COMPOSITION AND FUNCTIONAL PROPERTIES OF COMMERCIAL OAT FLOURS

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Judith Anne Emery

In Partial Fulfilment of the

Requirement for the Degree

of

Master of Science

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ABSTRACT

Six commercial oat flours, two from each of three Canadian oat mills, were analyzed alone or as composites with a bread wheat flour at oat flour dilutions of 5, 10, and 20% (w/w, as is moisture basis), for chemical composition, enzyme activity, colour, and functional properties, including breadbaking potential. The purposes were to investigate composition and functional properties of whole oat flour, examine the functional effects of bran removal from oat flour, and relate functional properties of oat-wheat composite flours to the breadbaking potential of oat-wheat composites.

Oat flours differed in chemical composition. Wide variations were found in levels of protein (12.6-15.0%, N x 5.5), ash (1.2-1.9%) and fibre (TDF, 5.6-14.1%; NDF, 2.9-9.0%; SDF, 2.5-5.1%; β-glucan, 1.8-4.0%). Little variation was observed in lipid content of the oat flours (TL, 9.9-10.7%; FL, 7.4-8.1%; BL, 2.1-2.9%), or fatty acid composition (palmitic:oleic:linoleic = 18:40:35). Starch levels (62.0-71.4%) were indirectly related to protein and fibre levels. Only one flour of five analyzed exhibited signs of starch damage (17.2 FU). All oat flours were similar in amino acid composition, with the exception of flours from one company which contained lower levels of cysteine. Flours high in protein were also high in ash and fibre. This allowed flours to be categorized into two groups (high or low) on the basis of protein, ash and fibre levels. One of the flours from the low protein, ash and fibre group was much lower than the other two flours in the group in ash and fibre levels, an indication of bran removal. Osborne solubility fractionation and PAGE analyses indicated flours varied, on a company of origin basis, in levels of protein denaturation and prolamin content. Flours lowest in prolamin exhibited signs of the most denaturation.

Oat flours were similar in enzyme activity levels (amylase, peroxidase and protease). These levels were depressed in comparison to unprocessed

groats, but were not zero.

Oat flours differed in colour. Flours highest in ash, protein and fibre content, were also the darkest - both in flour and slurry form. The flour lowest in ash content was brightest as flour or slurry.

Amylograph viscosities and farinograph water absorption varied according to company of origin. Farinograph dough strength properties (DDT, MTI) were greater in oat flours with higher levels of protein, ash and fibre. Bran removal (low ash and fibre) from one flour decreased farinograph absorption and Zeleny sedimentation volume, and increased lipid binding capacity of that oat flour.

Interactions between oat and wheat flours were identified. These interactions were related to storage, susceptibility to wheat amylases or non-disulphide protein polymerization or aggregation of the flours. Because of these interactions, most properties of oat-wheat composite flours were not related to the effects of dilution.

Properties of oat-wheat composite flours (amylograph, farinograph, extensigraph, sedimentation volume, gluten formation, loaf volume and loaf firmness after three days storage) varied according to company of origin or oat chemistry. Statistical analyses indicated oat flours differed in their response to functional properties when combined with wheat. Many of these differences (amylograph, farinograph absorption, extensigraph, loaf volume) were attributed to company processing variables, including the factors associated with protein denaturation or susceptibility to wheat amylase action; not to individual oat flour chemistry (protein, ash or fibre levels).

Loaf volumes were related statistically to extensibility ($r=0.89^{**}$), extensigraph area ($r=0.94^{**}$), wet gluten ($r=0.84^{*}$), Zeleny sedimentation ($r=0.94^{**}$) and loaf firmness ($r=-0.83^{*}$). At each dilution, the flours that were lowest in farinograph absorption produced the smallest loaves. Loaf firmness was statistically related to loaf volume ($r=-0.83^{*}$), wet gluten ($r=-0.78^{*}$), extensigraph area ($r=-0.87^{**}$) and Zeleny sedimentation ($r=-0.80^{*}$). Removal of bran from one sample produced the firmest bread at the 20% level of dilution only.

I. INTRODUCTION

Studies describing the hypocholesterolemic effects of oat bran (Kirby *et al.*, 1981; Anderson *et al.*, 1984; Van Horn *et al.*, 1988) created consumer demand for a new product - oat bran. Until this point, oat bran, which is an integral part of the oat groat, was ground along with the germ and endosperm to form oat flour. The demand for oat bran created two new products for the oat miller: oat bran, for which there was a market, and a low bran oat flour, for which there was no market. This project was conceived as a result of the lack of knowledge of the composition and functional properties of the low bran oat flour.

A report published by Swain *et al.* in 1990, suggested that the hypocholesterolemic effects of oat bran resulted only from fat replacement in the diet. This had the result of suddenly decreasing the consumer demand for oat bran, just as this study was initiated. The original purpose of the study was therefor redefined, in order to present sponsors of the study, the oat millers, with relevant information. The purpose of the study was broadened to include an investigation of the composition and functional properties of whole oat flour (including the bran), in order to identify factors affecting oat-wheat bread baking potential.

During the course of this study, Davidson *et al.*, in 1991, reported hypocholesterolemic effects of oat bran and oatmeal using diets equalized for fat. Although this provided clarification with respect to the nutritional debate over the benefits of consumption of oats and again increased utilization of oat bran, the market demand for this product did not return to the high level reached in 1989.

Despite the controversy over nutritional effects of oat bran, oats have long proved themselves as a highly nutritious cereal because of vitamin and mineral content, protein levels and quality, and also lipid levels and composition. Oats are a good source of B vitamins for the

diet; especially thiamine, biotin and pantothenic acid. In addition, they are considered a good source of the minerals manganese, phosphorus, magnesium and iron (Lockhart and Hurt, 1986). Pomeranz *et al.* (1973) reported that oat groats have the highest protein level among cereal grains. The presence of a high lysine content (unusual for cereals) provides oats with an excellent amino acid balance. In their review of oat nutrition, Lockhart and Hurt (1986) indicated that the protein efficiency ratio of oats is higher than that of most cereals including wheat, rice and rye. Oat lipids are similar in composition to lipids from other cereals, but higher in lipid concentration and are therefor a more concentrated energy source (Youngs *et al.*, 1977). In addition, oat lipids are highly unsaturated, including high levels of linoleic acid (Youngs, 1986).

Processing of oats for human consumption causes gelatinization of some starch granules as indicted by differential scanning calorimetry (Oomah, 1987), maltose production by amylase (Yui *et al.*, 1987), and structural fragmentation (Lookhart *et al.*, 1986). Thus, with only minimal heat treatment, the starch in oat products is ready for digestion, another nutritional benefit of oats.

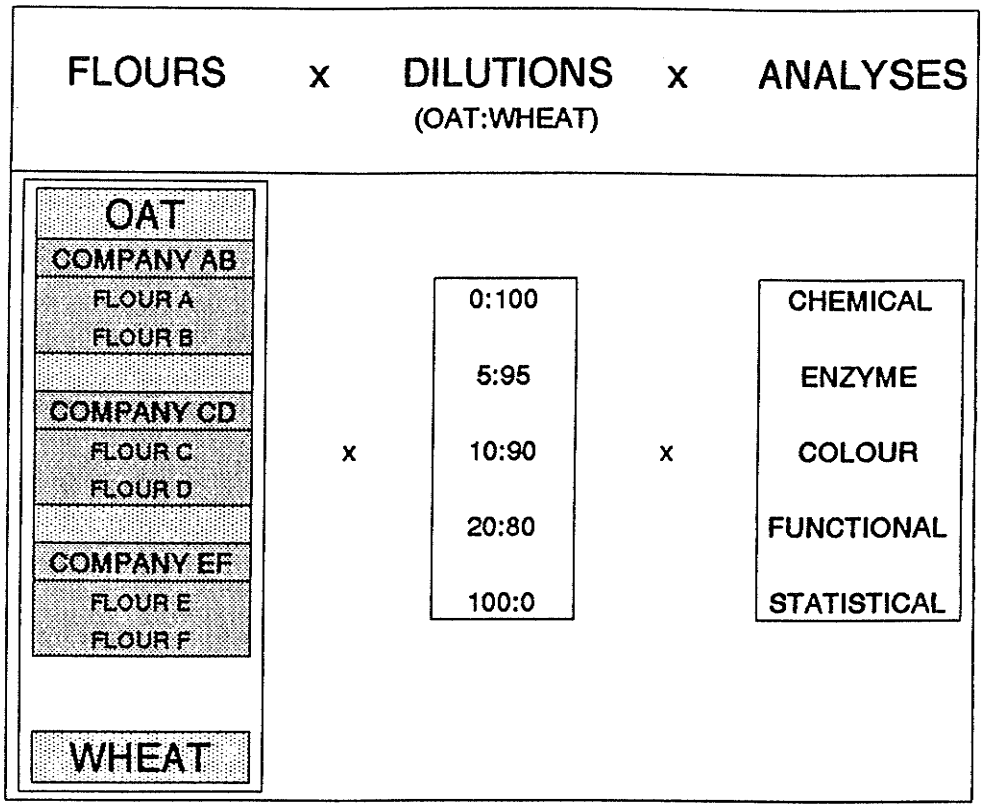
Oats provide a natural source of anti-oxidant. Their use as such in the form of oat flour has been demonstrated in several foods: added to margarine and oil, dusted on hot potato chips or peanuts, dusted on paper to wrap margarine or lard (Peters and Musher, 1937); added to milk, milk powder, ice cream, fish, bacon and sausage (Webster, 1986). This is a nutritional benefit to individuals wishing to avoid non-food based additives in their diet.

Because of the many nutritional values of oats, it is highly desirable to develop and expand their present use in the human diet further. For this reason, an examination of the composition and functional properties of commercial oat flours related to their breadbaking potential bears merit.

Experimental design for this project included chemical, enzyme, colour, functional and statistical analyses of seven flours (two oat flours from each of three companies: Company AB, Company CD, Company EF; plus a bread wheat flour) at five dilution levels (oat:wheat; 0:100, 5:95, 10:90, 20:80, 100:0) (TABLE 1). Not all analyses were conducted on all

dilutions.

TABLE 1. Experimental Design of Thesis



The wheat sample must be considered as a blank or control sample for the dilutions of the oat flours for the statistical analyses (regression analysis). As such, the wheat sample is referred to as the oat:wheat dilution, 0:100. In a similar manner, oat flour samples are referred to as the 100:0 (oat:wheat) dilution. When a dilution is expressed as a ratio, the oat flour is always indicated first, since oat flour is the focus of this thesis. When a dilution is expressed as a percentage, the number refers to the oat flour content of the composite, again because oat flour is the focus of this thesis. Reference to composite flours (5:95, 10:90, 20:80) always implies the presence of both oat and wheat flours in a sample. Reference to oat flour dilutions includes reference to wheat flour (0:100), oat flour (100:0) and all composites (5:95, 10:90, 20:80). The term oat flour dilutions simplifies the description of wheat flour, oat flour and oat-wheat composites. Even though these descriptions are only required for an understanding of the statistical analyses, they are

used throughout the thesis for the sake of consistency.

Analyses performed for the thesis are listed in TABLE 2. In general, an extensive chemical analysis was conducted on the oat flours including moisture, ash, protein (percent, amino acid composition, Osborne fractionation, electrophoretic mobilities), lipid (free, bound, neutral, glyco, phospho, and fatty acid composition), carbohydrate (fibre constituents including β-glucan, starch and damaged starch) and polyphenols. Some of these analyses were also conducted on the wheat flour as well as on some of the composite flours. Oat flours were analyzed for amylase, peroxidase and protease activities. All dilutions were examined for Falling Number value, a measurement of α-amylase activity. Where possible, all dilutions were examined for functional properties. Tests included amylograph, farinograph, extensigraph, sedimentation volume, gluten content, fat binding capacity and breadbaking potential, including loaf volume and firmness after three days. Since oat flours do not produce gluten in the presence of water, they were not analyzed for extensigraph, gluten content or breadbaking properties.

TABLE 2. Analyses Performed for Thesis

CHEMICAL	ENZYME	COLOUR	FUNCTIONAL	STATISTICAL
Moisture	Amylase	Flour	Amylograph	T-Test
Ash	Peroxidase	Slurry	Farinograph	Paired T-Test
Protein	Protease		Extensigraph	Regression Analysis
Lipid			Sedimentation	
Carbohydrate			Gluten Content	
Polyphenol			Fat Binding	
			Breadbaking	

Initial protein and ash results indicated Flours A and B were very similar. Therefor analysis of Flour B was not conducted by all procedures.

Results for functional properties of oat-wheat composites were analyzed statistically to determine differences in oat flour behaviour (*t*-test) and differences attributable to company of oat flour origin (paired *t*-test). Linear correlation coefficients were determined for functional properties.

Conclusions are presented regarding the effects of various chemical and functional properties of oat flours with respect to their breadbaking potential.

II. LITERATURE REVIEW

A. Introduction

Several concise publications exist about oats, including the book *Oats: Chemistry and Technology* (Webster, 1986) and reviews by McMullen (1991), Youngs *et al.* (1982) and Shukla (1975). These references include information about oat history, production, processing, morphology, chemistry and uses. Comparisons of oats with other cereals are also presented. The reader is referred to these publications for information on oats outside the subject area of this thesis.

Controversy over the nutritional benefits of oat fibre, and more concisely, soluble β-glucan, exists. One proposal suggests the benefits of oat fibre in the diet arise only as a fat replacement (Swain *et al.*, 1990). Other reports suggest oat fibre is capable of lowering plasma cholesterol (Davidson *et al.*, 1991; Shinnick *et al.*, 1988). Because the nutritional/health issue indirectly affects the market demand for oat bran and the resulting production of oat flour, recent publications referring to the role of oats in human nutrition are reviewed.

Oat composition, in terms of morphology and chemistry will be discussed. Included in morphology will be the difference between oat hulls and oat bran. Oat chemistry, in terms of protein, carbohydrate and lipid components will be discussed with respect to levels and constituents of each of these components. This information will provide a basis for a more complete understanding of the chemistry of oat bran and oat flour (with and without bran). Although many chemical comparisons have been made between oats and other grains, these comparisons are outside the realm of this literature review.

Many variations exist for the processing of oats for human

consumption. These operations have effects on oat-product chemistry which in turn can alter oat functionality. A brief review of processing, with respect to the physical changes of the oats and heat treatments encountered, is presented. The topic of equipment (options available for each procedure, descriptions of use, and flow of product) is not covered. The effects of processing on oat chemistry is also reviewed. A brief description of the various uses of finished oat products is included.

Very few studies have been conducted on the functional properties of oat flour or oat bran, either alone or as composites with wheat. Although the study of oat bran is not considered in this thesis, the literature related to its functional properties is included to broaden the understanding of oat properties.

B. Nutritional Aspects of Oat Fibre

1. Fibre Definitions

Fibre is a term that generally refers to polysaccharides and lignins, indigestible in the human small intestine, that compose cell wall components, storage polysaccharides or gum exudate of plants. Dietary fibre is a popular term, but carries several contradictory definitions. All definitions agree dietary fibre is the portion of plant cell walls, polysaccharides and lignin, that are not digested in the human small intestine. To some this excludes the storage polysaccharides of legumes. Others wish to include some other indigestible constituents including phytates, minerals, silica, cutins, waxes, enzyme resistant starch or proteins in their definition of dietary fibre. Pressures from marketing departments of industries to include dietary fibre labelling on food packages will force a legal definition of dietary fibre in the future.

Fibre can be further classified by solubility. Soluble, or more specifically water-soluble, fibre includes structures such as pectins, pentosans and gums. Insoluble fibre includes the structures cellulose and lignin, as well as some noncellulosic polysaccharides or hemicelluloses.

2. Physiological Effects of Plant Fibres

Anderson and Chen (1986) reviewed the physiological effects of plant fibre, and a summary of their discussion is presented here. Plant fibres affect the entire intestinal tract. Beginning in the mouth, high-fibre foods generally take longer to eat. In the stomach, soluble fibre delays gastric emptying and may also alter gastric acidity through buffering capacity. In the intestines, water-insoluble fibre decreases transit time, because of bulk; water-soluble fibre increases transit time, because of gel-forming ability. Effects of fibre intake are manifested in the entire body by decreased hormone secretions, including insulin and glucagon, which may also alter digestive enzyme activity.

Plant fibres possess chromatography-like properties in the small intestine. Gelatinous globules of water-soluble fibres can act as a gel filtration system, allowing large molecules to rapidly pass through the system and at the same time trapping smaller molecules in the pores for variable lengths of time. This affects the rate of digestion and absorption of nutrients. Minerals, bile acids, and potential carcinogens can be absorbed by plant fibres, either by ion exchange or adsorption properties.

By definition, plant fibres are indigestible by enzymes found in the small intestine. However, enzyme systems of microflora found in the colon are able to ferment all fibres but lignin into methane, carbon dioxide, water and short-chain fatty acids. Lignin is capable of adsorbing bile acids and may thus facilitate the removal of a form of cholesterol from the body. Lignin may also act as a free-radical scavenger and an antioxidant, and as such may alter the formation of carcinogens in the digestive tract.

Water-insoluble fibres increase fecal bulk, possibly by increasing the fecal content of water and bacteria. Water-soluble fibres, because they are almost completely degraded in the colon, do not increase fecal bulk. Colonic transit time is inversely related to stool weight.

These physiological effects have been the basis for claims of nutritional benefits of fibre in the prevention or control of diabetes, hypertension, intestinal disorders and coronary heart disease.

3. Proposed Mechanisms for Cholesterol Lowering Effects of Fibre

Although fibre found in oats has many nutritional benefits, it is the study of the cholesterol lowering effect of the soluble oat fibre, ß-glucan, that is responsible for many recent nutritional studies of oats. Through reduction of cholesterol levels, it is proposed that the rate of formation of atherosclerotic plaque can be decreased. Before examining some of these studies in detail, proposed mechanisms for the cholesterol lowering effects of fibre will be examined. The biochemical or physiological effects of fibre on cholesterol are not completely understood and conflicting data are reported in the literature. Anderson and Chen (1986) reviewed the literature and reported speculations for at least six mechanisms for the cholesterol lowering effects of soluble fibre. These mechanisms are not mutually exclusive in their applications. A summary of these possibilities is presented here.

1. Some fibres have been shown to bind bile acids, a form of cholesterol excreted into the intestine to facilitate the absorption of dietary fats. Thus fat absorption, including cholesterol, may be inhibited because of a decreased availability of bile acids; and cholesterol, in the form of bile acids, is excreted with the fibre.
2. Bile acids are normally conserved by the body. Removal of the acids by certain dietary fibres increases the synthesis of bile acids and thus depletes the level of cholesterol circulating in the body.
3. Most plant fibres are fermented to short-chain fatty acids (SCFA) in the colon. Absorption of the SCFA, especially propionate, may be responsible for a decrease in cholesterol synthesis by the liver: this may be mediated by SCFA feed-back on 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis.
4. Cholesterol circulates in the body in the form of lipoproteins. Low-density lipoproteins (LDL) carry most of the pool of cholesterol in the body and are considered the most atherogenic. High-density lipoproteins (HDL) carry a small, relatively fixed proportion of the body's cholesterol pool and are considered to be antiatherogenic. Plant fibres appear to alter the lipoprotein profiles, possibly by altering the composition of absorbed fats, the composition of lipoproteins, the rate of synthesis of apoproteins (the proteins associated with LDL and HDL), LDL receptors, or

LDL catabolic rates.

5. The presence of plant fibre in the diet may alter the colonic microflora balance, and thus affect the production of SCFA which in turn may alter the chain of events discussed in #3 altering cholesterol metabolism.

6. Plant fibres can modulate glucose absorption, thus decreasing postprandial levels of glucose, insulin and glucagon. Since insulin has been reported to increase cholesterol synthesis, plant fibres may decrease cholesterol levels in the body indirectly by inhibiting insulin release.

4. Nutritional Significance of Oat Fibre

DeGroot *et al.* (1963), who examined serum cholesterol levels of 21 healthy male volunteers fed diets which included 300 g per day of an experimental bread (containing 140 g of rolled oats), demonstrated depressed serum cholesterol levels in the participants of the study during the three weeks of the rolled oat diet, in comparison to the three weeks of control diet. Removal of the rolled oats from the diet led to an increase in serum cholesterol, although not to the same level the control diet generated.

Many subsequent papers have been published related to the effects of oat fibre on cholesterol and its metabolites. The designs of these studies vary in many ways, including initial cholesterol levels of patients, levels of fibre, fat and saturated fat in the diet, and control over diet. As a result, there are few conclusive remarks that can be made regarding the effects of oat fibre in the diet.

Significant increases in fecal bile acid excretion were reported in healthy individuals on diets which included oat bran (Kretsch *et al.*, 1979) or rolled oats (Judd and Truswell, 1981). However, in hypercholesterolemic men, the addition of oat-bran to diets, in addition to increasing fecal bile acid excretion, also lowered serum total cholesterol levels and LDL (Anderson *et al.*, 1984; Kirby *et al.*, 1981). Other studies have also shown decreased serum total cholesterol levels and LDL in hypocholesterolemic male patients fed diets which included oat bran as a source of dietary fibre (Anderson *et al.*, 1990 and Kestin *et al.*,

1990). Van Horn *et al.* (1988) presented data that indicated the inclusion of oatmeal in a fat-modified diet is helpful in lowering serum cholesterol. Ranhotra *et al.* (1989), in their examination of diets high in water-soluble fibres, including oat bran, fed to moderately hypercholesterolemic men demonstrated decreases in serum total cholesterol and increases in HDL.

