analyses such as Procrustes analysis, Principle coordinate analysis (Piggott, 1986), multidimensional scaling, factor analysis (Jambu, 1991) and Principal component analysis have been used to determine the relationships between parameters (Powers, 1984a).

A number of techniques have been used to isolate and characterize cereal proteins (Beitz, 1979). Electrophoresis is widely used to separate proteins on the basis of either molecular weight or net charge. In SDS gel electrophoresis, currently most popular, separations are based on molecular weight. More recently HPLC has proven an excellent technique for separation of polypeptides.

Proteins of MW of several hundred thousand can penetrate the silica matrices of wide bore HPLC columns and fully interact with suitable stationary phases (Hearn et al., 1982). In reverse-phase HPLC (RP-HPLC), proteins are resolved on the basis of differences in surface hydrophobicity, a characteristic largely independent of MW and charge. This is being applied for identification of wheat varieties (Beitz et al., 1984) and is independent of electrophoresis data. Automated data handling and sorting of elution profile is a distinct advantage of HPLC (Wrigley et al., 1987). The technology gives rapid analysis but the capital equipment is more expensive than that utilised for gel electrophoresis. Batey et al., (1991) reported that although HPLC technique was useful, a relatively rapid deterioration of column performance occurred with loss of resolution of the three major protein fractions. Lookhart et al. (1986) demonstrated that both HPLC and PAGE are equally suitable for separating and differentiating gliadin protein in wheat cultivars. However, for determining the causes of the variation in the amount of individual HMW glutenin subunits and for biochemical studies, the SDS-PAGE method is more beneficial. The identification of the HMW subunits is based on their molecular weights (Bushuk, 1991) and enables the quantification and identification of the glutenin subunits in one analysis. SDS-PAGE allows the separation of HMW glutenin subunits from the other proteins present in the crude protein extract (Payne et al., 1985). In contrast, because some HMW glutenin subunits are co-eluted with some of the other kernel proteins (Burnouf and Beitz, 1989), selective extraction or precipitation of the HMW subunits is required for RP-HPLC analysis which is a potential source of error.

Response surface methodology is a multivariate statistical technique which has proven useful in the development of food product. RSM has a variety of uses in research because it can simultaneously consider several factors. Introducing different levels to a product can provide information on interactions among these factors and levels. It has proven more attractive than traditional experimental procedures because it reduces the time and cost required to reach the optimal product if more than two factors are involved. In the present study, analysis of variance techniques were used to determine maximum levels of factors and this was followed by response surface methodology. This procedure proved more effective than simple response surface methods giving reduced time and costs by decreasing the size of experiments and also sharpened responses. When more than two factors are involved, an alternative approach is to vary the levels of factors simultaneously, determining appropriate levels using fractional factorial designs (Hunter and Muir, 1992).

7.5 Enrichment of flour

This study suggests that consumers preferred the taste of chapaties containing yeast extracts over those without these flavourings. Yeast extract enhances image in products by imparting savouriness without affecting chapati qualities. No technological problems were observed with doughs containing yeast extracts. Doughs exhibited no slackness or stickiness during preparation of chapaties.

Apart from introducing a savoury flavour, yeast extracts enhanced the nutritional quality of chapati. Substitution of yeast extract increased the concentration of crude protein from 13.1 % to 14.4 % in 2.0 % enriched chapati. Higher levels of yeast extract could be used, subject to a low content of NaCl for enrichment purposes in chapati or other bakery products. In the improvement of protein quality, an important role is played by the lysine content of the yeast extract which is moderately high. Yeast proteins also contain high levels of other essential amino acids except methionine (Dziezak, 1987a). The amino acid composition of yeast protein compares well with that considered desirable by FAO (except for low methionine; Table 7.2) (FAO/WHO, 1973). Yeast protein has been generally considered to be deficient in sulphur-containing amino acids, but a good source of lysine (Lipidsky and Litchfield, 1974).

The nutritional quality of protein also depends on its digestibility, related to its yield of intact amino acids (Eggum and Jacobsen, 1976). Protein efficiency ratio (PER) for bakers yeast is 1.8, whereas that for casein, a highly acceptable protein for food use is 2.5. The value for wheat gluten is 0.7 to 1.1 (Simmonds, 1981) and for typical chapati 1.65 (Shyamala and Kennedy, 1962).

Table 7.2 : Essential amino acid composition of yeast (*Saccharomyces cerevisiae*) and wheat protein (in %, amino acid content by weight).

