Pak. J. Food Sci., Vol. 6, No. 3-4, 1996.

REVIEW ARTICLE

FORTIFICATION OF WHEAT FLOUR

Javaid A. Awan, M. Ismail Siddique & Abrar H. Gilani*
Department of Food Technology, *Department of Animal Nutrition,
University of Agriculture, Faisalabad.

ABSTRACT

Food fortification is an effective public health technique that has been used to eradicate such adverse conditions as beriberi, iron deficiency anaemia, goiter, pellagra and protein-malnutrition. Wheat is the single largest source of energy and protein for millions of people in the world. This cereal is deficient in lysine while some other essential amino acids are also low. In order to improve the quality of wheat protein, efforts have been made by numerous workers. In this regard several protein rich materials have been used. Among these are cereals, tubers, milk, milk products, fish, fish protein concentrate, oil seed flour, oil seed meal, and different legumes. Foods in which thee composite flours have been employed include biscuits, cookies, *nans*, *chapati*, bread and miscellaneous products. This paper discusses the work conducted so far in this regard.

Key words: food fortification; wheat; cereal products; bread; public health; Pakistan

INTRODUCTION

Malnutrition is a common problem the world over caused by incorrect consumption of food and nutrients. In economically developed countries and rich communities, it results primarily from over intake of nutrients. In the developing countries, inadequate consumption of food and nutrients is the major cause. There are numerous factors associated for this type of malnourishment. In Pakistan, the poor state of nutrition is due to uneven income distribution, poverty, unhygienic environment, improper delivery of services, ignorance of nutritional practices, less availability of food in poor households and low level of mother's education (Anonymous, 1996). Bioavailability of nutrients is also an important factor. The prevalence of deficiency of micronutrients (mineral elements and vitamins) and proteins are common in populations of most developing nations. According to the United Nations International Children's Emergency Fund (UNICEF), malnutrition results in stunted mental and physical growth in one out of 3 children in developing countries. It is a factor in one third of the 13 million child deaths which occur annually (IAEA, 1995).

Among the various malnourishments, protein deficiency is a serious problem facing people whose diets consist mainly of cereals or other starchy foods (Chastain *et al.*, 1975; Carlson *et al.*, 1981). In Pakistan, nutritional surveys as well as the food balance sheets indicate varying degree of protein deficiency in some vulnerable groups of the population, due especially to the low quality and quantity of proteins intake. Although per capita food intake estimated at 2,570 calories per day for 1995-96 is 0.8 per cent above recommended dietary allowance (RDA) of 2,544 calories and likewise protein intake of 67.88 grams which is 13.1 per cent above the RDA of 60 grams, yet certain groups of the population like children particularly below 5 years and lactating mothers have high incidence of malnutrition (Anonymous, 1996). Food energy exerts a sparing effect on protein; energy deficiency widens the protein gap and most protein deficiency is conditioned by energy deficiency.

Wheat (*Triticum aestivum* L.) is the most ancient of the presently cultivated forms of cereals and is consumed throughout the world by more than 5 billion human beings. It is the single largest source of energy and protein for millions of people in the world. The production of wheat in Pakistan has increased progressively and was 17.57 million metric tonnes in 1995-966 (Anonymous, 1966). In the Indo-Pakistan subcontinent wheat is consumed in the form of flat breads such as *chapati*, *roti*, *paratha* and *nan* as well as bread, bun, biscuits, cakes, pastries, patties, *samosa*, pie, pan-cakes, *poori*, noodles, vermicelli, *sooji halwa*, *jalebi* and others. The flat breads are generally prepared from whole wheat flour or 95 per cent extracted flour, locally called *atta*, other commodities are prepared from white flour.

Lysine is the first limiting essential amino acid in wheat flour. Tryptophan and threonine are also low. Such deficiencies lead to poor utilization of proteins in wheat and thus contribute to malnutrition. Lysine deficiency in wheat products is aggravated by losses due to

browning reactions (Maillard) during baking which may be as high as 10 per cent (Saab *et al.*, 1981).

The four widely-recognized strategies for reducing malnutrition are supplementation, fortification, dietary diversification and disease reduction. Efforts are also being focussed towards introducing a fifth strategy of plant breeding (Bouis, 1995) to develop cereal varieties possessing high protein/lysine. The deficiency of essential amino acids in wheat protein may, however, be supplemented by the addition of amino acids either to wheat or the final product (Bressani *et al.*, 1966; Altschul 1970; Arao, 1976; De Tonella and Yepiz, 1986). The wheat grain may be tempered in the presence of the desired amino acids or the product may be fortified with lysine (Rashid, 1970). Such efforts have resulted in improved biological value of the product.

Food fortification is a useful and safe tool in the hands of food scientists and nutritionists to restore nutrients deficient in the raw material or lost during processing (Staff Report, 1996). This technique has a promising future and can be used to ensure that food supply will become ever healthier for the consumer. The concept is as relevant today as it was yesterday, as witnessed by the recent Food and Drug Administration of the United States ruling requiring that enriched bread, flour, pasta, and other grains be fortified with folate (0.34-1.4 mg/lb no later than January 1998) to reduce the risk of *Spina bifida* and other birth defects (Sloan & Stiedemann, 1996).

The quality of wheat protein can also be improved by fortifying with suitable edible proteinaceous materials relatively rich in such amino acids as lysine, tryptophan and threonine. Dried beef (Yousaf *et al.*, 1971), beef liver meal (Ahmad *et al.*, 1971), lamb liver tissue protein concentrate (Husain and Chaudhry, 1982) and other similar foods have been investigated with positive results. Although meat, milk, eggs and poultry have plenty of good quality proteins, yet these are expensive and often beyond the reach of common man. Edible plant products like legumes and oil seed meals/flours are comparatively cheaper sources of proteins. The international agencies like UNICEF and Food and Agriculture Organization (FAO) of the United Nations have continued programmes to save the world populace from protein malnutrition by financing research projects to enrich foods with locally produced proteinaceous materials.

Composite flour technology

Studies on the preparation of composite flours containing wheat and protein-rich materials have been carried out in many parts of the world. A comprehensive bibliography with 2 supplements has been published by the Tropical Products Institute London (Dendy *et al.*, 1975; Kasasian and Dendy, 1977; Dendy and Kasasian, 1979). In this regard numerous substances such as oil seeds, oil seed cakes, fish flour, leaf protein, milk solids, milk whey, single cell protein and edible legumes have been employed. The addition of edible materials to wheat is safe and practicable, especially in the less educated and developing societies. As a result of these efforts, products such as Multipurpose Food and *Bhal Ahar* in India, *Incaparina* in Central America, *Maisoy* in Bolivia, *Faffa* in Ethiopia, *Superamine* in Algeria, *Laubina* in Lebanon and many others have been produced and marketed successfully (Awan, 1995).

1. Biological evaluation of composite flours

Biological value (BV) of flours from different wheats varies between 50 to 55, while of pulses it ranges between 32-78 per cent (Patwardhan, 1962). However, when wheat is supplemented with other protein sources, the biological value is greatly enhanced. Husain and Chaudhry (1982) reported that when wheat and rice were supplemented with lamb liver tissue protein concentrate there was marked improvement in the biological value of the cereals. Weight gains were higher when wheat flour was supplemented with groundnut flour or with gram flour (Nawaz, 1979). Yasmin (1976) conducted metabolic studies on rats by feeding wheat flour supplemented with raw and cooked lentil flour at different levels of protein utilization. Biological value of wheat flour improved significantly. Bhatty and Chaudhry (1979) reported improvement in net protein utilization (NPU) and BV when wheat flour was supplemented with pea flour. In a similar study, Gilani *et al.* (1986) observed improved energy digestibility when wheat flour was supplemented with groundnut, gram and lentil flours.

The supplementation of wheat flour with different levels of gram flour has been studied by Khan *et al.* (1976). The authors reported that growth rate of albino rats increased with increasing levels of gram flour in the diet. The supplemented diets had significantly higher protein efficiency ratio than the basal diet. These results have also been confirmed by Anwar (1980) who reported a gain in body weight of rats fed on fortified samples of wheat with gram flour. All gram based diets had significantly higher protein efficiency ratio and digestibility. Supplementation improved the NPU and BV. However, Murthy and Urs (1985) observed that when Bengal gram was toasted, 12-13 per cent of lysine was rendered unavailable and *in vitro* digestibility decreased by 15-28 per cent.

2. Use of cereals and tubers

Cereals may be employed to blend wheat flour for specific purposes. Studies conducted on the use of sprouted wheat for blending wheat for the production of bread (Tariq, 1990) and biscuits (Rashid, 1994) have re-

vealed that good quality products could be prepared from the blended flour. Generally low amylase activity existing in Pakistani wheats (Ali, 1980) may be supplemented with malted barley flour (Latif, 1994). The bread prepared from this blend has an improved volume, crust colour, crumb colour and general appearance. Hedges and Tinklin (1969), satisfactorily substituted wheat protein concentrate (WPC) for part of whole wheat flour in *chapaties*. It was found that the use of 10 and possibly 20 per cent of WPC to replace equal weights of whole wheat flour in chapati would make acceptable products. The authors recommended use of some WPC to increase the nutritive value of chapati. Akhter (1994) produced bread of acceptable quality and higher fiber content by supplementing wheat flour with wheat, barley and rice brans.

Tubers are primarily a source of starch. However, where wheat is scarce or insufficient, these may be added. Potatoes, sweet potatoes and cassava have been studied. Jain and Sherman (1976) reviewed the influence of partial replacement of potato flour on bread texture and noted that wheat flour could be fortified with potato flour at the rate of 3-10 per cent. Elias *et al.* (1977) prepared potato flour with different treatments and used the same for blending wheat flour (93.3% extraction) at the rate of 3 and 6 per cent. The authors prepared *balady* bread from these blends with satisfactory results. However, Shekara and Shurpalekar (1983) found a decrease in dough stability and an increase in the mixing tolerance index with increasing levels of potato and cassava flours in the blends.

Sammy (1970) reported no difficulty in making bread from blends containing 20 per cent and pastries from 20-30 per cent sweet potato flour as a wheat flour substitute. Flour from peeled and unpeeled tubers differed little in their effect on baking properties. The author observed that the addition of sodium metabisulphite improved flour colour.

3. Use of milk and milk products

Milk is nutritionally a balanced and nearly complete food. It has, therefore, been the subject of interest for numerous investigators, especially with regard to its use in wheat flour-based products. Use of milk solids in baked products have helped to produce palatable and nutritious products (Henderson & Buchanan, 1969; Marston, 1971; Afzal, 1973; Bassi & De, 1973; Burnett, 1976; Anonymous, 1977). Recipes for baked products using milk and milk products have also been described (Hubbard, 1971; Van Gennip, 1977). Food value of cereals such as wheat and maize can be improved through the use of animal protein supplements like skimmed milk powder (Yousaf *et al.*, 1971).

Cheese whey has been extensively investigated and found suitable for use in baked goods (Chumachenko, 1973; Chumachenko *et al.*, 1973; Guy *et al.*, 1974; Mann, 1977). Ahmad (1981) used cheese whey powder at the rate of 1, 3 and 5 per cent in bread making and observed a positive effect on colour, flavour, texture and taste attributes. Lysine content in wheat bread increased by 46 per cent when whey solids at 5 per cent level were incorporated (Siddique *et al.*, 1986).

4. Use of fish protein

Fish is a cheap source of good quality protein in most parts of the world. Fish protein supplements at 5 or 10 per cent levels promote better growth as compared to vegetable protein supplements of guar meal (Chaudhry *et al.*, 1971). Yanez *et al.* (1976) evaluated the biological value of enzymatic fish protein hydrolyzate for wheat, rice and corn in growing rats. Fortification of these cereals at levels from 2-10 per cent brought about increase in both quantity and quality of dietary protein. Fish protein concentrates have been used to fortify wheat flour to produce baked products (Khan, 1976). Melinkova and Boyarkina (1973) observed that non-defatted dried concentrate from cod-type fish could be used at 10 per cent level for bread and 20 per cent level for pancakes. Riaz and Khan (1978) supplemented wheat flour with fish flour for the production of biscuits and reported an appreciable increase in protein and ash contents. Studies made by Ali *et al.* (1964) revealed that wheat flour containing 3-5 per cent shark meat flour satisfied the protein requirements of growing children.

Fish protein can also be used to enrich wheat flour for chapati making (Bass and Caul, 1972). Production of Arabic and Indian breads was studied by Nikkila *et al.* (1976). The results indicated that fish protein concentrate up to 10 per cent level produced products of acceptable quality with improved nutrition. Sharif and Bhatti (1971), on the basis of nutrition as well as sensory trials, recommended fortification of wheat flour with 10 per cent fish flour for the production of *nan*. However, while fish protein could help in combating malnutrition, problems were associated with consumer acceptance of such products (Moorjani, 1972; Moorjani & Lahiry, 1972).

5. Use of oil seed meals/flours

Oil seeds are comparatively rich sources of oils and proteins. After the extraction of oil, meal becomes a valuable source of protein. If properly processed, oil seed meals could be used to enrich wheat flour. Efforts have been made to utilize press cake from almond, coconut, cotton seed, groundnut, sesame, soybean and sunflower. The nutritive value of cereal products considerably improves with the addition of such combinations (Afzal, 1974; Haq, 1974; Parveen, 1979).

Harden (1974) observed that with one exception, liquid cyclone processed cotton seed flour increased protein efficiency ratio (PER) when used at various levels of substitution in wheat flour for bread and bakery products. At 10 per cent protein level, cotton seed flour increased PER from 1.6-1.9 when mixed in 50:50 ratio with Triticale flour and from 0.5-1.6 when used with wheat flour. Similarly, Mufti (1975) and Barque *et al.* (1979) utilized detoxified cotton seed flour to enrich wheat flour. Biological studies in rats revealed that PER, biological value (BV) and net protein utilization (NPU) were higher in enriched samples as compared to control containing wheat flour alone. Bass and Caul (1972) used cotton seed flour and soya protein concentrate to enrich wheat flour for chapati making. Qayyum (1976) employed glandless cotton seed flour in the production of chapati. High protein breads have been prepared by incorporation of oil seed protein concentrates (Sheikh *et al.*, 1970). Ghafoor (1979) reported that 10-15 per cent supplementation of wheat flour with cotton seed flour resulted in production of *nan* of acceptable quality with higher protein content.

Lawhon *et al.* (1972) and Lawhon and Cater (1975) conducted trials with different oil seed flours from cotton seed, soya and peanut and observed that various blends produced doughnuts that were satisfactory and acceptable. Jain *et al.* (1975) incorporated sunflower seed meal into bread flour at the rate of 10, 15 and 20 per cent. The loaves showed good crust, crumb, grain size, texture and improved taste at 10 and 15 per cent replacements. Robertson (1975) reviewed the uses of sunflower oil meal and concentrate in bread and other foods and pointed out that its use in the range of 10-20 per cent produced organoleptically acceptable food products. Bread (Chaistain *et al.*, 1975) and *nan* (Manzoor, 1976) produced by using coconut flour compared well with products from 100 per cent wheat flour. At 18 per cent supplementation, the experimental bread score was equal to wheat bread.

Studies on the preparation of high protein biscuits using sunflower protein isolate (Claughton & Pearce, 1989), mustard protein concentrate, cotton seed flour and cotton seed protein isolate (Rajput *et al.*, 1988) revealed that these could be conveniently used to increase the protein content of the product. The optimum level of incorporation was found to be 15 per cent for mustard protein concentrate or cotton seed protein isolate and 10 per cent for cotton seed flour.

6. Use of pulses

6.1. Production and significance of pulses

Pulses are recognized as rich sources of proteins, vitamins and mineral elements (Aykroyd & Doughty, 1969; Awan, 1993). It is well known that pulses form a valuable supplement to cereal diets especially when it lacks in animal proteins (Bressani *et al.*, 1974; Rajalakshami, 1976). Although deficient in sulphur containing amino acids, yet pulses are rich in lysine and thus natural and cheap sources of proteins for human nutrition. The quality of protein obtained from pulses is known to be superior to that of wheat, particularly in such amino acids as lysine, threonine and tryptophan. Many national and international agencies have advocated increased use of legumes in combating protein malnutrition. In Pakistan, several pulses are used in the form of whole or split grains for preparing curry to be consumed with chapati or rice. Numerous sweets and snacks are also prepared from pulses.

In addition to being nutritionally important, pulses are now recognized as having therapeutic/ medicinal properties. Atherosclerosis is characterized by massive accumulation of cholesterol in the arteries that forms the heart of an atheromatous plaque. Epidemiological studies have indicated a high level of plasma cholesterol as an important risk factor (Lewis *et al.*, 1974; Goldrick *et al.*, 1970). There is continued search for agents that can lower the blood cholesterol level. Some legumes are known to be hypocholesterolemic (Singh *et al.*, 1983). The hypocholesterolemic characteristics of these could be attributed to the nature of their carbohydrates, proteins and unsaturated fats. Involvement of dietary fibre in lowering the blood cholesterol level has also been reported (Kritchovsky & Story, 1974). Pulses when consumed as whole grains may provide significant quantity of fibre and thus prove good hypocholesterolemic agents. An earlier epidemiological survey revealed that people of low socioeconomic status consuming a staple diet of Bengal gram had a relatively low incidence of ischemic heart disease because it possesses a cholesterol lowering effect in man (Mathur *et al.*, 1964).

Considering the deficiencies in wheat and adequacies of pulses, efforts have been made to blend these to produce nutritionally balanced products. It is evident that a combination of wheat and a pulse in proper proportions could supply the required amino acids and mineral elements to the consumer. At the same time this blend will also increase the fibre content of the diet resulting in easy bowl movement, reduction in cholesterol level in serum and liver and beneficial effects to the diabetics (Murthy & Urs, 1985; Toma & Curtis, 1986).

6.2. Use in biscuit/cookie manufacture

Biscuits/cookies are important commodities used as snack food by all age groups. The commercially available biscuits are deficient in proteins, fiber and ash contents (Rauf, 1993). Efforts have been made to study the use of composite flour in the manufacture of bis-

cuits/cookies to improve their nutritional value (McWaters, 1978). Subrahmanyan *et al.* (1958) prepared biscuits from a blend of wheat flour (35-40), groundnut flour (25-30), sugar, shortening, salt, calcium carbonate and glucose with added vitamins. Biological studies showed no significant reduction attributable to baking in the utilization of protein, calcium, phosphorus and fat. Baking adversely affected the PER when biscuits formed 10% of the protein intake. Afzal (1973) and Tsen *et al.* (1973) fortified wheat flour using soya flour or soya protein isolates that resulted in considerable increase, to the extent of 100 per cent, in protein content of cookies. However, the spread factor reduced considerably which could be overcome by the use of sodium stearoyl-2-lactylate or sodium stearoyl fumarate. Less shortening was required with the use of the surfactants. Gill (1971) reported that biscuits could be made by supplementing pea powder at the level of 5 per cent. This improved especially the mineral and vitamin content of the product. Ullah (1990) fortified wheat flour with *matri* (*Lathyrus sativus*) and recommended a replacement level of up to 30 per cent though matri flour could be safely used in biscuits up to the level of 50 per cent without any deleterious effects. Similar studies were conducted by using moth beans (Rahman, 1990; Ahmad, 1993), raw Bengal gram (Hussain, 1993) and roasted Bengal gram (Shakoor, 1995). The results revealed that good quality, crispy and nutritious biscuits could be prepared when flour is fortified at 10-20 per cent levels with these legumes.

6.3. Use in bread making

Bread is the most important commercial product of wheat. It is consumed as a staple food by most wheat-eating people. Researchers have experimented with different pulses like faba, navy, pinto, mung, winged beans, lentils, cowpeas, soybeans and soya hulls. While working on the rheological and baking properties of flours obtained from various legumes, D'Appolonia (1977) observed that flour produced by dry milling from faba, navy, pinto and mung beans and lentils could be used for blending wheat flour. Blends of these with wheat flour at 5, 10 and 20 per cent levels were used for bread making. Improved crumb colour and decreased loaf volume with increased addition of the legume flours was reported.

Chatterjee and Abrol (1975) employed cereal-pulse combination for the production of bread. The authors observed that protein of the respective components complement each other, the optimum combination of pulse to wheat was determined as 10 per cent, while in rice, maize or barley the quantity of pulse could be increased to 20 per cent.

Kailasapathy and MacNeil (1985) enriched wheat flour with winged bean flour (0-2- per cent) and noted an increase of 63 per cent in phosphorus and 105 per cent in iron contents. The blends contained higher amounts of histidine and lysine as compared to wheat flour. Except methionine which decreased, all other essential amino acids increased with blending from 0-15 per cent. The authors observed significant differences between the value of 5 and 10 per cent winged bean full fat flour fortified bread diets. When dehulled winged bean flour at the rate varying from 0-20 per cent was substituted in wheat flour, water absorption, arrival time, development time and stability time increased with a rise in the level of substitution.