The results of Swain *et al.* (1990), refuted the results of the above studies. They concluded that oat bran had little cholesterol-lowering effect on healthy individuals and hypothesized it was only by replacement of dietary fat that high or low-fibre grain supplements reduced serum cholesterol levels. However, fibre levels of the base diet (23 g/d) and low fibre diet (18 g/d) were both higher than levels found in the average American diet (11-13 g/d) as determined by Lanza *et al.* (1987). In addition, diets were not controlled for fats: high fibre diets were higher in unsaturated fats than low fibre diets, and both diets were lower than the control diet in saturated fats. Leadbetter *et al.* (1991), also found no cholesterol lowering ability of oat bran, although this study examined the response of hypercholesterolemic subjects to the addition of up to 90 g oat bran per day to their usual diet (which was relatively high in saturated fatty acids).

Addition of oat products to fat-modified diets has shown cholesterol-lowering abilities in hypercholesterolemic individuals. Davidson *et al.* (1991) demonstrated that β-glucan, from oatmeal or oat bran, was effective in lowering serum cholesterol levels in a dose dependent manner. Van Horn *et al.* (1991), demonstrated reductions of serum total cholesterol and LDL-cholesterol by the addition of approximately 50 g of oatmeal to the diets of 80 men and women.

Although the details are not well understood, most researchers agree that the presence of oat bran in the diet can be beneficial in reducing serum cholesterol levels in some individuals. However, oat bran cannot be viewed as a medical miracle-food. Efforts to reduce serum cholesterol should include a well-balanced diet with less than 30% of daily calories from fats.

C. Oat Composition

1. Morphology

Fulcher (1986) reviewed aspects of oat morphology, including microscopic analyses, development of the oat kernel and microchemical organization of the mature groat. His analysis is summarized here, with emphasis on oat tissues and their chemical construction.

The oat kernel can be subdivided into hull (dehydrated, metabolically inactive fibrous structures) and groat (tissues capable of producing a plant) (FIGURE 1). The groat can be further subdivided into bran, endosperm and germ or embryo.

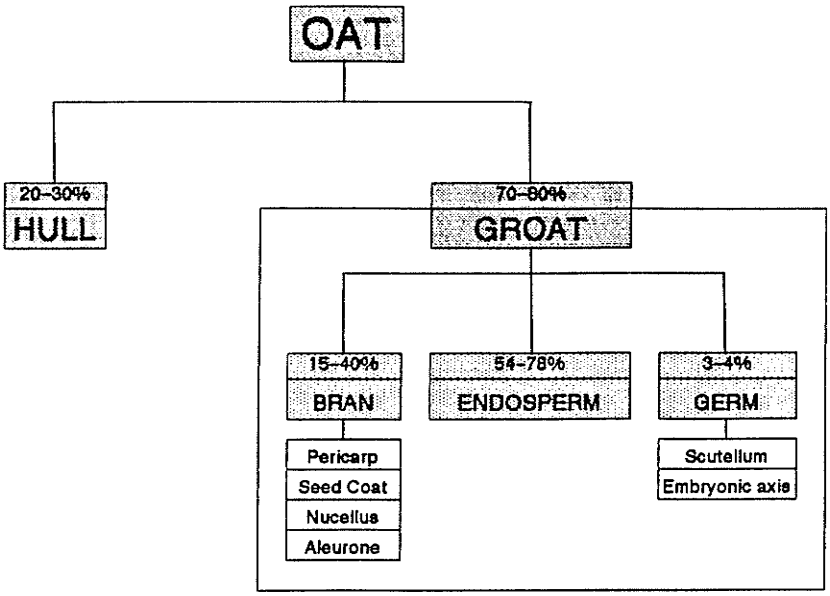


FIGURE 1. Morphological organization of oat tissues.

At harvest, most oat varieties are covered with hulls (FIGURE 2), the compressed layers of cell wall material once belonging to floral bracts of the developing kernel. The hull may be responsible for up to 30% of the dry weight of the oat kernel. Chemical composition of the hull is mostly cellulose, or hemi-cellulose and lignin polymers with minor amounts of protein, short-chain fatty acids and phenolic compounds.

Groats are elliptical in shape. A slight oval indentation on the dorsal surface indicates the location of the germ. The groat is attached

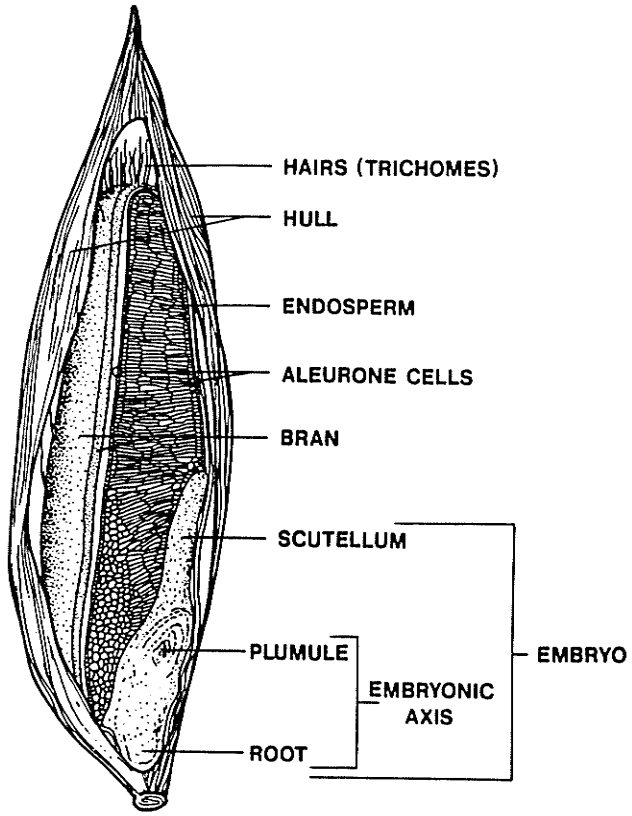


FIGURE 2. **Morphological structure of an oat kernel** (from Youngs, 1986).

to the plant at the embryo end. A crease is located on the ventral surface of the groat. Vascular tissues located in the deepest part of the crease transport nutrients into the grain during development. Although probably not functional at maturity, the vascular bundle remains in the crease as a thin strand of fibrous tissue.

Morphologically, the bran consists of three tissues which are remnants of the ovary in the developing seed: pericarp, seed coat and nucellus. Pressure from the developing grain compresses the tissues, expels the cytoplasm and leaves only tough, fibrous cell walls surrounding the mature groat. These cell walls contain an abundance of lignin or related phenolic compounds. Trichomes, or hairs, are extensions of the pericarp - the outer layer of bran - and surround the groat. These hollow, single-celled projections are composed of high levels of phenolic compounds.

Commercially, aleurone - the outer endosperm layer - is also included in the bran, because of a difficulty in separating the tissues. The

aleurone, a single layer of cells surrounding the endosperm and most of the germ, is 50-150 µm thick and the major constituent of bran. Like the cell walls of the pericarp, seed coat and testa, aleurone cell walls also have a high concentration of phenolics, probably in the form of ferulic acid. In addition, aleurone cell walls appear to contain high levels of mixed linkage ß-glucans. However, unlike the other bran tissues, aleurone cells contain a cytoplasm including nucleus, endoplasmic reticulum, mitochondria and plastids. Protein bodies (also called aleurone grains), composed of a protein matrix and embedded deposits of phytin, niacin-aromatic amine complexes, phenolics and carbohydrate are also found in the cytoplasm. These protein bodies are surrounded by lipid droplets. Granules of starch may also be found in the aleurone as well as the minerals potassium, calcium, magnesium and phosphorus. Bran tissues can be responsible for 15-40% of the dry weight of the groat. Higher levels of groat protein appear to be responsible for larger amounts of bran.

Endosperm cells are similar to aleurone cells. However, cells decrease in levels of protein, lipid and ß-glucan as they approach the midendosperm and at the same time, increase in starch content. Protein bodies of the endosperm vary in size from 0.2-0.6 µm; the larger ones are found in the subaleurone region. Endosperm protein bodies differ from those found in the aleurone in their lower levels of lysine, threonine, serine and alanine.

The embryo is responsible for only 3-4% of the weight of a mature, ungerminated groat. It can be subdivided into scutellum and embryonic axis. The scutellum can be further subdivided into parenchyma and epithelium. The embryonic axis can be further subdivided into plumule and root. Approximately 80% of the embryo is parenchyma, a tissue similar to aleurone in many ways. Parenchyma cells contain protein bodies that contain phytin, but no niacin; and are surrounded by neutral lipid droplets. Germ protein is higher in lysine and lower in glutamic acid than endosperm protein. Like aleurone cell walls, parenchyma cell walls appear to contain high concentrations of ferulic acid. Unlike aleurone, parenchyma tissue does not contain starch or ß-glucans. Epithelial cells cover the parenchyma and absorb nutrients released by hydrolysis of the endosperm during germination. The midpoint of the embryonic axis is

attached to the scutellum.

In summary, to clarify a common misconception, oat hulls are brittle, lignin-cellulosic structures that cover the groat. Oat bran, also mostly lignin and cellulose in composition by the morphological definition, is the outer layer of the groat. Commercial removal of oat bran from the groat also removes aleurone tissue and thus adds protein, niacin, lipid and β-glucans to the composition of commercial oat bran.

2. Chemistry

a. Proximate Composition. The proximate composition of some oat products is summarized in TABLE 3. McKechnie (1983) reported the typical analysis of commercial milled oat products. Lookhart *et al.* (1986), examined dehulled oats (groats), dried groats, and steamed and rolled dried groats (flakes) of three oat genotypes. Ranges of their results are reported. Seibert (1987) reported a typical analysis of commercial quick oats.

TABLE 3. Proximate Composition of Some Oat Products

	MILLED OAT PRODUCTS McKechnie, 1983 typical analysis	GROATS, DRIED GROATS AND FLAKES Lookhart et al., 1986 dry basis	QUICK OATS Seibert, 1987 typical analysis	OAT FLOURS Oomah, 1983 dry basis
MOISTURE	9.5	8.6–13.2	10.0	NR
PROTEIN	17.0	16.0–20.8 (N X 6.25)	15.3	11.6–13.9 (N X 5.7)
ASH	1.7	2.2–3.0	2.1	1.1–1.9
LIPID	6.5	4.8–8.3 petroleum ether	6.0	6.1–8.0
CRUDE FIBRE	1.3	NR	NR	1.1–2.0
CARBOHYDRATE	65.0	71.0–75.4 by difference	66.6 by difference	NR

%

NR, not reported

Oomah (1983) analyzed three oat flours (commercial, 42% extraction and whole oat flour) and the ranges are recorded.

Differences exist in methods of reporting values. Analyses are reported on an as is or dry basis. Protein determinations are calculated with different nitrogen factors, which may or may not be reported with the results. Solvents used for lipid extractions vary and are not always reported. Crude fibre or carbohydrate may not be determined by analysis, and instead, carbohydrate, including fibre, calculated by difference. These differences present difficulties in comparing data on a scientific basis. However, these tabulated data present a general profile of the chemical composition of oats.

b. Protein.

i. Content. Various levels of protein have been reported in oats. Pomeranz *et al.* (1973) determined concentrations of 18-37% (N x 6.25, dry basis) for 11 oat species studied. Wu and Stringfellow (1973) studied two oat varieties: Garland, considered a high-protein variety (17% protein, N x 6.25, dry basis) and Sioux, considered an average variety (13% protein). Welch *et al.* (1991) examined six oat cultivars which ranged from 9-23% protein (N x 6.25, dry basis) as well as eight wild oat species which were found to vary between 18-40% protein.

ii. Osborne fractions. Several studies have analyzed the Osborne solubilities of oat proteins (TABLE 4). Although albumins were always extracted in distilled water, and prolamins were always extracted in 70% ethanol, various solvents and solvent concentrations were used for extraction of globulins (salt soluble proteins) and glutelins (acid or base soluble proteins).

Several workers have included an analysis of the nitrogen contained in the residue with the solubility results of their Osborne fractions, and their results can be expressed in terms of percent total recovered nitrogen. Ewart (1968) analyzed oat flour of unexplained origin and recovered 83% of the nitrogen in his fractions. Wu *et al.* (1972) examined the solubility fractions and residue of defatted (n-butanol) milling fractions of the oat variety, Wyndmere, with 71-86% nitrogen recovery.

TABLE 4. Osborne Fractionation Studies of Oat Proteins

	ALBUMIN	GLOBULIN	PROLAMIN	GLUTELIN	RESIDUE	%N RECOVERED
EXPRESSED AS % TOTAL RECOVERED NITROGEN:						
Ewart, 1968		(0.04M NaCl)		(0.1N acetic acid)		
OAT FLOUR	14	16	4	0	66	83
Wu et al., 1972		(1M NaCl)		(0.05N NaOH) (0.1N acetic acid)		
BREAK FLOUR	5	38	ND	45 ND	12	78
REDUCTION FLOUR	15	43	ND	21 ND	21	86
SHORTS FLOUR	8	51	ND	27 ND	14	71
SHORTS	15	41	10	6 15	13	71
BRAN	14	46	10	1 15	14	72
ND not determined						
Ma & Harwalkar, 1984		(0.5M CaCl2)		(0.1N acetic acid)		
SENTINEL	13	42	15	15	14	91
EXPRESSED AS % TOTAL SOLUBLE NITROGEN:						
Draper, 1973		(5% K2SO4)		(0.05N NaOH in EtOH)		
CONDOR	3	11	13	72		
Peterson, 1976		(1M NaCl)		(0.1N NaOH)		
LOCATION W.	18	49	10	23		73
LOCATION R.	20	47	9	24		75
Peterson & Smith, 1976						
MATURE GOODLAND	11	56	9	23		83
MATURE ORBIT	10	54	13	23		93
Ma, 1983		(0.5M CaCl2)		(0.05N NaOH)		
HINOAT	16	53	12	20		
SENTINEL	16	52	15	17		

Their results have been converted from percent total nitrogen to percent total recovered nitrogen. Ma and Harwalkar (1984) prepared fractions from defatted (hexane), ground groats of the variety Sentinel with over 90% nitrogen recovery. Their results have also been converted from percent total nitrogen to percent total recovered nitrogen.

Other workers have not analyzed nitrogen in the residue, and their results can be expressed as percent total soluble nitrogen. Draper (1973) did not defat the ground groats before extraction and used a method that required a separate extraction for glutelins, not a sequential extraction

as performed by other workers. The numbers in the table are calculated from his results for percent nitrogen and yield for each fraction. Peterson (1976) examined the nitrogen solubilities of six oat cultivars, grown in two locations. Values in the table are calculated from his results for percent protein (fractions and total). Peterson and Smith (1976) examined solubility changes occurring with the maturation of two oat cultivars. Protein solubility results for mature, defatted (cold acetone) ground groats are presented in TABLE 4.

Some studies have determined oat proteins are greater than 50% globular in nature (Peterson, 1976; Peterson and Smith, 1976; and Ma, 1983). Other studies have determined that glutelin, comprising approximately 70% of recovered protein, is the largest protein solubility fraction (Ewart, 1968, and Draper, 1973). The large differences in Osborne solubilities reported in the literature probably result from source of oats as well as method variations: solvent to solute ratio, order of extraction, type and concentration of salt, and acid or base extraction of glutelins. In addition, temperature of extraction affects globulin solubility and unextracted globulin will extract as glutelin (Peterson and Brinegar, 1986). Ma (1983) suggested that defatting groats did not significantly affect the protein content of solubility fractions.

Albumins. According to studies by Ma and Harwalkar (1984), albumins are water soluble in all pH ranges and exhibit higher fat-binding and water hydration capacities than other solubility fractions. As a result, they also exhibit better foaming properties (percent foam formation, foam stability, pH range of foam formation). Differential scanning calorimetry (DSC) revealed an endothermic peak at 87°C. Isoelectric focusing (IEF) patterns of albumins covered a wide range, with most components displaying a pI between 4.0 and 7.5. Column chromatography results indicate most albumins have a molecular weight of about 15 kDa, although components with molecular weights of approximately 6, 22 and 36 kDa were also detected. These results are in general agreement of the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) results of Brinegar, which also indicate a major band at approximately 15 kDa and minor bands of various intensities between 5 and 66 kDa (as reported by Peterson and

Brinegar, 1986). Values for albumin amino acid compositions in the literature vary, but they agree that in comparison with other solubility fractions, albumins are highest in lysine, glycine and alanine and lowest in glutamate. (Wu *et al.*, 1972; Draper, 1973).

Globulins. Brinegar and Peterson (1982) described oat globulin as a hexamer (327-369 kDa) composed of six acid-base, disulphide-linked dimers (53-58 kDa); each dimer contains at least one free thiol group. The acidic subunit has a pI of 5-7 and molecular weight of 32.5-42 kDa; the basic subunit has a pI of 8-9 and molecular weight of 19-25 kDa (Brinegar and Peterson, 1982, Burgess *et al.*, 1983). Comparing amino acid profiles of the subunits, the acidic subunit is higher in glutamate/glutamine and glycine, the basic subunit is higher in aspartate/asparagine, alanine, histidine, lysine and arginine (Peterson, 1978, Brinegar and Peterson, 1982, Burgess *et al.*, 1983). Additional oat globulin subunits have been demonstrated by SDS-PAGE with estimated molecular weights of 12-19, 50-55, and 59-65 kDa (Burgess *et al.*, 1983); and by column chromatography with estimated molecular weights of 6 and 150 kDa (Ma and Harwalkar, 1984).

Oat globulin can be also be classified by Svedberg sedimentation. This separates the globulin into 3S, 7S and 12S fractions (Burgess *et al.*, 1983; Adeli and Altosaar, 1984). In addition, a fraction with a sedimentation greater than 12S is visible on sedimentograms (Peterson, 1978; Bikbov *et al.*, 1986). This fraction was identified as 15S by Bikbov *et al.* (1986).

In addition to separation by sedimentation, globulin fractions can also be separated by solubility and thermal properties. Solubility of oat proteins was found to increase with increasing NaCl concentrations to a maximum at 0.8 M NaCl (Peterson, 1978). Oat globulin has been observed to precipitate from a 1M NaCl solution at 4^{o}C (Peterson and Brinegar, 1986). The 3S and 7S fractions have been separated from the 12S and 15S fractions by heating a globulin solution (0.5M NaCl) to 80^{o}C, centrifuging and removing the precipitate; 3S and 7S globulins precipitate and 12S and 15S fractions remain in solution. The 15S fraction can be precipitated out of this solution by dilution with water to decrease the NaCl

concentration to 0.3M (Bikbov *et al.*, 1986). Dilute salt (0.2M NaCl) has been shown to preferentially extract 3S and 7S globulins (Danielson, 1948; Adeli and Altosaar, 1984).

The 3S fraction exhibits acidic pI's with molecular weights from 14-25 kDa (Burgess *et al.*, 1983; Adeli and Altosaar, 1984; Robert *et al.*, 1985a), although Western blot analysis indicates the lower molecular weight proteins (14-20 kDa) are not actually 3S proteins (Robert *et al.*, 1985a). The 20 kDa band is glycosylated (Adeli and Altosaar, 1984). In contrast to subunits of the 3S fraction, subunits of the 7S fraction are mostly basic with high molecular weights (four bands of 35-67 kDa) (Burgess *et al.*, 1983; Adeli and Altosaar, 1984; Robert *et al.*, 1985a) and glycosylated (Adeli and Altosaar, 1984). Descriptions of 12S proteins by several workers (Peterson, 1978; Burgess *et al.*, 1983; Adeli and Altosaar, 1984; Robert *et al.*, 1985b) are similar to the description of Brinegar and Peterson (1982) of oat globulin. Small molecular weight proteins (13-20 kDa) have appeared with reduced 12S globulin on SDS-PAGE (Burgess *et al.*, 1983; Robert *et al.*, 1985b). Western blot analysis indicates these proteins are not globulins (Robert *et al.*, 1985b). Examination of 12S oat globulin revealed the possibility of low levels of glycosylation (Peterson, 1978; Adeli and Altosaar, 1984).

Oat globulins exhibit high thermal stability; differential scanning calorimetry reveals an endothermic peak at approximately 110°C. (Arntfield and Murray, 1981, Ma and Harwalkar, 1984, Ma *et al.*, 1988). An additional, broad peak was visible between 60-90°C, under the conditions described by Ma and Harwalkar (1984). Some denaturation also occurred at 100°C and caused insolubility of the globulin (Ma and Harwalkar, 1987, 1988). Upon heating at this temperature, a soluble, high molecular weight aggregate of hexamers, not acid-base dimers or monomers, was evident with separation by HPLC, indicating disulphide bonds are not disrupted at 100°C (Ma and Harwalkar, 1987). This aggregate was not present at 110°C, possibly because of precipitation or dissociation. According to the studies of Bikbov *et al.* (1986), this precipitate results from thermal denaturation of 3S and 7S globulins. Formation of this soluble aggregate was prevented by the thiol blocking agent, N-ethylmaleimide (NEM), indicating thiol-disulphide interchanges may be involved. However, since

NEM did not prevent the formation of the insoluble aggregates and the reducing agent, dithiothreitol (DTT) increased the formation of insoluble aggregates, it appears that reduction of disulphides is involved in the desolubilization process. This disulphide reduction did not result in the segregation of acidic and basic polypeptides into soluble and insoluble fractions (Ma and Harwalkar, 1988).

Thermal gelation of oat globulin, that is the formation of an ordered, three-dimensional network, has been produced at temperatures of 95-100°C, below the thermal denaturation temperature (Ma *et al.*, 1988). Gel hardness was decreased by the addition of DTT, NEM, SDS or urea, indicating thiol-disulphide interchanges as well as electrostatic, hydrogen-bonding and hydrophobic interactions are involved in the process of gelation. Lowering the thermal transition temperature by the addition of specific anions, ethylene glycol or fatty acid salts improved the gel-forming ability of oat globulin.