Amino acid	Yeast*	Wheat**	FAO/WHO* provisional pattern
Isoleucine	5.5	3.57	4.0
Leucine	7.9	6.98	7.0
Lysine	8.2	2.91	5.5
Methionine	2.5	1.75	(M+C) 3.5
+ Cystine	1.6	2.60	
Phenylalanine	4.5	4.66	(P+T) 6.0
+ Tyrosine	5.0	3.28	
Threonine	4.8	2.99	4.0
Tryptophane	1.2	1.18	1.0
Valine	5.5	4.79	5.0

Sources : *Reed and Peppler, 1973
**Eggum and Jacobsen, 1976

Accordingly, in relation to casein the nutritional values of bakers yeast, gluten and chapati are 72 %, 28 % - 44 % and 66 %, respectively. The addition of 0.5 % by weight of methionine has been reported to increase the PER value of bakers yeast proteins to 2.77 (Reed and Nagodawithana, 1991). Hence, although native yeast proteins are inferior to animal proteins, such deficiencies could be minimized without major difficulty.

Wheat flour, by comparison, is adequate in methionine and cystine but low in lysine. Maleki and Djazayeri (1968), in a study of effects of amino acid supplementation on protein quality of Arabic bread, showed that addition of 0.3 % L-lysine to flour significantly improved nutritional quality by increasing the absorption of protein in the body. Supplementation of the lysine-fortified flour with 0.62 % DL-threonine yielded a further improvement whereas addition of methionine had no effect. Hussein et al. (1973) reported that the PER rose significantly with the amino acid supplement, from 1.28 to 2.19 with DL-methionine supplementation, and to 3.32 with L-lysine. Since the amino acid composition of yeast and wheat proteins are complementary in terms of essential amino acids, there is a potential for the effective use of yeast protein in cereal product supplementation (Mateles and Tannebaum, 1968). As chapati forms a staple in the diet in Pakistan, these breads would be a practical vehicle for nutritional enrichment of diet and a potentially good protein source.

Yeast contains predominantly the vitamins of the B complex, important in human nutrition. Essential vitamins present in yeast extract include B1, B2, B6, niacin, folic acid, pyridoxine and biotin (Table 7.3).

Yeast products do not contain vitamins C, B12 and fat soluble vitamins such as A, E, K and D. Nevertheless, yeast products function as a valuable vitamin source when used in combination with other food ingredients as in the case of this study.

Yeast products are also rich in minerals in comparison with wheat (Table 7.4). Bioavailability of minerals from wheat is influenced by many factors such

Table 7.3: Amount of vitamins in yeast and wheat.

Vitamin	Dry yeast* mg/100 g	Wheat** mg/100 g (db)	US (RDA)* (mg)	% of RDA*** in 100 g wholemeal bread
Thiamine (B1)	1.20	0.40	1.50	22
Riboflavin (B2)	0.40	0.16	1.70	5
Niacin	3.00	6.95	20.00	22
Pyridoxine	0.28	0.79	2.00	7
Pantothenic acid	0.70	1.37	10.00	-
Biotin	0.013	0.016	0.30	-
Folic acid	0.13	0.049	0.40	-
Vitamin B12	0.00001		0.006	-

Sources : * Reed and Nagodawithana, 1991.
** Anon., 1985.
*** Bender, 1985.
RDA : Recommended Daily Allowance
db : dry basis

as nutritional status of the subjects, wheat type, production and milling effects and the presence of other food components in the diet, which may improve (protein, ascorbic acid) or impair (phytate, fiber, oxalate) bioavilability of minerals and also the form and level of minerals (Erdman, 1981; Harland and Morris, 1985; Health and Welfare Canada, 1985). In general, the minerals in cereals are not readily absorbed (INACG, 1982) whereas those in yeast are readily available and supplementation of cereal-based diets with yeast products can partially or wholly alleviated dietary problems associated with mineral deficiencies.

Table 7.4: Mineral composition of Yeast (*S. cerevisiae*) and wheat.