Selvaraj and Shurpalekar (1982) improved the nutritional quality of bread by fortification with soya flour. Though at 12 per cent level, the addition of soya flour adversely affected the bread quality, a highly acceptable bread comparing well with wheat bread was obtained by including 5 per cent sugar and 5 per cent fat in the recipe. Farinographic water absorption was 64.8, 68.0, 69.1, 70.1, 71.0 and 72.2 per cent, while mixing tolerance index was 80, 50, 50, 40 and 40 BU. in case of 0, 8, 10, 12, 14 and 16 per cent soya fortified flours, respectively. The use of soybean hulls for fortification at 5 per cent level increased the iron content in baked products without affecting the loaf volume, cross sectional area, tenderness and overall acceptability (Johnson *et al.*, 1985). Shoaib (1986) concluded that 8 per cent soy flour supplemented bread was ideal as observed by chemical and organoleptic evaluations.

Preparation of mung bean flour and its use in bread making was studied by Thompson *et al.* (1976). Mung beans were steam conditioned, dehulled and processed into flour. Dehulling improved the food value of the flour, which was found suitable as a protein supplement in bread at substitution level up to 15 per cent with 0.5 per cent sodium stearoyl lactylate.

Functional properties of legume and wheat flours are important in baked products. Padmashree *et al.* (1987) studied the effect of traditional processing on the functional properties of cowpea flour. The authors found that all processing treatments increased its water absorption capacity. Heat treatment increased the bulk density, while other processing treatments did not have marked effect on the functional properties of cowpea flour. Okaka and Potter (1977) studied blends of cowpea and wheat flour and observed that levels of cowpea flour up to 20 per cent in wheat flour produced acceptable bread. Loaf volume decreased when cowpea flour above 10 per cent levels was used; higher levels needed improvers for getting acceptable quality. Bread prepared from blends containing 20 per cent cowpea flour deteriorated rapidly

during storage at 30°C but was acceptable even after 12 weeks when stored at 22°C.

Polysaccharides do not exhibit surface activity, but confer thermal stability on the foam formed by the protein and prevent its disruption by heat. A surface-active principle of the nature of a globulin and an arabogalactan type polysaccharide has been shown to occur in black gram (Susheelamma and Rao, 1974). These two components together appear to be responsible for the characteristic soft, spongy texture of leavened foods containing this legume. It has been reported (Bains and Tara, 1967) that 5 per cent addition of lentil and gram flour adversely affect the grain and volume of bread. Loss of added lysine in leavened bread was far more than in chapati. Sanchez *et al.* (1971) added chickpea and horse bean flour to a base flour available in Morocco and baked bread according to the local formula and procedure. Soya flour was used for comparison. The Moroccan bread was able to carry higher level of fortification without objectionable effect than the American bread.

6.4. Use in *chapati/nan* Making

In Pakistan, India and some other countries, flat bread or chapati and *roti* are major wheat-based staple food products. *Nan*, leavened flat bread, is also common. These are the least expensive and nutritionally most important. Often, the terms chapati and *roti* are synonymously used to mean unleavened breads, produced from a dough prepared by mixing ground whole wheat flour and water. Though, it is not a good reserve of nutritionally balanced protein, it is nonetheless, a principal source of both protein and energy for the consumer. Chapati is normally consumed with a curry dish. In well-to-do families, the curry may consist of meat, vegetables and/or legumes, while in the very poor class often an onion or a piece of pickle with or without butter milk (*lassi*) may suffice. Since majority of the population in the Indo-Pakistan sub-continent belongs to the low income group, their diet, therefore, comprises primarily of chapati with less of the other nutritious foodstuffs.

Safdar (1971) employed groundnut flour for supplementation of wheat flour for the production of *nan* and found an increase in the protein content without adverse effects on the sensory qualities of the product. Biological studies revealed a gain in body weight and NPU. Gill (1971) concluded that *nans* of satisfactory quality could be prepared by the addition of 5 per cent pea powder. The product was richer in calcium phosphorus, iron, vitamin A, Vitamin B and niacin.

The use of soya bean flour for fortification of wheat flour has also been investigated (Hallab *et al.*, 1974). Imtiaz (1962) reported that chapati prepared from whole wheat pastry flour, with the addition of 15 per cent medium-fat soya flour and 10 per cent dry milk, gave the best balanced diet. Rathod and Williams (1970) incorporated up to 20 per cent soya flour in chapati made from wheat flour without appreciable adverse reactions from consumers. Acceptability trials conducted on chapati fortified with 10 per cent defatted toasted soya flour or chickpea flour revealed that 60 per cent of the panel preferred soya-fortified chapati, while 50 per cent considered chickpea-fortified chapati inferior to the control (Robinson, 1974).

Chaudri and Muller (1970) successfully used decorticated chickpea flour for chapati and *nan* production. Shehata and Fryer (1970) supplemented wheat flour with 10-20 per cent chickpea flour and observed that it did not alter the overall acceptability of the Egyptian bread and produced a significantly higher PER. Firdous *et al.* (1977) studied the influence of varying levels (10, 15 and 20 per cent) of added gram flour (chickpea) on the improvement of wheat chapati digestibility and retention of nitrogen in adult agricultural workers. The supplementation had little influence on the digestibility of crude protein, ether extract and nitrogen free extract. The highest nitrogen was retained by persons fed on diet containing 80 per cent wheat flour and 20 per cent gram flour.

Khan *et al.* (1972) used various proportions of soybean, horse bean, chickpea and groundnut flours, with sodium stearoyl lactylate in wheat flour for making chapati. In softness, 5 per cent soya flour plus 0.5 per cent SSL proved most satisfactory blend. The organoleptic tests showed that chickpea, soya bean, horse bean and groundnut were suitable for blending wheat flour.

Work had also been conducted on protein supplemented cereal based Pakistani diet by Ilahi (1978). The diet containing chapati was supplemented with decorticated lentil, mung, gram and groundnut flour and was used in cooked as well as uncooked forms. The nutritive value of wheat flour in terms of NPU, BV, NPR and PER both in the uncooked and cooked states, was improved by supplementation with the protein sources. The enhanced nutritive value indicated that the pattern of limiting amino acids was modified by supplementation. The digestibility of wheat flour, however, decreased by supplementation with these sources.

Siddique (1989) employed chickpea, Kabligram, lentils, moth beans, mash, mung beans, cowpeas and Haricot beans (*rajmah*) for the production of composite flours for chapati production. The results revealed that while NPU, BV, NPR, PER and feed efficiency increased with supplementation of wheat flour, digestibility decreased. The author concluded that Kabligram ranked highest for the supply of limiting amino acids followed by cowpea, mung, mash, lentils, rajmah, moth and chickpea. In overall acceptability, *chapaties* con-

taining Kabligram and lentil were judged superior as compared to others. However, all the chapaties were acceptable at 10 per cent level of replacement with different legumes. Sarojni *et al.* (1996) studied the utilization of *rajmah* (*Phaseolus vulgaris*) flour in some Indian traditional products including *phulkka*, *puri* and *chapati*. The workers concluded that the products made with the composite flour were well accepted and the flour could be stored for 12 weeks.

REFERENCES

Afzal, M., 1973. Effect of addition of wheat soy blend (WSB) and milk on quality of cookies. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Afzal, M., 1974. Effect of supplementation of sunflower seed oil meal on nutritive value of cereals. M.Sc. Thesis, Dept. Nutr., Univ. Agri., Faisalabad.

Ahmad, M.M., 1981. Production, characterization and utilization of cheese whey powder in various food products. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Ahmad, M., 1993. Effect of supplementation of treated moth beans on the quality of biscuits. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Ahmad, S.U., 1973. The effect of cooking on the essential amino acids content of common pulses. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Akhtar, S., 1994. Use of wheat bran, barley bran and rice bran in bread manufacturing. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Akhtar, S., 1972. Nutritive value of cereals as influenced by guar and jantar meal supplementation. M.Sc. Thesis, Dept. Nutr., Univ. Agri., Faisalabad.

Ali, M.A., 1980. Effect of supplementation of flour from Pakistan wheats with amyloytic enzymes on quality of bread and *roti*. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Ali, S.M., A.S. Alvi and M. Hanif, 1964. Protein value of wheat flour supplemented with shark meal flour. Pak. J. Sci. Res., 2: 41-44.

Altschul, A.M., 1970. Amino acid fortification of foods. Proc. 3rd Int. Cong. Food Sci. Technol., pp: 208-212; Inst. Food Technol., Chicago.

Anonymous, 1977. Protein powered biscuits. Food Eng. Int., 2 (4): 42-43, 45.

Anonymous, 1996. Economic Survey of Pakistan, 1995-96. Finance Division, Economic Advisor's Wing, Govt. of Pakistan, Islamabad.

Anwar, M., 1980. Studies on the determination of optimum level of gram flour supplementation on the nutritive value of wheat protein. M.Sc. Thesis, Dept. Nutr., Univ. Agri., Faisalabad.

Arao, O., 1976. Fortification of cereals with lysine. Cereal Foods World, 21 (8): 282-286.

Awan, J.A., 1993. Elements of Food and Nutrition. Virgos, Faisalabad.

Awan, J.A., 1995. Elements of Food Science and Technology. Virgos, Faisalabad.

Aykroyd, W.R. and J. Doughty, 1969. Legumes in Human Nutrition. FAO Nutritional Studies No. 19, Food & Agri. Org. of the United Nations, Rome. 138 p.

Bains, G.S. and A.K. Tara, 1967. To develop improved types of bread. Annual Report, Central Food Technol. Res. Inst., Mysore.

Barque, A.R., T.A. Mufti, A.H. Gilani and M.I. Siddique, 1979. Supplementation of wheat flour with detoxified cotton seed flour. Pak. J. Agri. Sci., 16 (1-2): 133-138.

Bass, J.K. and J.F. Caul, 1972. Laboratory evaluation of three protein sources for use in chapati flours. J. Food Sci., 37 (1): 100-102.

Bassi, R. and S. De, 1973. Protein enriched milk biscuits. J. Food Sci. Technol., 10 (4): 181-183.

Bhatty, N. and H.M. Chaudhry, 1979. Supplementation of wheat flour with pea flour. Pak. J. Agri. Sci., 16 (1-2): 25-28.

Bouis, H., 1995. Enrichment of food staples through plant feeding: A new strategy for fighting micronutrient malnutrition. SCN News, 12: 15-19.

Bressani, R., B. Murillo and L.G. Elias, 1974. Whole soybean as a means of increasing protein and calories in maize based diet. J. Food Sci., 39: 197-199.

Bressani, R., D. Wilson, M. Chung, M. Behar and N.S. Schrinshaw, 1966. Supplementation of cereal protein with amino acids. J. Nutr., 80 (2): 80-84.

Burnett, K.R., 1976. Milk products for the bakery trade. Food Technol., 11 (9): 27-29.

Carlson, B.L., D. Knoor and T.R. Watkins, 1981. Influence of tomato seed addition on the quality of wheat flour bread. J. Food Sci., 46: 1029-1013.

Chastain, M.F., S.J.S. Sheen, T.J. Cooper and D.R. Strength, 1975. Coconut bread as a means of improving protein nutrition. J. Food Sci., 40 (5): 1614-1617.

Chatterjee, S.R. and Y.P. Abrol. 1975. Amino acid composition of new varieties of cereals and pulses and nutritional potential of cereal-pulse combinations. J. Food Sci. Technol., 12 (5): 221-227.

Chaudhry, G.A., A.H. Gilani and M.B. Sial, 1971. Comparative biological value of different protein supplements. Pak. J. Biochem., 4 (1): 29-32.

Chaudri, A.B. and H.G. Muller, 1970. Chapati and Chapati flour. Milling, 152: 22-25.

Chumachenko, N., 1973. The effect of dried whey on the quality of semi-products and bread. Khlebopekarnaya i konditerskaya Promyshlennost. No. 6, pp: 16-18.

Chumachenko, N., O.I. Demchuk and M.I. Roiter, 1973. Use of dried whey in production of bread from flour of the first quality. Kharchova Promislovist. No. 5, pp: 45-47.

Claughton, S.M. and R.J. Pearce, 1989. Protein enrichment of sugar-snap cookies with sunflower protein isolate. J. Food Sci., 54 (2): 354-56.

D'Appolonia, B.L., 1975. Rheological and baking studies of flours obtained from various legumes. Cereal Foods World, 20 (9): Abstracts only.

D'Appolonia, B.L., 1977. Rheological and baking studies of legumes-wheat flour blends. Cereal Chem., 54 (1): 53-63.

Dendy, D.A.V., R. Kasasian, A. Bent, P.A. Clarke and A.W. James, 1975. Composite Flour Technology Bibliography. Trop. Products Inst., London.

Dendy, D.A.V., and R. Kasasian, 1979. Composite Flour Technology Bibliography. Supp. 2, Trop. Products Inst., London.

De Tonella, M.L.F. and M.S. Yepiz, 1986. Effects of lysine and methionine fortification on dough and bread characteristics. J. Food Sci., 51 (3): 637-639.

Elias, A.N., M.M. Morad and S.K. El-Samahy, 1977. Use of potato flour for the production of *balady* bread in Egypt. Lebensm. Wiss. U. Technol., 10 (1): 42-44.

Firdous, R., A.H. Gilani, M.B. Sial and A.R. Barque, 1977. Effect of supplementation of wheat flour with gram flour on the improvement of wheat chapati. Pak. J. Agri. Sci., 14 (4): 55-58.

Ghafoor, A., 1979. Effect of different levels of supplementation with cotton seed flour on quality of some baked products. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Gilani, A.H., M. Asif, S.A. Nagra, 1986. Energy utilization of supplemented cereal diet in human volunteers. Archivos Latino-Americanos de Nutricion, 36 (3): 373-378.

Gill, I.A., 1971. Enrichment of bakery products: effect of addition of guava and pea powder on quality of *nan* and biscuits. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Goldrick, R.B., P.F. Sinnet and H.N. Whyte, 1970. Atherosclerosis: Proc. Second Int. Symp., (Jones, R.J., ed.), Springer Verlag, New York.

Guy, E.J., H.E. Vettel and M.J. Pallansch, 1974. Effect of cheese whey protein concentrates on the baking quality and rheological characteristics of spongi Cereal Sci. Today, 19 (12): 551-556.

Hallab, A.H., H.A. Khatchadourian and I. Jabr, 1974. Nutritive values and organoleptic properties of wheat Arabic bread supplemented with soybean and chick pea. Cereal Chem., 51 (1): 106-112.

Haq, M.Y.I., 1973. Studies in supplementation of wheat flour with gram flour. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Haq, R., 1974. Nutritive value of wheat flour (Maxi Pak) as affected by supplementation with defatted soyflour. M.Sc. Thesis, Dept. Nutr., Univ. Agri., Faisalabad.

Harlan, J.R., 1974. The use of glandless cottonseed flour in protein fortification of selected foods. IV. Int. Cong., J. Food Sci. Technol., 8: 75-76.

Harlan, J.R., 1976. The Plants and Animals that Nourish Man. "A Scientific American Book". H.W. Freeman & Co., San Francisco.

Hedges, N.J. and G.L. Tinklin, 1969. Chapati quality as affected by wheat protein concentrate. Cereal Sci. Today, 14 (10): 344-346.

Henderson, J.O. and R.A. Buchanan, 1969. Home-baked high protein milk biscuits. Aust. J. Dairy Technol., 24 (3): 116-118.

Hubbard, R., 1971. Bread making with milk powders. Food Technol. N.Z., 6 (2): 22-25.

Husain, S.J. and H.M. Chaudhry, 1982. Studies on preparation, composition, bioevaluation of liver tissue protein concentrate. J. Anim. Sci. Pak., 4 (3-4): 1-8.

Husain, M.A., 1973. A fresh look at the incidence of protein deficiency in Pakistan. Brit. J. Nutr., 29 (2): 211-219.

Hussain, A., 1993. Protein enriched biscuits from composite flour of gram and wheat. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

IAEA, 1995. IAEA Technical Cooperation Activities in the 1990s. Div. Public Inf., Int. Atomic Energy Agency, Vienna.

Ilahi, A., 1978. Studies on protein supplements for cereal (wheat) based Pakistani diet. Ph.D. Thesis, Dept. Nutr., Univ. Agri., Faisalabad.

Imtiaz. Z., 1962. Fortification of Pakistani bread recipe with animal protein and calcium, and the determination of its biological value. M.Sc. Thesis, Oklahoma State Univ., Stillwater, Oklahoma.

Jain, N.C., N.D. Shiralkar and B.Y. Rao, 1975. Incorporation of sunflower seed meal in bread. J. Food Sci. Technol., 12 (3):

Jain, S. and P. Sherman, 1976. The influence on bread texture of partially replacing wheat with potato products. J. Texture Stud. 7 (12): 297-311.

Kailasapathy, K. and J.H. MacNeil, 1985. Baking studies with winged bean (*Psophocarpus tertagololobus* L. DC) flour wheat flour blends. J. Food Sci., 50: 1672-1675.

Kasasian, R., D.A.V. Dendy, 1977. Composite Flour Technology Bibliography. Supp. 1, Trop. Products Inst., London.

Khan, M.A., K. Almas, A.R. Abid and M. Yaqoob, 1976. The effect of gram flour on the quality of wheat protein. Pak. J. Agri. Sci., 13 (2):167-172.

Khan, M.N., R.J. Robinson, M. Wang and W.J. Hoover, 1972. Effect of protein-rich flour and sodium stearoyl lactylate on chapati (Indian non-leavened bread). Cereal Sci. Today, 17 (9): 315-319.

Khan, T.H., 1976. Effect of enrichment with fish flour on quality of various baked products. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Khan, Z.N., 1975. Studies on preparation and suitability of sesame-seed flour for addition to *nans*. M.Sc, Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Kritchovsky D., and J.A. Story, 1974. Binding of bile salts *in vitro* by non-nutritive fiber. J. Nutr., 104: 458-460.

Latif, S., 1994. Effect of different types of barley malt supplementation on the quality of bread. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Lawhon, J.T., C.M. Cater, 1975. Sensory analytical evaluation of cake doughnuts fortified with protein from oilseed flours. Food Prod. Dev., 9 (4): 110-118.

Lawhon, J.T., L.W. Rooney, C.M. Cater and K.F. Mattil, 1972. Evaluation of protein concentration produced from glandless cottonseed flour by a wet extraction process. J. Food Sci., 37: 778-783.

Lewis, B., A. Chatti, O.M.O. Oakley, I.D.P. Wooton, D.M.A. Onitri, G. Sigurdson and A. February, 1974. Serum lipoprotein abnormalities in patients with ischemic heart disease comparison with a control population. Brit. Med. J., 3: 489-491.

McWatters, 1978. Cookie baking properties of defatted peanut, soybean and field pea flour. Cereal Chem., 55 (6): 853-863.

Mann, E.J., 1977. Whey utilization in food: general, infant food and bakery products. Dairy Ind. Int., 42 (7): 26-27.

Manzoor, M.A., 1976. Studies on preparation and suitability of coconut flour for additions to *nans*. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Marston, P.E., 1971. The use of dairy products in bread and other baked products. Food Technol. Aust., 23 (10): 506-509.

Mathur, K.S., S.S. Singhal and R.D. Sharma, 1964. Effect of Bengal gram in experimentally induced high levels of cholesterol in tissues and serum in albino rats. J. Nutr., 84: 201-203.

Melinkova, O.M. and L.G. Boyarkina, 1973. The use of fish protein concentrate from cod type fish in flour and bakery products. Rybonoe Khozyaistovo No. 6: 72-73.

Moorjani, M.N., 1972. Fish protein concentrate in combating malnutrition. Ind. Food Packer, 26 (1): 47-49.

Moorjani, M.N. and N.H. Lahiry, 1970. The fish protein concentrate story. Efforts in India. Food Technol., 24: 56-59.

Mufti, T.A., 1975. Supplementation of wheat flour with detoxified cottonseed flour. M.Sc. Thesis, Dept. Anim. Nutr., Univ. Agri., Faisalabad.

Murthy, K.S., and M.K. Urs, 1985. Effect of Bengal gram (*Cicer arietinum*) proteins and lipids on serum and liver cholesterol levels in rats. J. Food Sci. Technol., 22 (1): 54-56.

Nawaz, S., 1968 Comparative nutritive value of different cereals on growth performance of rats as affected by type of cereal. M.Sc. Thesis, Dept. Anim. Nutr., Univ. Agri., Faisalabad.

Nikkila, E.M., S.M. Constantinides and T.L. Meade, 1976. Supplementation of Arabic and India breads with fish protein concentrate. J. Agri. Food Chem., 24 (6): 1144-1147.

Okaka, J.C. and N.N. Potter, 1977. Functional and storage properties of cowpea powder wheat flour blends in bread making. J. Food Sci., 42 (3): 828-833.

Padmashree, T.S., L. Vijayalakshmi and S. Puttaraj, 1987. Effect of traditional processing on the functional properties of cowpea (*Vigna catjang*) flour. J. Food Sci. Technol., 24 (5): 221-225.

Parveen, N., 1979. Supplementation of wheat flour with defatted groundnut (*Arachis hypogaea* L.) flour. M.Sc. Thesis. Dept. Anim. Nutr., Univ. Agri., Faisalabad.

Patwardhan, V.N., 1962. Pulses and beans in human nutrition. Amer. J. Clin. Nutr., 11: 12-14.

Qayyum, A., 1976. Influence of glandless cotton seed flour on quality, acceptability and amino acid content of flat bread. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Rahman, A., 1990. Use of composite flours for the production of biscuits. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Rajalakshami, R., 1976. Pattern and prevalence of malnutrition. Baroda J. Nutr., 3: 1-3.