Prolamins. Although Osborne fractionation studies use 70% ethanol for oat prolamin extraction, a lower concentration (52%, v/v) more completely extracts protein (Kim *et al.*, 1978) and is frequently used for extraction of prolamins for PAGE (Fabijanski *et al.*, 1988; Hansen *et al.*, 1988; Robert *et al.*, 1983a, 1983b, 1985c). According to Robert *et al.* (1983a), a mixture of isopropanol and mercaptoethanol (ME) (55%, v/v, isopropanol; 2%, v/v, ME) more completely extracted prolamins than ethanol (52% v/v) and increased the estimated weight of the protein bands by 1-2 kDa; presumably a result of the breakage of intramolecular disulphides, since no new bands appeared.

Molecular weights of oat prolamins, estimated by amino acid composition or SDS-PAGE are generally grouped into three ranges: 14-16 kDa, 20-30 kDa, and 30-35 kDa (Fabijanski *et al.*,1988; Hansen *et al.*, 1988;), although bands with subunits greater than 43 kDa have also been observed (Robert *et al.*, 1983a, 1985c). Amino acid sequencing of prolamin fractions has identified two proteins with similarity to oat globulin: one approximately 22 kDa and fast-moving on Acid PAGE gels (Egorov, 1988; Kim *et al.*, 1978); and the other, 16 kDa, rich in sulphur-containing amino acids (Fabijanski *et al.*, 1988). Globulin contamination of prolamin

fractions has been identified (Kim *et al.*, 1978; Wieser and Belitz, 1989).

Lookhart and Pomeranz (1985) studied the electrophoretic and high performance liquid chromatography (HPLC) profiles of ethanol extracts of twenty-five oat samples representing fourteen oat species. An increased ploidy level was generally associated with an increase in the number of electrophoretic bands. Different accessions of a species did not always exhibit identical PAGE patterns, possibly because of a general lack of polymorphism in the proteins analyzed. Diploid species showed the greatest diversity in electrophoretic patterns. Hexaploids showed as much variation in patterns among accessions of one species as among species. HPLC results were consistent with those from PAGE.

Hansen and coworkers (1988) extracted prolamins from oat flour samples and single seeds. Cultivars of established purity whose prolamin patterns were not affected by growing conditions were chosen. Unlike the results reported by Lookhart and Pomeranz (1985), analysis by SDS-PAGE provided unique patterns that could be used to distinguish cultivars. A rapid, reproducible procedure using ultra-thin gels was reported.

Literature reports of IEF patterns of prolamins vary. Ma and Harwalkar (1984) identified three pH ranges: 6.0, 7.6 and 9.0. Robert *et al.* (1983a) reported values of 3-10, but most prolamins were in the range of 4.5-8.0. In comparison with the other solubility fractions, prolamins are highest in glutamate, proline and leucine; and lowest in lysine, aspartate and glycine (Wu *et al.*, 1972; Draper, 1973).

Glutelins. Glutelins can be described as soluble or insoluble: soluble glutelins are the proteins soluble in acid or base, following the sequential extraction of albumins, globulins and prolamins; insoluble glutelins are the proteins remaining in the residue following the extraction of soluble glutelins. Soluble glutelins exhibit acidic (pH 4.5-6.4) and basic (pH 9.0-10.0) isoelectric ranges, are largely insoluble below pH 9.5 and exhibit molecular weights of 10, 18 and 150 or greater kDa by column chromatography (Ma and Harwalkar, 1984). The quantity of soluble glutelin increases with the number of acid extractions and also the ionic strength of the acid (Wieser *et al.*, 1980). Complete extraction of all glutelins was accomplished at neutral pH with SDS and ME (Robert

et al., 1983b). SDS-extracted residual glutelin exhibited molecular weights of 14, 25, 30-45 and 50-70 kDa, as estimated by SDS-PAGE (Robert *et al.*, 1985c)

It has been shown that only a small portion of glutelin protein is unique in nature: glutelins are mostly composed of unextracted globulins in addition to small amounts of unextracted albumin and prolamin (Robert *et al.*, 1983b, 1985c). SDS-PAGE mobilities of insoluble glutelin proteins are similar to those of albumins, globulins and prolamins (Robert *et al.*, 1983b, 1985c); two-dimensional analysis (iso-electric focusing, pH 3.5-10.0 followed by SDS-PAGE) of glutelin proteins resulted in a pattern similar to the sum of globulin and prolamin patterns (Robert *et al.*, 1985c); Western blot analysis of glutelins revealed the major proteins in the residue were globulins (Robert *et al.*, 1985c).

c. Lipid. In his review of oat lipids, Hammond (1983) reported that 90% of oats contain between 5-9% lipid, with a mean value of seven percent. Karunjeewa *et al.*(1989), in their studies of 11 oat cultivars, found a range of 4-6% free lipid and 3-9% bound lipid. Sahasrabudhe (1979), in his study of six oat cultivars, determined that most oat lipids are in the form of neutral lipids (66-80%) or phospholipids (12-26%). The largest fraction of neutral lipids is triglyceride (43-56%), but it also includes partial glycerides (4-9%), free fatty acids (4-11%), steryl esters (2-4%) and free sterols (2-6%). Glycolipids were found to contribute 6-10% of the lipid fraction.

Several workers have examined fatty acid profiles of oat lipids. Variations exist in method of lipid extraction, type of lipid extracted, concentration of lipids, and fatty acid concentrations. Some of these variables are presented in TABLE 5.

Saastamoinen *et al.* (1989) compared the genetic and environmental variation in chloroform-methanol (2:1, v/v) extracted lipid content and fatty acid composition of oats grown in Finland. Although oleic (18:1) and linoleic (18:2) acid levels were very similar (37-43%), a negative correlation existed between them. Negative correlations were also observed between stearic (18:0) and eicosenoic acids (20:0). Linoleic and linolenic acids were positively correlated.

TABLE 5. Lipid Content and Fatty Acid Profiles of Oats

	14:0 %	16:0 %	18:0 %	18:1 %	18:2 %	18:3 %	20:1 %	22:1 %
FINNISH BREEDING TRIALS Saastamoinen et al., 1989								
chloroform-methanol (6.1-7.8% lipid)	ND	13.2-17.4	0.8-1.4	38.5-42.1	38.6-42.5	1.3-2.0	0.7-1.0	0.1-0.5
THREE CULTIVARS Karow et al., 1984								
chloroform-methanol-water (3.0-8.0% lipid)	ND	18.6-21.4	1.2-2.8	26.1-30.2	35.4-48.3	2.0-2.9	ND	ND
petroleum ether (3.0-8.0% lipid)	ND	20.0-22.3	1.7-3.8	28.9-42.7	31.4-43.6	2.0-3.0	ND	ND
11 AUSTRALIAN CULTIVARS Karunajeewa et al., 1989								
diethyl ether (3.8-6.1% free lipid)	ND	15.9-19.0	1.3-2.7	37.7-47.1	33.0-39.1	1.1-2.1	ND	ND
water saturated butanol (3.3-8.6% bound lipid)	ND	30.7-39.4	1.4-2.7	21.2-27.6	33.3-38.8	0.7-3.0	ND	ND
SIX CULTIVARS Sahasrabudhe, 1979								
chloroform-methanol (4.6-11.7% lipid)								
triglycerides (43.1-56.4% of lipid)	1.5	14.8	2.2	43.3	35.0	2.0	0.5*	0.1**
glycolipids (5.8-9.6% of lipid)	4.3	22.1	4.4	25.1	36.2	4.0	3.0*	1.2**
phospholipids (11.6-26.0% of lipid)	2.2	28.1	4.2	21.3	38.1	2.8	2.0*	0.3**

ND not determined

* includes 20:1-20:5

** includes 22:0, 22:1 and 24:0

Karow *et al.* (1984) examined the effects of different fat extraction procedures on the fatty acid profiles of three oat cultivars varying in lipid concentration. Greatest variations among cultivars were noted in linoleic and linolenic components. These fatty acids also varied the most with extraction procedure.

Karunajeewa and Tham (1989) examined the lipid and fatty acid compositions of Australian oats. Free lipids were soxhlet extracted with diethylether for 16 hours. Bound lipids were soxhlet extracted from the residue with water-saturated butanol for five hours. Lipid fatty acid compositions were examined. Free lipid values ranged from 3.8-6.1% with oleic, linoleic and palmitic acids predominating, in that order. Bound

lipid values ranged from 3.3-8.6% with high levels of the same fatty acids as found in free lipids, but with slightly lower oleic and higher palmitic levels.

Sahasrabudhe (1979) examined many aspects of oat lipids, including fatty acid profiles of triglycerides, glycolipids and phospholipids found in chloroform-methanol (2:1, v/v) soluble lipids of six oat cultivars. Mean fatty acid values are reported in TABLE 5. Approximately 50% of oat lipids were identified as triglycerides, less than 10% as glycolipids, up to 26% as phospholipids, and the remaining lipids were identified as partial glycerides or unsaponifiables. Levels of palmitic and oleic acids varied the most among the fractions studied.

d. Carbohydrate.

i. Fibre. Several methods exist to measure fibre. Crude fibre analysis was originally developed to analyze animal forages and involves digestion of the sample with sulphuric acid and sodium hydroxide. This was modified by Van Soest (1963) to eliminate the digestions, and instead extracted non-fibrous components with acid detergent, leaving cellulose, hemicellulose and lignin; or neutral detergent, leaving cellulose and lignin. As knowledge of dietary fibre has increased, so has the number of analytical methods. These methods can be generally categorized into either gravimetric or chemical identification.

Insoluble, soluble and total. Levels of dietary fibre (insoluble, soluble and total) in some oat products determined by gravimetric or chemical analysis are listed in TABLE 6.

Pak *et al.* (1989) examined the fibre levels of whole grain oats. The very high levels of insoluble fibre is an indication that this group of workers is unfamiliar with cereal science and considers whole grain oats to include hulls. Prosky *et al.* (1988) conducted an interlaboratory study of dietary fibre levels in various foods, including quick cooking flaked oats. Analyses of this product by the 13 collaborators resulted in a considerable range of results. Williams *et al.* (1991) have developed a near-infrared reflectance spectroscopy (NIR) calibration for oat bran using this method. Standard deviations for the wet chemistry fibre

TABLE 6. Dietary Fibre Profiles of Some Oat Products

	DIETARY FIBRE		
	INSOLUBLE	SOLUBLE	TOTAL
GRAVIMETRIC			
WHOLE GRAIN			
Pak et al., 1989	17.1	3.9	21.0
QUICK OATS			
Prosky et al., 1988	5.0–7.3	2.5–6.2	8.2–16.1
CHEMICAL			
OATMEAL			
Shinnick et al. 1988	7.2	4.9	12.1
Englyst & Cummings, 1988	3.5	5.0	8.5
ROLLED OATS			
Chen & Anderson, 1981	6.8	8.5	15.3
Anderson & Bridges, 1988	5.1	5.4	10.5
OAT BRAN			
Chen & Anderson, 1981	15.1	15.3	30.4
Anderson & Bridges, 1988	8.2	7.8	15.7
Shinnick et al., 1988	11.4	7.2	18.6

g/100g dry weight

components used to calibrate the NIR were less than 0.3, indicating the precision of the method that is possible when conducted by an experienced technician. The NIR gave highly reproducible results (standard error not greater than 0.3), with minimal sample preparation (only sample grinding required). The accuracy, precision and speed of this method makes it ideal for quality assurance purposes in oat mills. Commercially prepared oat bran, from four oat mills, contained a range of dietary fibre levels, as measured by NIR: insoluble fibre, 3.4-12.6%; soluble fibre, 3.8-7.7%; and total dietary fibre, 7.3-19.1%. Some of these commercial products would not be considered oat bran under the AACC definition which requires a minimum level of 16% total dietary fibre, of which at least one-third is soluble (Anonymous, 1989).

Shinnick *et al.* (1988) examined the chemical profile of dietary fibre of a sample of oatmeal and a sample of oat bran. The oat bran was higher

in insoluble, soluble and total dietary fibre. Total neutral sugar profiles of both products were similar: both containing high levels of glucose, and low levels of xylose, arabinose and mannose in the soluble fibre; and moderate levels of glucose, xylose and arabinose and minor levels of galactose/rhamnose and mannose in the insoluble fibre. Soluble fibre was mostly in the form of ß-glucans, whereas insoluble fibre was mostly in the form of neutral sugars and klason lignin.

Chen and Anderson (1981) compared the dietary fibre levels of a sample of oat bran with a sample of rolled oats. The oat bran contained fibre levels approximately twice as high as rolled oats. Insoluble fibre for both products contained high levels of insoluble polysaccharides, with cellulose and lignin also present. Anderson and Bridges (1988) also examined fibre levels of rolled oats and oat bran. All fibre levels for these products were lower than the fibre levels for products studied by Chen and Anderson (1981). The oatmeal sample examined by Englyst and Cummings (1988) contained low levels of dietary fibre.

ß-glucan. In his review of oat ß-glucan, Wood (1986) described a large (MW 250 kDa) unbranched polysaccharide chain composed of multiple tri- or tetra- (1-3)-linked ß-D-glucopyranosyl units joined by (1-4)-linkage. Minor features of this fibre may possibly include branching and longer runs of consecutive (1-4)-linkages or (1-3)-linkages. Oat ß-glucan is primarily located in endosperm cell walls, especially in the subaleurone layer where cell walls are thickest, but is also located in aleurone tissue. These glucans are possibly linked to protein. The molar ratio of tri- to tetrasaccharide of 2.1 has been calculated for oats and oat bran prepared either by dry sieving or sieving in aqueous ethanol (Wood *et al.*, 1991a). Groat or bran ß-glucans appear to be similar in size (3,000 kDa) and become smaller with increased levels of processing for cereals (2,500-600 kDa) or purification steps of oat gum into ß-glucan (2,200-350 kDa) (Wood *et al.*, 1991c).

The ß-glucan contents of some oat products are listed in TABLE 7. Except where otherwise indicated, all values are for water-soluble ß-glucans. Those marked total ß-glucan are soluble in NaOH (1N).

TABLE 7. Beta-glucan Content of Some Oat Products

	WHOLE GROAT	BRAN	ENDOSPERM	ROLLED	PROCESSED BRAN CEREALS
11 CULTIVARS Wood et al., 1991b	4–7	6–9			
COMMERCIAL PRODUCTS Wood et al., 1991c		6–7		3	2–4
6 CULTIVARS Welch et al., 1991	3–5				
2 CULTIVARS Henry, 1987	4		1–2		
COMMERCIAL PRODUCTS Carr et al., 1991		3–4 total beta-glucan 7–9		2 total beta-glucan 2–5	1–2 total beta-glucan 2

water soluble beta-glucan, unless otherwise indicated
g/100g dry weight

Wood *et al.* (1991b) examined 11 cultivars and found the bran, which included subaleurone, to contain a higher concentration of β-glucan than the whole groat. There was only a weakly significant correlation between groat β-glucan and bran β-glucan (r^2=0.823). Some whole groats contained levels of β-glucan as high as bran, indicating they could replace oat bran in diets designed to decrease cholesterol level. Wood *et al.* (1991c) examined β-glucan levels of commercial products. Rolled oats and processed bran cereals were lower than oat brans in β-glucan content. Welch *et al.* (1991) examined six cultivars at two levels of nitrogen fertility. β-glucan levels showed a positive correlation to protein levels. Henry (1987) dissected oat samples of two cultivars to determine endosperm β-glucan levels. Carr *et al.* (1990) examined different commercial products for soluble and total β-glucan. There was a considerable difference between the two values in oat brans, indicating the presence of insoluble β-glucan; but with increased processing of some rolled oats and bran cereals, the level of insoluble β-glucan decreased.

Several methods and many variations of methods exist to measure β-

glucans. In general, ß-glucans are broken down by enzymic digestion and measured as glucose. However, possible contamination of starch must be considered. This has been accomplished in several ways.

The AACC method (32-22) (based upon the experiments of McCleary and Glennie-Holmes (1985) and McCleary and Nurthen (1986)) requires starch gelatinization, lichenase digestion (endo-1,3;1,4-ß-D-glucan-4-glucanohydrolase) to release the tri- or tetrasaccharides, ß-glucosidase digestion to free the glucose units, and measurement of the glucose released by absorption after glucose oxidase/peroxidase digestion. The effects of starch contamination are eliminated by subtraction of results from a duplicate control, not digested with lichenase or ß-glucosidase. Procedural steps of this method have been modified by McCleary and Codd (1991) to increase efficiency and also include a possible extraction with sodium hydroxide. Results of ß-glucan content were unchanged by the manner of extraction.

Åman and Hesselman (1985) chose another route to eliminate the contaminating effects of starch. Starch was depolymerized after gelatinization by the use of α-amylase and amyloglucosidase and separated from the residue by washing and filtering. A ß-glucanase, very low in cellulase activity, depolymerized ß-glucans in the residue to glucose, which was then measured by the glucose oxidase method.

Henry and Blakeney (1988), reduced all endogenous reducing sugars to alditols with sodium borohydride, hydrolysed ß-glucans with (1-3),(1-4)-ß-glucanase and measured the reducing sugars produced by the coloured reaction with p-hydroxybenzoic acid hydrazide.

Carr *et al.* (1990) removed sugars by refluxing with ethanol, extracted ß-glucans from the residue with either boiling water or sodium hydroxide, reduced the solubilized ß-glucans to glucose with cellulase and measured the glucose with the glucose oxidase method.

Two methods have been reported based on the use of automated flow injection systems. The method of Munck *et al.* (1989) is based upon the ability of high molecular weight ß-glucans (>100 kDa) to complex the fluorochrome, Calcofluor, in solution; thus increasing the fluorescence intensity, which can be measured. The method of Wood *et al.* (1991a) used a flow injection system linked to an HPLC to measure the methylated

oligosaccharides produced following lichenase digestion. (Starch was first removed by α-amylase digestion and ethanol extraction.)

Pentosans. Oat pentosans have not been extensively researched. Henry (1987) examined arabino-xylans in groats and endosperm. Groats contained higher levels (6%) than endosperm (1%). This study also examined β-glucans and found the ratio of pentosan to β-glucan was less in the endosperm (0.4) than in the groat (1.5). Various levels of pentosans were identified by MacArthur and D'Appolonia (1980) in ammonium sulphate fractions of water-soluble nonstarchy polysaccharides. Pentosan content increased with increasing concentrations of ammonium sulphate.

ii. Starch. Up to seventy percent of the oat groat is starch (Paton, 1977; MacArthur and D'Appolonia, 1979). In his review of oat starch, Paton (1986) described the following characteristics. Starch granules are in the size range of 7-10μ, irregular in shape and often polyhedral. Weakly birefringent, they tend to exist intracellularly as clusters (30-60μ in diameter) neighbouring proteins. In their review of oats, Youngs *et al.* (1982) reported the amylose concentration of oat starch varies between 19-28%.

3. Enzymes

Lipase catalyses the removal of free fatty acids from lipids - primarily triglycerides, the major component of oat lipids. In his review of oat lipids and enzymes, Youngs (1986) described optimum lipase activity conditions as 35-39°C and pH 7.4. Activity is influenced by moisture; lipids show little change at moisture levels below 10%. Most lipase activity (70-80%) is associated with the outer layers of the groat (Urquhart *et al.*, 1983). At moisture contents of 50-100%, macerated oat groats achieved maximal free fatty acid production within 30 min of incubation at 37.5°C (Sahasrabudhe, 1982).

Lipoxygenase catalyzes the oxidation of polyunsaturated free fatty acids (eg linoleic acid) to the more saturated and unstable hydroperoxides in the presence of moisture and oxygen. This reaction can also occur non-

enzymically by the autoxidation of free fatty acids in the presence of oxygen, light, or free radical-producing substances. Lipoxygenase, in the presence of moisture, continues its catalytic activity on the hydroperoxides to form bitter hydroxy acids (Youngs, 1986; Vorwerck, 1988).

Like lipoxygenase, peroxidase is able to catalyze the breakdown of hydroperoxides. In this process, an electron donor (ascorbate, phenol, amine or other organic compound) is oxidized, often to a coloured product (Vorwerck, 1988; Youngs, 1986).

Maltase and α-amylase are found in very low levels in oats which have been properly harvested and stored (Fulcher, 1986). The presence of several glucanases has been determined in oats (Youngs *et al.*, 1982).

D. Oat Processing

1. Methods

Oat processing is the subject of numerous publications (Western and Graham, 1961; Webster, 1983; Deane and Commers, 1986; Hoseney, 1986; Pomeranz, 1987; Seibert, 1987; Von Werner Rohde, 1987; Vorwerck, 1988; Caldwell *et al.*, 1990; Stauffer and Caldwell, 1990; McMullen, 1991; Tribelhorn, 1991; Vorwerck, 1991). These processes can be summarized into four steps: clean oats, remove hulls, condition groats, and reduce particle size (FIGURE 3). Although they generally occur in the order listed, many variations exist among companies as well as within a single company for each of these steps.

Oats are cleaned to remove foreign material such as straw, insect parts, stones, weed seeds and other grains. A variety of separators exist to accomplish this process. As they are cleaned, the oats are also separated according to length and width for ease of further processing. Cleaned oats may be dried at this point to reduce the moisture content from approximately 12% to 7-10%. This drying process reduces the lipase activity 60-80% and also causes the hull to become more brittle to facilitate its removal.