Mineral	Yeast* (per gram) (db)	Wheat** (per gram) (db)	RDA** of Adult
Calcium	0.75 mg	0.45 mg	800 mg
Phosphorus	13.00 mg	4.33 mg	800 mg
Magnesium	1.65 mg	1.83 mg	350 mg
Iron	20.00 mcg	43.00 mcg	10 mg
Zinc	170.00 mcg	35.00 mcg	15 mg
Selenium	5.00 mcg	-	70 mcg
Copper	8.00 mcg	5.30 mcg	1.5 - 3.0 mg
Manganese	8.00 mcg	46.00 mcg	2.0 - 5.0 mg
Chromium	2.20 mcg	-	50 - 200 mcg
Molybdenum	0.04 mcg	0.48 mcg	75 - 250 mcg
Potassium	21.00 mg	4.54 mg	-

Source : * Anon, 1991c
** Reed and Nagodawithana, 1991.
db : dry basis

Yeast extracts can be produced within the country at reduced cost by utilising industrial byproducts as yeast growth substrates. Molasses (a byproduct of the sugar industry), cheese whey from the dairy industry, sulfite waste liquor from the paper pulp industry, corn steep and other wastes of the maize products industry, and wood hydrolysates are all potentially fermentable.

It may become economically practical to improve the nutritional value of yeast protein by changing the composition of growth media with respect to carbon and nitrogen sources, or by selection of mutant strains. Considerable increases in the L-lysine content of yeast are possible through adding a lysine precursor, 2-oxoadipic acid, to growth media (Rieche et al., 1966;

Jensen and Shu, 1961). A maximal lysine content of 16.5 % was reached in 35 hr with growth of *Saccharomyces cerevisiae* on molasses containing 2-oxoadipic acid (Broquist and Stiffey, 1959). Chiao and Peterson (1953) have also attempted to increase the methionine content of yeast. Addition of various amino acids to the growth medium can also significantly influence the amino acid composition of yeast but are not economical (Kautzmann, 1969).

7.6 Suggestions for future research

The present study suggests that protein contents of chapaties were increased by incorporation of yeast extracts. Biological studies could be conducted to evaluate quality of protein, measuring protein efficiency ratios of composite flour chapaties. Amino acids, vitamins, and minerals could also be quantified in flours.

Oxidative changes may affect the flavour profile and nutritional qualities of yeast extracts on prolonged storage and effects of storage on composite flours at ambient temperature should be investigated.

Acceptability studies of yeast extracts supplemented chapaties using low income groups in Pakistan should be conducted. Yeast extracts could also be incorporated into snack biscuits to enhance nutritional quality for children using response surface methodology.

Triticale possesses a high protein content and an improved amino acid profile over wheat. However, its flour does not possess the gluten-forming proteins of wheat and doughs are less dry, smooth and pliable than properly developed wheat dough (Chen and Bushuk, 1970). In Pakistan, triticale is receiving some attention as a novel crop and is currently passing through experimental evaluation of yield. Production of composites of triticale with wheat in flours for chapati, biscuits, naan and other flat breads should be studied using response surface methodology.

Matri (*Lathyrus sativus*), a legume considered to be an important source of protein, energy, minerals and vitamins (Morrison, 1959; Kay, 1979), is widely cultivated in winter in south western parts of Pakistan. In southern Punjab and northern Sind, matri is sown to form a wind break for cotton. This legume is available abundantly and is comparatively cheap in comparison with other beans. The current major end usage is in adulteration of chickpea flour. A series of studies should be made to incorporate matri into wheat in order to enhance nutritional qualities of chapati, roti, naan and biscuits employing response surface methodology.

Response surface methodology can be more widely used in development of new food products or to modify existing products with minimal experimentation, time and use of raw materials. It should be more widely implemented for research and development of food products in food industry.

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9. APPENDICES

Example

APPENDIX 1

SCORE SHEET

Name________________________________Date 25.4.92

You are provided with Four samples of chapaties. Please check their texture (softness/hardness) at three intervals and score them on a scale running from one to ten. Here "1" refers to soft, "5" refers to neither soft nor hard and "10" refers to hard.

Date	Interval (time)	Sample code			
		425	730	520	635
25.4.92	**0930**	____	____	____	____
25.4.92	**1930**	____	____	____	____
26.4.92	**0930**	____	____	____	____

Instructions:

1. Take a small piece of chapati, fold it to form a scoop, bite and chew it. If the scoop is smooth to touch, showing no tearing and provides a sensation of softness in the mouth, it is considered as soft, otherwise hard.
2. Score it according to the intensity of these properties.
3. Repeat the above procedure (step 1 and 2) for subsequent storage intervals.
4. After each testing, put samples back into their corresponding bags and then put them into another polyethylene bag provided with the samples.
5. Store them in a cool place for subsequent analysis.