Rajput, L.P., P.H. Rao and S.R. Shurpalekar, 1988. Use of unconventional protein sources in high protein biscuits. J. Food Sci. Technol., 25 (1): 31-34.

Rashid, A., 1994. Effect of sprouting on the quality characteristics of wheat. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Rashid, T., 1970. Study on Enrichment of `nans' with lysine, certain vitamins and minerals. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Rathod, K.L. and S.W. Williams, 1970. Consumer acceptance of *chapatis* containing soybean flour. Indian J. Agri. Economics, 25 (4): 68-72.

Rauf, M.A., 1993. Chemical and biological evaluation of some commercially available biscuits. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Riaz, R.A. and T.H. Khan, 1978. Development of high protein foods in Pakistan. Some studies on the supplementation of cakes and biscuits with fish flour. J. Agri. Res., 16 (1): 90-100.

Robertson, J.A., 1975. Use of sunflower seed in food products. Crit. Rev. Food Sci., 6 (2): 201-240.

Robinson, R.J., 1974. Acceptability tests of fortified *roti* and bread products in Pakistan. Cereal Sci. Today, 19 (9): 357-361.

Saab, R.M.C.B., C.S. Rao and R.S.F. DeSilva, 1981. Fortification of bread with L-lysine HCl: losses due to the baking process. J. Food Sci., 46: 662-664.

Safdar, M.A., 1971. Preparation and suitability of groundnut flour for enrichment of Pakistani *nan*. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Sammy G.M., 1970. Studies in composite flour Part-I. The use of sweet potato flour in bread and pastry making Trop. Agri., 47(2): 115-125.

Sanchez, C., M. Kahn, C.C. Tsen and R.J. Robinson, 1971. High protein Moroccan bread. Cereal Sci. Today, 16 (9): 351-358.

Sarojini, G., G. Nirmala and A. Chaturvedi, 1996. Studies on utilization of Rajmah (*Phaseolus vulgaris*) flour in some Indian traditional products. J. Food Sci. Technol., 33 (2): 159-161.

Selvaraj, A. and S.R. Shurpalekar, 1982. On improving the quality of soya-fortified bread. J. Food Sci. Technol., 19 (6): 242-245.

Shakoor, A., 1995. Production of biscuits using composite flour containing roasted gram flour. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Sharif, M. and M.B. Bhatti, 1971. Preparation and suitability of fish flour for the enrichment of Pakistani bread or nan. Agri. Pak., 22 (3): 409-415.

Shehata, N.A. and B.A. Fryer, 1970. Effect of protein quality of supplementing wheat flour with chickpea flour. Cereal Chem., 47 (6): 663-667.

Sheikh, I.A., R.I. Ishaq and S.M. Ali, 1970. Preparation and nutritional evaluation of high protein breads containing oil seed protein concentrate. Pak. J. Sci. Ind. Res., 12 (1-2): 1-7, 111.

Shekara, S.C. and S.R. Shurpalekar, 1983. Some chemical, pasting, rheological and textural characteristics of composite flours based on wheat and tubers. J. Food Sci. Technol., 20 (6): 3-8, 312.

Shoaib, M., 1986. Study on supplementation of soyflour with wheat flour for bread making. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Siddique, M.I., 1989. Physico-chemical properties of composite flour for chapati production. Ph.D. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Siddique, M.I., S. Rehman, M.M. Ahmad and A.A. Khan, 1986. Production, characterization and utilization of cheese whey powder. Pak. J. Agri. Sci., 23 (2): 93-96.

Singh, R., M. George, and G.L. Soni, 1983. Role of dietary fibre from pulses as hypocholesterolemic agent. J. Food Sci. Technol., 20 (5): 228-230.

Sloan, A.E. and M.K. Stiedemann, 1996 Food fortification: from public-health solution to contemporary demand. Food Technol., 50 (6): 100-108.

Staff Report, 1996. Food fortification roundtable. Food Technol., 50 (6): 86-94.

Subrahmanyan, V., D.A.V. Dandy, R. Kasrsian, A. Bent, P.A. Clark and A.W. James, 1958. The effect of baking on the nutritive value of fortified biscuits. Food Sci., 7 (4): 83-86.

Susheelamma, N.S. and M.V.L. Rao, 1974. Surface-active principle in black gram and its role in the texture of leavened foods containing the legumes. J. Sci. Food Agri., 25: 665-673.

Tariq, M.S., 1990. Utilization of sprouted wheat for the production of bread. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Thompson, L.U., L. Hung, N. Wand, V.F. Rasper and H. Gade, 1976. Preparation of mungbean flour and its application in bread making. Canadian Inst. Food Sci. Technol. J., 9 (10): 1-5.

Toma, R.B. and D.J. Curtis, 1986. Dietary fibre: effect on mineral bioavailability. Food Technol., 40 (2): 111-116.

Tsen, C.C., E.M. Peters, T. Schaffer and W.J. Hoover, 1973. High protein cookies. I. Effect of soya fortification and surfactants. Baker's Dig., 47: 34, 36-39.

Ullah, E., 1990. Effect of supplementation of *matri* (*Lathyrus sativus*) on the quality of biscuits. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Van Gennip, A.H.M., 1977. Protein enrichment of biscuits. Ind. Aliment., 16 (4): 71-76.

Yanez, E., D. Ballester and F. Moncheberg, 1976. Enzymatic fish protein hydrolysate: chemical composition, nutritive value and use as a supplement to cereal protein. J. Food Sci., 41 (6): 1289-1292.

Yasmin, S.F., 1976. Supplementation of wheat flour with lentil. M.Sc. Thesis, Dept. Nutr., Univ. Agri., Faisalabad.

Yousaf, M., A.H. Gilani and M. Akram, 1971. Nutritive value of cereals as effected by blending with fish flour, skimmed milk powder and dried beef. Pak. J. Sci., 23 (1-2): 6-11.

Pak. J. Food Sci., Vol. 6, No. 3-4, 1996.

PRODUCTION OF ENHANCED NUTRITIONAL VALUE SAVOURY CHAPATIES USING RESPONSE SURFACE METHODOLOGY: TASTE

Salim-ur-Rehman, Alistair Paterson* & J.R. Piggott*
Department of Food Technology,
University of Agriculture, Faisalabad.
**Department of Bioscience & Biotechnology,*
(Food Science Division), University of Strathclyde, Glasgow, UK.

ABSTRACT

Response surface methodology was used to analyze the effect of blending yeast extracts with chapati flour on the quality characteristics of chapaties. Good-fit models were developed for taste judged by sensory evaluation. Results revealed that a good savoury product could be produced by incorporating 1.5 to 2.0 per cent yeast extract. Yeast extract enhanced the concentration of protein from 13.1 to 14.4 per cent and imparted a desirable savoury flavour to chapati.

Key words: response surface; savoury; yeast extract; chapati; nutritional value; blends

INTRODUCTION

Wheat in the form of chapati is the major source of protein and energy in the Pakistani diet and contributes as much as 90 per cent of the total intake (Chaudhry, 1968). The recommended dietary allowance in Pakistan was 2550 calories and 60 g protein per day per capita in 1995-96 (Anonymous, 1996). As wheat proteins are deficient in some amino acids and have low levels of certain others, consumption of chapati as sole protein source leads to protein-malnutrition. Such a situation exists in villages and among the poorest class as consumers cannot make up the deficiencies in wheat protein from other sources. Moreover, lysine deficiency in wheat protein is aggravated by losses arising during baking of chapati (Saab *et al.*, 1981).

In the past, attempts have been made to enhance nutritional quality of chapaties with synthetic amino acids, fish protein concentrate, oil seed meals, legumes and other materials (Awan and Siddique, 1996). However, the success of such strategies appears to be limited by several factors including high cost, low availability and convenience and poor product image in respect of colour and taste (Bass and Caul, 1972; Ali *et al.*, 1964). A further problem is low protein digestibility and flatulence (Siddique, 1989).

Response surface methodology uses quantitative data to determine and simultaneously solve multivariate equations that specify the optimum product for a specified set of factors through mathematical models (Giovani, 1983). Moreover, RSM is more efficient than traditional experimental procedures because it decreases both the time and cost required to determine the optimum product (Khuri, 1992). This technique has also been used in studies of protein denaturation (Nielsen *et al.*, 1973), whipping properties of ultrafiltered soybean product (Lah *et al.*, 1980), optimization of textural attributes of cakes (Frye and Setser, 1991), analysis of the effects of peak drying temperature and blending of hard red spring farina with Durum semolina on spaghetti quality (Malcolmson *et al.*, 1993), processing conditions improvement (Malundo *et al.*, 1992; Floros *et al.*, 1992) and optimization of pH and temperature for pasteurization of citrus juice (Ulgen and Ozilgen, 1993). In the present study, yeast extracts were employed using response surface methodology (RSM) to enhance nutritional quality of chapati and to impart savoury flavour.

MATERIALS AND METHODS

Studies were carried out in the Food Science Division, Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow, UK. A commercial chapati flour was used for preparation of chapati. 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 used to quantify the flavour potentiators and salt (as chloride) in yeast extracts. High performance liquid chromatography was performed using a LKB 2150 HPLC pump (LKB-Produkter AB. 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. Chromatography was performed using Partisil SAX (25 cm x 4.6 mm) with a 10 cm x 4.6 mm silica gel precolumn. Protein was determined with microbiuret method (Itzhak and Gill, 1964).

Production of chapati

Dough was prepared by mixing flour (100 g) with 60 - 70 per cent water (6 to 7% more 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 from 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 cooking range. After 30 seconds 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 seconds, it was turned for the second time and 30 seconds later it was removed from the heat. The total baking time was 90 seconds. The chapati was left to cool and packed in individual polyethylene bags at 32°C.

Moisture, ash and protein contents of flour, yeast extract (%N) and composite flour chapaties were determined by AACC methods (1983).

Sensory evaluation

A panel of 12 Pakistanis (students and housewives) was used. Samples of chapati, packed individually in coded polyethylene bags along with score sheets, were presented to assessors. Each bag contained 1/2 a chapati. Taste was evaluated on Hedonic scale of 1 - 9 where 1 represents extremely dislike and 9 indicates extreme likeness.

Statistical analysis

Response surface methodology was applied to analyze the data (Minitab Version 8.2, Minitab Inc., 3081 Enterprise Drive, State College, PA 16801-3008, USA). It was possible to fit second-order polynomial equations which contained linear, quadratic and interaction terms for two variables. Two dimensional contour plots for quality parameters were generated to determine the effects of the variables on the selected response (Gacula and Singh, 1984).

RESULTS AND DISCUSSION

Chemical composition of yeast extracts

Yeast extracts were analyzed for moisture, total solids, protein, nitrogen, ash content, and sodium chloride (Table 1). The total solids were determined by difference.

Samples were free flowing dried powders which could be mixed easily in flour. Protein content ranged between 22 to 37 per cent as determined by the method of Itzhak and Gill (1964). The nitrogen content, from Kjeldahl method, ranged between 6.62 and 7.28 per cent. 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 (1987) reported that Brewer's dried yeast contained 48 per cent 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 per cent, primarily influenced by the level of sodium chloride.

Chromatographic analysis of yeast extracts

Inosine 5'monophosphate, guanosine 5'monophosphate, monosodium glutamate and sodium chloride were quantified in yeast extracts by HPLC (Table 1). Measurement of peak areas gave a linear responses for all compounds quantified. The retention times of IMP, GMP, MSG and sodium chloride were found to be 17.8, 29.8, 6.8 and 12.9 minutes, 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).

Optimization of acceptability of chapaties

Response surface methodology (RSM) was used to optimize a flour supplemented with yeast extract for chapati production. Yeast extract ranged from 1.0 - 3.0 per cent for YEP 77 and 0.5 - 2.5 per cent for YEP L in increments of 0.5 per cent. 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 regression coefficients within each equation show the direction of the effect of each independent variable, the squares and the interaction (Table 2). The significant F values provided guidelines for further model building (Table 3). Increasing

Table 1. Chemical composition and flavouring potentiators in yeast extracts

Parameter	YEP L	YEP 77	Std AYP	Standard deviation
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
Inosine 5' monophosphate (%)	0.026	4.48	0.48	0.05
Guanosine 5' monophosphate (%)	0.00	1.72	0.00	0.06
Monosodium glutamte (%)	5.52	0.00	0.00	0.07
Sodium chloride (%)	39.87	43.48	9.53	0.08

* = Itzhak and Gill method, ** = Kjeldahl method.

yeast extract (A) had evidently the most important single influence. Both cases indicated that acceptability of taste improved with increasing yeast extract content.

Table 2. Models for selected attributes used in response surface optimization

YEP L

$Y = 7.9^{**} + 0.5\,A^{**} + 0.2\,B - 1.3\,AA^{**} - 1.4\,BB^{**} - 0.2\,AB$

YEP 77

$Y = 7.9^{**} - 0.01\,A - 0.8\,B^{**} - 1.0\,AA^{**} - 0.5\,BB^{**} - 0.3\,AB$

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

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 findings (Shelke *et al.*, 1990).

Table 3. Analysis of variance for evaluation of models for quality parameters of optimally prepared chapaties

Source	F	P	CV	R_2
YEP L				
Model	54.4	0.000	7.7	0.96
YEP 77				
Model	18.3	0.001	7.5	0.89

To analyze 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. A. The region of optimum response was central, with 1.5 per cent yeast extract and 0.4 per cent 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. B. The region of optimized response was defined by yeast extract 2.0 per cent and salt 0.3 per cent. 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 per cent containing 40 per cent NaCl. Previously, Dziezak (1987) reported addition of 2.0 to 4.0 per cent yeast extract in formulation of baking powder biscuits. The chapaties containing YEP L was found to rank higher than those with YEP 77. A possible explanation is the presence of the chicken note in the product which was ranked highest. 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 (Kawamura, 1990; Schoenberg, 1992).

Nutritional impact of yeast extract on chapaties

The chapati samples were freeze-dried and analyzed for ash and protein (Table 4). Moisture content of fresh chapati ranged from 36 to 38 per cent. Chapaties containing yeast extracts retained less moisture than control possibly through the lower initial water content of dough. The ash content of yeast-containing samples was found to increase due to higher salt content. The crude protein content derived from Kjeldahl determination of YEP L (1.5%), YEP 77 (2.0%) and control samples resulted in

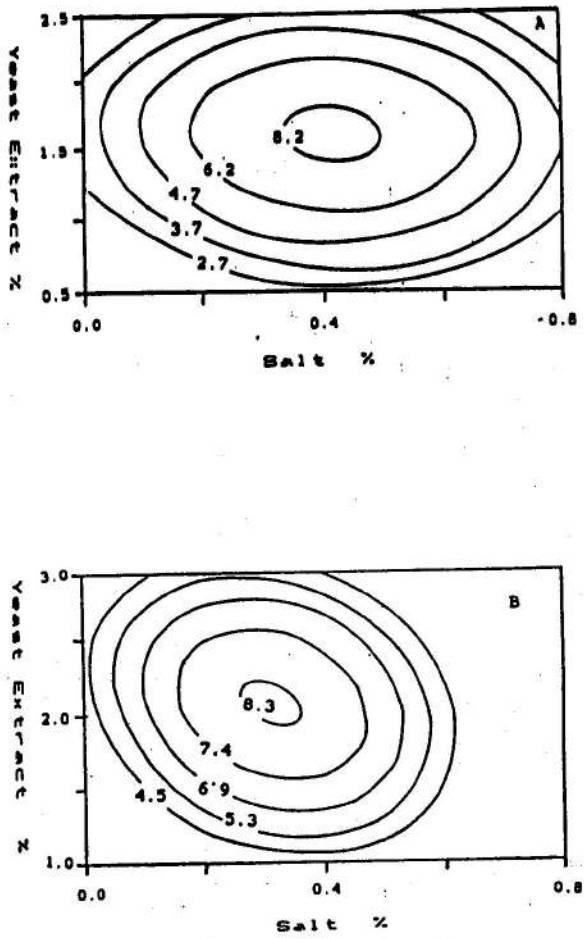

Fig. 1. Contour plots for taste in chapaties containing (A) YEP L (B) YEP 77.

0.88 - 1.33 per cent increase 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, 1987).

Table 4. 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
Standard deviation (pooled)	0.1	0.06	0.07

db = dry basis.

ACKNOWLEDGMENTS

We are very grateful to the Ministry of Education, Government of Pakistan for financial assistance to undertake this research.

REFERENCES

A.A.C.C., 1983. Approved Methods of American Association of Cereal Chemists. Vol I & II. Amer. Assoc. Cereal Chemists Inc., Pilot Knob, Minnesota.

Ali, S., A.S. Alvi and M. Hanif, 1964. Protein value of wheat flour supplemented with shark meat flour. Pak. J. Sci. Res., 16 (2): 41-44.

Anonymous, 1996. Economic Survey, 1995-96. Finance Div., Economic Advisor's Wing, Govt. of Pakistan, Islamabad.

Awan, J.A. and M.I. Siddique, 1996. Fortification of wheat flour. Pak. J. Food Sci., 6 (3-4): 39-49.

Bass, J. and J.F. Caul, 1972,). Laboratory evaluation of three protein sources for use in chapati flour. J. Food Sci., 37: 100-104.

Chaudhry, M.S., 1968. Preparation and evaluation of atta and chapaties from US wheats. Ph.D. Thesis, Dept. Grain Sci. Ind., Kansas State Univ., Manhattan.

Dziezak, J.D., 1987. Yeasts and yeast derivatives: Applications. J. Food Technol., 41: 122-125.

Floros, J.D., A. Ekanayake, G.P. Abide and P.E. Nelsen, 1992. Optimization of a diced tomato calcification process. J. Food Sci., 57 (5): 1144-1148.

Frye, A.M. and C.S. Setser, 1991. Optimization texture of reduced-calorie yellow layer cakes. Cereal Chem., 69 (3): 338-343.

Gacula, Jr. M.C., and J. Singh, 1984. Statistical Methods in Food and Consumer Research. Academic Press, Inc., New York.

Giovanni, M., 1983. Response surface methodology and product optimization. Food Technol., 37 (11): 41-45, 83.

Itzhak, R.F., and D.M. Gill, 1964. A microbiuret method for estimating proteins. Anal. Biochem., 9: 401-410.

Johnson, J.C., 1983. Other food additives: Yeast and related products. In: Food Additives - Recent Developments. Noyes Data Corp., Park Ridge, NJ.

Kawamura, Y., 1990. "Umami: One of the basic tastes". Food Technol. Int., Europe. pp: 151-155.

Khuri, A.I., 1992. Response surface models with random block effects. Technometrics, 34 (1): 26-37.

Lah, C.L., M. Cheryan and R.E. Devor, 1980. A response surface methodology approach to the optimization of whipping properties of an ultrafilter soy product. J. Food Sci., 45: 1721-1726.

Law, L.M., 1991. Comparison of preference for salty and umami flavours between two ethnic groups of different habits. A Practical Project Report, B.Sc (Hons.) in Food Science, Dept. Biosci. Biotechnol., Univ. Strathclyde, Glasgow.

Maase, I.W.J.L., 1991. Yeast extracts, discovering the fifth taste "Umami". Food Mark. Technol., 5 (3): 16,18.

Malcolmson, L.J., R.R. Matsuo and R. Balshaw, 1993. Effects of drying temperature and farina blending on spaghetti quality using response surface methodology. Cereal Chem., 70 (1): 1-7.

Malundo, T.M., A.V.A. Resurrenccion and P.E. Koehler, 1992. Sensory quality and performance of spray-dried coffee whitener from peanut. J. Food Sci., 57 (1): 223-226.

Minitab, 1991. Release 8.2. Minitab, Inc., 3081 Enterprise Drive, State College, PA.

Nguyen, T.T. and P. Sporns, 1984. Liquid chromatographic determination of flavour enhancers and chloride in food. J. Assoc. Off. Anal Chem., 67 (4): 747-751.

Nielsen, M.A., S.T. Coulter, C.V. Merr and J.R. Rosenabe, 1973. Four factors response surface experimental design for evaluating the role of processing variables upon protein denaturation in heated whey system. J. Dairy Sci., 45 (1): 76-80.

Saab, R.M.C.B., C.S. Rao and R.S.F. DeSilva, 1981. Fortification of bread with L-lysine HCL: Losses due to the baking process. J. Food Sci., 46: 662-664.

Schoenberg, E., 1992. The science of flavour enhancement. Int. Food Ingredients, 2: 36-38.

Shelke, K., J.W. Dick, Y.F. Holm and K.S. Loo, 1990. Chinese wet noodle formulation: A response surface methodology study. Cereal Chem., 67 (4): 338-342.

Siddique, M.I., 1989. Physico-chemical properties of composite flours for chapati production. Ph.D. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Ulgen, N., and M. Ozilgen, 1993. Determination of optimum pH and temperature for pasteurization of citrus juices by response surface methodology. Z. Lebensm. Unters. Forsch., 196: 45-48.

Yang, H.H., S.P. Yang and D.W. Thayer, 1977. Evaluation of the protein quality of single cell protein produced from mesquite. J. Food Sci., 42: 1247-1252.