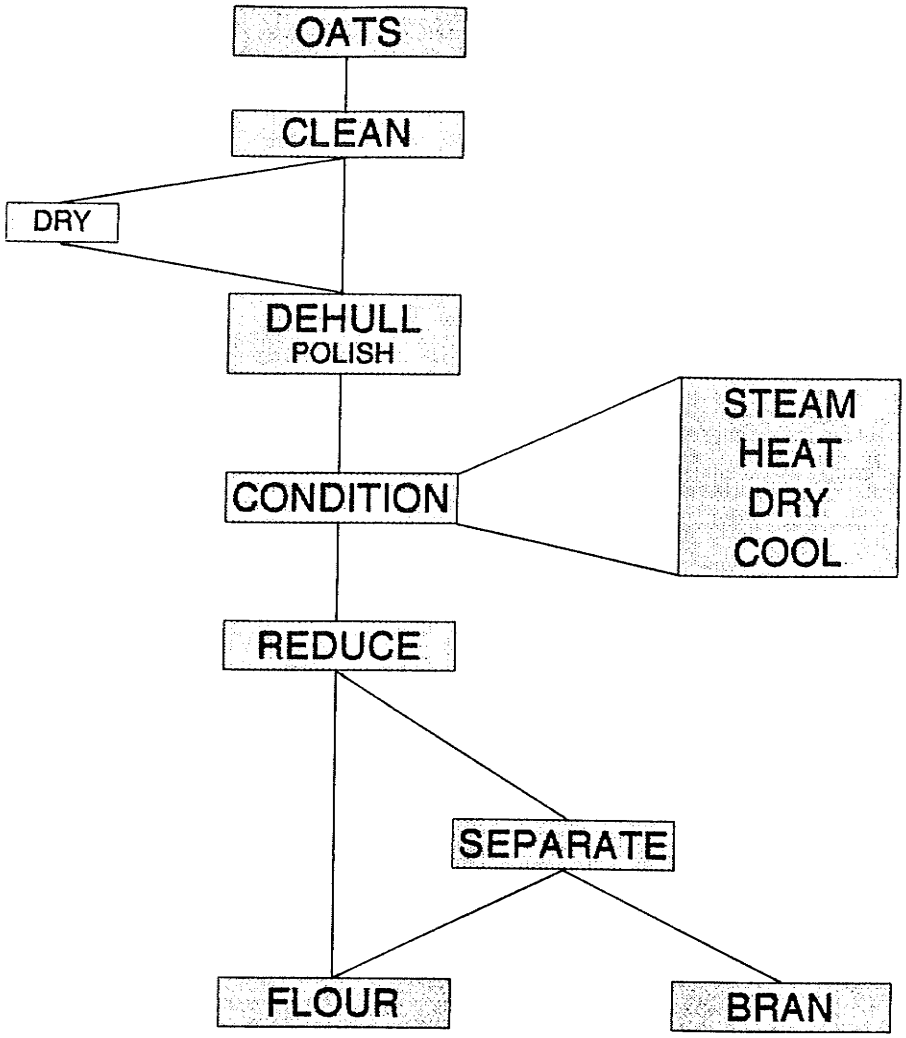


FIGURE 3. Processing oats into flour or bran.

The hull is the seed coat of the oat kernel. Because of its dry, brittle, cellulosic nature, it is not included in oatmeal products for human consumption and must be removed before further processing of the oat. This can be achieved by either an abrasive or an impact process. Following the removal of the hull, the oat is referred to as a groat. Before the conditioning process, the groat is sometimes referred to as a green groat - green referring to unconditioned, not the colour. The next step is usually to polish the groat and remove the trichomes.

Conditioning of green groats usually includes four steps: steam, heat,

dry and cool. During conditioning, enzymes are inactivated and groats develop a roasted flavour and appearance. Time, temperature, and moisture content are variables that can be manipulated in the conditioning process.

Many variations exist in the particle reduction stage of processing, depending on the product desired (FIGURE 4). To produce oat flakes, the groats are usually cut into smaller pieces called cutmeal. Depending on the type of flakes desired, the cutmeal may be graded according to size before being rolled into flakes. Usually, the cutmeal is steamed before

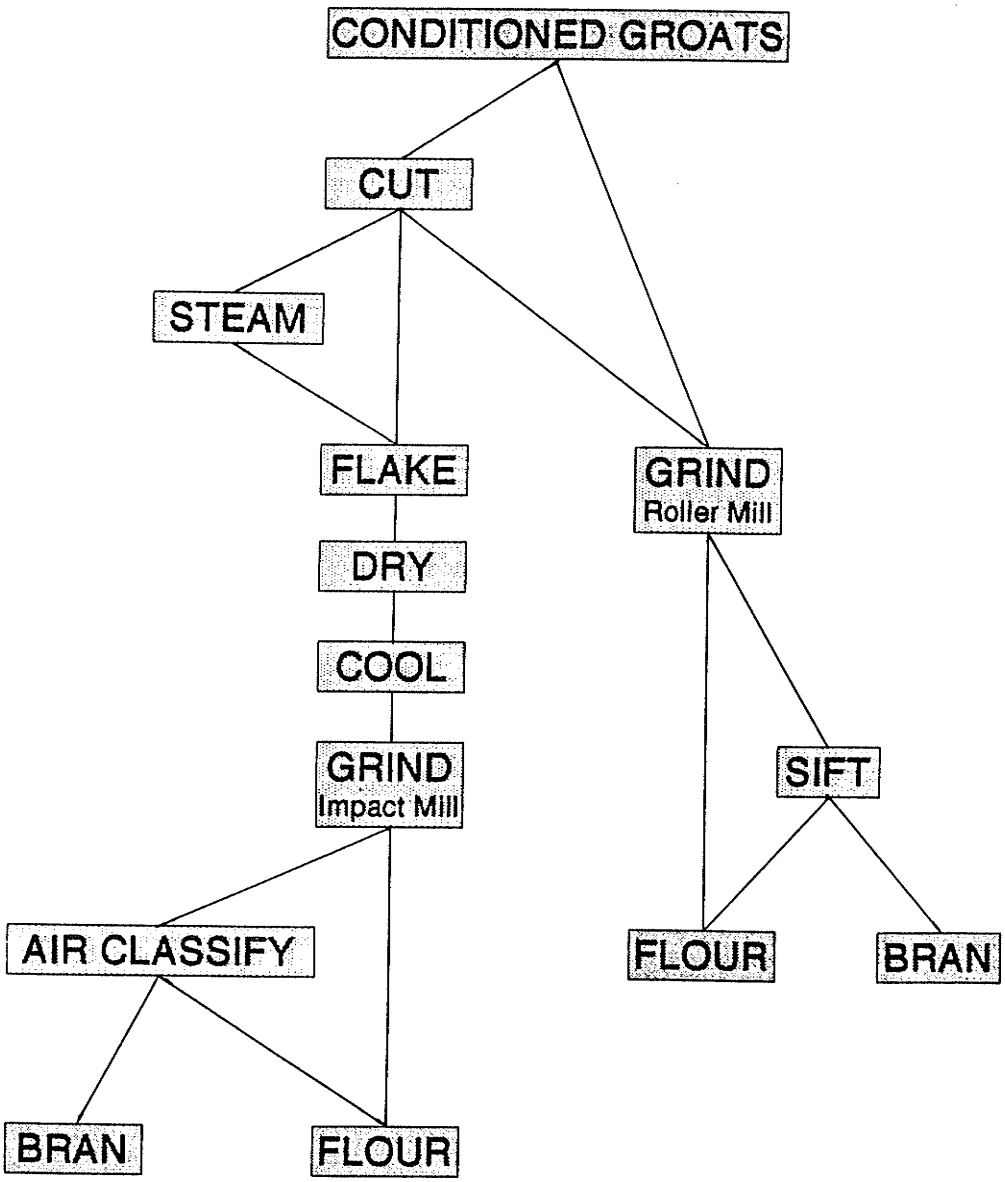


FIGURE 4. Reduction of conditioned groats into flour or bran.

rolling to soften the groats and permit the formation of flakes with minimal breakage. Steamed flakes are dried and cooled before packaging.

Because oat bran is an integral part of the oat kernel, the production of oat bran is not just a simple separation procedure. Two methods exist for the production of oat bran. In one method, groats or cutmeal may be passed through a roller mill, or series of roller mills, and the largest pieces removed by sifting for use as oat bran. In the other method, flakes are ground with an impact mill and an air classification system removes the bran. In either case, by the definition of oat bran, up to 50% of the oat groat may be included as bran with at least a level of 16% total dietary fibre (TDF) on a moisture free basis. At least one-third of the fibre must be soluble (Anonymous, 1989). Oat bran includes the aleurone and may include some attached endosperm.

The product not included in oat bran becomes flour, with the possibility that further size reduction processes may be required for specifications of the finished product. The production of whole oat flour begins with cutmeal or oat flakes and may include the fines produced during flaking. Size reduction is accomplished with either an impact mill or a roller mill. A series of sieves insures particle sizes of generally less than 850μ.

2. Chemical Changes

The processing of oats includes heat treatment to inactivate lipase, and thereby inhibits the development of lipid rancidity. This heat may be moist or dry. Oomah (1987) studied the effects of heat and steam on processing milling oats into oat flour. Oats were steamed, then dried before being cut, flaked and roller milled into flour. Lookhart *et al.* (1986) examined the effects of drying, then steaming in the production of oat flakes.

Heat treatments decrease enzymatic activity. Vorwerck (1988) examined lipase and peroxidase activity in response to different heat treatments. At 100°C and 16% moisture, steam treatment for 10 min inactivated lipase. Under the same conditions, peroxidase was inactivated in half the time - 5 min. Enzyme inactivation is related to oat moisture content: at 80°C,

in oats of 10% moisture, little change was noticed in lipase activity, even after two h; in oats of 14% moisture, two h almost eliminated lipase activity; and in oats of 20% moisture, lipase was inactivated after 10 min of heat treatment. Von Werner Rohde (1987) described a process in which the moisture of groats was increased to 17%, and groat temperature was rapidly increased to 80°C and then gradually to 100°C. In this process, lipase was inactivated in less than 60 min and peroxidase inactivated in less than 45 min. Although a test for peroxidase activity is frequently performed in oat mills as an indicator of lipase activity, it is evident from these tests (Von Werner Rhode, 1987; Vorwerck, 1988) that lipase may still be active, even though peroxidase is inactive.

Heat treatments denature oat proteins. As a result of solubility studies with SDS, Oomah (1987) proposed that steam treatment of oats promoted the aggregation of high molecular weight protein subunits through non-covalent bonds. Further heat, in the form of a drying stage, formed covalent bonds that decreased protein solubility, even at high soap concentrations. Protein denaturation was confirmed through DSC studies. Lookhart *et al.* (1986), through scanning electron microscopy (SEM), noted that protein bodies of groats remained intact after the initial drying step. Fewer protein bodies were visible following the flaking procedure. Electrophoretic bands of avenins in this study were unaltered by drying or flaking, suggesting they are not involved in the covalent bonds described by Oomah (1987).

Heat treatments alter oat carbohydrates. Lookhart *et al.* (1986) noted that cell wall structures remained intact following dry heat treatment, but steaming and flaking led to fragmentation and separation from other cell components. The drying process fractured some compound starch granules. This effect was enhanced by flaking. Oomah (1987) noted a decrease in peak pasting viscosity following steaming, but an increase after drying. DSC thermograms indicated steamed groats were 24% gelatinized, and dried groats, 26%. Heat treatment of groats, moist or dry, decreased both susceptibility to shear and setback properties of oat pastes.

Heat treatments alter oat lipids. Extraction of petroleum ether soluble lipids varied with the heat treatment and oat genotype in the

study by Lookhart *et al.* (1986). This may be explained in part by the behavior of amylose-lipid complexes explored by Oomah (1987). Steam treatment of milling oats led to a change in the DSC enthalpy peak related to the amylose-lipid complex. The one, broad peak found in the milling oat was found as three peaks in the steamed groat. The drying process applied to the steamed groat led to a reappearance of a smaller version of the enthalpy curve displayed by the milling oat. Enthalpy was the least in dried groats and greatest in oat flour. It was demonstrated that amylose-lipid complexes dissociate at high temperatures and reform on cooling. Rerunning the DSC endotherms led to increases in enthalpy for groats and steamed groats, but decreases for dried groats and cutmeal, implying changes had occurred in the amylose-lipid complexes.

3. End Product Uses

a. Flakes. McKechnie (1983) reviewed the use of oat products in bakery foods. Oat flakes are used in cookies, granola, whole grain breads and snack foods. The size and thickness of the flake as well as the method used to add the oat product to the dough or batter affects water absorption properties and the appearance and texture of the finished product.

Oat flakes may also be included as a thickening agent in soups, gravies and sauces; added to meat loaf and meat patties as a meat extender or cooked as a hot cereal (Webster, 1986). The method of cooking oat flakes can alter the functional behavior of oat flakes: a slow cooking method induced more structural damage to cell walls and increased the release of ß-glucan from the cooked oatmeal than rapid cooking (Yiu *et al.*, 1987). The slow-cooked product was therefor of creamier texture. Microwave cooking has been found to produce a more grainy and less viscous porridge than that produced by conventional cooking (Yiu *et al.*, 1991).

b. Bran. Oat bran may be cooked as a porridge, or used as an ingredient in hot or cold cereals. In addition, oat bran may be used as an ingredient in breads, cookies and muffins (Seibert, 1987).

c. Flour. Oat flour is an ingredient in some breads, cookies, infant foods and breakfast cereals (McKechnie, 1983;Webster, 1986). It is a main ingredient in frescavena, a South American beverage (Webster, 1986). In addition, both debranned and whole oat flour act as substrates for the production of Oatrim, a soluble oat fibre containing β-glucan in amounts similar to that in oat bran (Duxbury, 1990). Oatrim is bland in flavour and odour, readily dispersible in cold water, and stable at baking and pasteurization temperatures. However, the product is unstable at temperatures used for frying. When heated to form a gel, it has potential as a fat substitute for use in reduced-fat cheese spreads and low-fat frozen dairy desserts. Other food applications of Oatrim include its use in breads, breakfast drinks and diet drinks.

E. Functional Properties of Oat Flour or Oat Bran

1. Pasting

A commercial oat flour, steam-treated and hammer-milled was compared to a laboratory-prepared oat flour, roller-milled and bran removed (Oomah, 1983). Steamed flour demonstrated earlier swelling, higher peak viscosity and increased setback. Upon cooling, the viscosity of steamed flour plateaued for ten degrees beginning at 70°C, before climbing to a higher plateau at 45°C. Unsteamed flour plateaued earlier, at 75°C, and varied only slightly for the duration of cooling. As 25% oat-wheat composites, both oat flours were very similar in response to pasting, but the steamed flour was more viscous. Steam treatment of oats was found to increase the formation of mucilaginous compounds and thereby increase viscosity. Similarly, unsteamed wild and domestic oats, in levels up to 5% with wheat, also increased peak viscosity (Dexter *et al.*, 1984).

2. Farinograph and Mixograph

Farinograph properties of oat-wheat flour blends have been reported for a variety of experimental conditions. Heat treatment and particle size have been shown to affect farinograph characteristics. Steam-treated oat brans increased water absorption and produced stronger, more stable doughs in comparison with unsteamed oat brans (D'Appolonia and Youngs, 1978). In the same study, steamed bran with water-solubles removed was the most stable. Wild and domestic oats, in wheat flour at levels up to 5%, like the unsteamed oat brans, decreased water absorption and produced weaker and less stable doughs than wheat flour alone (Dexter *et al.*, 1984).

Krishnan and coworkers (1987) studied oat bran separated from commercial oat flour. Like the steam-treated brans of D'Appolonia and Youngs, an increased farinograph absorption and a more stable dough resulted with increasing concentration of oat bran or decreasing size of bran particles. Dough strength, as measured by dough development time, was inversely related to particle size of the oat bran but directly related to the concentration of small and medium brans, unlike large sized brans which exhibited varying effects with bran concentrations.

Oomah (1983) reported that mixograph studies of oat flours, mixed with wheat at levels up to 50%, resulted in dough weakening. An increased mixing tolerance also noted was suggested to be related to the presence of oat lipids.

3. Extensigraph and Wet Gluten

The response of oat-wheat composites to extensigraph and wet gluten evaluations have also been conducted. Both wild and domestic oats, at levels up to 5% with wheat, were found to decrease extensigraph maximum height and area; a more extensive decrease was noted for domestic oats (Dexter *et al.*, 1984). Domestic oats increased extensigraph length and wild oats decreased it, in comparison to the control. Both oat varieties were also found to depress wet gluten content.

4. Fat Binding Capacity

Fat binding has been studied in commercially steam-treated wild or domestic oats, defatted and full fat (Chang and Sosulski, 1985); deamidated oat protein isolates (Ma and Khanzada, 1987); and heat modified (5 min, 75°C) or enzyme modified, lab-prepared, oat flour (Ponnampalam *et al.*, 1987). These authors all used the same method to examine this quality (Lin *et al.*, 1974), which is based on the ability of flour to bind corn oil (oleic:linoleic approximately 2:1). In this method, flours are mixed with excess corn oil, allowed to settle and centrifuged. The amount of oil bound is measured by difference. A comparison of these papers allows an examination of the effects of protein, water holding capacity and colour on the fat binding capacity (FBC) of oats.

FBC of oat protein isolate (182%) was higher than oat flour (62%), indicating that protein is associated with FBC. Examination of flours prepared from fractions of commercially steam-treated groats (domestic, wild or defatted wild groats) showed bran to be the fraction highest in FBC. Brans were also higher in protein. Although bran from wild groats was higher in protein than bran from domestic groats, wild groat bran was not higher in FBC, indicating other factors, in addition to protein levels, are associated with FBC.

In addition to achieving the highest levels of FBC, the bran fraction from domestic, wild or defatted wild groats was also highest in water holding capacity (WHC). Within each groat classification, FBC and WHC appeared to be directly related: the fraction highest in FBC was also highest in WHC, the fraction lowest in FBC was also lowest in WHC. Mild heat treatment of oat flour (5 min, 75°C) increased both FBC and WHC. FBC and WHC were also directly related in deamidated oat protein isolates: both increased with increasing deamidation to a maximum, beyond which further deamidation decreased both FBC and WBC. This relationship of FBC and WHC was reversed in oat flours treated with protease and glucanase: enzyme hydrolysis decreased FBC but increased WHC, in comparison to the untreated flour. Treatment with protease only, no glucanase, revealed a different relationship between FBC and WHC: enzyme hydrolysis had no effect on FBC, but increased WHC, in comparison to the untreated flour.

FBC of commercially heat treated flours is directly related to the

darkness of flour colour, as measured by an Hunterlab (Chang and Sosulski, 1985). In wheat based breakfast cereals, it was found that Hunter L values were directly related to available lysine (Maillard browning decreased L and available lysine) and inversely related to lignin content (an increase in Maillard reaction products) (McAuley *et al.*, 1987).

5. Breadbaking

The effects of the addition of oat products to wheat flour on the breadbaking potential of the composite flour has been studied. Wild oat flour (Dexter *et al.*, 1984), wild oat bran (Krishnan *et al.*, 1987), oat bran (D'Appolonia and Youngs, 1978; Krishnan *et al.*, 1987 and Sosulski and Wu, 1988) and oat flour (Oomah, 1983; Dexter *et al.*, 1984 and Oomah and Lefkovitch, 1988) have a depressing effect on bread loaf volume.

Several variables can be manipulated to optimize loaf volumes. Prehydrating wild oat bran for 20 h at 4°C increased loaf volume and water absorption (Sosulski and Wu, 1988). Sodium stearoyl lactylate, added at a 0.05% level, was shown to increase the loaf volume of breads containing oat bran (D'Appolonia and Youngs, 1978), oat flours (Oomah, 1983), and wild oat bran (Sosulski and Wu, 1988). Loaf volumes of bread from wheat flour containing up to 25% roller-milled or hammer-milled oat flour were improved by the addition of ascorbic acid (a common bread improver); optimum loaf volume was obtained at 75 ppm (flour basis) addition (Oomah, 1983). In contrast, loaf volumes of bread from wheat flour containing up to 10% commercial oat flour (as opposed to roller-milled or hammer-milled oat flour) were not optimal at 75 ppm ascorbic acid, but instead required decreased ascorbic acid levels with increasing oat flour dilution (Oomah and Lefkovitch, 1988).

The effect of potassium bromate (an approved bread dough improver) on breads baked from oat-wheat composite flours has been studied, and exhibited a variety of effects on loaf volumes presumably as a result of the diversity of oat products tested and manipulation of other factors concomitantly. Steam-treated, hammer-milled oat flour added to wheat produced breads that were negatively affected by potassium bromate, but roller-milled oat flour, bran removed and no heat treatment could handle

10 ppm bromate with no deleterious effects (Oomah, 1983). Oat brans of small, medium and large particle size added to wheat flour and including 15 ppm bromate, produced breads with improved loaf volume, as well as crumb grain and texture in comparison with breads produced without bromate (Krishnan *et al.*, 1987). Wheat breads baked with bran from wild oats at levels up to 15% dilution demonstrated higher loaf volumes with the addition of 45 ppm potassium bromate (Sosulski and Wu, 1988). Breads baked from wheat with added commercial oat flour demonstrated no set pattern of effects with potassium bromate when both ascorbic acid levels and water absorption were also varied (Oomah and Lefkovitch, 1988).

F. CONCLUSIONS

The above literature review indicates that a considerable amount of nutritional, chemical and technological research has been carried out in oats. However, there has been essentially no reported work on commercial oat flours produced as a by-product of the oat bran milling industry. Such a study forms the basis of this thesis.