APPENDIX 2

SCORE SHEET

Name__________________________________Date______________

Rinse mouth with water. Chew small piece of chapati, moving it around in the mouth so that it reaches all internal surfaces. Score how much you like or dislike its taste according to the scale given below. Rinse mouth again, wait approximately for one minute and taste the next sample. Continue through the series using the same procedure.

1 Dislike extremely
2 Dislike very much
3 Dislike moderately
4 Dislike slightly
5 Neither like nor dislike
6 Like slightly
7 Like moderately
8 Like very much
9 Like extremely

Sample code	Score
__________	______
__________	______
__________	______
__________	______
__________	______
__________	______
__________	______
__________	______
__________	______
__________	______
__________	______

APPENDIX 3

MARKET RESEARCH QUESTIONNAIRE FOR CHAPATI QUALITY AND SAVOURY FLAVOURS

DEPARTMENT OF BIOSCIENCE AND BIOTECHNOLOGY (FOOD SCIENCE) JAMES P.TODD BUILDING, UNIVERSITY OF STRATHCLYDE, 131 ALBION ST. GALSGOW G1 1XQ.

Please respond to the following questions as accurately as possible. Thank you for your cooperation and willingness to assist in our research project. Please encircle the appropriate number in all the questions.

1. Are you Pakistani in origin?
   1). Yes
   2). No
2. Where are you living?
   1). Urban
   2). Rural
3. Sex
   1). Male
   2). Female
4. Age (Years)
   1). Less than 25
   2). 26-40
   3). 41-60
   4). More than 60
5. What is your profession? ____________________.
6. What is your education? ____________________.
7. How long you have been here________________years?
8. Marital status.
   1). Unmarried
   2). Married
   3). Widowed
9. How many children are there in your household_________?
10. Do you buy chapaties from a shop or make at home?
    1). Buy
    2). Make
    3). Both
11. Do you buy wheat flour or prepare yourself?
    1). Buy
    2). Prepare
    3). Both
12. How much flour do your family consume?_________Kg/month.

13. At what time do you eat chapaties? Please encircle the appropriate.

1). Morning
2). Afternoon
3). Evening

14. What flavouring dishes do you more prefer?

1). Savoury
2). Sweet

15. In savoury flavour dishes which do you prefer? Here is the scale running from one to five; "5" refers to MOST PREFER and "1" refers to LEAST PREFER. Please encircle the appropriate numbers.

	Least prefer				Most prefer
1). Gram flour like	1	2	3	4	5
2). Vegetable (specify)	1	2	3	4	5
3). Meaty (Beef/Mutton)	1	2	3	4	5
4). Yeasty	1	2	3	4	5
5). Fishy	1	2	3	4	5
6). Cheesy	1	2	3	4	5
7). Spicy	1	2	3	4	5
8). Chicken meat	1	2	3	4	5
9). Garlicky	1	2	3	4	5
10). Herby	1	2	3	4	5

16. What flavouring dishes do your children more prefer?

1). Savoury
2). Sweet

17. In savoury flavouring dishes do your children prefer? Here is the scale running from one to five; "5" refers to MOST PREFER and "1" refers to LEAST PREFER. Please encircle the appropriate numbers.

	Least prefer				Most prefer
1). Gram flour like	1	2	3	4	5
2). Vegetable	1	2	3	4	5
3). Meaty (Beef/Mutton)	1	2	3	4	5
4). Yeasty	1	2	3	4	5
5). Fishy	1	2	3	4	5
6). Cheesy	1	2	3	4	5
7). Spicy	1	2	3	4	5
8). Chicken meat	1	2	3	4	5
9). Garlicky	1	2	3	4	5
10). Herby	1	2	3	4	5

18. In chapati, what is important? Here is a scale running from one to five; "5" refers to MOST IMPORTANT ans "1" refers to NOT IMPORTANT. Please encircle the appropriate numbers.

	Not important				Most important
1). Price	1	2	3	4	5
2). Availability	1	2	3	4	5
3). Tradition	1	2	3	4	5
4). Colour	1	2	3	4	5
5). Taste	1	2	3	4	5
6). Flavour	1	2	3	4	5
7). Texture	1	2	3	4	5
8). Nutritional quality	1	2	3	4	5

19. Any suggestion____

____

____

Note: Questions No 2, 5, 6, 11 and 12 were not included in questionnaire for Glasgow.