Pak. J. Food Sci., Vol. 6, No. 3-4, 1996.

BREAD MAKING QUALITIES OF PAKISTANI WHEAT VARIETIES

Salim-ur-Rehman, Riaz Ahmad, M. Ismail Siddique & Muhammad Aslam Shaheen*
Department of Food Technology, University of Agriculture, Faisalabad.
**Nutrition Cell, P & D Section, Engineer's Building, Islamabad.*

ABSTRACT

Studies were undertaken to evaluate bread making qualities of two commercial wheat varieties, Faisalabad 85 and Punjab 85 at 60, 70, 80, 90 and 100% extraction rates. Moisture, crude protein, and fibre contents were higher in Faisalabad 85, whereas crude fat and ash contents were higher in Punjab 85. Punjab 85 was found better quality wheat possessing higher dough development time, dough stability and resistance of the dough. Water absorption capacity enhanced as the rate of extraction increased. Maximum peak viscosity values increased with a decrease in the rate of extraction. It was concluded that both varieties at 60 and 70% extraction rates were suitable for bread making. However, Punjab 85 possessed better dough behaviour and bread making qualities.

Key words: bread; qualities; wheat; dough behaviour; varieties; peak viscosity; extraction rate

INTRODUCTION

The production of wheat has increased significantly during the past two decades as a result of improvements in breeding and agricultural practices. White flour is an important milled product used for the production of bread. The protein of flour is important both in quality and quantity. For bread preparation, water insoluble proteins are required which are more important than water soluble (Matz, 1972; Musarat and Kausar, 1978). The composition of wheat flour is influenced by the variety, class, soil, weather conditions and cultural practices. It is also influenced by extraction rates of flour as the protein content decreases from periphery to the centre of the grain (Kent, 1975). Farinographic values are improved due to increase in protein with increasing extraction rates (Zunic *et al.*, 1978; Rehman *et al.*, 1988).

In addition to small bakeries, semi-automatic plants producing thousands of bread loaves daily, often face constraints in the procurement of wheat flour from roller mills. Moreover, in the absence of technical expertise and quality control facilities, these plants are confronted with the problems arising out of the usage of flour of widely varying characteristics for the preparation of quality bread (Chaudhry, 1991). Informations are scanty as to the baking response of newly evolved wheat varieties with respect to the flour of different extraction rates. The physical and chemical characteristics including baking quality of newly evolved wheat are, therefore, needed to be assessed.

MATERIALS AND METHODS

Samples of wheat varieties namely Faisalabad 85 and Punjab 85 were collected from the Wheat Research Institute, Faisalabad. Proximate composition of grains and different extraction rate flours were carried out according the methods described in AACC (1983). The cleaned wheat was conditioned in batches of 3 Kg by placing in air-tight bottles, adding calculated amount of water and allowing to rest for 48 hours. They were milled in Quadrumat Senior Experimental Mill to collect high grade flour, low grade flour, shorts and bran. Sixty, 70, 80, 90 and 100 % extraction flours were prepared by blending bran and shorts into fine flours after passing through fine grinder.

Rheological studies such as farinographic and amylographic were carried according to the methods given in AACC (1983). Index of gluten strength and baking tests were also performed (AACC, 1983). Dough was prepared by straight dough method. Sensory analysis was performed by a penal of 7 judges for external and internal characteristics of bread (Rehman *et al.*, 1988). Data were analyzed statistically by using the analysis of variance technique and Duncan's Multiple Range Test as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Chemical composition

It is obvious from the data (Table 1) that moisture, crude protein and crude fiber contents were higher in Faisalabad 85, whereas crude fat and ash contents were higher in Punjab 85. The proximate composition of these varieties are in close agreement with the results reported by Rehman *et al.* (1988). Proximate analysis of extraction rates of flour of the two varieties revealed that all the components increased progressively with an increase in extraction rate in both cases. This increase is due to the increase in constituents from centre to the periphery of the grain (Mahmood, 1985).

Table 1. Proximate composition of wheat varieties Faisalabad 85 and Punjab 85 at different extraction rates (on dry basis)

Components			Extraction Rates (%)				
			60	70	80	90	100
Faisalabad 85							
Moisture	(%)	8.30	12.80	13.00	13.20	13.40	13.62
Crude protein	(%)	13.59	12.32	12.65	12.96	13.25	13.50
Crude fat	(%)	2.10	1.10	1.10	1.38	1.67	2.00
Crude fibre	(%)	3.60	0.90	1.00	1.60	2.65	3.56
Total ash	(%)	1.68	0.60	6.70	0.88	1.25	1.65
Nitrogen free extract	(%)	79.10	85.10	84.54	83.18	81.18	79.30
Punjab 85							
Moisture	(%)	8.00	12.90	13.10	13.30	13.53	13.70
Crude protein	(%)	12.60	11.70	11.84	12.00	12.30	12.56
Crude fat	(%)	2.30	1.05	1.14	1.48	1.77	2.20
Crude fibre	(%)	3.45	0.82	0.90	1.90	2.71	3.42
Total ash	(%)	1.90	0.62	0.74	0.98	1.36	1.83
Nitrogen free extract	(%)	79.78	85.82	85.38	83.54	81.86	80.13

Milling performance

The data on milling fractions is given in Table 2. It was observed that Punjab 85 yielded higher quantity of high grade flour than Faisalabad 85, whereas for low grade flour the case was vice versa. The total flour yield was higher in Punjab 85 than Faisalabad 85. Punjab 85 was found to be lower in bran and short yield than Faisalabad 85. This might be due to the varietal, soil and climatic effects. The results are similar to those reported by Mahmood (1985).

Table 2. Milling performance of Faisalabad 85 and Punjab 85

Composition		Faisalabad 85	Punjab 85
High grade flour	(%)	27.15	30.30
Low grade flour	(%)	40.50	39.46
Total flour	(%)	67.72	69.76
Shorts	(%)	4.30	4.10
Bran	(%)	27.45	25.26
Milling loss	(%)	0.52	0.88

Rheological characteristics

It is evident from the data in Table 3 that Punjab 85 showed slightly higher values for dough development time, dough stability and resistance of the dough than Faisalabad 85 except for water absorption and softening of dough. The water absorption capacity increased with an increase in the rate of extraction. It was concluded that Faisalabad 85 is stronger than the Punjab 85. Kent (1975) reported that flour from stronger wheat had the ability to absorb and retain higher quantity of water. The increase in water absorption might be due to higher level of protein of the high extraction rates. Merritt and Stamberg (1941) showed an increase in water absorption of approximately 1.5 percent for each 1 percent increase in the protein content of flours. It is due to the quality of gluten in wheat which is affected by variety, soil location and other environment factors. These results agree with the findings of Mahmood (1985).

Values of peak viscosity in Punjab 85 ranged from 980 to 1900 BU (Table 3). As the rate of extraction decreased, the viscosity increased in both the varieties. This phenomenon is due to increasing concentration of starch and decreasing concentration of bran with fall in the extraction rate. Balint and Momirovic (1976) found that viscosity maxima was a function of starch concentration in slurry.

Pelshenke value

The quality of the Faisalabad 85 was better than the Punjab 85 in respect of Pelshenke value and may be considered strong under the classification of soft wheat as compared to the Punjab 85 (Table 3). The fermentation time is affected by quantity and quality of gluten which is affected by the wheat variety, soil and climatic conditions (Kausar *et al.*, 1976).

Table 3. Effect of extraction on the rheological characteristics of flour (14% moisture basis)

Characteristics		Extraction Rates (%)				
		60	70	80	90	100
Faisalabad 85						
Water absorption	(%)	55.0	58.4	63.4	64.5	67.0
Dough development	(Min)	3.5	4.0	4.0	4.5	4.5
Dough stability	(Min)	5.0	5.0	4.5	3.5	3.0
Resistance of dough	(Min)	7.0	7.0	6.5	6.5	6.0
Softening of dough	(BU)	100	100	90	70	60
Peak Viscosity	(BU)	1340	1240	1160	980	880
Test No.		170	155	132	122	115
Punjab 85						
Water absorption	(%)	55.0	56.0	59.0	62.5	65.0
Dough development	(Min)	4.0	4.5	4.5	4.5	5.0
Dough stability	(Min)	9.5	7.5	5.5	4.0	3.0
Resistance of dough	(Min)	11.0	9.0	7.0	6.5	6.5
Softening of dough	(BU)	40	40	40	60	60
Peak Viscosity	(BU)	1900	1780	1380	1040	980
Test No.		100	95	84	80	75

BU = Brabender unit; Min. = Minutes.

Table 4. Effect of extraction rates on the baking characteristics of bread

Extraction rate (%)	Volume (mL)	Weight (g)	Weight to volume ratio
Faisalabad 85			
60	443	141	1:3.14
70	464	141	1:3.30
80	432	152	1:2.84
90	388	151	1:2.60
100	355	145	1:2.50
Punjab 85			
60	456	139	1:3.30
70	438	139	1:3.20
80	433	150	1:2.88
90	388	150	1:2.60
100	381	151	1:2.50

Baking characteristics

It was observed that the loaf volume was higher for bread prepared from the Punjab 85 at all extraction rates except 70 percent which was lower than the Faisalabad 85 (Table 4). The results were similar to the findings of Kausar *et al.* (1976). Weight to volume ratio ranged from 1:2.5 to 1:3.3 in case of the Faisalabad 85. Weight to volume ratios were observed to be higher in case of Punjab 85 than the Faisalabad 85 except 70 and 80% extractions. Bread quality in terms of loaf volume or loaf specific volume is positively correlated with flour protein content, but is also related to protein quality (Kent, 1978).

Sensory evaluation

It is evident from the analysis of variances (Table 5) that there are highly significant differences among the extraction rates and in some cases between the varieties. The extraction rates of the two wheat varieties affected the volume of bread significantly ($P<0.05$). In case of the wheat varieties, a significant difference was noted for volume of bread in Faisalabad 85 and the Punjab 85. The volume of bread is affected by wheat variety and extraction rates (Mahmood, 1985). The effect of extraction rate on colour of crust, character of crust, crumb of bread, aroma and symmetry of form was highly significant. The extraction rates in case of Faisalabad 85 did not affect these parameters at 60 and 70% as well as at 90 and 100% levels. Significantly lower mean scores were obtained at 90 and 100% extraction rates as compared to higher mean score at 60 and 70% extraction rates. Somewhat similar results were obtained in case of wheat variety Punjab 85. The mean scores for these parameters at different extractions were slightly lower in case of Punjab 85 than Faisalabad 85. The effect of ex-

Table 5. Analysis of variances showing the effect of extraction rate on sensory characteristics of bread

Characteristics	Total score	Extraction rate (%) 60	70	80	90	100
Faisalabad 85						
Volume	10	7.40 a	6.90 b	6.80 b	5.50 c	5.40 c
Crust colour	8	6.50 a	6.20 a	5.70 b	4.30 c	4.30 c
Symmetry	5	3.90 a	4.30 a	3.20 b	2.70 c	2.50 c
Evenness of bake	3	2.40 a	2.50 a	2.40 a	1.60 b	1.60 b
Crust of bread	4	3.30 ab	3.40 a	2.70 b	2.00 c	2.00 c
Grain of bread	15	11.70 a	11.10 a	11.00 a	9.20 b	8.60 b
Colour of crumb	10	8.10 a	8.00 a	7.10 b	6.00 c	5.20 c
Aroma	10	7.70 a	7.70 a	6.40 b	6.00 b	5.20 c
Taste	20	15.10 a	14.60 a	13.90 a	11.50 b	11.20 b
Texture	15	12.10 a	12.00 a	11.10 a	9.50 b	9.40 b
Punjab 85						
Volume	10	7.80 a	8.10 a	6.10 b	5.10 b	5.60 c
Crust colour	8	6.50 a	6.30 a	5.70 ab	5.10 c	4.70 c
Symmetry	5	3.70 a	3.60 a	3.10 b	2.80 c	2.70 c
Evenness of bake	3	2.60 a	2.50 a	2.50 a	2.10 b	1.80 c
Crust of bread	4	3.10 a	2.70 a	2.50 b	2.10 b	2.00 b
Grain of bread	15	12.20 a	12.10 a	10.30 bc	9.20 b	9.40 c
Colour of crumb	10	7.70 a	7.90 a	6.40 b	5.70 bc	5.20 c
Aroma	10	7.50 a	7.20 a	6.50 b	6.00 bc	5.40 c
Taste	20	15.50 a	15.70 a	13.40 a	12.70 bc	11.50 c
Texture	15	12.50 a	12.40 a	11.00 b	10.10 bc	9.60 c

traction rate on evenness of bake, grain, taste and texture in Faisalabad 85 was not significant at 90 and 100% extraction rates with mean score of 1.6 as well as at 60 and 70 and 80% extraction rates with mean scores from 2.4 - 2.5. These characters differed significantly in case of Punjab 85 but with a slightly higher scores, showing a significant difference between the varieties. The evenness of bake of bread is affected by the baking time and temperature (Faridi and Rubenthaler, 1984).

REFERENCES

AACC, 1983. Approved Methods of American Association of Cereal Chemists. Vol. 1 & II, Amer. Assoc. Cereal Chemists Inc., St. Paul, Minnesota.

Balint, L. and C.J. Momirovic, 1978. Effect of wheat protein components on the amylograph viscosity of flour. Bull. Sci. Sec. A (Yugoslavia) No. 10-12: 218.

Chaudhry, M.S., 1991. Baking industry in Pakistan. Food Sci. News, 1 (2): 1-4.

Faridi, H.A. and G.L. Rubenthaler, 1984. Effect of baking time and temperature on bread quality, starch gelatinization and staling of Egyptian balady bread. Cereal Chem., 61 (2): 151-154.

Kausar, P., H.M. Chaudhry and B. Musarat, 1976. Correlation between wheat protein fractions and loaf volume. Pak. J. Agri. Sci., 13 (2): 47-50.

Kent, N.L., 1975. Technology of Cereal with Special Reference to Wheat. 2nd Ed., Pergamon Press, New York.

Kent, N.L., 1978. Technology of Cereal with Special Reference to Wheat. 2nd Ed., Pergamon Press, New York.

Mahmood, A., 1985. Response of new wheat varieties to some dough improving agents. M.Sc. Thesis, Dept. Food Technol., Univ. Agri., Faisalabad.

Matz. S.A., 1972. Bakery Technology and Engineering. AVI Pub. Co., Inc., Westport.

Merritt, P.P. and O.C. Stamberg, 1941. Some studies on flour absorption. Cereal Chem., 18: 632.

Musarat, B. and P. Kausar, 1978. A comparative study of important wheat varieties in Pakistan for their protein characteristics and baking behaviour. Int. Cong. Food Sci. Technol., Abst. p. 127. (FSTA, 11 (2): M1905/8; 1979).

Rehman, S., A. Mahmood, M.I. Siddique and S.A.H. Gilani, 1988. Rheological and baking properties of wheat in relation to dough improving agents. Sarhad J. Agri., 4 (5): 619-631.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. McGraw Hill Book Co. Inc., New York.

Zunic, G., D. Stanimirovic and S. Stanimirovic, 1978. Content and composition of protein in the flours of some high yielding types of wheat and quality of their dough. Harana i Ishrana, 19 (2): 3-12. (FSTA, 11 (4): M421; 1979).

Pak. J. Food Sci., Vol. 6, No. 3-4, 1996.

QUALITY IMPROVEMENT OF LEGUMES THROUGH THE APPLICATION OF VARIOUS PHYSICO-CHEMICAL TREATMENTS

Zia ur Rehman & W.H. Shah
Biotechnology & Food Research Centre,
PCSIR Laboratories Complex, Lahore.

ABSTRACT

Protein digestibility of the legumes significantly improved on soaking at 40°C in tap water, 1% NaCl solution or 1% $NaHCO_3$ solution for 2 hours. Total polyphenols including tannins reduced as a result of these treatments. Sodium bicarbonate soaking process was the most effective for improving protein digestibility and reducing the polyphenols in the legumes. Cooking time of the legumes also markedly reduced upon the application of these treatments.

Key words: legumes; polyphenols; protein digestibility; soaking treatments; Pakistan

INTRODUCTION

Legumes serve as the most important source of protein in the diet of rural and urban families in Pakistan. Legumes contain about 25 per cent protein but the digestibility of protein is very low due to the presence of certain polyphenols (Oyenuga, 1978). During the past, many attempts have been made to minimize these polyphenols from the legumes using different physical and chemical methods (Chavan, 1979; Bagepalli *et al.*, 1982; Deshaande *et al.*, 1982). Legumes are usually soaked in simple water to reduce the cooking time and for the partial removal of the polyphenolic compounds (Onayemi, 1986). Tannin is one of the major polyphenolic compounds in legumes which has the ability to combine with protein and reduces the digestion of protein to some extent (Bressani & Elias, 1980). The present work was undertaken to study the effect of different physico-chemical treatments on the reduction of polyphenols including tannin and improvement in protein digestibility.

MATERIALS AND METHODS

Samples of lentils (*Lens esculenta*) and red kidney beans (*Phaseolus vulgaris* L.) were obtained from Ayub Agricultural Research Institute, Faisalabad. The samples were cleaned to remove broken seeds, dust and foreign matters manually.

Physico-chemical treatments

Lentils and red kidney bean samples were soaked at 40°C in simple water, 1% sodium chloride solution and 1% sodium bicarbonate solution separately, keeping bean to water ratio of 1:5 for 2 hours. After soaking, excess of water was drained off, rinsed three times with distilled water and cooked.

The soaked seeds were put in a round bottom flask fitted with a condenser. Tap water (three times the weight of dry seeds) was added and samples were cooked on a hot plate until they became soft as felt between the fingers.

Chemical analysis

Protein contents of the legume samples were estimated after digestion with concentrated sulfuric acid according to micro-Kjeldhal method (AOAC, 1984). Total polyphenols were determined by the Vanillin-H_2SO_4 method as described by Wilson and Blunden (1983). Tannin contents of the samples were estimated using Folin Denis reagent on spectrophotometer at 760 nm (AOAC, 1984). Protein digestibility was estimated *in vitro* after digestion with pepsin-HCl solution at 37°C for 24 hours (Price *et al.*, 1979). All determinations were carried out in triplicate and standard deviations were calculated (Steel & Torrie, 1980).

RESULTS AND DISCUSSION

Table 1 indicates that the amount of total polyphenols of raw red kidney beans and lentils was 1.77 and 1.59 per cent whereas tannin contents were 1.13 and 1.03 per cent, respectively. *In vitro* protein digestibility (IVPD) of raw kidney beans and lentils was 41.20 and 41.93 per cent, increased to 58.60 and 63.00 per cent after cooking, respectively. However, the amount of protein in both these legumes was almost the same.

Effect of soaking on the reduction of cooking time

Cooking time of red kidney beans and lentils markedly decreased on soaking (Table 2). Cooking time

of unsoaked kidney beans and lentils was 105 and 90 minutes, decreased to 90 and 70 minutes after soaking in water, respectively. It is apparent from these results (Table 2) that minimum time i.e. 30 minutes for kidney beans and 20 minutes for lentils were required to cook sodium bicarbonate soaked legumes. reduction in cooking time could be the result of absorption of sufficient water from the soaking media which ultimately decreased the hardness of the legume. Onayemi *et al.* (1986) reported a reduction of 23-25 per cent in cooking time of cowpea as a result of 9 hours pre-soaking treatment in tap water.

Effect of soaking on tannin contents of legumes

Results given in Table 3 indicate the removal of tannin contents from the legumes to various extent on soaking in different salt solutions. Water soaking process removed about 40 per cent tannins from red kidney beans and lentils whereas 66 per cent tannins were removed on soaking in 1% NaCl solution. On soaking in sodium bicarbonate solution, 72 per cent tannins from red kidney beans and 87.50 per cent tannins from lentils were removed. Sharma and Sehgal (1992) also reported the removal of tannin from various food materials as a result of water soaking process. Reduction in tannin

Table 1. Percentage of polyphenolic compounds and protein digestibility of legumes prior to treatments

Parameter	Kidney beans	Lentils
Protein (N x 6.25)	24.77 ± 1.21	24.00 ± 1.32
Total polyphenols (%)	1.77 ± 1.09	1.59 ± 0.60
Tannin (%)	1.13 ± 0.98	1.03 ± 0.76
In vitro protein digestibility (IVPD) before cooking (%)	41.20 ± 1.11	41.93 ± 0.95
In vitro protein digestibility after cooking (%)	58.60 ± 0.51	63.00 ± 0.51

Table 2. Effect of physico-chemical treatments on cooking time of legumes

Treatment	Kidney beans		Lentils	
	Cooking time (minutes)	Reduction in cooking time (%)	Cooking time (minutes)	Reduction in cooking time (%)
Control (untreated)	105	-	90	-
Tap water treatment	90	14.28	70	22.22
1% NaCl treatment	75	28.57	55	38.88
1% $NaHCO_3$ treatment	30	71.42	20	77.77

Effect of soaking on total polyphenols of legumes

It is apparent from the results (Table 3) that significant amount of polyphenols was removed from red kidney beans and lentils during various soaking processes. About 31 and 37 per cent total polyphenols were removed on soaking red kidney beans and lentils in tap water at 40°C for 2 hours, respectively. On soaking in 1% NaCl solution, about 60 per cent total polyphenols were removed from both the legumes. However, maximum amount of total polyphenols was removed from these legumes on soaking in sodium bicarbonate solution at 40°C for 2 hours. Removal of polyphenols from these legumes could be attributed to easy penetration of solvent molecules in the tissue cells which helped to leach out the anti-nutrients to varying extent.

contents of beans soaked in sodium bicarbonate solution has also been reported by many other workers (deLumen & Salamat, 1980; DeLeon *et al.*, 1992). It seems that some soluble salts of tannic acid were formed under alkaline conditions which might be responsible for reduction of tannin content. However, the exact mechanism of tannin leaching is still unknown.