III. MATERIALS and METHODS

A. Materials

Samples of oat flour were provided by Ogilvie Mills Ltd., The Quaker Oats Company of Canada Ltd. and Robin Hood Multifoods Inc.; each providing two samples milled to company specifications. They are identified only as Flour A, B, C, D, E or F in the text to provide anonymity for the mills. Flours A and B were provided by the same mill, Company AB, Flours C and D are from Company CD and Flours E and F are from Company EF. Composite oat-wheat flours were prepared in 600 g batches by blending a hard red spring wheat flour (protein, 15.8%, N x 5.5; ash, 0.67%; dry basis) at 5%, 10% and 20% oat flour levels on an as-is basis for each of the six oat flours. A sample of unsteamed groats (G) was provided by Ogilvie Mills Ltd. for Osborne fractionation and comparative enzyme analyses.

B. Chemical Analyses

1. Moisture

All sample dilutions (oat:wheat; 0:100, 5:95, 10:90, 20:80, 100:0) were analyzed for moisture in a Brabender, semi-automatic, Rapid Moisture Tester using the AACC standard method 44-15A (AACC, 1986) in which flours are heated (130°C, one h) for a gravimetric determination of moisture.

2. Ash

All sample dilutions were incinerated (560°C) according to the AACC method 08-01 (AACC, 1986) for gravimetric ash determination.

3. Protein

a. Percent. AACC method 46-12 (AACC, 1986) was used to determine Kjeldahl nitrogen in all sample dilutions. In this method, amino nitrogen is converted to ammonium sulphate in the presence of sulphuric acid. During digestion, an ammonium complex is formed and decomposed into ammonia which is then distilled from an alkaline medium into a boric acid solution. The concentration of ammonia is determined by titration and this value used to calculate the concentration of nitrogen. According to Tkachuk (1969), the conversion factor of 5.5 was applied to the nitrogen value to calculate protein. The means of duplicate or triplicate analyses are reported.

b. Amino acid composition. The method of Andrews and Baldar (1985) was used to analyze all oat flours for amino acid levels. Samples were hydrolysed in 6N HCl:phenol (1:1, v/v) under reflux (110°C, 23 h). The cooled hydrolysate was diluted with sodium citrate buffer, and adjusted to pH 2.2 with sodium hydroxide. The filtered samples were analyzed for amino acids on an LKB 4151 Alpha Plus Amino Acid Analyzer with the modified buffer sequence and column temperature of Mills *et al.* (1989) to reduce elution times. This system utilizes a cation exchange column. Amino acids were detected by ninhydrin reaction (absorbance at 570 nm for amino acids, 440 nm for imino acids).

Acid hydrolysis causes partial destruction of sulphur containing amino acids. For this reason, the flour is oxidized prior to analysis of these acids and cysteine and cystine are quantified as cysteic acid. Methionine is quantified as methionine sulphone and methionine sulphoxide. The oxidation step begins with incubation (30°C, one h; 0°C, 16 h) of the flour with hydrogen peroxide (30%, v/v) and formic acid (88%, v/v). Excess reagent is degraded by adding sodium metabisulphite. The sample is then prepared for acid hydrolysis as described above. The means of

duplicate analyses are reported.

c. Osborne fractionation. A modification of Chen and Bushuk (1970) was used to determine the albumin, globulin, prolamin and acid soluble and insoluble glutelin contents from oat flours A, C, D, E, F, and ground, unsteamed groats, G. A 10:1 solvent to flour ratio (v/w) was used according to Ma (1983) for extractions (4°C, two h, centrifuge (19,690*g*, 20 min) and decant) which were repeated (modification; one h extraction).

Samples were extracted first with 0.5 M sodium chloride and then with distilled water (4°C, 30 min). The collected solutions were dialysed, to remove the salt, against numerous changes of distilled water (4°C, 3 d) in tubing with a molecular weight cutoff of 12-14 kDa. Centrifugation (19,690*g*, 20 min) separated the globulins, as residue; from the albumins, contained in the supernatant. The residue remaining after salt extraction was extracted with 70% ethanol (v/v) and the ethanol removed from the centrifuged supernatant with a rotary evaporator. The salt and alcohol extracted residue was further extracted with 0.05 M acetic acid solution. Centrifugation separated the acid-soluble glutelins, found in the supernatant from the acid-insoluble glutelins, contained in the residue. The five fractions were freeze-dried, analyzed for protein using the Micro-Kjeldahl method (AACC method 46-13, 1983), and protein contents of fractions expressed as a percentage of recovered protein. The means of duplicate or triplicate analyses are reported.

d. Acid polyacrylamide gel electrophoresis. Oat flour proteins soluble in 70% ethanol (v/v), the prolamins, were analyzed by polyacrylamide gel electrophoresis (PAGE) on a 7.5% acrylamide gel at pH 3.1 (aluminum lactate buffer) according to the method of Lookhart (1985). A constant voltage of 500 volts was applied to the gels for two hours before staining overnight with a solution of trichloroacetic acid (50%, w/v) and Coomassie Brilliant Blue. Prolamins from a wheat sample of the variety, Neepawa, were analyzed concurrently to provide a pattern for comparison.

e. Sodium dodecyl sulphate polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) was performed according to the method of Ng and Bushuk (1987) on all oat flours, and composites of Flour D. Because prolonged contact with SDS is capable of dissociating oat globulin, even without the addition of reducing agents, (Harwalker *et al.*, 1989), only freshly prepared protein samples were analyzed. Flour proteins were extracted into Tris buffer (pH 6.8) containing glycerol, SDS and Pyronin Y. Reduced protein samples were prepared by the addition of mercaptoethanol to the extraction solution. Unreduced and reduced protein extracts were plated on 17.3% gels (acrylamide and bisacrylamide) with a 3% stacking gel (acrylamide and bisacrylamide) and electrophoresed at 20°C at a constant current (5 mA, two h; 10 mA, 18 h; 15 mA, 2 h). Gel proteins were stained overnight with an acidic solution of Coomassie Brilliant Blue G-250. Molecular weights of flour proteins were estimated by comparison of mobilities of stained bands to a standard curve prepared from the mobilities of standard proteins of known molecular weights. All oat flour, wheat and composites of Flour D were examined.

4. Lipid

a. Free lipid. A 20.000 g flour aliquot, on an as is moisture basis, was dried (103°C, three h) before soxhlet extraction (16 h) with 175 mL hexane in a dried, tared flask. The residue remaining following solvent removal with a rotary evaporator was defined as free lipid. This method was based on that of Sahasrabudhe (1979). All oat flours were analyzed.

b. Bound lipid. Following the extraction of free lipid, a similar process was conducted on the residue. Aqueous methanol (85%, v/v) replaced hexane as the solvent for the extraction of bound lipids. All oat flours were analyzed.

c. Total lipid. Total lipid is estimated as the sum of free lipid and bound lipid.

d. Non-polar, glyco- and phospholipids. Based on the methods of Békés *et al.* (1983), and Sebedio *et al.* (1986); a weighed aliquot of free lipid (0.15 g) was dissolved in 1 mL of chloroform and applied by pipette to the surface of a florisil sep-pak (Waters Associates), previously wetted with 5 mL of chloroform. The lipid-containing glassware was rinsed with 1 ml of chloroform and applied to the sep-pak by pipette. Chloroform (8 mL) was forced through the sep-pak column by means of a syringe (taking precautions to preclude air bubbles) into a dried, tared flask. In a similar manner, acetone (10 mL) and methanol (10 mL) were applied to the column and eluted into separate, tared flasks. The flasks were evaporated to dryness over a steam bath, vacuum dried (35°C, overnight), transferred to a desiccator and weighed when cool. The contents of the chloroform flask were identified as non-polar lipids, the contents of the acetone flask as glycolipids, and those of the methanol flasks as phospholipids. Duplicate analyses were performed for each lipid sample. Oat Flours B, C, D and E were analyzed.

e. Fatty acid composition. Free lipid extracts of all oat flours were dissolved in heptane. Fatty acids, bound to glycerol and contained in the free lipid, were transesterified to methyl ester derivatives by sodium methoxide methanolysis (Houghen and Bodo, 1973). In this method, sodium in methanol (0.02N) is added to the lipid sample, refluxed (10 min), cooled to room temperature, and neutralized by the addition of acetic acid in petroleum ether (0.2N). Water is added to wash the ether layer by swirling. An aliquot of the ether layer, containing methyl esters of fatty acids, is decanted and evaporated. This procedure does not release amide-bound fatty acids, such as those attached to sphinogolipids, and does not esterify free fatty acids.

The resulting methyl esters of fatty acids were identified according to AACC method 58-18 (AACC, 1986), with modifications. Samples were automatically injected into a Perkin-Elmer 8320B Capillary Gas Chromatograph equipped with a 10 m fused silica capillary column which contained the stationary phase DB225 (J. and W. Scientific, Inc.). Helium was used as the mobile phase. Detection was by hydrogen flame ionization. Comparison of peak retention times of fatty acid esters to those of known

standards allowed identification of peaks. Quantification of fatty acids was achieved by a mathematical formula involving the integrated peak areas and calibration factors for each fatty acid.

5. Carbohydrates

a. Neutral detergent fibre. All oat flours were analyzed for neutral detergent fibre (NDF) with modifications to the method of Mongeau and Brassard (1986) using the Tecator Fibertec E system. Flour samples were refluxed with neutral detergent solution (sodium lauryl sulphate, 2-ethoxyethanol, disodium ethylene diamine tetra-acetate, sodium tetraborate and sodium phosphate dibasic) and then filtered. The filtrate was digested with α-amylase for five min at 55°C, filtered and washed with hot distilled water. The filtrate was again digested with α-amylase at 55°C, this time for a period of one h, filtered, washed with hot distilled water and then washed with acetone. The residue was dried and the weight reported as NDF, the water insoluble fibre. The means of duplicate analyses are reported.

b. Soluble dietary fibre. The water soluble dietary fibre (SDF) content of each oat flour was analyzed with modifications to the method of Mongeau and Brassard (1986) using the Tecator Fibertec E system. Samples were autoclaved in an acetate buffer (pH 4.5) to gelatinize the starch, cooled to 55°C, digested with heat stable amylase, and filtered. The filtrate was digested by amyloglucosidase and then protease. Addition of anhydrous ethanol (80%) precipitated soluble fibre. The solution was filtered, and the precipitate dried and the weight reported as SDF. The means of duplicate analyses are reported.

c. Total dietary fibre. According to Mongeau and Brassard (1986), total dietary fibre (TDF) was the sum of NDF and SDF.

d. β-glucan. Extraction and analysis of all oat flours for β-glucans was accomplished with a Biocon kit according to the method of McCleary and Nurthen (1986). Aqueous ethanol (50% v/v) and sodium phosphate buffer (pH

6.5) were added to flour samples before boiling water bath treatment (five min). Lichenase digestion (40°C, one h) was followed by centrifugation (2,000*g*, 10 min). A known aliquot of supernatant was digested with β-glucosidase (40°C, 15 min). Modifications of the method were made to analyze the glucose by absorbance at 340 nm after hexokinase digestion (40°C, 15 min) instead of absorbance at 510 nm after glucose oxidase/peroxidase digestion. It was assumed that the concentration of glucose (calculated from comparison of absorbance readings to a standard glucose curve) was directly related to the β-glucan composition of the flour. The means of duplicate or triplicate analyses are reported.

e. Starch. The enzymatic method of Kim and Williams (1990) was used for starch analysis of the oat flours. Oat flours were extracted with 80% ethanol (v/v) in a boiling water bath (30 min), centrifuged (2,000*g*, 10 min) and decanted. Sodium acetate buffer (pH 5.0) and thermostable α-amylase were added to the residue for further boiling water bath treatment (30 min). Cooled samples were further digested with amyloglucosidase (60°C, overnight), and centrifuged (2,000*g*, 10 min). Glucose concentration was determined by comparison of an aliquot of solution to a standard curve after hexokinase digestion followed by absorbance readings at 340 nm. It was assumed that the starch content of flour was directly related to the calculated glucose concentration. The means of duplicate analyses are reported.

f. Damaged starch. A method based on AACC method 76-30A (AACC, 1986) was used for the determination of starch damage in Farrand Units of oat Flours A, C, D, E and F. Flours were digested (30°C, one h) in an α-amylase extract containing sodium chloride and acidic calcium acetate. Sulphuric acid (10%) and sodium tungstate (12%) were then added followed by a settling period (two min) and filtration. An aliquot of filtrate was added to 0.1 N alkaline ferricyanide reagent in sodium acetate buffer (pH 4.7) and incubated (boiling water bath, 20 min). Acetic acid and a starch-iodide solution were added to the cooled sample and titrated against thiosulphate. Released maltose, calculated from the comparison of the thiosulphate titre to a standard maltose curve, was assumed to be

directly related to the damaged starch content.

6. Total Polyphenols

Total polyphenols were determined by modifications to the method published by Bendelow (1977). According to this method, the polyphenols are extracted by stirring (40 min) a 10% slurry (w/v) of oat flour and dimethyl formamide. Phenols in the filtered extract (Whatman #4) were identified at a wavelength of 505 nm in a Technicon AutoAnalyzerII using aqueous ammonia (20%, v/v), 4-aminoantipyrene and potassium ferricyanide reagents. Comparison of absorbances to a gallic acid standard curve allowed quantification of total polyphenols. All oat flours were analyzed and means of duplicate analyses are reported.

C. ENZYME ANALYSES

1. Amylase Activity

a. α-amylase. All oat flours and ground, unsteamed groats were analyzed for α-amylase activity according to the method of Bendelow (1977). Samples were extracted in an acetate buffer (pH 5.2; 10%, w/v; 20 min), filtered (Whatman #4), and an aliquot analyzed in a Technicon AutoAnalyzerII. Phenyl mercuric acid was added to inhibit β-amylase activity. Reducing sugars were identified by their reaction with neocuproin and copper sulphate at a wavelength of 460 nm. Comparison of values to a glucose standard curve allowed quantification, which was assumed to be related to β-amylase activity. The means of duplicate analyses are reported.

b. Diastatic power. The α-amylase method (modified by the elimination of phenyl mercuric acetate) was used to determine diastatic power. Diastatic power is defined as the sum of α- and β-amylase activity. The means of duplicate analyses are reported for all oat flours and ground, unsteamed groats.

c. Falling Number value. AACC method 56-81B (AACC, 1986) was used to determine Falling Number values using the single sample apparatus from the Falling Number Co. of Sweden. All sample dilutions (oat:wheat; 0:100, 5:95,10:90, 20:80, 100:0) were analyzed. In this method, seven g of flour is combined with 25 mL distilled water in a test tube and mixed for one min in a boiling water bath. A plunger is then allowed to move to the bottom of the test tube by the action of gravity through the gelatinized mixture. The Falling Number value is defined as the time, in seconds, from the start of mixing in the boiling water bath to the completion of the plunger's fall. Action of α-amylase on starch granules decreases the viscosity of gelatinized flour mixtures and thus decreases the Falling Number value.

2. Peroxidase Activity

Based on the method of Bendelow (1977), all oat flours and ground, unsteamed groats were examined for peroxidase activity. Seven percent slurries (oat flour/distilled water, w/v) were extracted (67°C, 40 min). Cooled solutions were filtered (Whatman #4) and an aliquot of filtrate analyzed in a Technicon AutoAnalyzerII with phosphate buffer (pH 6.6), catechol and hydrogen peroxide. Absorbance at 505 nm was used as a relative indicator of peroxidase activity. The means of duplicate analyses are reported.

3. Protease Activity

A 10% slurry (w/v) of flour in sodium acetate buffer (pH 4.4) was extracted (30°C, 17 h) and filtered (Whatman #4). The amino nitrogen contained in the filtrate was identified at a wavelength of 420 nm in a Technicon AutoAnalyzerII containing picrylsulphonic acid (0.1%) and phosphate buffer (pH 6.6). Protease activity is assumed to be directly related to the quantity of amino nitrogen. All oat flours and ground, unsteamed groats were examined. This method is based on that of Bendelow (1977). The means of duplicate analyses are reported.

D. COLOUR ANALYSIS

Hunter Lab measurements L, a and b were obtained for flour and 50% flour slurries (w/v) in distilled water for all oat flours. With this instrument, L values, which measure brightness, vary from 0 (black) to 100 (white). The a values, which measure increasing red responses in the positive direction and increasing green responses in the negative direction have no numerical boundaries. The b values, which measure increasing yellow responses in the positive direction and increasing blue responses in the negative direction also have no numerical boundaries. The Hunterlab, model D5-2, was used and standardized with the C2-12418, white tile. Means of duplicate readings are reported.

E. FUNCTIONAL ANALYSES

1. Amylograph

A 12% flour slurry (w/v) in distilled water and AACC method 22-10 (AACC, 1986) was used to obtain pasting curves from the Brabender Visco-Amylograph. The slurry was heated, with stirring, at a rate of 1.5°C per minute from 30°C to 95°C, maintained at 95°C for 20 min, and then cooled to 55°C at a rate of 1.5°C per min. Peak viscosity is the highest viscosity achieved during initial heating, 20-min viscosity occurs at the end of the 95°C heating period, and setback is the difference between the final cooled viscosity and the 20-min viscosity. All dilutions (oat:wheat; 0:100, 5:95, 10:90, 20:80, 100:0) were examined.

2. Farinograph

The constant weight procedure of AACC method 54-21 (AACC, 1986) was used to determine farinograph water absorption, dough development time, and mixing tolerance index from all oat flour dilutions. The 50 g bowl of the Brabender Farinograph was used for dough mixing. Absorption is described as the amount of water required to centre the farinograph curve

peak on the 500 Brabender Unit (BU), resistance line. Dough development time is the mixing time required, after the correct amount of water is added, to centre the curve peak on the 500 BU resistance line. The mixing tolerance index is the loss of resistance, measured in BU, five min after the curve peaks.

3. Extensigraph

The 300 g bowl of the Brabender Farinograph was used to mix 200 g flour doughs to their dough development time with 2% less water than absorption in the Farinograph. Sodium chloride (2%) was added to the distilled water (w/v). Doughs were shaped and stretched with the Brabender Extensigraph, and rested in a humidified proofing cabinet at 30°C. Extensibility, a measurement of strain and also the distance in mm the dough was stretched; resistance at 5 cm, a measure of stress and also the force exerted by the dough expressed in Brabender Units (BU); and area under the curve, a measurement of energy exerted by the dough during deformation, measured in cm^3, were determined according to AACC method 54-10 (AACC, 1986). Doughs were tested at 45 and 135 min, and measurements recorded for the 135 min curves. Wheat flour and oat-wheat composites were examined.

4. Zeleny Sedimentation Volume

AACC method 56-61A (AACC, 1986) was used to calculate the Zeleny Sedimentation Volume of all flour dilutions. In this method, 3 g flour and 50 mL water containing bromophenol blue (0.4%, w/v) are mixed for five min before the addition of 25 mL of an isopropanol-lactic acid mixture and a further five min mixing period. After settling for exactly five min, the volume of sediment in the cylinder is read and reported as the sedimentation volume.

5. Gluten Content

Gluten was prepared with the Falling Number Glutamatic system according to ICC method 137 (1980). A dough, prepared by automatically mixing 10 g flour and 5 mL of 2.0% NaCl (w/v), was washed with 200 mL of the NaCl solution on a fine screen (80µ) before a final wash over a coarse screen (800µ) to remove bran particles not incorporated in the gluten. The gluten was centrifuged at 6000 rpm in the Falling Number Centrifuge 2012 before weighing to determine percentage wet gluten. The Falling Number Glutork 2020 removed the moisture by heating for four minutes at 150°C to produce the percent dry gluten value. Gluten moisture (%) was calculate as [(wet gluten - dry gluten) / wet gluten] * 100. Wheat flour and oat-wheat composites were analyzed.

6. Fat Binding Capacity

The method of Lin *et al.* (1974) was used to determine the amount of canola oil (oleic:linoleic approximately 1:2) bound by the oat flours, wheat flour and composites of Flour D. Excess canola oil (3 mL) was added to the flour (0.5 g), stirred (1 min), and held (30 min) before centrifuging (1610 *g*, 25 min). The volume of free oil was read from the centrifuge tube. Fat binding capacity of the flour was expressed in percentage as the amount of oil bound by a 100 g sample on a dry basis.

7. Breadbaking

Pup loaves were baked from wheat and oat-wheat composites according to the method of Kilborn and Tipples (1981). Doughs were prepared by mixing flour(s) (100 g), compressed yeast (3%), salt (1%), sucrose (2.5%), malt syrup (0.6%), ammonium phosphate (0.1%), potassium bromate (15 ppm) and water (farinograph absorption minus two percent) in a high speed, open bowl mixer (3.5 min); fermented (2 h and 45 min), remixed (2.5 min), rested (25 min), sheeted, molded, proofed (55 min) and baked (25 min., 223°C). Composites from oat samples A, B, C and D were baked on one day; composites from oat samples E and F on another.

a. Appearance. Loaves were examined visually for colour and crumb structure. Pictures were taken with a Pentax camera, 1:1.4, 50 mm lens and black and white film.

b. Loaf volume. Loaf volume was calculated by means of rapeseed displacement.

c. Firmness. The Baker Compressimeter, according to the AACC method 74-10 (AACC, 1986), was used to measure the resistance to compression of bread slices after three days storage at room temperature. Three middle slices from each pup loaf, each approximately 12 mm thick, were compressed to 3.00 mm and the applied force, measured in g, read from the appropriate scale.

F. STATISTICAL ANALYSES

1. *t*-Test

t-tests were performed to identify differences in oat flours for all composite functional tests. For each functional test, a mean and standard deviation was calculated for the three composites of each oat flour. Each mean was divided by the corresponding standard deviation and divided by the square root of three. This value (t) was compared to critical values of t to determine levels of significance. For P=0.05, t=2.92. For P=0.01, t=6.97. Only levels of significance are recorded.