Effect of soaking on protein digestibility of legumes

Significant improvement in protein digestibility of legumes was observed on soaking in different salt solutions (Table 3). *In vitro* protein digestibility of red kidney beans and lentils increased from 41.21 to 75.66 per cent and 41.93 to 73.06 per cent, respectively as a result of water soaking process. After soaking in NaCl solu-

Table 3. Effect of physico-chemical treatments on polyphenolic compounds and protein digestibility of legumes

Treatment	Kidney beans			Lentils		
	Total polyphenols (%)	Tannin (%)	IVPD* (%)	Total polyphenols (%)	Tannin (%)	IVPD* (%)
Control (untreated)	1.32 ± 0.11	0.75 ± 0.07	58.60 ± 0.51	1.25 ± 0.09	0.80 ± 0.05	63.00 ± 0.51
Tap water treatment	0.91 ± 0.08	0.44 ± 0.08	75.66 ± 0.91	0.79 ± 0.07	0.48 ± 0.09	73.06 ± 0.42
1% NaCl treatment	0.53 ± 0.09	0.25 ± 0.03	77.77 ± 0.77	0.49 ± 0.09	0.27 ± 0.03	74.41 ± 0.59
1% $NaHCO_3$ treatment	0.42 ± 0.07	0.21 ± 0.02	84.33 ± 0.73	0.37 ± 0.06	0.10 ± 0.01	79.21 ± 0.43

*IVPD = *In vitro* protein digestibility.

tion, IVPD of red kidney beans and lentils was found to be 77.71 and 74.41 per cent, respectively. However, maximum IVPD of red kidney beans and lentils was 84.33 and 79.21 per cent as a result of soaking in sodium bicarbonate solution. Improvement in protein digestibility could be attributed to partial removal of polyphenols including tannins from the legumes during soaking process. These results are in consistent with the findings of other workers (Sathe & Salunkhe, 1981; Singh, 1993) who reported an improvement in protein digestibility of winged beans and pigeon peas due to the partial removal of tannin during soaking process.

REFERENCES

AOAC, 1984. Official Methods of Analysis. 14th Ed., Association of Official Analytical Chemists, Washington, D.C.

Bagepalli, S., R. Naransinga and P. Tatineni, 1982. Tanin contents of food commonly consumed in India and its influence on ionizable iron. J. Sci. Food Agri., 33: 89-96.

Bressani, R. and L.G. Elias, 1980. The nutritional role of polyphenols in beans, polyphenols in cereals and legumes. (Hulse, E.D.J.H., ed.). 61 p, IDRC, Canada.

Chavan, J.K., S.S. Kaelam, C.P. Ghonsikar and D.K. Salunkhe, 1979. Removal of tannins and improvement of *in vitro* protein digestibility of sorghum seeds by soaking in alkali. J. Food Sci., 44: 1319-1321.

deLeon, L.F., L.G. Elias and R. Bressani, 1992. Effect of salt solutions on the cooking time, nutritional and sensory characteristics of common beans (*Phaseolus vulgaris*). Food Res. Inst., 25: 131-136.

deLumen, B.O. and L.A. Salamat, 1980. Trypsin inhibitor activity in winged beans and the possible role of tannin. J. Agri. Food Chem., 28: 533-536.

Deshaande, S.S., S.K. Sathe, D.K. Salunkhe and D.D. Cornforth, 1982. Effect of dehulling on phytic acid, polyphenol and enzyme inhibitors of dry beans. J. Food Sci., 47: 1846-1849.

Onayemi, O., O.A. Osibogun and O. Obemleis, 1986. Effect of different storage and cooking methods on some biochemical, nutritional and sensory characteristics of cowpea (*Vigna unguicultata* L. Walp). J. Food Sci., 51: 153-156.

Oyenuga, V.A., 1978. Nigeria's Food and Feeding Stuffs: Their Chemistry and Nutritive Value. Ibadan Univ. Press, Ibadan, Nigeria.

Price, M.L., L.G. Butler, J.C. Rogler and W.R. Featherston, 1979. Overcoming the nutritionally harmful effects of tannin in sorghum grain by treatment with inexpensive chemicals. J. Agri. Food Chem., 27: 441-443.

Sathe, S.K. and D.K. Salunkhe, 1981. Investigations on winged bean proteins and anti-nutritional factors. J. Food Sci., 46: 1389-1392.

Sharma, A. and S. Sehgal, 1992. Effect of domestic processing, cooking and germination on the trypsin inhibitor activity and tannin content of faba bean (*Vicia faba*). Plant Foods Human Nutr., 42: 127-133.

Singh, U., 1993. Protein quality of pigeon pea as influenced by seed polyphenols and cooking process. Plant Foods Human Nutr., 43: 171-179.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. McGraw Hill Book Co. Inc., New York.

Wilson, M.F. and C.A. Blunden, 1983. Changes in the level of polyphenols in three pear varieties during bud development. J. Sci. Food Agri., 34: 973-978.

Pak. J. Food Sci., Vol. 6, No. 3-4, 1996.

DETERMINATION AND CHARACTERIZATION OF FAT IN ANIMAL TISSUES

Nuzhat Huma and Amjad Ali
Department of Food Technology,
University of Agriculture, Faisalabad.

ABSTRACT

For the determination of fats two methods, dry (time consuming) and wet (rapid) were compared. The correlation of 0.9925 was found highly significant. The regression equation of the dry method (Y) on the wet method (X) was Y = a + bx. After extraction, the fat was characterized for chemical constants. Acid value of the beef, mutton, poultry and fish fats was 2.4, 2.6, 1.7 and 7.0 respectively. Saponification value of the fats from these animals was 188, 181, 184 and 186. Iodine values were 46.1, 47.7, 56.5 and 111.8, respectively. Similarly, the unsaponifiable matter found was 1.6, 2.6, 3.3 and 3.6, respectively.

Key words: fat; animal tissue; saponification value; acid value; iodine value

INTRODUCTION

Meat and fish are good natural sources of dietary proteins, fats, vitamins and minerals. Considerable amount of energy in an average diet is supplied by animal fats, which also provide protection, insulation, essential fatty acids, as well as the fat-soluble vitamins A, D, E and K. Chemical analysis of meat is required for several reasons such as quality control, stability evaluation, conformity with governmental regulations, assistance in interpreting results and assessing the nutritive value.

The free lipid content, consisting essentially of neutral fats (triglycerides) and free fatty acids, can be conveniently determined in foods by extracting the dried, ground materials with diethyl ether in a continuous or intermittent type extraction apparatus (Subbulakshmi and Chitra, 1996). In meat, lipids are strongly associated with proteins (Schain, 1949) and these complexes are usually insoluble in lipid solvents. Such complex chemical bondings can be broken down by hydrolyzing the sample with dilute HCl in a boiling solution, thereby liberating the fat and permitting its extraction by fat solvents.

There are a few standard methods of fat determination, among which wet rapid method (Schain, 1949) and the dry AOAC method (AOAC, 1984) are commonly employed. The former method requires 15 to 30 minutes, while in the latter the operator spends up to 6 hours. This present study was planned to compare these two methods and to find a correlation between the results. Moreover, some chemical constants of meat and fish fats were also determined.

MATERIALS AND METHODS

For the quantitative determination of fat in animal tissues, ten samples of round cut of freshly slaughtered beef and five samples each of mutton (goat meat), poultry and fish were purchased from different local butchers and markets on different days. The animals were of unknown pedigree, history and age. After removing bones, fillets and fatty tissues by means of a sharp stainless steel knife, the prepared samples were cut into small pieces and passed through an electric mincer. Each sample was thoroughly mixed and kept in clean, dry, airtight jar, properly labelled and stored in a refrigerator until used.

Moisture percentage in the animal tissues was determined by placing weighed quantities of each sample in hot air oven at 95^{o}C and drying to a constant weight (AOAC, 1984). Fat was estimated by wet method of Schain (1949) and the dry method according to AOAC (1984).

Chemical constants of the fats such as acid value, saponification value, iodine value and unsaponifiable matter were studied according to AOAC methods (AOAC, 1984). Acid value was determined by neutralizing the free fatty acids in one gram of fat. The saponification value was determined by saponifying one gram of fat with KOH, while Iodine value was estimated by calculating the number of milligrams of iodine absorbed by 100 g of fat. The unsaponifiable matter was obtained by determining the residue after saponifying 5 g of fat with KOH.

Statistical analysis of the data was applied to calculate the correlation of the two methods following Steel and Torrie (1984).

RESULTS AND DISCUSSION

Moisture is one of the most variable component of animal tissues and varies among species and animals. It is also dependent upon the age, feed and degree of fatness of the animal. The calculated mean values of moisture in beef, mutton, poultry and fish tissues given in Table 1 are 77.68, 74.23, 76.19 and 80.17 percent, respectively. The moisture percentage in beef and mutton were close to the results reported by Chichester et al. (1980). Forrest et al. (1975), and Lawrie (1979). Moisture percentage in poultry and fish are comparable with the results given by Essary (1979) and Jafri (1973).

Table 1. Moisture and fat content (percent) in different animal tissues

	Beef	Mutton	Poultry	Fish
Moisture	77.68	74.23	76.19	80.17
Fat (dry weight basis)	22.49	26.27	29.40	14.25
Fat (wet weight basis)	5.02	6.77	7.00	2.85

Table 2. Comparison of fat content in various animal tissues by wet (X) and dry (Y) methods

Sample No.	1	2	3	4	5	6	7	8	9	10
Wet method	4.91	3.17	5.46	4.27	2.06	7.04	5.29	6.62	9.36	8.73
Dry method	6.01	4.18	6.58	5.33	3.00	8.26	6.45	7.82	10.65	9.70

Correlation = 0.9925 ** ** = Highly correlated

Y = 0.949 + 1.0299x

Fat content, like moisture, is also a variable component in the animal tissues. The amount of fat depends upon the meat cut and the quantity of fat left after cutting and trimming. Two methods, wet and dry, were adopted for fat determination in selected animal tissues. The calculated average fat percentage in beef, mutton, poultry and fish are given in Table 1. The fat percentage in beef tissues is close to that reported by Forrest et al. (1975), Romans and Ziegler (1979) and Ono et al. (1986). The fat content in mutton and poultry tissues are close to the values obtained by Forrest et al. (1975). But the fat percentage in poultry reported by Ziauddin (1996) is quite different. The average fat percentage in fish is in agreement with the values reported by Jafri (1973) and Lawrie (1979). Minor variations depend upon the breed, nutrition, age, weight physical conditions, sex and environmental factors.

Table 2 shows the fat content in 10 beef tissue samples as determined by the wet and the dry methods. A cursory look at the data reveals that the dry method gives higher fat content as compared with the wet method. This data was compared statistically following the methods described by Steel and Torrie (1980). Correlation of r = 0.9925 was found highly significant. A regression equation Y = a + bx was also obtained from the data which permits the conversion of values yielded by fast method into the more accurate and precise dry method consuming about 6 hours (Graph I). These results were in agreement with those of Pettinati and Swift (1977).

Characterization of the fat

Oils and fats always contain a certain amount of free fatty acids. As rancidity is usually accompanied by the formation of free fatty acids, this determination is often used as an index of freshness. The acid value depends upon the degree of rancidity. The acid value in beef, mutton, poultry and fish fats as given in Table 3 is 2.4, 2.6, 1.7 and 7.0 mg KOH/g fat, respectively. Acid value of beef fat compared favourably with the values reported by Hood and Allen (1971). The acid values of mutton, poultry and fish were in agreement with the data reported by Pearson (1976). The acid value of fish fat was higher because it hydrolyzed easily on storage. The saponification value of beef, mutton, poultry and fish fat as given in Table 3 are 188, 181, 184 and 186 mg KOH/g fat, respectively. These values fall within the British standard range (Cocks and Rede, 1960; Pearson, 1976). Variations between the saponification values of different animal fats may be attributed to the variations in their fatty acid composition.

The iodine value of the analyzed samples of beef, mutton, poultry and fish were found to be 46.1, 47.7, 56.5 and 111.8 g iodine/100 g fat, respectively (Table 3). Variations in the iodine values within different sam-

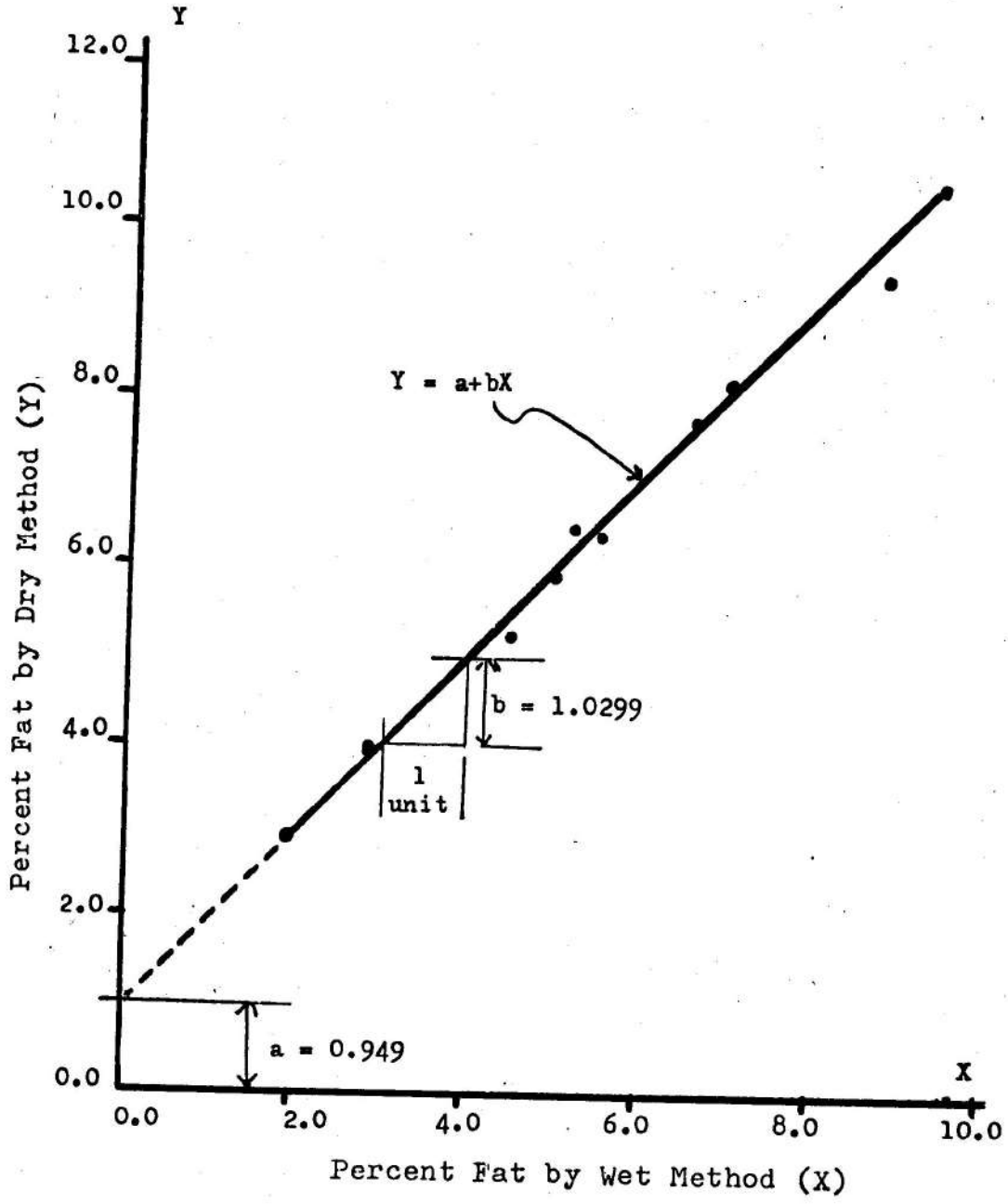

Fig. 1. Regression graph of dry method (Y) on wet method (X) for fat (%) in animal tissues.

ples of the animal are due to the different degree of unsaturation, which depends upon the diet of the animal. Westerling and Hedrick (1979) studied the effect of feed on lipid composition of animals. They reported that grass-fed animals contained more saturated fatty acids as compared to grain-fed animals, which contain higher proportion of unsaturated fatty acids. George et al (1971) reported that the degree of unsaturation of fatty acids of body fat was influenced by the degree of unsaturated fatty acids in the lipid fraction of the diet. Fish muscles contain substantial amounts of unsaturated fatty acids which are readily oxidized to form lipid hydroperoxides (Watanabe et al., 1996).

The iodine value of beef fat is close to the results reported by Lawrie (1979). American Meat Institute Foundation (1960) also reported 40 to 48 and 35 to 48 ranges of iodine values of beef and mutton fats respectively. The iodine value of fish is near to the value re-

Table 3. Characterization of fats from different animal tissues

Constituents	Beef	Mutton	Poultry	fish
Acid value (mg KOH/g fat)	2.4	2.6	1.7	7.0
Saponification value (mg KOH/g fat)	188	181	184	186
Iodine value (g I/100 g fat)	46.1	47.7	56.5	111.8
Unsaponifiable matter (%)	1.6	2.6	3.3	3.6

ported by Lawrie (1979). The unsaponifiable matter in beef, mutton, poultry and fish fats is given in Table 3. These values for the above mentioned animals are 1.6, 2.6, 3.3 and 3.6 percent, respectively. These results compare well with the British standards (Cock and Rede, 1960). The variations may be due to impurities or less or more concentration of cholesterol, hydrocarbons and other higher alcohols.

REFERENCES

American Meat Institute Foundation, 1960. The Science of Meat and Meat Products. W.H. Freeman & Co., San Francisco.

AOAC, 1984. Official Methods of Analysis. Assoc. Agri. Chemists, Arlington.

Chichester, C.O., E. M. Mark and G.F. Stewart, (eds.), 1980. Advances in Food Research, Vol. 26. Academic Press, New York.

Cocks, L.V. and C.V. Rede, 1960. Laboratory Handbook for Oil and Fat Analysis. Academic Press, London.

Essary, E.O., 1979. Moisture, fat, protein and mineral content of mechanically deboned poultry meat. J, Food Sci., 44 (4): 1070-1073.

Forrest, J.C., E.D. Aberle, H.B. Headrick, M.D. Judge and M.A. Markel, 1975. Principles of Meat Science. W.H. Freeman & Co., San Francisco.

George, A., G.A. Schuler and E.O. Essary, 1971. Fatty acid composition of lipids from broiler fed saturated and unsaturated fat. J. Food Sci., 36 (3): 431-34.

Hood, R.L. and E. Allen, 1971. Analysis of animal tissues. J. Food Sci., 36 (5): 786-790.

Jafri, A.K., 1973. Fat and water distribution pattern in the flesh of common cat fish (*Wallago atta*). Fishery Technol., 10 (2): 138-141.

Lawrie, R.A., 1979. Meat Science. Pergamon Press, Oxford.

Ono, K., B. W. Berry and L.W. Douglass, 1986. Nutrient composition of some fresh and cooked retail cuts of veal. J. Food. Sci., 51 (5): 1352-1357.

Pearson, D., 1976. The Chemical Analysis of Food. Churchill Livingstone, Edinborough.

Pettinati, J.D. and C.E. Swift, 1977. Collaborative study of accuracy and precision of rapid determination of fat in meat and meat products. JAOAC, 60 (4): 853-858.

Romans, J.R. and P.T. Ziegler, 1977. The Meat We Eat. The Interstate Printers & Publishers Inc., Danvill.

Schain, D., 1949. The use of detergent for the quantitative determination. 1. Determination of fat in milk. Sci., 110: 121-122.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc., New York.

Subbulakshmi, G. and L. Chitra, 1996. Methods for determining nutrients in foods: A critical appraisal. J. Food Sci. Technol., 33 (4): 267-84

Watanabe, F., M. Goto, K. Abe and Y. Yakano, 1996. Glutathione peroxidase activity during storage of fish muscle. J. Food Sci., 61 (4): 734-35, 782.

Westerling, D.B. and H.B. Hedrick, 1979. Fatty acid composition of bovine lipid as influenced by diet, sex and anatomical location and relationship to sensory characteristics. J. Anim. Sci., 38 (6): 1343-1348.

Ziauddin, K.S., K.C. Singh, H. Subba Rao and N. Fairoze, 1996. Comparative study of meat quality of `giriraja' and broiler birds. J. Food Sci. Technol., 33 (3): 229-30.

Pak. J. Food Sci., Vol. 6, No. 3-4, 1996.

THE POTENTIAL OF MICROALGAL POLY-SACCHARIDES IN FOOD INDUSTRY

Muhammad Iqbal, Zahida Nasreen & Saeed I. Zafar
Biotechnology & Food Research Centre,
PCSIR Laboratories Complex, Lahore.

ABSTRACT

Growth of *Porphyridium cruentum* for the purpose of polysaccharides and biomass production was investigated. Main purpose was to demonstrate the potential of *P. cruentum* polysaccharides in food industries. Laboratory studies on the rheology of the polysaccharides indicate their thermostability, resistance to pH variation and compatibility with mono- and divalent salts. The comparative studies, on the basis of their viscosity, with other commercially available biopolymers such as kappa-carrageenan, lambda-carrageenan and xanthan gum confirmed the superiority of *P. cruentum* polysaccharides as compared with these biopolymers.

Key words: *Porphyridium cruentum*; polysaccharides; biomass; rheology; biopolymers

INTRODUCTION

Hydrophilic colloidal polysaccharides, such as agar, algin and carrageenan have been traditionally extracted from marine macroalgae (seaweeds) since long (McLachlan, 1985). These polysaccharides are extensively used as thickeners, gelling agents, emulsion stabilizers, suspending agents, and additives in food, cosmetics and pharmaceutical products (Sandford & Baird, 1983).

Recovery of these polymers from macroalgae is, however, attended by numerous difficulties (Borowitzka & Borowitzka, 1988). Artificial culture of macroalga on large scale for polysaccharide production is not practicable due to their large size and the space required for the purpose. The macroalgae, accordingly, must be harvested from their natural sites in shallow water near sea coasts. Many of the coasts whereon the macroalgae naturally occur are rocky and subject to severe storms at certain times of the year. The labour involved in harvesting these macroalgae in the nature, furthermore, is difficult, arduous and expensive.

In view of the difficulties associated with commercial production of the polysaccharides from macroalgae, their extraction has been attempted from microalgae. Some species of a unicellular red alga (*Porphyridium*) have been proposed as a potential source of polysaccharides (Thepenier & Gudin, 1985; Vonshak *et al.*, 1985; Iqbal & Zafar, 1993). The present study reports a series of laboratory tests performed to evaluate the quality of *Porphyridium* polysaccharides and its comparison with other commercially available polysaccharides.

MATERIALS AND METHODS

The red alga used in this study, *Porphyridium cruentum* strain *Porphyridium cruentum* were obtained from the Culture Collections for Algae and Protozoa, Cambridge. Cultures were maintained axenically in 250 mL Erlenmeyer flasks containing artificial seas water (ASW) medium (Jones *et al.*, 1963) at photon flux density of 50 $\mu E\ m^{-2}\ s^{-1}$ and temperature of 25 ± 2°C.

Cultivation of *P. cruentum* for the production of polysaccharides was done in triplicate in 21 custom built V-shaped flat-sided photonioreactors (Iqbal *et al.*, 1993) and maintained for 21 days at a temperature of 25 ± 2°C. The bioreactors were inoculated with uniform inoculum (approximately 0.95×10^6 cells mL^{-1}) taken from two week old cultures in exponential phase of growth. The cultures were illuminated continuously at a PFD of 75 μmole photons $m^{-2}\ s^{-1}$ and aerated with air containing 2.5 per cent CO_2. Growth of *P. cruentum* was monitored by cell count in a WSI counting chamber (Weber Scientific Int. Ltd., Lancing, England) as well as by spectrophotometric measurement of absorbance at 760 nm. Viscosity of the cell-free medium and EPs solution was measured by Contraves Rheomat 115 (Contraves AG, Schaffauserstrasse 580, Zurich, Switzerland). The level of polysaccharides in the medium was measured by Alcian blue reagent (Ramus, 1977), using carrageenan as the standard. For the measurement of dissolved polysaccharides (DPs), cells were removed from the medium by centrifugation, whereas for cell wall bound polysaccharides (BPs) centrifuged cell pellet was suspended in known volume of distilled water and autoclaved for 10

minutes at 121°C followed by centrifugation. The supernatant was used to measure the content of BPs. The sugar component of polysaccharides was determined by the method of Albersheim *et al.* (1967). For isolation of polysaccharides from the liquid medium, an equal volume of cold industrial ethanol was mixed and the precipitated polysaccharides were separated by straining and squeezing through 2 layers of cheese cloth. The polysaccharides thus recovered were dissolved in water to remove any traces of salt and precipitated again with cold ethanol. The product was dried in an oven at 70°C for 24 hours.

RESULTS AND DISCUSSION

Extensive laboratory studies on the rate of growth and polysaccharides production as a function of photon flux density, CO_2 and nitrogen concentration, aeration rate and inoculum density were completed before initiating further work on polysaccharide quality. The results of these studies have been reported elsewhere (Iqbal *et al.*, 1992; Iqbal & Zafar, 1993 a, b). Similar studies performed by other workers have also been previously reported (Arad *et al.*, 1985; 1988; Rezanka *et al.*, 1987).

A typical growth curve with polysaccharides production is shown in Figure 1. During the course of growth, algal cells become encapsulated in an envelop of sulphated polysaccharides (Fig. 2). This envelop is known as bound polysaccharides, the external part of which gradually dissolves in the medium to form what is referred to as dissolved polysaccharides.

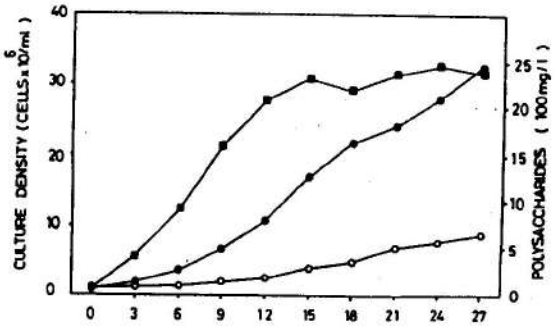

Fig. 1. Growth and polysaccharides production by *Porphyridium cruentum* cultures grown in 8 cm depth plastic pots under outdoor natural conditions: (■) cell number, (●) total polysaccharides and (□) extracellular polysaccharides.

The potential of *Porphyridium* polysaccharides in the food industry was investigated in terms of their rheological behaviour. For rheological studies the extracted extracellular polysaccharides were dissolved in distilled water and viscosity measurements were performed at 25°C.

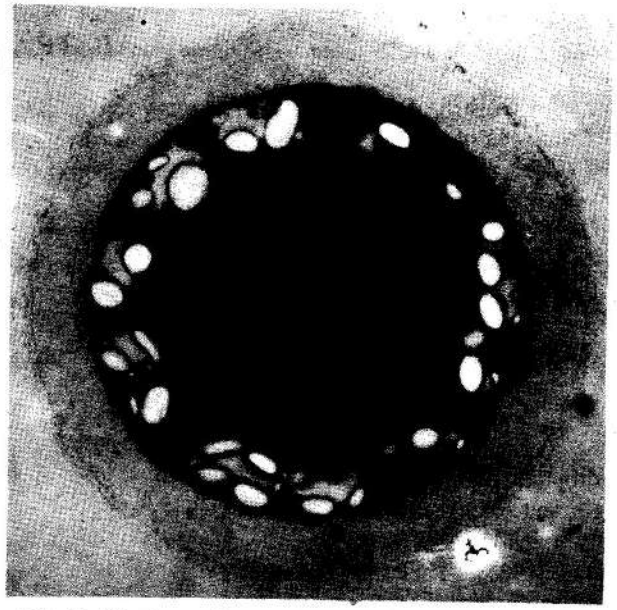

Fig. 2. Electron photomicrograph of *Porphyridium cruentum* cell showing extracellular polysaccharide capsule.

Viscosity measurements of the polysaccharides produced during different stages of growth revealed an interesting aspect. The polysaccharides produced at early stages of growth exhibited lower viscosity than that produced at the stationary phase (Fig. 3). As there were no major changes in the composition of sugars in samples of polysaccharides from different stages of growth (Table 1), it was possible that differences in their rheological behaviour were caused by altered sugar sequences in the biopolymer.

Table 1. Composition of extracellular polysaccharide of *P. cruentum* extracted at different stages of growth

Sugars*	Culture age (weeks)		
	1	2	3
Xylose	38.82	38.06	36.48
Glucose + Galactose	47.39	46.98	49.22
Rhamnose	15.76	15.82	14.33

*Amounts of sugars are presented as a percentage of total sugars.

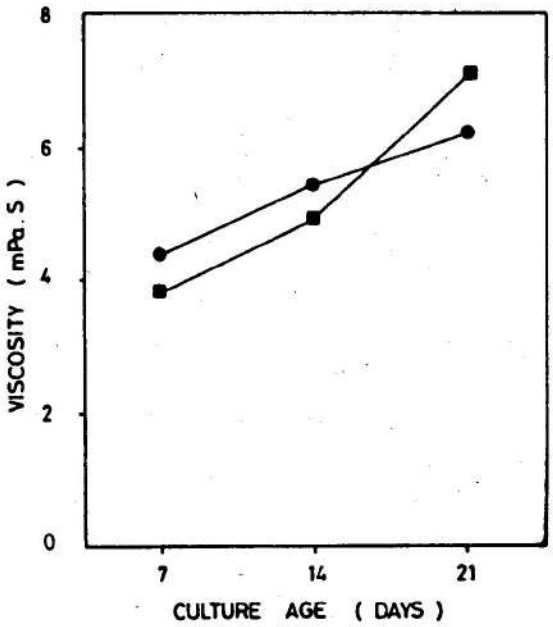

Fig. 3. Viscosity of 0.1% solution of *Porphyridium cruentum* extracellular polysaccharide extracted during various stages of growth.

Effect of temperature measured at a range of 10-80°C on viscosity showed a decrease in viscosity with the increase in temperature (Fig. 4 a). This change in viscosity was transient as the original viscosity was restored after cooling. This indicates that the polysaccharides of *P. cruentum* are quite thermostable and their chemical nature is relatively resistant to rise in temperature.

The viscosity of the polysaccharides was also studied at different ionic strengths ranging from highly acidic (pH 3) to highly alkaline (pH 12). The polysaccharides were observed to be stable to changes in the pH and no significant differences in the viscosity were observed (Fig. 4b). Sodium (monovalent) and calcium (divalent) slats at a concentration range of 1-5 per cent were used to assess their effect on the viscosity of polysaccharides at 25°C. After an initial slight increase in viscosity, no further changes were observed at the different salt concentrations used (Fig. 4c).

Studies were also carried out on the viscosity of the polysaccharides obtained from *P. cruentum* with that of the commercially available biopolymers, such as kappa-carrageenan, lambda-carrageenan and xanthan gum. At 25°C, the viscosity of the *P. cruentum* polysaccharides, throughout the selected range of concentrations, was considerably higher than all the commercial polymers investigated (Fig. 5). These results are in accordance with the findings of Arad (1988), who compared the viscosity of polysaccharides produced by *P. aerugineum*, *Porphyridium* sp. and another unicellular red alga, *Rodella reticulata* with the aforementioned commercial biopolymers and reported that the viscosity of the polysaccharides from *R. reticulata* and *P. aeruginosum* was higher than that of the other polymers studied. However, viscosity of the polysaccharide from the *Porphyridium* sp. was found to be higher than carrageenan but lower than xanthan gum.

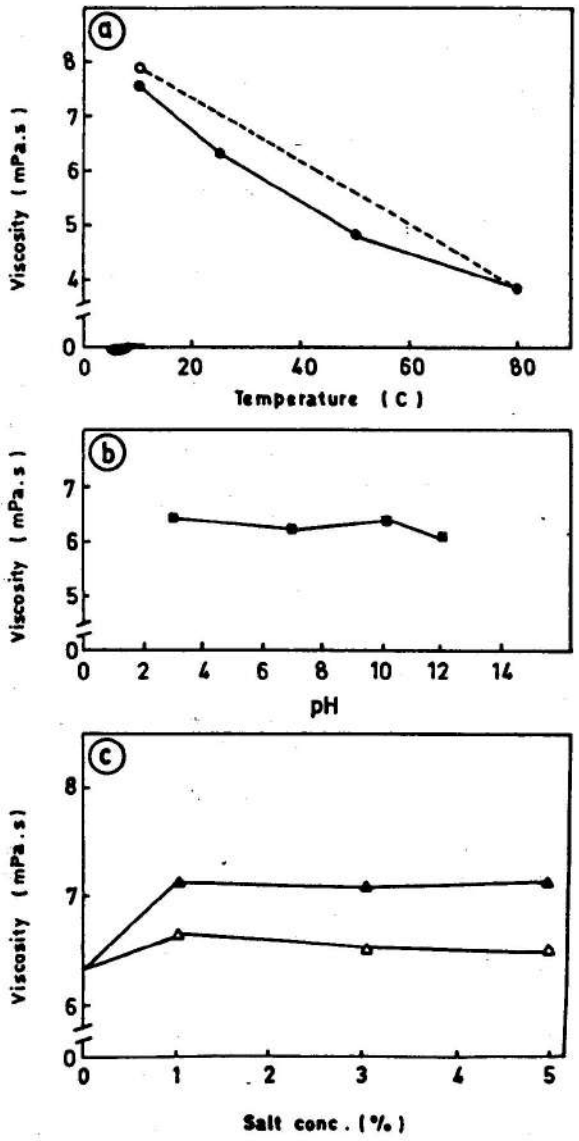

Fig. 4. Effect of temperature (a), pH (b) and salt (c) on the viscosity of a 0.1% solution of *Porphyridium cruentum* extracellular polysaccharide. Dotted lines in (a) indicate viscosity of a solution when cooled back to 10°C.

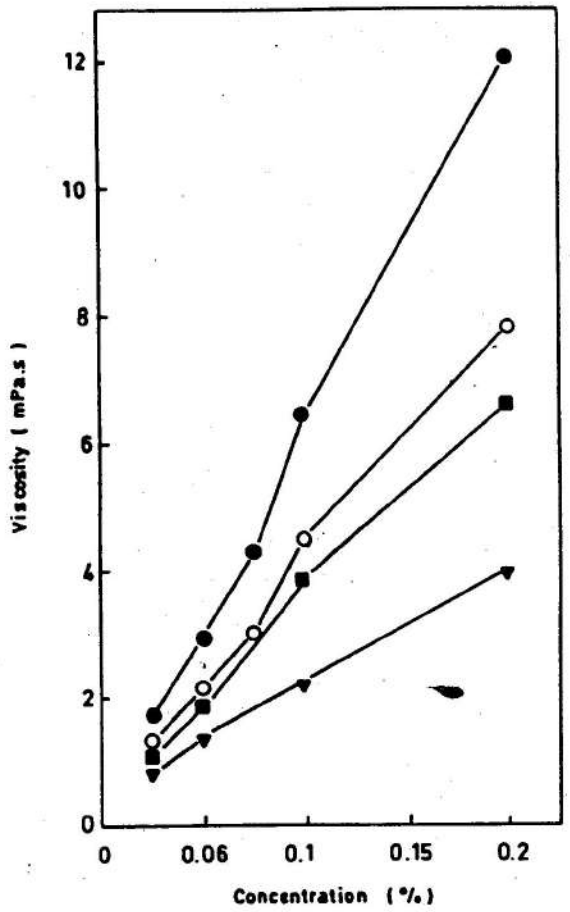

Fig. 5. Viscosities of the polysaccharides from *Porphyridium cruentum* cultures and commercially available biopolymers. *Porphyridium cruentum* polysaccharides (●), kappa-carrageenan (▲), lamda-carrageenan (o) and xanthan gum (□).

In conclusion, superiority of *Porphyridium* polysaccharides over the presently used commercially available gelling agents, on the basis of higher viscosity, their stability in wide range of temperature, pH and different salt concentrations, confirms their significant potential in the food industry.

REFERENCES

Albersheim, P., D.J. Nevins, P.D. English and A. Karr, 1967. A method for the analysis of sugars in plant cell wall polysaccharides by gas-liquid chromatography. Carbohydrate Res., 5: 340-345.

Arad, S., M. Adda and E. Cohen, 1985. The potential of production of sulphated polysaccharides from *Porphyridium*. Plant Soil, 89: 117-127.

Arad, S., O. Friedman and A. Rotem, 1988. Effect of nitrogen on polysaccharide production in a *Porphyridium* sp. App. Environ. Micrbiol., 54: 2411-2414.

Borowtizka, M.A. and L.J. Borowitzka (eds.), 1988. Microalgal Biotechnology. Cambridge Univ. Press, Cambridge.

Iqbal, M., D. Grey and G. Stepan-sarkissian, 1992. Effect of nitrogen on growth, extracellular polysaccharide and intracellular phycoerthrin reduction by the unicellular alga, *Porphyridium cruentum*. Acta Microbiol. Poly., 41: 65-73.

Iqbal, M., D. Grey, G. Stepan-sarikissian and M.W. Fowler, 1993. A flat-sided photobioreactor for culturing of microalga. Aquacultural Engg., 12: 183-190.

Iqbal, M. and S.I. Zafar, 1993. Strategies toward optimization of cultural conditions of *Porphyridium cruentum* for higher polysaccharide production. Acta Microbiol. Poly., 42: 71-82.

Jones, R.F., H.L. Speer and W. Kury, 1963. Studies on the growth of the red alga, *Porphyridium cruentum*. Physiol. Plant, 16: 636-643.

McLachlan, J., 1985. Microalgae (seaweeds): Industrial resources and their utilization. Plant Soil, 89: 137-157.

Ramus, J., 1977. Alcian Blue: A quantitative aqueous assay for algal acid and sulphated polysaccharides. J. Phycol., 13: 345-348.

Rezanka, T., J. Doucha, P. Mares and M. Podojil, 1987. Effect of cultivation temperature and light intensity on fatty acid production in the red alga, *Porphyridium cruentum*. J. Basic Micrbiol., 27: 275-278.

Sandford, P.A. and J. Baird, 1983. Industrial utilization of polysaccharide. In: The Polysaccharide. Vol. 2 (Aspinall, G.O., ed.). Academic Press, London. pp: 411-490.

Thepenier, C. and C. Gudin, 1985. Studies on optimal conditions for polysaccharide production by *Porphyridium cruentum*. J. App. Microbiol. Biotechnol., 3: 257-240.

Vonshak, A., Z. Cohen and A. Richmond, 1985. The feasibility of mass cultivation of *Porphyridium*. Biomass, 8: 13-25.

Pak. J. Food Sci., Vol. 6, No. 1-2, 1995.

COLD ENRICHMENT TECHNIQUE AS A TOOL FOR THE ISOLATION OF PSYCHROPHILIC BACTERIUM, *LISTERIA MONOCYTOGENES* FROM REFRIGERATED FOODS

Amna Bibi, Ali Abbas Qazilbash & Abdul Bari
Department of Biological Sciences,
Quaid-i-Azam University, Islamabad.

ABSTRACT

The incidence of *Listeria monocytogenes*, a foodborne psychrophilic pathogen, in refrigerated and frozen foods was investigated. The isolation of this microbe was carried out using a two-stage technique of "cold enrichment". Out of the 185 samples of frozen foods examined in this study, only 2 i.e. 1.08 per cent were found to carry *L. monocytogenes*, whereas in case of refrigerated foods, the incidence was higher i.e. 2.16 per cent (13 out of 600 samples). The highest level of incidence i.e. 3 per cent was observed in case of raw milk.

Key words: *Listeria monocytogenes*; food contamination; cold enrichment; incidence; isolation

INTRODUCTION

Listeria monocytogenes, a psychrophilic organism (Beuchat *et al.*, 1986; McLauchlin, 1987), can grow at refrigeration temperature (Brackett, 1988). The optimum temperature for growth is 37°C but growth occurs over a wide range of temperatures, down to 2.5°C (Willet, 1988). The organism can grow best in slightly alkaline medium with a pH range of 6.0-9.6 (Jenkins & Watkins, 1971; Khan *et al.*, 1973; Leighton, 1975). *L. monocytogenes* is a salt-tolerant bacterium capable of surviving for 8 weeks in 20% NaCl at 4°C (Conner *et al.*, 1986). It is not a fastidious organism (Willet, 1988) and can grow mostly on non-selective media like tryptose agar, but growth is enhanced when blood serum or fermentable carbohydrates are added (Jenkins & Watkins, 1971; Khan *et al.*, 1973).

L. monocytogenes, a human and animal pathogen, has a mortality rate of up to 33 per cent. Both sporadic and epizootic cases occur in cattle, sheep, goats and pigs. It has been isolated from 37 species of mammals, 17 avian species, fish, ticks and crustaceans (Pirie, 1927).

L. monocytogenes has emerged as a foodborne pathogen of major concern in the United States and in many developing countries. The organism possesses many traits which make it a particularly challenging safety problem for the food industry. These traits include: the ability to grow at temperatures as low as 4°C, its wide distribution both in nature and food processing environments and its resistance to intracellular digestion by phagocytes (Schlech *et al.*, 1983; Beaman & Beaman, 1984; Hayes *et al.*, 1986). *L. monocytogenes* has recently been a major concern in the dairy industry as well. Contaminated food and dairy products have been the source of disease leading to severe illness and death (Kerr *et al.*, 1990). Schlech *et al.* (1983) suggested that dairy products enhance the growth ability of *L. monocytogenes*. Post-pasteurization contamination of dairy products has also been documented. In a survey by the US Food and Drug Administration in 1986, 2.5 per cent of the 357 dairy processing plants were found to have products contaminated with *L. monocytogenes* (Prentice & Neaves, 1988).