2. Paired *t*-Test

Paired *t*-tests were performed to identify differences related to the processing influences of the company of oat flour origin on composite functional properties. In the paired *t*-test, the samples are paired (in this case, according to dilution level), the difference between each pair calculated, and the *t*-test as described above performed on the differences. However, since six comparisons were made for each test, the square root of six was used instead of the square root of three in the

above equation. For P=0.05, t=2.02 and for P=0.01, t=3.37.

Two paired t-tests were made for each comparison of companies to ensure differences were attributable to company of origin and not just due to the method of pairing. For example, in comparing Companies AB and CD, the first t-test compared the three dilutions (5, 10 and 20%) of Flour A with those of Flour C and the dilutions of Flour B with those of Flour D. The second t-test made similar comparisons with Flours A and D and Flours B and C. Only if both methods of pairing were significant was the conclusion drawn that the difference was due to company of origin and not individual flour differences.

3. Regression Analysis

To calculate correlation coefficients (r), linear regression analyses of wheat and oat-wheat composite functional properties were performed using Lotus 1-2-3. Levels of significance were based on the factorial design of the experiment (Company x Flour x Dilution) + Wheat = (3x2x3)+1. This allowed six degrees of freedom for the model and 12 degrees of freedom for the error term. For P=0.05, r=0.78 and for P=0.01, r=0.84. This design minimizes Type II Error (false acceptance) at the expense of Type I Error (false rejection).

The effects of dilution, with and without the wheat control, were also examined. Comparisons of the values were made. Large differences between values are attributable to oat-wheat chemical interactions.

In the design including wheat as a control, the value for wheat was included as zero dilution for each flour, so (Company x Flour x Dilution) = (3x2x4); allowing six degrees of freedom for the model and 17 degrees of freedom for the error term. For P=0.05, r=0.70 and for P=0.01, r=0.77. Although the dilutions were 0, 5, 10 and 20% oat flour, the value of one was added to each dilution (1, 6, 11 and 21%) in order to avoid mathematical errors evolving from the multiplication of a term by zero. The resulting error in x-intercepts was determined to be of lesser significance for the purposes of this analysis.

In the design excluding wheat, (Company x Flour x Dilution) = (3x2x3); allowing five degrees of freedom for the model and 12 degrees of freedom for the error term. For P=0.05, r=0.75 and for P=0.01, r=0.82.

IV. RESULTS and DISCUSSION

A. Chemical Analyses

1. Proximate Composition of Oat Flours

a. Moisture. Oat flour moistures range from 9.2-10.1% (TABLE 8). It is desirable to keep moistures below 10.0% to prevent oxidative rancidity in oat flour (Youngs, 1986).

TABLE 8. Proximate Composition of Oat Flours

	A	B	C	D	E	F
Moisture	9.3	9.2	9.0	9.8	10.1	10.1
Ash**	1.6	1.6	1.9	1.2	1.8	1.9
Protein** N X 5.5	12.6	12.8	14.3	13.0	14.5	15.0
Lipid** hexane extraction	7.4	7.8	7.9	7.8	7.6	8.1
Fibre** TDF	8.4	8.5	14.1	5.6	12.0	13.1
Starch**	68.5	67.3	62.0	71.4	63.9	62.4

%, ** dry basis (db)

b. Ash. Oat flour ash results (TABLE 8), range from 1.2-1.9%. Flours from Company AB are similar in ash content, as are flours from Company EF. Flours from Company CD differ in this respect. Flour D contains the least ash of all six commercial oat flours.

c. Protein. The Kjeldahl protein content of oat flours (TABLE 8) is divided into two groups: Flours A, B, and D with a range of 12.6-13.0% and Flours C, E, and F, of higher protein content, with a range of 14.3-15.0%.

d. Lipid. An individual flour response, unrelated to company of origin or protein content is identified in the hexane solubles (free lipids) (TABLE 8). Flour A contains the least free lipid (7.4%) and Flour F the most (8.1%).

e. Fibre. Fibre levels (TABLE 8), measured as total dietary fibre (TDF), is divided into the same two groups as the protein levels: Flours A, B and D with a TDF range of 5.6-8.4%, and Flours C, E, and F, of higher fibre content, with a range of 12.0-14.1%. In addition to containing the least ash, Flour D also contains the least fibre; two indications that more bran has been removed from this oat flour than from the others.

f. Starch. As expected from fibre and protein results, Flours A, B and D are higher in starch content than Flours C, E and F (TABLE 8).

2. Proximate Composition of Oat Flour Dilutions

a. Moisture. Moisture levels of all dilutions are depicted in bar graph form in FIGURE 5. The moisture value for the one wheat sample analyzed is

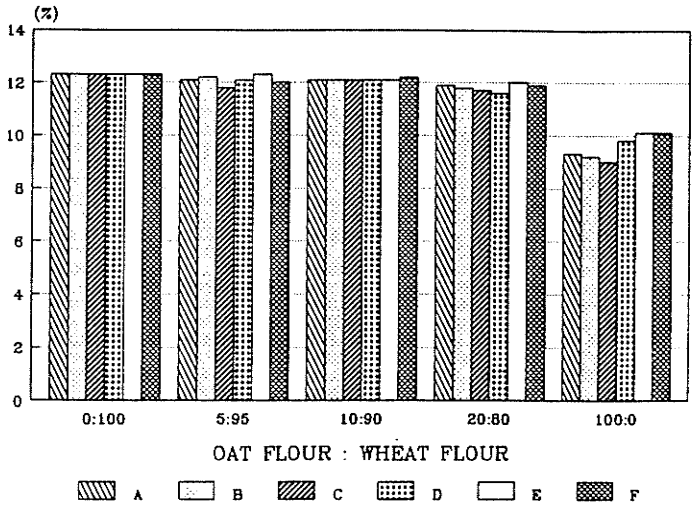


FIGURE 5. Moisture content (%) of oat flour dilutions.

represented in the graph by six bars identified as oat:wheat dilution (0:100). This is to represent wheat as a control or blank for each of the oat flour dilutions; not to suggest the wheat sample has been analyzed six times, with perfect analytical replication. (This is also the case for all subsequent bar graphs.) Values for the oat flours (oat:wheat, 100:0) are repeated from TABLE 8.

By comparing the patterns of the bars at each dilution, it can be seen that the moisture contents of composite flours are not equal to the weighted sum of the moistures of the individual flours. All oat flour composites at the 10% dilution level are very similar in their moisture content, indicating that at this level, the dilution of wheat flour with oat flour has caused the redistribution of bound and free water.

b. Ash. The ash content of oat-wheat dilutions is found in FIGURE 6. Values for oat flours (oat:wheat, 100:0) are repeated from TABLE 8. As with moisture, it is evident that the ash level of composite flours is not directly related to the effects of dilution. Ash values are generally higher in the composites than calculated values that would arise only from the effects of dilution.

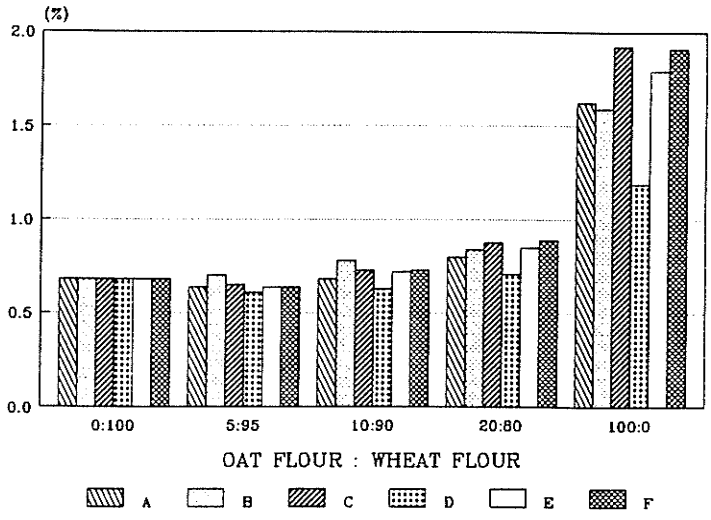


FIGURE 6. Ash content (%) of oat flour dilutions.

c. Protein. Protein content (FIGURE 7) of composite flours, like moisture and ash contents, is not directly related to dilution, but instead exhibits unique oat flour responses. Protein levels of composite flours are lower than values that would arise strictly through the effects of dilution. These findings indicate that an interaction exists between oat and wheat flours and causes the disappearance of flour proteins.

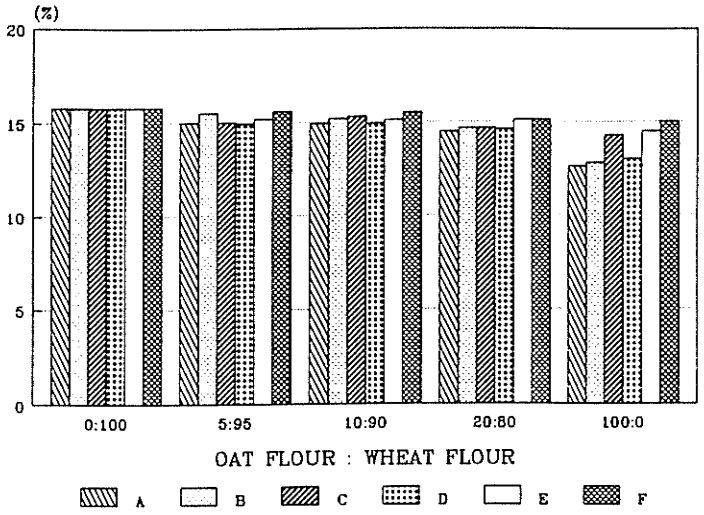


FIGURE 7. Protein content (%) of oat flour dilutions.

3. Protein

a. Amino acid composition. Bran, comprising approximately 35% of the oat groat (Youngs *et al.*, 1982), is higher in proline and lysine than the whole groat (Pomeranz *et al.*, 1973). Therefor, it is expected that differences in these amino acids would be observed between flours that differ substantially in ash (fibre) content. However, only small differences in amino acid composition are noted among the oat flours (TABLE 9), and differences in proline and lysine are inconsequential. Cysteine levels are lower in flours of Company CD (2.4, 2.8) than in flours from the other companies (3.0-3.2). Glutamate, histidine, arginine and valine vary more than the other amino acids, with the exception of cysteine. Proline, leucine, tyrosine and phenylalanine vary the least.

TABLE 9. Amino Acid Composition of Oat Flours

	A	B	C	D	E	F
Lys	4.1	4.3	4.2	4.0	4.0	4.2
His	2.3	2.6	2.3	2.3	2.2	2.9
Ammonia	1.3	1.3	1.3	1.3	1.3	1.3
Arg	6.7	6.3	6.8	6.9	6.7	6.8
Asp	8.6	8.5	8.5	8.4	8.6	8.3
Thr	3.5	3.5	3.5	3.5	3.7	3.4
Ser	5.4	5.3	5.3	5.1	5.3	5.1
Glu	23.1	22.9	23.2	23.5	23.0	22.7
Pro	6.3	6.3	6.3	6.3	6.2	6.3
Cys	3.0	3.2	2.4	2.8	3.1	3.2
Gly	5.4	5.3	5.3	5.0	5.3	5.2
Ala	4.9	4.7	4.7	4.6	4.7	4.5
Val	4.2	4.5	4.6	4.8	4.6	4.6
Met	1.9	2.0	2.0	1.9	2.0	2.1
Ile	3.1	3.2	3.3	3.4	3.4	3.3
Leu	7.6	7.6	7.8	7.7	7.6	7.6
Tyr	3.2	3.1	3.1	3.0	3.1	3.1
Phe	5.4	5.3	5.4	5.4	5.3	5.3

% amino acid recovered

b. Osborne fractionation. The proteins of five oat flours, and the ground, unsteamed groat sample were fractionated on the basis of solubility by the modified Osborne procedure (TABLE 10). Preliminary

analysis (protein and ash) indicated Flours A and B were very similar, and so Flour B was not analyzed in this test. Nitrogen recovery for the procedure was low, ranging from 42.4-52.7%. Protein was not analyzed for sample G, and so nitrogen recovery was not calculated.

Several conclusions can be drawn by comparing flour solubilities. The unsteamed groats contain more globulin and prolamin, and less insoluble glutelin than the commercial oat flours, confirming the results of Oomah (1987) that oat milling processes decrease the solubility of oat proteins. Flour D (low in fibre and ash) differs from the other commercial flours (highest soluble glutelin and lowest insoluble glutelin); indicating that fibre may aggregate with flour protein to form insoluble glutelin. Flours from Company EF are much lower than flours from Company CD in prolamin content and higher in insoluble glutelin content; indications of more extensive protein denaturation in the flours from Company EF than in flours from Company CD.

TABLE 10. Protein Content of Oat Flour Osborne Fractions

	A	C	D	E	F	G
Albumin	5.2	4.9	5.7	3.8	3.6	5.7
Globulin	2.5	4.0	6.5	1.5	2.0	8.6
Prolamin	3.4	11.0	9.4	0.5	0.4	13.1
Soluble Glutelin	9.3	4.5	27.1	5.4	4.3	3.6
Insoluble Glutelin	79.8	77.3	49.9	89.0	89.9	69.0
Total N recovered	47.0	44.4	52.7	47.7	42.4	ND

% N recovered

ND, not determined

c. Acid PAGE. The standard electrophoretic procedure at pH 3.1 was applied to the oat flour prolamins (alcohol soluble fraction) (FIGURE 8). Unlike the report of Oomah (1987), who reported that oat prolamins are unaltered during processing (drying or flaking), a company-based processing effect is evident in the intensity of stained protein bands. Using equal flour aliquots, only faint bands appear for oat Flours E and F, and bands for Flours C and D appear darker than those for Flours A and B. Band intensities correspond to Osborne prolamin concentrations, supporting the preliminary conclusion that the proteins of Flours E and F were extensively heat denatured during processing.

Prolamin patterns for Flours A and B are the same. The patterns for Flours C and D differ; Flour C displays two intermediate bands, whereas Flour D displays only one. In addition, Flour D possesses an additional, very faint, faster moving band. Since it has already been concluded that bran has been removed from Flour D, differences between Flours C and D may result from differences in composition of the proteins of bran and endosperm (Donhowe and Peterson, 1983; Peterson *et al.*, 1985). It is also possible that Flours C and D were produced from different oat cultivars.

The pattern for Neepawa wheat was included in FIGURE 8 (outside lanes) as a standard reference and as a check of the method. The patterns for oat prolamins (avenins) differ from those of wheat prolamins (gliadins).

d. SDS-PAGE.

i) Unreduced oat flour protein. All oat flours are similar in high molecular weight SDS-PAGE patterns, (FIGURE 9); faint bands at 112-215 kDa and dark bands at 55-72 kDa. The very dark bands correspond to apparent molecular weights of unreduced globulins (Robert *et al.*, 1985c). Lower molecular weight regions are distinct in company response: Flours E and F, faint but similar bands; Flours A and B, slightly darker, similar bands, but different from Flours E and F; and Flours C and D, darkest in band intensity, differing from each other and other flours in band composition. The unreduced electrophoretic pattern of Flour D displays two bands about 30 kDa that are slightly slower in mobility than those found in the patterns of Flours A, B and C. These bands are not found in Flours E and F.

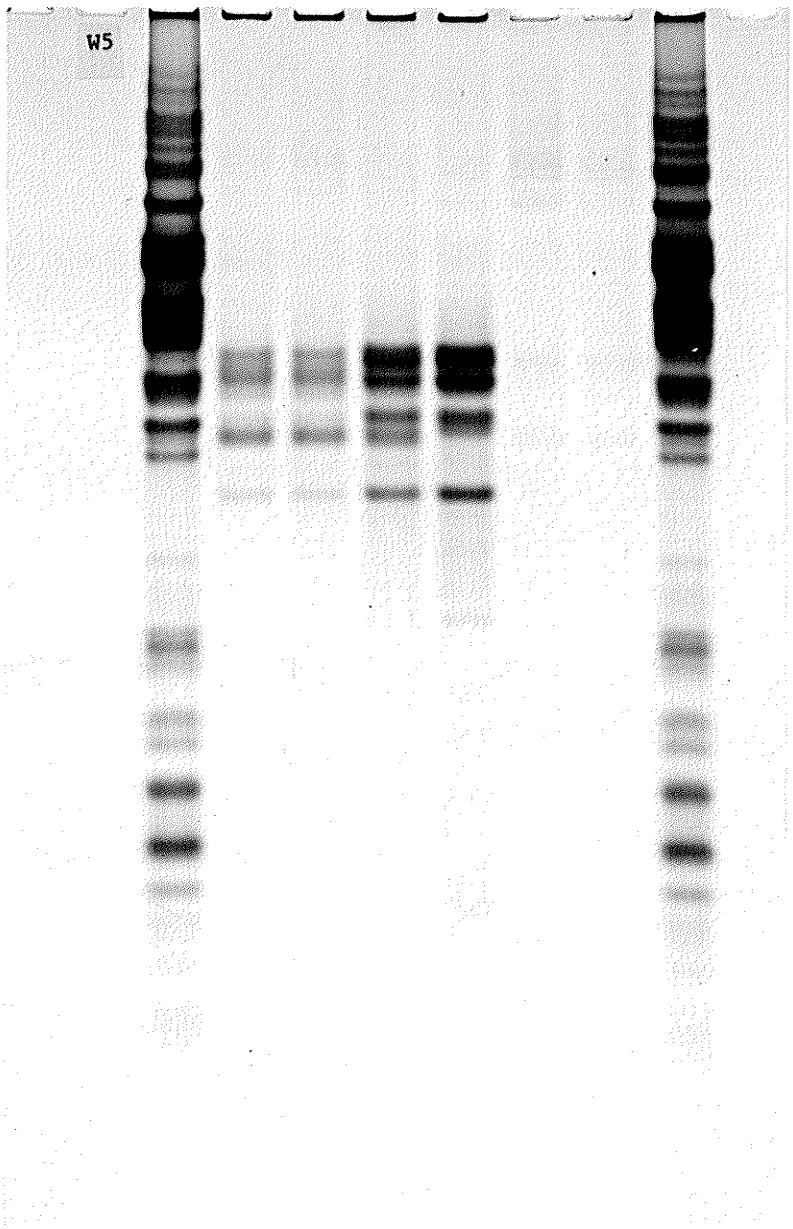


FIGURE 8. Acid PAGE patterns of flours (outer lanes, Neepawa; inner lanes, left to right, oat Flours A, B, C, D, E, F)

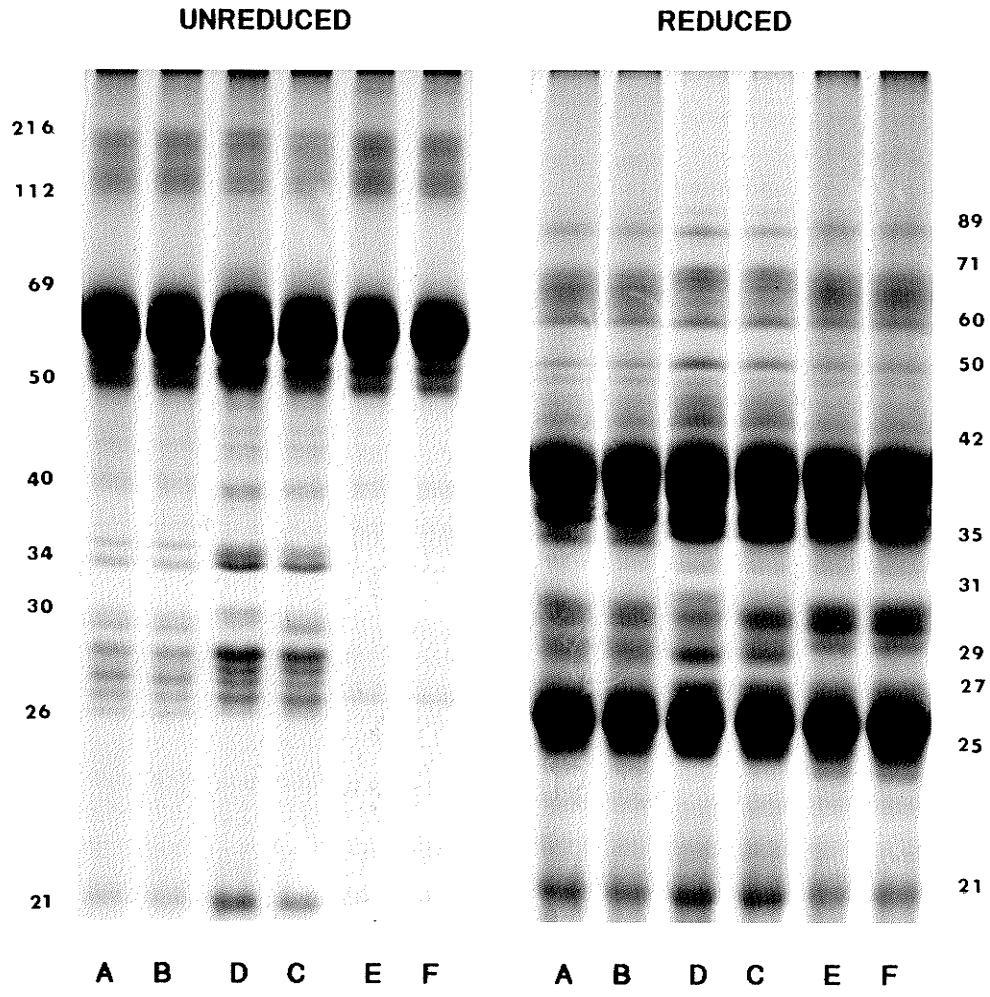


FIGURE 9. Oat flour SDS-PAGE patterns, unreduced and reduced.