Due to the ability of the organism to grow at refrigeration temperatures, cold storage, provided for most dairy products, cannot be relied upon to prevent the growth of bacterium if it was present as a consequence of post-pasteurization contamination (Seeliger & Jones, 1986; Hunghey *et al.*, 1989).

The problem of listeriosis in Pakistan perhaps does exist in a sizable scale, but due to the intricate isolation techniques and time consuming procedures, it is possible to skip the isolation of *L. monocytogenes*. Hence, it is imperative that the distribution/occurrence of this pathogen be investigated in developing countries like Pakistan.

MATERIALS AND METHODS

Collection of samples

Samples of various foods were randomly collected from Rawalpindi, Islamabad and adjoining areas. Commodity-wise distribution of food samples was:

a.	**Frozen**	
	Vegetables	54
	Meat	70
	Ice Cream	61
	Total	185
b.	**Refrigerated**	
	Vegetables	106
	Meat	77
	Curd	67
	Cheese	50
	Sweets	30
	Milk	270
	Total	600
	Grand Total	785

The liquid samples were collected in sterile vials/flasks, whereas the solid samples were collected in sterile polythene bags. These were transported to the laboratory in ice packs and were processed immediately. In case, immediate processing was not possible, the samples were stored in refrigerator till the time of processing.

A total of 785 samples were analyzed for the presence of *Listeria monocytogenes*. Each sample was enriched using selective enrichment broth and was subsequently cold enriched, cultured on selective agar medium. The isolates were confirmed on the basis of their cultural, morphological and biochemical characteristics.

Isolation from milk and milk products

Three types of milk samples were tested:

a. Thirty samples (50 mL each, pooled from 10-15 cows) were taken from milk cans (100 L capacity) from a dairy farm in Rawalpindi, where bovine listeriosis had been reported.
b. Two hundred raw milk samples of individual animals were collected from domestic refrigerators.
c. Forty samples of boiled and UHT-sterilized milk were also collected from hostel and market refrigerators. The sample size was 30-50 mL.

Each sample was mixed thoroughly by shaking the vial and 0.1 mL of each samples was directly plated on tryptose agar.

Two-step cold enrichment procedure

a. 25 mL of each sample was added to 100 mL of tryptose broth and was placed in the refrigerator at 4°C for primary cold enrichment.
b. After four weeks of cold enrichment, 1 mL of enriched broth was transferred into 9 mL of a secondary selective broth.
c. Secondary enrichment broth was incubated for 24 hours at 35°C.
d. The preparation was then streaked on acridine-nalidixic acid agar and incubated at 37°C for 24 hours and subsequently incubated for 48 hours at room temperature (15-20°C).

Isolation from vegetables and meats

10 g of each beef, mutton, poultry and vegetable sample was blended for 2 minutes in 90 mL of enrichment broth. The blended sample was dispensed in sterilized flasks, which were then placed in a refrigerator at 4°C for four weeks (cold enrichment).

The enriched samples were cultured on acridine-nalidixic acid agar medium, at weekly intervals up to 4 weeks, and incubated at 37°C for 24 hours and subsequently incubated for 48 hours at room temperature (15-20°C).

RESULTS

A total of 785 samples including 600 samples of refrigerated foods and 185 samples of frozen foods from 7 food groups were examined for the incidence of *Listeria monocytogenes*. The results of examination of 185 samples of frozen foods are given in Table 1 which showed that only 2 i.e. 1.08 per cent samples contained *L. monocytogenes*. The highest incidence (1.85%) was observed in the case of vegetables, followed by meat (1.42%). Ice cream samples were found to be negative for *L. monocytogenes*.

Table 2 shows the incidence of *L. monocytogenes* in refrigerated foods. Six hundred (600) food samples comprising of milk and milk product, vegetables, meat and sweets were examined for the presence of *L. monocytogenes*. Thirteen (2.16%) samples were found positive for *L. monocytogenes* with vegetables (2.8%) showing the highest incidence rate, followed by milk (2.5%), cheese (2.00%), curd (1.40%) and meat (1.30%).

Three kinds of milk samples were tested. Only raw milk was observed to be positive for *L. monocytogenes*. Boiled and pasteurized milk samples were totally negative for *L. monocytogenes*. The overall isolation incidence for milk was 2.50 per cent.

Table 1. Incidence of *Listeria monocytogenes* in frozen foods

Food	Number of samples	Number of isolates	Percentage
Ice cream	61	0	0.00
Vegetables	54	1	1.85
Meat	70	1	1.42
Total	185	2	1.08

Table 2. Incidence of *Listeria monocytogenes* in refrigerated foods

Food	Number of samples	Number of isolates	Percentage
Milk			
Raw milk	230	7	3.00
Boiled milk	25	0	0.00
Pasteurized milk	15	0	0.00
Curd	67	1	1.40
Cheese	50	1	2.00
Vegetables	106	3	2.80
Meat	77	1	1.20
Sweets	30	0	0.00
Total	600	13	2.16

DISCUSSION

Refrigerated and frozen foods may be important in the multiplication and pathogenicity of *Listeria monocytogenes*. Therefore, a number of such foods were screened to record the incidence of this pathogen.

It is believed that refrigerated foods constitute a greater hazard of foodborne illnesses as compared to non-refrigerated foods and *Listeria* sp. are the most common psychrophilic pathogens isolated from such foodstuffs. The reason being that *Listeria* sp. produce more hemolysin at 4°C as compared to 37°C (Durst, 1975; Wood & Woodbine, 1979).

The two-step enrichment isolation procedure yielded the greater number of *L. monocytogenes*. In this method, milk samples were diluted after cold enrichment at 4°C for 4 weeks, secondary enrichment was made in a selective broth and plated on acridine-nalidixic acid agar medium. The technique thus dilutes the sample and provides cold treatment and inhibitors to check the growth competition (Ralovich *et al.*, 1971).

Moreover, the purpose of diluting milk was to impede spoilage and to provide a bacteriological medium supportive of the growth of *L. monocytogenes*. Another reason for diluting the milk was to reduce the lactose concentration and prevent a drop in the pH level, since it is reported that the growth of *L. monocytogenes* ceases at low pH levels (below 5), thus it is difficult to isolate *L. monocytogenes* from specimens with high lactic acid contents (Wramby, 1944). The present study, therefore, substantiates the above views regarding the two-step enrichment technique. Hayes *et al.* (1986) also reported the advantage of two-step enrichment procedure over direct plating and one-step cold enrichment procedure.

Two main groups i.e. frozen and refrigerated foods were included in the present study. Out of 785 samples, 185 were frozen, while 600 were refrigerated foods, 15 (1.91%) samples were found to contain *L. monocytogenes*. Two isolates were recovered from frozen foods and 13 from refrigerated samples. Isolation from frozen foods is possible because *L. monocytogenes* can survive freezing, but does not multiply even after thawing (Lehnert, 1960), however, under refrigeration conditions, *L. monocytogenes* can both survive and multiply. Refrigeration temperatures facilitate the multiplication of *L. monocytogenes* by lowering the population of the competing microflora (Beuchat *et al.*, 1986; McLauchlin, 1987; Brackett, 1988). The results regarding high percentage of isolation from refrigerated foods are in line with the results of previous workers (Schlech *et al.*, 1983).

Highest incidence of *L. monocytogenes* was recorded in refrigerated raw milk samples, 7 out of 230 (3.00%) samples were found positive for *L. monocytogenes*. Three different procedures i.e. direct plating, one step enrichment and two-step cold enrichment were tried for the isolation of *L. monocytogenes* from milk samples.

The ability of *L. monocytogenes* to grow at low temperatures, coupled with the appearance of the pathogen in raw milk, meat/poultry and vegetables makes this bacterium a serious threat to susceptible consumers and the entire food industry especially in a developing country such as Pakistan. The widespread distribution of *L. monocytogenes* in nature and its association with domestic livestock makes its occasional presence in raw meat unavoidable.

Currently, the developed nations are adopting the following procedures for avoiding contamination of food by *L. monocytogenes*; excellent sanitary procedures, proper pasteurizing procedures, use of active cultures to produce cultured milk products and the avoiding of post-pasteurization contamination.

The problem of listeriosis in Pakistan perhaps does exist at a large scale, but due to the intricate isolation

procedure used and time consuming procedures, it is not being reported to its right magnitude. In view of the public health implications of *L. monocytogenes*, the results of this study suggest that more work be continued on this important aspect of food hygiene and a protocol be set up to ensure the use of the two-step enrichment for more precise isolation of this foodborne pathogen.

REFERENCES

Beaman, L. and B.L. Beaman, 1984. The role of oxygen and its derivatives in microbial pathogenesis and host defence. Ann. Rev. Microbiol., 38: 27-48.

Beuchat, L.R., R.E. Brackett, D.Y.Y. Hao and D.E. Conner, 1986. Growth and thermal inactivation of *Listeria monocytogenes* in cabbage and cabbage juice. Canadian J. Microbiol., 32: 791-795.

Brackett, R.E., 1988. Presence and persistence of *Listeria monocytogenes* in food and water. Food Technol., 42: 162-164, 178.

Conner, D.E., R.E. Brackett and L.R. Beuchat, 1986. Effect of temperature, sodium chloride and pH on the growth of *Listeria monocytogenes* in cabbage juice. App. Environ. Microbiol., 52: 59-63.

Durst, J., 1975. The role of temperature factors in the epidemiology of listeriosis. Zentr. Bakteriol. Parasitenkd. Infektionskor. Hyg. Abt. 1 Orig. Reihe A 223: 72-74.

Hayes, P.S., J.C. Feeley, L.M. Graves, G.W. Ajello and D.W. Fleming, 1986. Isolation of *Listeria monocytogenes* from raw milk. App. Environ. Microbiol., 51: 438-440.

Hunghey, V.L., P.A. Wilger and E.A. Johnson, 1989. Antibacterial activity of hen egg white lysozyme against *Listeria monocytogenes* Scott A in foods. App. Environ. Microbiol., 55: 631-638.

Jenkins, E.M. and B.B. Watkins, 1971. Extracellular antigens from *Listeria monocytogenes*. Infect. Immunol., 3: 589-594.

Kerr, K.G., N.A. Rotowa, P.M. Hawkey and R.W. Lacey, 1990. Evaluation of the Mast ID and API 50CH systems for identification of *Listeria* sp. App. Environ. Microbiol., 56: 657-660.

Khan, M.A., A. Seaman and M. Woodbine, 1973. Differential media in the isolation of *Listeria monocytogenes*. Zentr. Bakteriol. Mikrobiol. Hyg. Abt. 1 Orig. A. 224: 362-376.

Lehnert, C., 1960. The tenacity of *Listeria monocytogenes* in the external environment. Zentra. Bacteriol. Parasitenk. Abt. 1 Orig. 180: 350-456.

Leighton, I., 1975. Use of selective agents for the isolation of *Listeria monocytogenes*. Med. Lab. Sci., 36: 283-288.

McLauchlin, J., 1987. *Listeria monocytogenes*: Recent advances in the taxonomy and epidemiology of listeriosis in humans. J. App. Bacteriol., 63: 1-11.

Perraudin, J.P. and J.P. Prieels, 1982. Lactoferrin binding to lysozyme treated *Micrococcus luteus*. Biochem. Biophysics Acta, 718: 42-48.

Pirie, J.H., 1927. A new disease of wild rodents, `Tiger River Disease'. Pub. South African Inst. Med. Res., 3: 317-326.

Prentice, G.A. and P. Neaves, 1988. *Listeria monocytogenes* in food: Its significance and methods for its detection. Bull. Int. Dairy Federation, 223: 1-16.

Ralovich, B., A. Forray, E. Mero, H. Malovics and Szizados, 1971. New selective medium for isolation of *Listeria monocytogenes*. Zentr. Bakteriol. Parasitnek. Inferkionskr. Hyg. Abt. 1 Orig., 216: 88-91.

Rosenow, E.M. and E.H. Marth, 1987. Growth of *Listeria monocytogenes* in skim, whole and chocolate milks and in whipping cream during incubation at 4, 8, 13, 21 and 35°C. J. Food Protec., 50: 452-459.

Schlech, W.F., P.M. Lavigne, R.A. Borolussi, A.C. Allen, E.V. Haldane, A.J. Wort, A.W. Hightower, S.E. Johnson, S.H. King, E.S. Nichols and C.V. Broom, 1983. Epidemic listeriosis: Evidence for transmission by food. N. Eng. J. Med., 308: 203-206.

Seeliger, H.P.R. and D. Jones, 1986. Genus *Listeria*. In: Bergey's Manual of Determinative Bacteriology (Sneath, P.H.A., N.S. Mair & M.E. Sharpe, eds.). 8th Ed., pp: 593-596. Williams & Wilkins, Baltimore, MA, USA.

Willet, H.P., 1988. *Listeria* and *Erysipelothrix*. In: Zinsser Microbiology (Joklik, W.K., H.P. Willet, D.B. Amos & C.M. Wilfert, eds.). 19th Ed., pp: 409-413. Prentice-Hall Int. Inc., USA.

Wood, L.V. and M. Woodbine, 1979. Low temperature virulence of *Listeria monocytogenes* in the avian embryo. Zentr. Bacteriol. Parasitenk. Infektiockor. Hyg. Abt. 1 Orig. Reihe A243: 74-81.

Wramby, B.O., 1944. O m *Listeria monocytogenes* bacteriologi och fore Komst av Listeria infections hos djur. Skand. Vet. Tidskr., 34: 277-279.

Pak. J. Food Sci., Vol. 6, No. 3-4, 1996.

EXTRACTION, PURIFICATION AND STORAGE STABILITY OF BOVINE PEPSIN

Zia-ur-Rehman & W.H. Shah
Biotechnology & Food Research Centre,
PCSIR Laboratories Complex, Lahore 54600.

ABSTRACT

Optimum conditions for the extraction and purification of bovine pepsin from young animals were studied. The yield of pepsin was maximum when minced gastric tissue of buffalo, adjusted at pH 2.2, was incubated at 40°C for 18 hours. Pepsin from the filtrate was precipitated by the addition of sodium chloride at the rate of 200 g L^{-1}. A gradual decrease in pepsin activity was observed during storage of gastric tissue at -10°C for six months from different animals.

Key words: pepsin; bovine; extraction; buffalo; gastric tissue; Pakistan

INTRODUCTION

Pepsin is a proteolytic enzyme which is present in the gastric tissue of all vertebrates (Kageyama & Takahashi, 1972; El-Abbasay & Wehba, 1986; Kastka, 1992). The gastric tissue of young animals contains high amount of pepsin than those of older ones (Valles & Furet, 1981). Pepsin has many useful industrial applications. It is used as digestive enzyme in pharmaceutical preparations. It is also used for making cheese and protein hydrolysates (Emmons *et al.*, 1976; Husek & Debek, 1981; Chorbanor *et al.*, 1986). On commercial scale, pepsin is usually extracted from gastric tissues of pig, cattle and sheep (Valles & Furet, 1977; Daytyarev & Degtyrava, 1981; Guilotea *et al.*, 1982). As the animal tissues are generally spoiled at room temperature, therefore, these are preserved in cold storages before processing (Djordjevic *et al.*, 1985). At present, pepsin is being imported to meet the demand of various local industries particularly pharmaceutical industry. Keeping in view the extensive use of this enzyme, present study was undertaken to optimize the conditions for the extraction and purification of bovine pepsin from gastric tissues of young animals.

MATERIALS AND METHODS

Gastric tissues of young buffalo (approximately 2 years old) and other animals (cow, goat, sheep, camel and chicken) were collected from the local slaughterhouse soon after slaughtering. The stomach contents were removed and washed with chilled water immediately. The washed tissues were put in ice box and brought to the laboratory for processing. The offals and fatty layers were removed before mincing. Minced tissues were packed in plastic bags and then preserved in a freezer for later use.

Extraction of pepsin

Minced gastric tissues were mixed well with water of different pH containing 1.0% boric acid having different substrate to water ratio i.e. 1:1.00, 1:1.25, 1:1.50, 1:1.75 (w/v). The desired pH of the mixture was adjusted with dilute HCl on pH meter and then incubated at different temperatures for different time periods with frequent stirring. After incubation, the contents were filtered and pepsin activity was assayed in the filtrate.

Precipitation of pepsin

Sodium chloride and ammonium sulphate were used as precipitating agents. These were added in different amounts slowly with continuous stirring into the filtrate and allowed to stand for about 20 minutes to complete the precipitation. Crude pepsin precipitate was collected by filtration and then dried at room temperature.

Purification of pepsin

Dried crude pepsin was redissolved in water containing boric acid and allowed to stand at ambient temperature. After 60 minutes, the mixture was centrifuged and pepsin was reprecipitated from the supernatant with sodium chloride.

Assay of pepsin

Pepsin activity in the tissue extract was measured by the standard method as described in British Pharmacopeia (1968). The volume of the tissue extract required to digest completely 12.5 g of egg albumin in 4 hours was determined and the amount of pepsin (mL^{-1}) of the extract was calculated.

Protein concentrate

The offals were minced, mixed with water and autoclaved for 30 minutes. Fat was skimmed off. Excess of water was removed by filtration through cheese cloth and the tissues were dried at 60 ± 5°C . Protein concentrate was obtained by mixing the autoclaved dried offals with the residue left after extraction of pepsin from gastric tissues. Chemical analysis of protein concentrate was carried out according to AOAC (1984).

RESULTS AND DISCUSSION

Optimum conditions for the extraction of pepsin

It is evident from the results (Table 1) that the change in pH, temperature and time period of incubation significantly affected the extraction of pepsin from gastric tissue of buffalo. With the extraction period of 6 hours, only 2.6 mg mL^{-1} pepsin was obtained, when minced gastric tissue was thoroughly mixed with acidic solution (pH 1.2) at 30°C. The yield increased as the extraction time increased and was maximum (4.2 mg mL^{-1}) after 18 hours at 30°C in case of pH 1.2. There was no change in the amount of pepsin after 18 hours incubation. Variable amount of pepsin was extracted from the material at various temperatures ranging from 30-45°C. Maximum pepsin extraction was 6.3 mg mL^{-1} at 40°C on mixing the tissue with acidic solution (pH 1.2) for 18 hours. However, significant reduction in the rate of extraction was observed when temperature was raised from 40-50°C. The findings of Valles and Furet (1977) revealed that maximum pepsin was obtained when minced tissues were incubated in 0.2 M HCl solution at 42°C.

cubation pH (1.2, 1.7, 2.2 and 2.7). Highest amount of pepsin was found to be 15.3 mg mL^{-1} at pH 2.2 after incubation at 40°C for 18 hours (Table 1). At pH 2.7, extraction rate of pepsin was adversely affected in all cases.

The effect of different material extractant ratios on the extraction of pepsin was studied at 40°C for 18 hours. The extraction of the pepsin was maximum (14.93 mg mL^{-1}) when the material extractant ratio was 1:1.25. The extraction of the enzyme decreased as this ratio was further increased. When the material extractant ratio was increased to 1:1.75, the pepsin extraction decreased by about 50 per cent.

Extraction of pepsin using different solutions

Maximum pepsin (16 mg mL^{-1}) was extracted after incubation in HCl solution at 40°C for 18 hours. However, least amount of pepsin (12 mg mL^{-1}) was obtained with citric acid solution. The HCl buffer also extracted pepsin (12.8 mg mL^{-1}) which was comparatively less than the HCl solution. It indicates that presence of potassium ions in HCl buffer inhibited the extraction of pepsin to some extent. Therefore, HCl solution of pH 2.2 can be effectively used for the extraction of pepsin from gastric tissues of the animals.

Precipitation and purification of pepsin

Crude pepsin was precipitated from the filtrate by the addition of variable amounts of sodium chloride and ammonium sulphate. Sodium chloride was found to be significantly better precipitating agent as compared to ammonium sulphate. Maximum crude pepsin was obtained when sodium chloride at the rate of 200 g L^{-1} was

Table 1. Effect of pH, temperature and time period on the extraction of pepsin* (mg mL^{-1})

Extraction time (hours)	pH (1.2)				pH (1.7)				pH (2.2)				pH (2.7)			
	30**	35	40	45	30	35	40	45	30	35	40	45	30	35	40	45
6	2.6	4.4	5.1	3.7	4.0	4.9	6.0	5.5	4.6	5.4	7.6	6.0	3.9	5.0	5.7	5.5
12	3.9	4.9	5.9	4.2	5.3	5.7	7.6	7.0	5.8	6.3	8.9	7.6	5.0	5.9	7.0	6.7
18	4.2	5.6	6.3	4.9	7.2	9.0	9.3	8.4	7.7	9.6	15.3	12.8	6.9	9.1	9.0	8.8
24	4.1	5.5	6.4	5.0	7.0	8.8	9.0	8.1	7.2	9.2	12.0	11.9	6.0	8.8	9.2	8.5
30	4.2	5.5	6.3	5.1	7.1	8.7	9.0	8.1	7.0	9.0	11.8	11.8	5.8	8.7	9.2	8.3

* = Average of three determinations. ** = Extraction temperature (°C).