ii) Reduced oat flour protein. All oat flours reveal two thick banded regions: 36-42 kDa and 24-27 kDa (FIGURE 9). These approximately correspond to the acidic (33-43 kDa) and basic (20-25 kDa) globulin subunits described by Robert *et al.* (1985c). Unlike the results of Robert and co-workers, a single band at 20 kDa is also very prominent in patterns of all flours. Company related differences are demonstrated. The 52 kDa band is more prominent in the electrophoretic patterns of Flours C and D than the other oat flours. In addition, all protein-related material in these flours (Flours C and D) enters the gel, unlike that of the other four flours. Company differences among flours are also evident in the distribution of bands in the 28-32 kDa region. Flours C and D also display differences in this region with Flour D revealing an extra band at 32 kDa.

iii) Unreduced oat-wheat composite proteins (Flour D). The presence of oat flour is almost unnoticed in the composite electrophoregrams of Flour D. The unexpected proximate analysis (moisture, ash, protein) of the composite flours is demonstrated electrophoretically to be based upon the interaction of oat and wheat proteins. The presence of oat flour adds only one new band to the wheat flour electrophoregram, at 28 kDa (FIGURE 10). The intensity of this very faint, almost invisible band is directly related to oat flour concentration. With increasing oat flour concentration, the diffuse band at 29-30 kDa becomes darker and narrower, the faint bands at 25-26 kDa become slightly darker, and the faint band at 66 kDa becomes more prominent.

iv) Reduced oat-wheat composite proteins (Flour D). Upon initial comparison, the oat flour composites appear to be replicates of the wheat sample (FIGURE 10). However, upon closer examination, it can be seen that only the dark, broad, electrophoretic bands found in Flour D find expression in the composite flours; as faint, narrow bands. As oat flour concentration increases, a band at 39 kDa becomes more clearly defined, bands at 28 and 26 kDa appear and become more prominent and bands from 24-30 kDa become darker. The very narrow, well defined band in the 147 kDa area is present in the wheat flours and all composite flours except

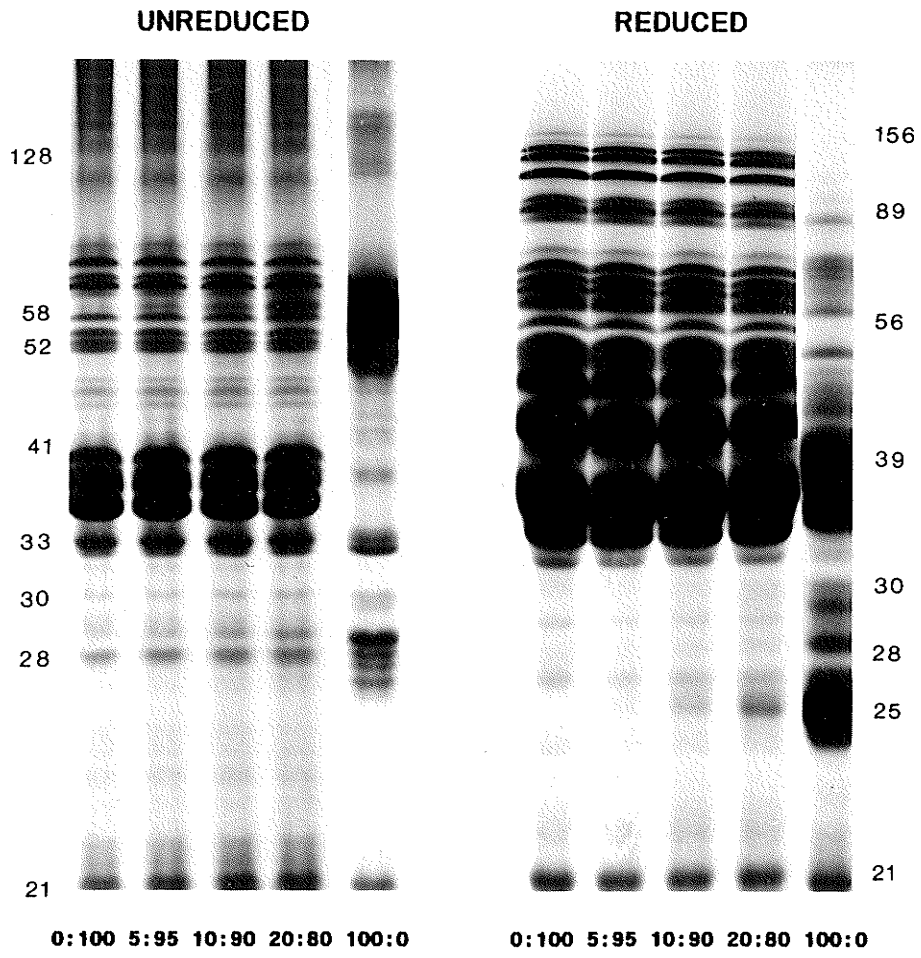


FIGURE 10. SDS-PAGE patterns, (Flour D dilutions), unreduced and reduced.

D-20%. These results indicate the oat-wheat flour protein interchange observed in unreduced composite flours does not result from disulphide interactions of proteins.

4. Lipid

a. Bound lipid. The bound lipid content, that is the difference between methanol and hexane solubles, is similar for all oat flours (TABLE 11). In comparing the bound lipid contents of flour companies, Flours from Company CD exhibit the largest variation (Flour C, 2.9%, Flour D, 2.1%).

b. Total lipid. The sum of free and bound lipids, identified as total lipid, is very similar for all oat flours - approximately 10% (TABLE 11).

c. Non-polar, glyco- and phospholipid. Most hexane extractable lipids are non-polar lipids (81.9-84.1%) (TABLE 11). Glycolipids range from 4.7-8.7% and phospholipids from 4.6-6.0%. Oat flours exhibit unique levels of non-polar and glycolipid, unrelated to company of origin or protein levels. Flours C and D are higher in phospholipid than Flours B and E.

TABLE 11. Lipid Composition of Oat Flours

	A	B	C	D	E	F
Free*	7.4	7.8	7.9	7.8	7.6	8.1
Bound*	2.5	2.5	2.9	2.1	2.4	2.2
Total*	9.9	10.3	10.7	9.9	10.0	10.3
Non-polar**	ND	82.7	81.9	83.2	84.1	ND
Glycolipid**	ND	8.7	4.7	6.6	6.0	ND
Phospholipid**	ND	4.8	5.0	6.0	4.6	ND

* % flour, db

** % free lipid

ND not determined

Free lipid, as reported in TABLE 8

d. Fatty acid composition. Despite variations in free lipid, oat flours are very similar in free fatty acid composition (TABLE 12). Largest amounts, and largest variations are exhibited in palmitic, oleic and linoleic acids. These acids are found, on average, in the proportion of 18:40:35 (palmitic:oleic:linoleic).

TABLE 12. Fatty Acid Composition of Oat Flours

	A	B	C	D	E	F
Palmitic	17.4	17.6	18.0	17.6	18.5	18.4
Stearic	1.5	1.5	2.1	1.7	1.6	1.6
Oleic	38.1	38.1	40.7	39.9	40.3	40.7
Linoleic	36.6	36.1	32.7	34.9	34.0	33.9
Linolenic	1.1	1.1	0.8	0.9	1.1	1.1
Arachidic	0.1	0.1	0.2	0.2	0.1	0.1
Eicosanoic	0.9	0.9	0.9	0.8	0.9	0.9
Behinic	2.2	2.2	2.5	2.2	1.7	1.6
et al.	0.8	0.8	0.8	0.7	0.5	0.5

% fatty acid recovered

5. Carbohydrate

a. Neutral detergent fibre. Flours A and B are similar in neutral detergent fibre (NDF) content, (4.9% and 5.2%) as are Flours E and F (7.3% and 8.2%) (TABLE 13). Flour D is the lowest (2.9%) and Flour C the highest (9.0%). NDF contents of oat flours follow the same trend as protein and ash values: Flours C, E and F (7.3-9.0%) are higher than Flours A, B and D (2.9-5.2%).

TABLE 13. Fibre Composition of Oat Flours

	A	B	C	D	E	F
TDF	8.4	8.5	14.1	5.6	12.0	13.1
NDF	4.9	5.2	9.0	2.9	7.3	8.2
SDF	3.6	3.3	5.1	2.6	4.7	4.9
beta-glucan	2.8	2.6	3.4	1.8	3.3	4.0

% db

TDF, as reported in TABLE 8

b. Soluble dietary fibre. Results for soluble dietary fibre (SDF) (TABLE 13), indicate trends similar to those of NDF, with Flours C, E and F (4.7-5.1%) containing higher levels than Flours A, B and D (2.6-3.6%).

c. β-glucan. As with the other fibre analyses, β-glucan results (TABLE 13) follow trends similar to those of NDF, and Flours C, E and F contain higher levels (3.3-4.0%) than Flours A, B and D (1.8-2.8%).

d. Damaged starch. Of the flours examined, only Flour A gave evidence of damaged starch (17.2 Farrand Units). Flour B was not analyzed for starch damage.

6. Total Polyphenols

Oat Flours A, B, E and F are equal in polyphenol content (0.08%, as gallic acid). Flours C and D contain lower amounts of polyphenols (Flour C, 0.07%; Flour D, 0.06%).

B. ENZYME ANALYSES

1. Amylase Activity

a. α-amylase. α-amylase activities vary only slightly among oat flours (TABLE 14). Processing reduced the α-amylase activity by approximately 50%, as indicated by comparing the commercial oat flour values with the unsteamed ground oat sample, G. These results, as well as the other enzyme activities listed in TABLE 14, are much lower than the automated system (used for malted barley analysis) was designed to handle. Because of the very low activities, they were calculated from extrapolated portions of the standard curve and therefor are inherently inaccurate.

b. Diastatic power. Diastatic power, which includes α- and β-amylase activities, is more variable among the oat flours than α-amylase activity (TABLE 14). In comparison to the unsteamed groat, G, the diastatic power

TABLE 14. Enzyme Activity of Oat Flours

	A	B	C	D	E	F	G
Alpha-amylase alpha-amylase activity, units	1.3	1.3	1.1	1.1	1.3	1.3	2.4
Diastatic Power saccharifying activity, units	9.6	6.3	6.0	6.9	6.3	5.1	10.8
Peroxidase transmittance at 505 nm	3.0	2.5	2.3	2.5	3.5	4.0	8.0
Protease transmittance at 420 nm	0.1	0.1	0.2	0.1	0.3	0.4	1.2

of Flour A has not been affected by processing. Flours B, C, D, E and F are all similar in diastatic power, and reduced in activity in comparison with the unsteamed groat.

c. Falling Number value. All oat flour Falling Number values are greater than 450 seconds (FIGURE 11); values for Flours A, B, C and D are lower than values for Flours E and F. The Falling Number value for wheat flour is 579 seconds. The variations in the composite results indicate the presence of an interaction between the oat and wheat flours that contributes to the Falling Number value. The action of wheat amylases

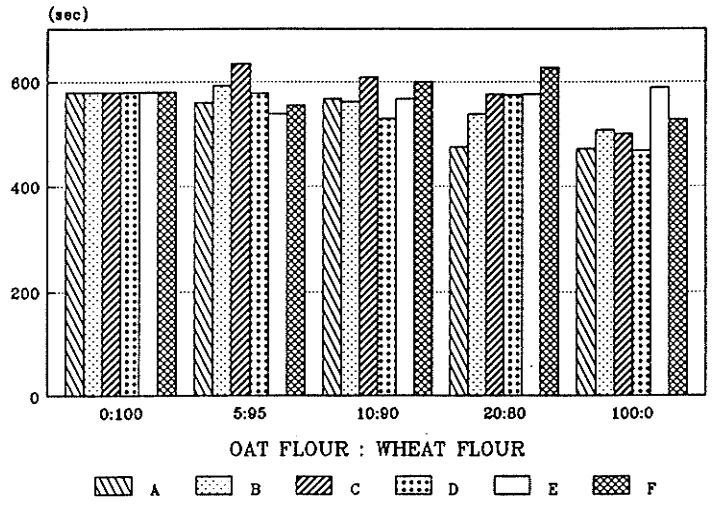


FIGURE 11. Falling Number values (sec) for oat flour dilutions.

acting on oat flour starch may be involved in this interaction. Oat flour composites A, B, C and D decrease Falling Number values with increasing oat flour concentration. Wheat amylases acting on oat starch would decrease viscosity and demonstrate this response. Flours E and F demonstrate the opposite response; Falling Number values are directly related to oat flour concentration, indicating that wheat amylases have very little effect on flours from Company EF.

2. Peroxidase Activity.

By comparing the commercial oat flours to the unsteamed groats, it is evident that oat flour peroxidase activity was reduced by processing to 30-50% of the activity of flour from unprocessed groats (TABLE 14). The activities of the six commercial flours are approximately the same.

3. Protease Activity.

As a result of the heat treatment of commercial oat flours with the purpose of inactivating lipase, protease activity is also diminished to approximately 8-30% of the activity of the unprocessed groats (TABLE 14). Slight variations among oat flours appear to be related to oat flour protein levels, although because the values are calculated from an extrapolated curve, no significant difference in protease activity is attributed to this difference.

C. COLOUR ANALYSIS

Flours C, E and F, those higher in protein, ash and fibre, are also darker (lower L values) than the other oat flours (TABLE 15). Flour D (lowest in ash and fibre) is the brightest of the oat flours; both as a flour and a slurry. Mixing with water to form a slurry darkens all oat flours. A comparison of a values indicates oat flours are slightly red in colour and became redder as a slurry. Oat flours differ in their

yellow-blue intensities (b values); Flour D, lowest in ash and fibre, is the least yellow, both as a flour and as a slurry. Upon addition of water, Flours E and F are the most yellow, and Flour D the least yellow.

These results indicate that commercial flours can vary widely in colour. The variation would be significant in food applications where white colour is desired. Because colour variations are somewhat related to oat flour chemistry, it may be possible to use colour as a screening indicator for oat flour protein or fibre levels.

TABLE 15. Hunterlab Colour Analysis of Oat Flours

	A	B	C	D	E	F
Dry Flour:						
L	82.1	82.0	81.6	84.3	79.4	80.4
a	0.9	0.8	1.2	0.8	0.9	0.8
b	10.0	10.0	9.4	7.9	10.7	10.6
Slurry:						
L	72.3	72.2	71.7	74.3	69.8	69.6
a	1.3	1.2	1.7	1.5	1.4	1.6
b	13.2	13.3	13.2	11.5	14.2	14.4

D. FUNCTIONAL ANALYSES

1. Amylograph

Amylograph analysis of Flour D at the 5% level, was conducted several weeks after the other amylograph analyses. Results were not consistent with those for the 5% dilutions of the other samples. Other flours were very similar in amylograph properties for 5% and 10% dilutions; 5% values only slightly lower than 10% values. Flour D-5% was much lower in viscosity than the corresponding 10% sample. It is assumed that oxidative changes in the composite flour resulted in this change of functional properties. Therefor, reported results are estimates obtained by means of linear regression analysis of the 10% and 20% composites of Flour D.

a. Peak viscosity. Peak viscosity (PV) is generally attributed to the thickening of a starch paste as a result of gelatinization (starch granule swelling and leaching of amylose). Oat flour PVs (FIGURE 12) are much greater than the PV displayed by the wheat flour. Oat flours exhibit a company effect in their PV response (Company AB > Company CD > Company EF).

Again, an oat-wheat interaction is demonstrated in composite flours. If the PV of composite flours were to result strictly from the effects of dilution, the addition of highly viscous oat flours to wheat flour would increase the viscosity of the composite. However, the addition of oat flour to wheat flour does not increase the viscosity of wheat at low dilutions (5% and 10%), confirming the presence of an oat-wheat interaction. The action of wheat amylase (demonstrated in the Falling Number analysis) on the oat flours could be responsible for these results and cause the reversal of the trend observed in oat flours. Therefor, in the composites, flours from Company EF generally are the most viscous and flours from Company AB, the least.

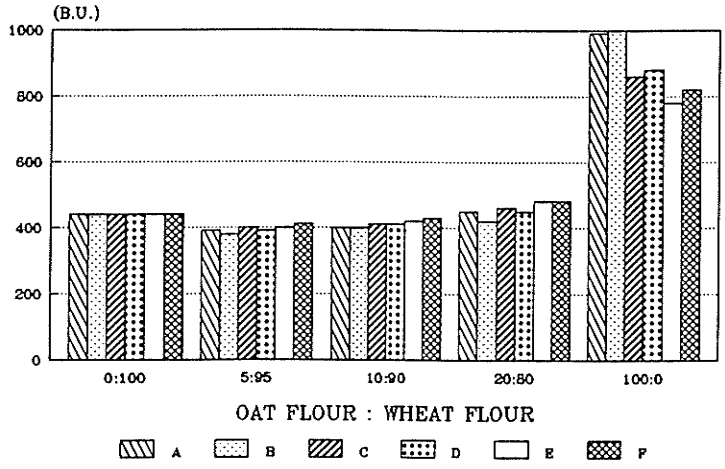


FIGURE 12. Amylograph peak viscosity (BU) of oat flour dilutions.

b. 20-min viscosity. The 20-min (20-MIN) viscosity, the viscosity achieved after heating a paste at 95°C for 20 minutes, can indicate a shear-thinning behavior (the decrease in viscosity of a solution as highly viscous, globular-shaped molecules are elongated into molecules of lesser viscosity by a shearing action). As with the PV values, after 20 min at 95°C, oat flour viscosities also exhibit a company effect and have values

greater than the wheat viscosity (FIGURE 13). However, the 20-MIN viscosities exhibit a different trend than found for PV and Company CD > Company AB > Company EF. This company relationship is similar to that found in protein denaturation: flours from Company CD are the least denatured, and those from Company EF the most denatured, thus indicating an oat protein effect in paste shear-thinning. Protein denaturation makes oat flour pastes more susceptible to the effects of shear thinning.

As with PV, the addition of oat flour to wheat has a thinning effect on 20-MIN viscosities; that is, the addition of oat flour to wheat depresses the 20-MIN viscosity of composites to levels below the wheat value. At the 20% dilution level, oat flours of higher protein content (Flours C, E and F) also display higher 20-MIN viscosities than the other composites. Protein levels of oat flours have no influence on 20-MIN viscosity values of oat flours. This change in properties between oat flour and composite again indicates an interaction between oat and wheat flours.

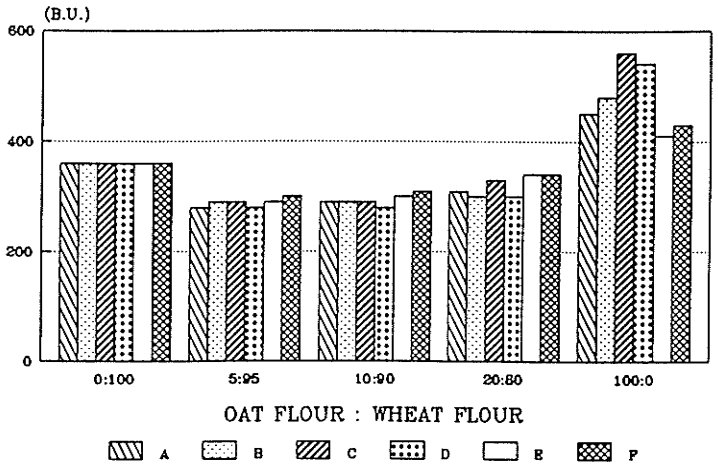


FIGURE 13. Amylograph 20-min viscosity of oat flour dilutions.

c. Set-back. Set-back (SB), the difference between hot and cooled paste viscosities, is related to the ability of a paste to form a firm network upon cooling. This network is generally attributed to the actions of amylose in the dispersed phase, and amylopectin in the solid phase. Like PV and 20-MIN viscosity values, SB for the oat flours from Company EF is also the lowest of the six oat flours (FIGURE 14). However, unlike other

amylograph properties, SB does not exhibit a company effect. Flours A and B differ in SB, despite their similarity in terms of chemical properties; and yet despite the numerous chemical differences of Flours C and D, their SB properties were similar. It is possible that the chemical variable(s) responsible for SB has (have) not been analyzed in this thesis. It is also possible that company processing effects are ultimately responsible for SB characteristics of an oat flour.

As with PV and 20-MIN viscosity, the addition of oat flour to wheat decreases SB of wheat. However, unlike flours from the other companies, SB of flours from Company EF are not depressed beyond the level exhibited at the 100:0 (oat:wheat) dilution. In fact, a synergistic effect is demonstrated with these flours at the 20:80 (oat:wheat) dilution and results in higher SB than exhibited by either the oat flour or wheat flour alone. This synergy is another indication of oat-wheat flour chemical interactions. It is expected that this synergistic response of Flours E and F is related to their resistance to wheat amylase activity. This resistance would result in longer amylose chains in these flours (E and F), thereby building a stronger network and higher SB than flours susceptible to wheat amylase.

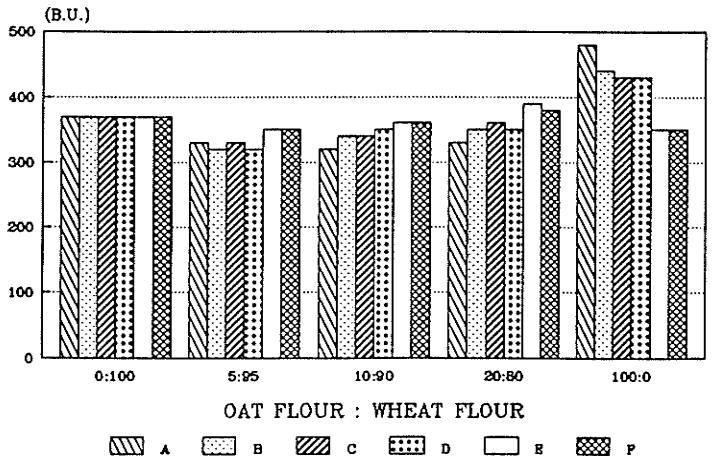


FIGURE 14. Amylograph set-back (BU) for oat flour dilutions.