The pH of the solution also played a great role in the extraction of enzyme, pepsin from the gastric tissue. It is apparent from these results that the conversion of pepsinogen to pepsin varied appreciably at different in-

added slowly with constant stirring in the filtrate (Table 2). Crude pepsin was then purified by reprecipitation method. About 0.45 per cent purified pepsin was obtained which had an activity 3-4 times more than crude

pepsin. The purified pepsin was tested for its microbiological purity and it was found free from pathogenic microorganisms.

Table 2. Effect of different salts on the precipitation of pepsin from the filtrate

Precipitating agent	Amount added (g L^{-1})	Crude pepsin yield* (g kg^{-1})
Sodium chloride	50	8.20
	100	16.50
	200	18.70
Ammonium sulphate	50	4.40
	100	8.91
	200	10.29

*Average of three determinations.

months storage (Table 3). Similar trend was observed when cow gastric tissue samples were kept at -10°C for 6 months. Hence, it is obvious that under these conditions, buffalo and cow gastric tissues should not be stored for more than 2 months.

Sheep and goat fresh gastric tissue contained 17.39 and 18.25 mg pepsin mL^{-1} extract, respectively. It also decreased but at a slower rate as compared to buffalo and cow gastric tissues. So, it seems feasible to extract pepsin from sheep and goat tissues up to 4 months storage. Fresh gastric tissue of camel contained 6.06 mg mL^{-1}, on 6 months storage, this level decreased to 1.01 mg mL^{-1}. therefore, it is advisable that camel gastric tissue may be extracted for pepsin soon after slaughtering.

Out of various animal gastric tissues examined, chicken gastric tissue was found to contain maximum amount of pepsin (24.60 mg mL^{-1}) that decreased only to 15.67 mg mL^{-1} after 6 months storage. This means that chicken pepsin was more stable as compared to

Table 3. Effect of storage on pepsin content of different animal's gastric tissue

Animal	mg mL-1						
	0	1	2	3	4	5	6
Buffalo	14.73	12.57	8.71	5.06	3.09	2.71	2.29
Cow	13.92	11.01	7.49	4.22	3.11	2.53	1.89
Goat	18.25	16.53	15.22	14.01	12.32	8.05	5.55
Sheep	17.39	16.00	15.05	14.25	13.23	7.69	6.44
Camel	6.06	3.65	2.51	1.73	1.51	1.32	1.01
Chicken	24.68	21.70	19.69	18.21	16.78	15.92	15.67

* = Average of three determinations. ** = Storage time in months.

Utilization of protein concentrate

Protein concentrate was obtained as a byproduct during extraction of pepsin from gastric tissue of the cattle. The protein concentrate contained 68.4 per cent protein, 13.8 per cent fat, 3.05 fibre, 5.35 per cent ash and was free from all kinds of pathogenic microorganisms. Therefore, it is suggested that this protein concentrate can be used safely as a rich source of animal protein in poultry feed.

Effect of frozen storage on pepsin activity

The minced gastric tissue samples of different animals were stored at -10°C in a deep freezer for 6 months. The samples were taken out at an interval of one month and analyzed. Initially, the buffalo tissue contained 14.73 mg mL^{-1} pepsin which decreased to 8.71 mg mL^{-1} after 2 months. However, pepsin degradation took place rapidly as the storage progressed and the sample contained only 2.29 mg mL^{-1} at the end of 6

pepsin obtained from other sources studied during this work. Hence, chicken stomach can be a potential source of this precious proteolytic enzyme.

REFERENCES

AOAC, 1984. Official Methods of Analysis. Assoc. Off. Analyt. chemists, Washington, DC.

British Pharmacopeia, 1968. 11154 p.

Chorbanor, B., M. Bozhiova, Kh. Dilov and V. Licher, 1986. Protein hydrolysate from green algae. Nahrung, 30: 405-408.

Daytyarev, V.P. and T.V. Degtyarava, 1981. Species characteristics of proteolysis in the ruminant stomach and intestine. Doke TSKHA, 265: 42-48.

Djordjevic, M., B. Selivanoski, B. Mihajlovic, D. Dimitrijevic, D. Perovic and N. Gnjatovic, 1985. Pepsin of yak and camel: Isolation and molecular characterization. Technol. Mesa, 26: 374-376.

El-Abbasay, F. and A. Wahba, 1986. Studies of camel pepsin. Effect of some additives. J. Dairy Sci., 14: 181-186.

Emmons, D.B., B. Raiser, R.N. Girous and D.W. Stanle, 1976. Isolation and molecular characterization of avian pepsin. Canadian Inst. Food Sci. Technol., 9: 189-200.

Guilotea, P., R. Delensorne and A. Tovillec, 1982. Distribution of enzyme concentration in the abomasal mucosa of the pre-ruminant calf. Reprod. Nutr. Dev., 22: 511-522.

Husek, V. and M. Debek, 1981. Experiments with chicken pepsin and possibility of its use in cheese making. Netherlands Milk Dairy J., 35: 302-306.

Kageyama, T. and K. Takahashi, 1972. Pepsinogen C and pepsin C from gastric mucosa of Japanese monkey. J. Biochem., 80: 983-992.

Kastka, V., 1992. Chicken pepsin and other avian aspartic proteinase. Scandinavian J. Clin. Lab. Invert, Suppl., 52: 121-125.

Valles, E. and J.P. Furet, 1977. Study of bovine abomasum in the ruminant state to obtain bovine pepsin. Lait, 57: 601-618.

Valles, E. and J.P. Furet, 1981. A study of bovine abomasum for obtaining bovine pepsin extract. Effect of breed, age, sex on enzyme extract. Lait, 61: 590-618.

EXTENSION ARTICLE

APPLICATIONS AND ADVANTAGES OF EXTRUSION TECHNOLOGY IN FOOD INDUSTRY[1]

Mian Nadeem Riaz, Edmund W. Lusas & Ghulam Mohy-ud-Din*
Food Protein Research & Development Centre,
Texas A & M University, College Station, Texas, USA.
**Department of Food Technology,*
University of Agriculture, Faisalabad.

ABSTRACT

Basic extruder technology has been around for a long period of time. It has been used in one form or another in many industries. New designs of extrusion equipment have increased the range of their application in food processing. Today's consumers are demanding a broader selection of foods. Extrusion processing equipment has become the touchstone operating equipment in most snack food companies throughout the world. One big advantage of extrusion cooking is the capability to produce a wide range of finished products with minimum processing time using inexpensive raw material. The verb "extrude" characterizes a process of shaping by forcing softened or plasticized material through dies or holes by pressure. A food extruder is a device that expedite the shaping and restructuring process for food ingredients. Extrusion is a highly versatile unit operation that can be applied to a variety of food products. Extruders can be used to cook, form, mix, texturize and shape food products under conditions that would favour quality retention, high productivity and low cost.

Key words: food extrusion; cost-effectiveness; food processing; preservation; Pakistan

INTRODUCTION

Food extrusion is a process in which a food material is forced to flow, under one or more of a variety of conditions of mixing, heating and shear, through a die which is designed to form and/or puff-dry the ingredients (Rossen & Muller, 1973). Extrusion, therefore, combines several unit operations such as mixing, cooking, keading, shearing, shaping and forming.

Food extrusion has been exercised for nearly 55 years. Initial role of extruders was mixing and forming of macaroni and pasata products (Harper, 1989). Now, the food extruder is visualized as a high temperature short time (HTST) bioreactor that can transform a variety of raw ingredients into intermediate and finished products. During extrusion, cooking temperature could be as high as 200°C, but the residence time is usually 5-15 seconds. For this reason, extrusion cooking is called HTST process.

During extrusion of food, several processes occurred i.e. conveying, mixing, homogenization, deaeration, heating/cooling, sterilization, forming/ shaping, expansion, texturization, flash drying and center-filling/striating. The food extruder has seen increased utility because it has many attributes that manufatcturers can apply to both expand and increase their product lines (Harper, 1981).

Advantages of extrusion

The principal advantages of the food extrusion technology as compared to the other food processing techniques with modification include:

Adaptability

A variety of products are feasible by changing the minor ingredients and the operation conditions of the extruder. Extrusion procession is remarkably adaptable in being able to accommodate the demand by consumers for new products.

Product characteristics

A variety of shapes, texture, colour and appearances can be produced, which is not easily formed using other production methods.

Energy efficient

Extruders operate at relatively low moisture while cooking food products, so less re-drying is required.

[1]Paper presented at the 6th Annual General Meeting of Pakistan Society of Food Scientists & Technologists held on December 20, 1995 at PCSIR Laboratories Complex, Lahore.

Low cost

Extrusion has lower processing cost than other cooking and forming processes. According to Darrington (1987), a saving of up to 19 per cent raw material, 14 per in labour and 44 per cent capital investment is possible. Extrusion processing also requires less space per unit of operation than other cooking systems.

New foods

Extrusion can modify proteins (vegetable and animal), starches (almost all sources) and other food materials to produce a variety of new and unique food products.

High productivity and automated control

An extruder provides a continuous high throughput processing and we can have a fully automated controls for these extruders.

High product quality

Since extrusion is HTST process, it minimizes degradation of food nutrients, while improving the digestibility of proteins (by denaturing) and straches (by gelatinizing). Extrusion cooking at high temperature also destroys the antinutritional compounds i.e. trypsin inhibitors, gossypol, haemagglutinins and undesirable enzymes such as lipases, lipoxidases, and microorganisms.

No effluent

Very low levels of process effluents are produced in this processing method.

Classification of Extruders

Single screw extruders

Single screw cooking extruders have compressive screws with decreasing channel depth turning at high speeds to increase shear and mechanical energy input for heating. Heating of product is induced by the resulting friction. The barrel is jacketed for steam to allow additional contact heating in the metering section. To increase capacity and efficiency, it is common to preheat ingredients in a preconditioner by adding steam before they enter the extruder (Fig. 1). Categories of single screw extruders, based on Harper (1981) with modification (Lusas & Riaz, 1994) includes:

Cold forming (Pasta-type) extruder

The extruders have deep flight, smooth barrel, and low shear speed. There is little or no cooking. These are used for pasta, pastry dough, cookies, egg-rolls, ravioli, processed meat and certain types of candies.

High pressure forming extruder

These are characterized by grooved barrels to prevent slip at the wall and greater compression in the screw design. Such extruders are used for pregelatinized cereals and fried snack foods.

Low shear cooking extruders

There are moderate shear machines with high compression and grooved barrels to enhance mixing. Soft-moist foods and meat-like snacks such as simulated jerky are manufactured using them.

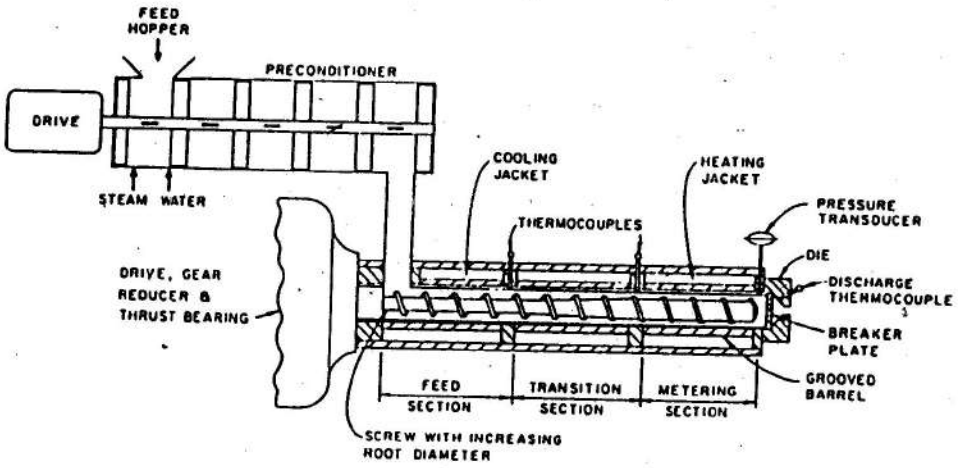

Fig. 1. Mechanical features of a single-screw extruder (Harper, 1989).

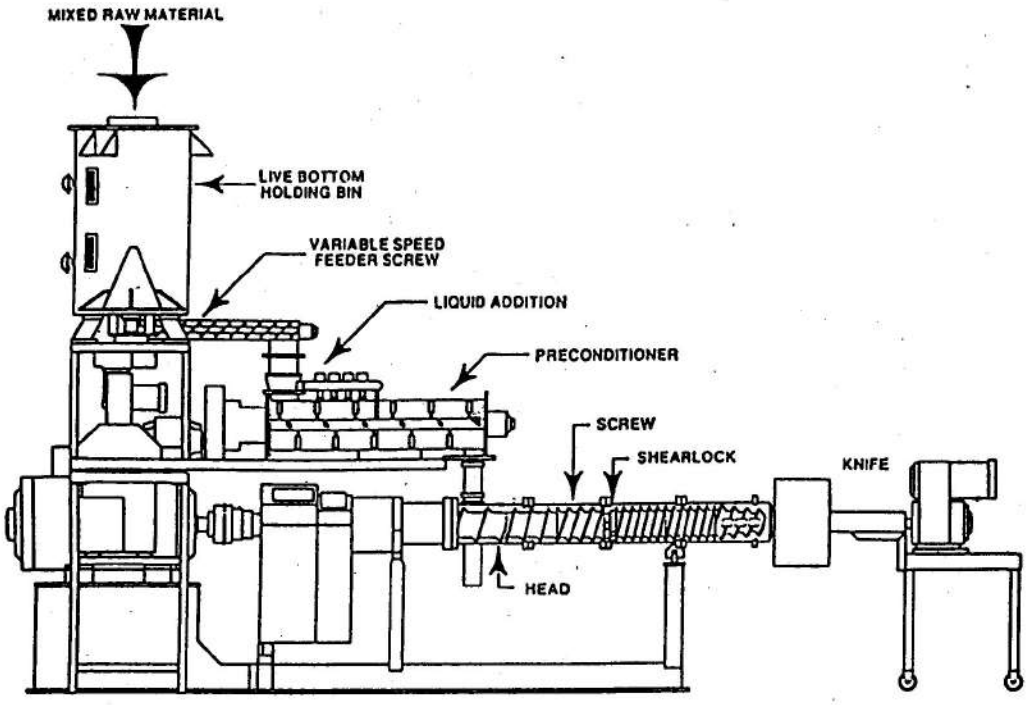

Fig. 2. Cutaway view of a typical twin-screw extrusion cooking system (Courtesy of Wenger Mfg., Co., Sabetha, KS)

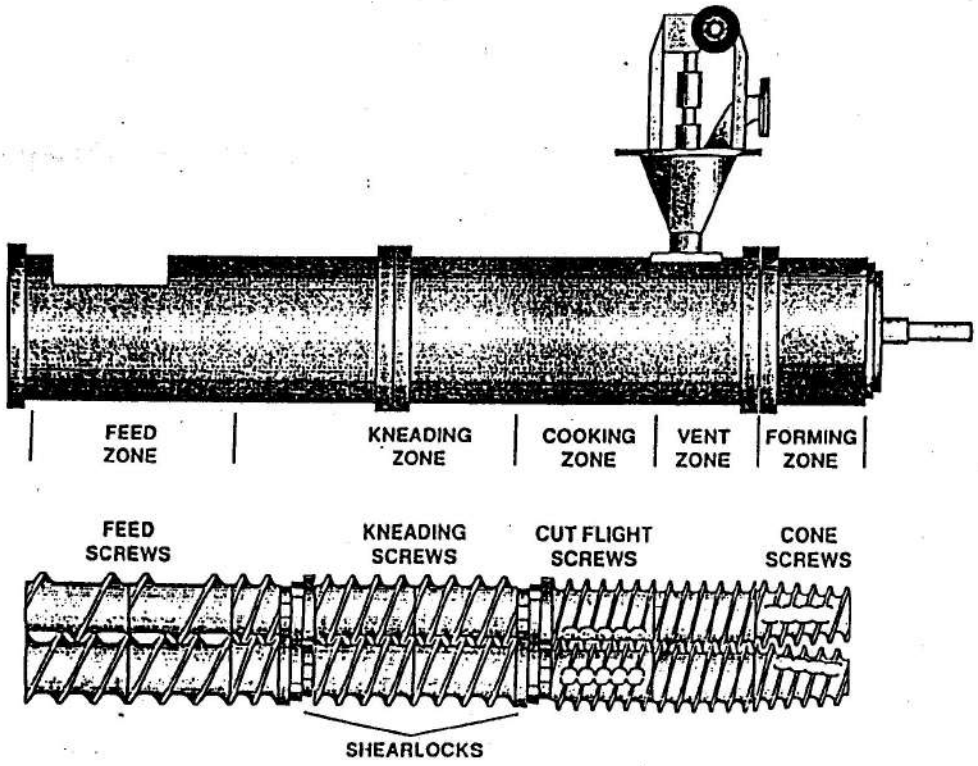

Fig. 3. Extruder components (Courtesy of Wenger Mfg., Co., Sabetha, KS)

Collet extruders

Such extruders are high shear machines with grooved barrels and screw with multiple shallow flights. These are suitable for puffed snacks and expanded curls or collets.

High shear cooking extruders

These extruders are high shear machines, with screws of changing flight depth, HTST devices, employed for pet foods, manufacture of ready-to-eat cereals (RTE), candies, crisp breads, precooked food ingredients, pregelatinized corn flour, dried food mixes, instant beverage powders, croutons and breading, crackers and wafers, enzymes, deactivation of full fat soy flour, imitation nuts, famine relief feeding, texturized vegetable protein (TVP) and deactivation of enzymes in cereals and oil seeds.

Twin screw extruders

Twin screw consist of two parallel screws in a barrel with a figure-eight cross section (Fig. 2 & 3). The use of twin-screw extruders for food processing started in the 1970s, with an expanding number of application in the 1980s (Harper, 1989). Twin screw extruders are generally one and one-half times or more expensive than single screw machines of the same capacity (Lusas & Riaz, 1994). Yet the degree of quality control and processing flexibility they offer, can make them attractive to food industries. Twin screws produce a more uniform flow of product through the barrel due to the positive pumping action of the screw flights. Some other advantages of twin screw are:

- It can handle viscous, oily, sticky or very wet material and some other products which will slip in a single extruder (it is possible to add up to 25% fat in a twin screw extruder).
- The wear in smaller part of the machine is less than in single screw extruder.
- It is possible to use wide range of particle size (from fine powder to grains) whereas, single screw extruder is limited to a specific range of particle size.
- Because of the self-wiping characteristics, its clean up is very easy.

Four types of twin screw extruders are possible:

1. Non-intermeshed, co-rotating
2. Non-intermeshed, counter rotating
3. Intermeshed, co-rotating
4. Intermeshed, counter-rotating

From these four types of twin-screw extruders, co-rotating, intermeshed screw type has found the widest acceptance in food industry (Harper, 1989).

Based on thermodynamic characteristics:

Autogenous (Adiabetic) extruders

All the heat is essentially produced through friction and little if any heat is removed through the barrel.

Isothermal extruders

Operates at essentially constant product temperature throughout the entire length of the barrel, and are used mainly for forming. Water-cooled jackets are sometimes used.

Polytropic

These have provision for alternately adding or removing heat as required by the specific process.

Application of extruders in the food industry

Following are areas of food industries where extrusion technology can be employed or is being used presently. Beverage powders, boiled sweets, bran stabilization (general, rice bran, wheat bran, oat bran), breads (miscellaneous, expanded, dense), breading substitutes, breakfast cereals (expanded, flakes/pellets, bran), candy sticks, caramel, chewing gum, chocolate, cocoa and crump, crisp bread, croutons, confectionery (miscellaneous), cooked grain (barley, corn, milo/sorghum, mixed), cookies and cracker, corn chips and tortilla, dairy products, dried food mixes, egg rolls, fabricated potato chips, flavouring, food additives, frozen confectionery, fudges, full-fat and partially defatted soy flour, gums, imitation nuts, industrial products (general, dehydrated), infant foods, jellies, legumes (miscellaneous, precooked), meat products (snacks, jerky), pasta products (noodle, spaghetti, macaroni), pastry dough, precooked and modified starches, pressed tablet, pretzels, proteins (textured and gluten), ravioli, ready-to-eat cereals, rice (miscellaneous, precooked), semi-moist foods, snacks (mixed or other, corn, fruits/nuts, potato, rice, wheat, co-extruded), soup and gravy mixes, sugar crust liqeurs, three dimensional confections and toffees.

REFERENCES

Darrington, H., 1987. A long running cereal. Food Manufacturing, 3: 47-48.

Harper, J.M., 1981. Extrusion of Food. Vol. 1. CRC Press Inc., Boca Raton, Florida, USA. pp: 127-128.

Harper, J.M., 1989. Food extruders and their applications. In: Extrusion Cooking (Mercier, C., P. Linko & J.M. Harper, eds). Amer. Assoc. Cereal Chemists, Inc., St. Paul, Minnesota, USA. pp: 1-2.

Lusas, E.W. and M.N. Riaz, 1994. An introduction to extruders and extrusion principles. Extrusion Communique, 7: 9-24, 34.

Rossen, J.L. and R.C. Miller, 1973. Food Extrusion. Food Technol., 27: 46-53.