2. Farinograph

Farinograph curves are depicted in FIGURE 15. Curve shapes for Flours A, B and D are similar as are the shapes for Flours C, E and F; corresponding to chemical similarities (protein, ash and fibre) of the oat flours. At the 5:95 (oat:wheat) dilution, all oat flours have a weakening effect on the wheat flour, and the dough develops more quickly. At the 10:90 (oat:wheat) dilution, a difference in flours is noted and both weakening and strengthening are demonstrated, with respect to dough development time. At this dilution (10:90), dough strength is unrelated to oat flour protein levels. At the 20:80 (oat:wheat) dilution, all oat flours strengthen the wheat dough and dough development time is increased; and Flours E and F produce the largest strengthening effect. A thickening (ballooning) effect is evident on some curves, especially at the 20% level.

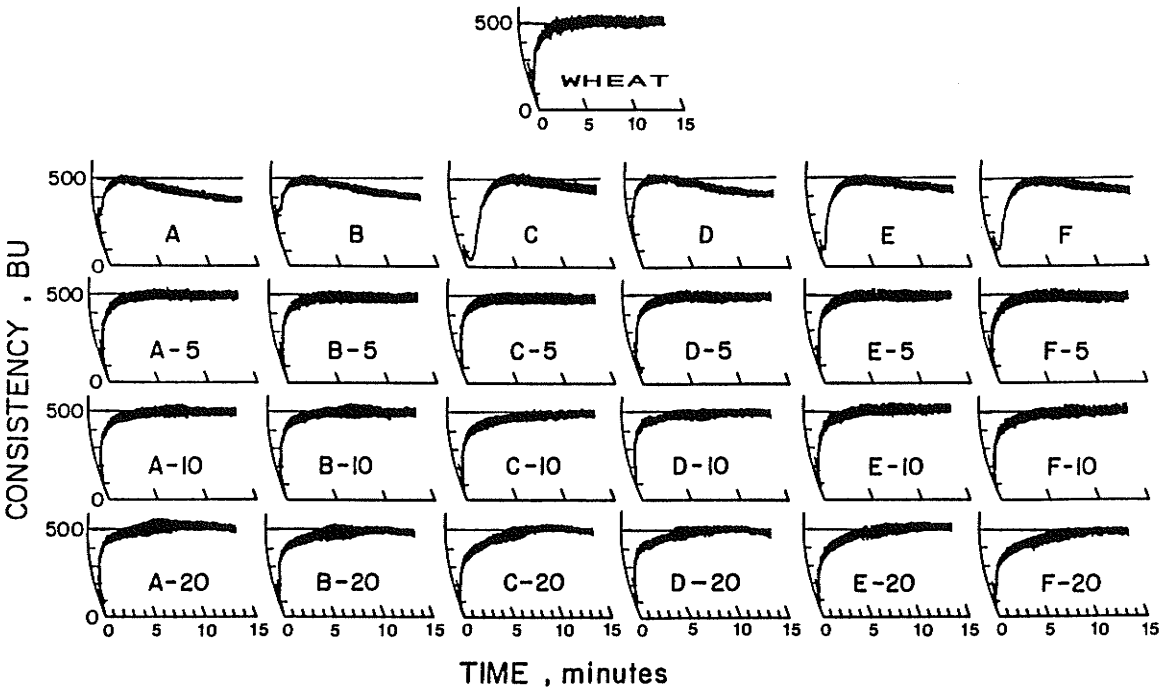


FIGURE 15. Farinograph curves of oat flour dilutions.

a. Dough development time. Dough development time (DDT) is the mixing time required for a dough to reach the peak resistance of 500 BU. In oat flours, DDT is related to oat flour protein content; Flours C, E and F, which are higher in protein content, display longer DDT than Flours A, B and D (FIGURE 16). This pattern is disrupted when oat flours are combined with wheat. At the 20% dilution level, flours from Company EF take longer to develop than Flours A, B, C and D. An oat-wheat synergy is demonstrated at 10% and 20% dilution levels, and DDT is greater than either oat or wheat flours by themselves. At the 20% dilution level, DDT is related to the Kjeldahl protein content of the composites (FIGURE 7).

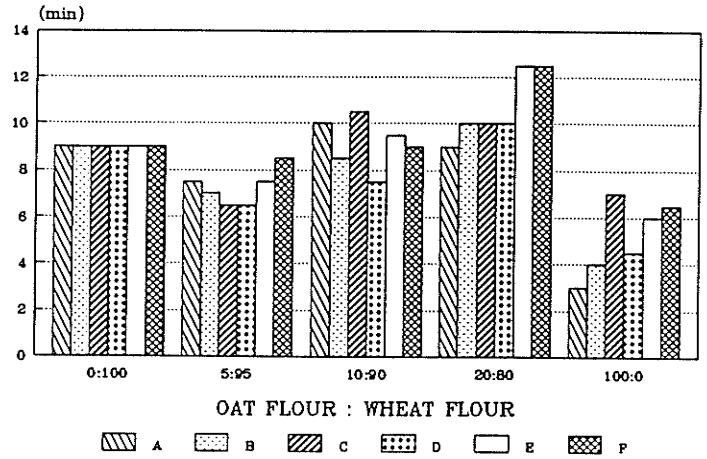


FIGURE 16. Farinograph dough development time (min) for dilutions.

b. Mixing tolerance index. The mixing tolerance index is the loss of resistance (BU) of the dough five minutes after DDT. As with DDT, MTI of oat flours is related to oat flour protein levels, however, the relationship between oat flour proteins and MTI is inverse, high protein oat flours demonstrate low MTI (FIGURE 17).

MTIs for composite flours exhibit unique flour responses, unrelated to oat flour concentration, protein or company processing. These differences between the functional behavior of oat and composite flours for DDT and MTI is again interpreted as a further indication of an interaction between oat and wheat flours.

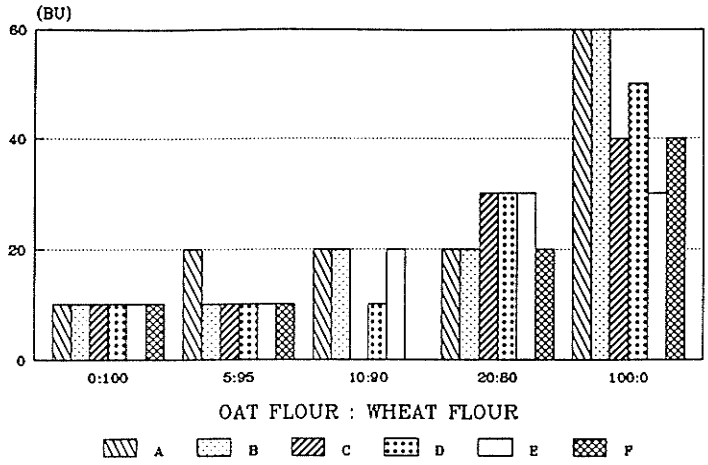


FIGURE 17. Farinograph mixing tolerance index (BU) for oat flour dilutions.

c. Absorption. Farinograph water absorption (ABS) is the amount of water required to produce a dough with a peak resistance of 500 BU. The ABS results for oat flours (FIGURE 18) demonstrates a company effect (Company AB > Company EF > Company CD). This is apparently unrelated to fibre levels of the oat flours, although the influence of fibre on water absorption may be overshadowed by another factor (for example, starch damage).

With the addition of increasing levels of oat flours to wheat to form the oat-wheat composite flours, there is generally an increase in ABS, however, absorptions of the composites of Flour D, the oat flour lowest in fibre, varied little with dilution level.

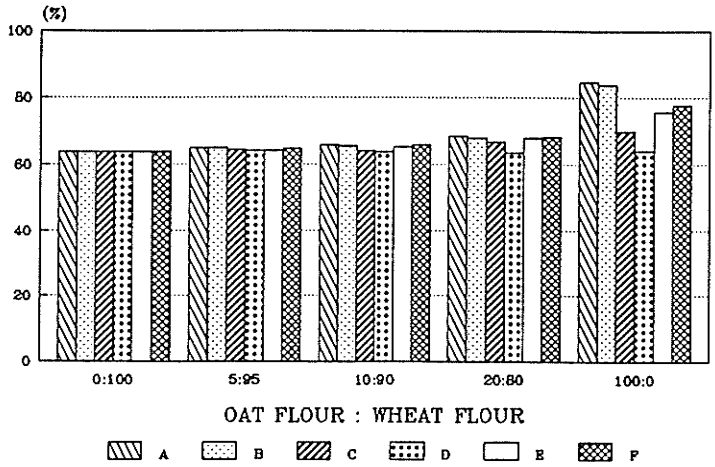


FIGURE 18. Farinograph water absorption (%) for oat flour dilutions.

3. Extensigraph

Extensigraph curves are shown in FIGURE 19. Dough prepared in the farinograph for a time corresponding to DDT was formed into two cylinders to be tested on the extensigraph at 45 and 135 min after mixing. Curves for the two cylinder-shaped doughs tested at 45 min demonstrate less resistance or greater extensibility than the same doughs, reformed into cylinders, and tested again at 145 min. Slight variations (if present) in the duplicate curves produced for each test (45 min or 135 min) are attributed to slight variations in dough handling procedures. Both curves formed during the 135 min extension were measured and averages reported.

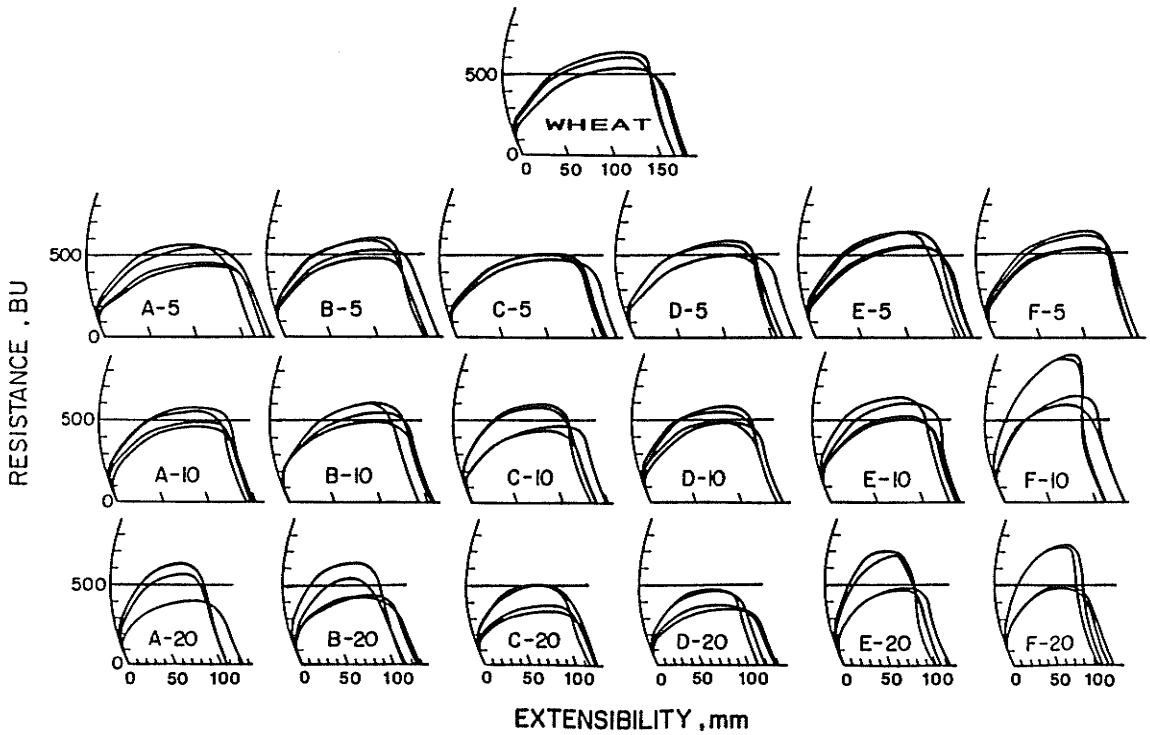


FIGURE 19. Extensigraph curves for oat flour dilutions at 45 and 135 min. (135 min curves are identified by higher resistance than 45 min curves)

a. Extensibility. Extensibility (EXT), a measurement of strain, is the distance (mm) dough stretches before breaking. With the exception of Flour A at the 5% dilution level, the composite flours are less extensible than the wheat sample (FIGURE 20). Extensibility (EXT) decreases as oat flour dilution level increases. At the 20% level, EXT is inversely related to the degree of protein denaturation of oat flour; and flours from Company EF, exhibiting signs of the most protein denaturation of the six flours analyzed, are also the least extensible.

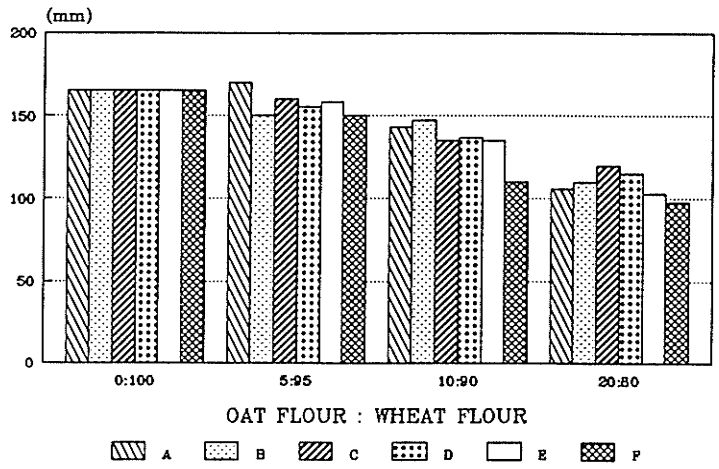


FIGURE 20. Extensigraph extensibility (mm) for oat flour dilutions.

b. Resistance. Resistance to extension (RES), a measurement of stress, is the force exerted by the dough (BU) opposing extension. In FIGURE 21, it is shown that RES generally increases with increasing oat flour dilutions. However, the RES of flours from Company CD is non-linear in behavior: there is an increase in RES as oat flour dilution increases from 5% to 10%, and then a decrease with further oat flour addition (20%). At the 20% level, the extent of protein denaturation is directly related to dough resistance to extension; flours from Company EF, containing proteins with the highest degree of denaturation of the six flours analyzed, also exhibit the greatest RES. At the 10% level, Flour F exhibits the greatest RES of all flours at any dilution. An explanation for this behavior is not available.

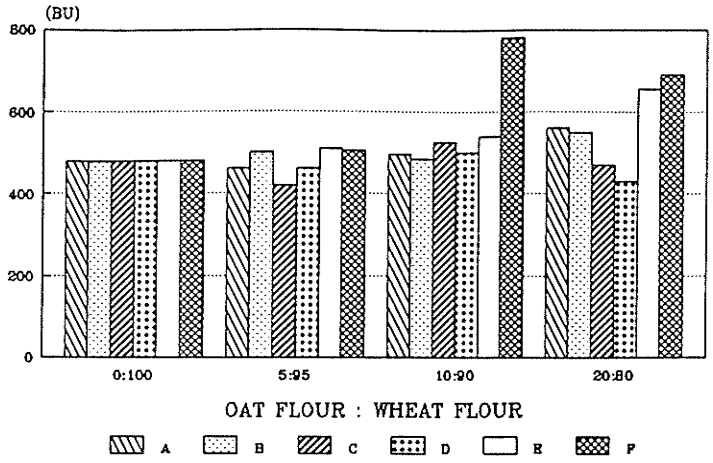


FIGURE 21. Extensigraph resistance (BU) for oat flour dilutions.

c. Area. The area under the curve (AREA), in units of cm^2, is a measurement of energy exerted by dough during deformation. For the composite flours, AREA generally decreases with increasing oat flour dilutions (FIGURE 22). However, at the 5% level, the area of Flour E composite is increased. At both the 10% and 20% dilution levels, the extent of protein denaturation is directly related to AREA; flours from Company CD (showing fewer signs of protein denaturation than the other four oat flours) also exert the least energy during deformation (AREA).

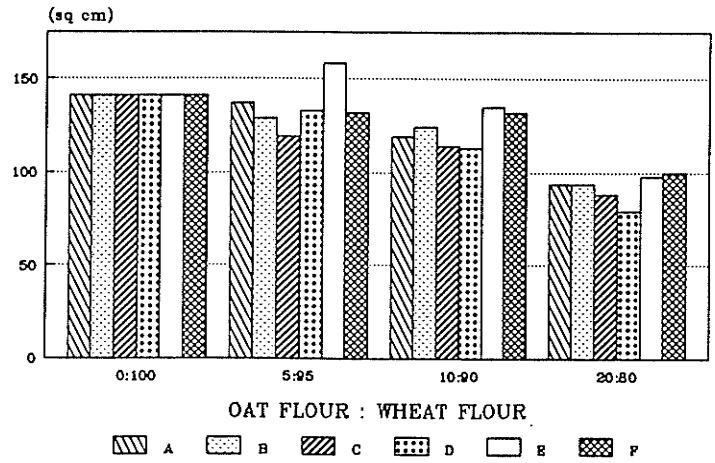


FIGURE 22. Extensigraph area (cm^2) for oat flour dilutions.

4. Gluten Content

It was not possible to wash out any gluten from oat flour doughs using the Glutamatic automatic gluten washer and 2% NaCl solution (w/v). Therefor, only wheat and oat-wheat composites were analyzed for gluten content. Several weeks after the initial testing, an attempt was made to duplicate results. Flour D-20% was unable to produce gluten at that time, although the earlier attempt was successful. However, when the mixing time of the Glutamatic was increased, gluten was washed out of the flour. As with the amylograph, a storage related oat-wheat interaction was evident.

a. Wet gluten. Flours A, B, C and D, (flours that exhibit susceptibility to wheat α-amylase in the Falling Number analysis) are all able to increase the wet gluten (WET) yield of wheat at the 5% dilution level (FIGURE 23). The effects of Flour D, the oat flour lowest in fibre, were the greatest in increasing the wet gluten yield. Increasing dilutions of oat flour decrease wet gluten content. At the 20% dilution level, wet gluten content is directly related to the protein content of the 20% oat-wheat composite flours (FIGURE 7).

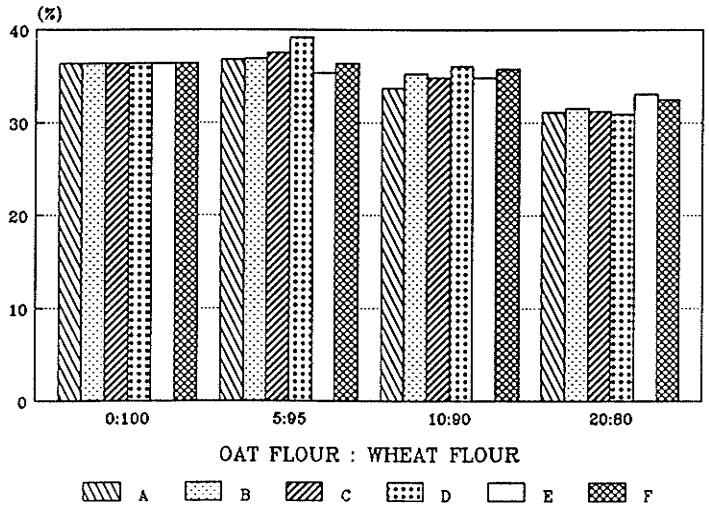


FIGURE 23. Wet gluten content (%) of oat flour dilutions.

b. Dry gluten. Unlike the wet gluten content of composite flours, all oat flours increase the dry gluten content of wheat at the 5% or 10% dilution levels (FIGURE 24). However, as with wet gluten, at the 20% dilution, dry gluten is directly related to protein content of the 20% oat-wheat composites.

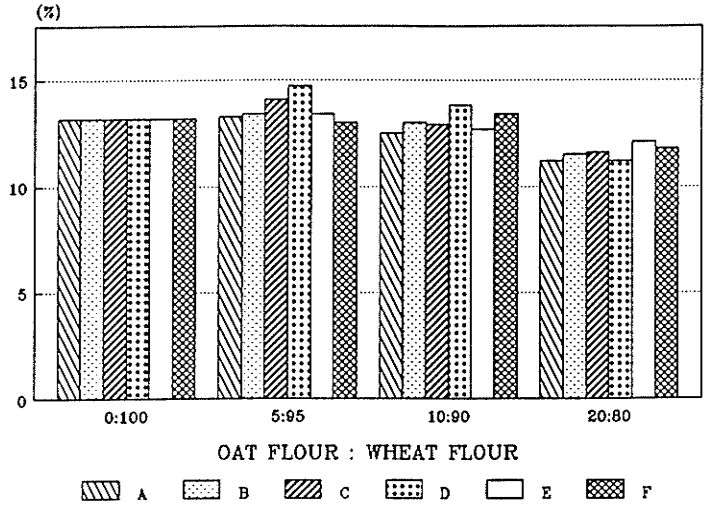


FIGURE 24. Dry gluten content (%) of oat flour dilutions.

c. Gluten moisture. Variations in gluten moisture content (FIGURE 25) indicate that composite flours vary slightly in their ability to bind water. These variations appear to be unrelated to dilution level, company of origin of oat flour or oat flour chemistry (protein, fibre). The gluten moisture content of the composite flours is very similar to the

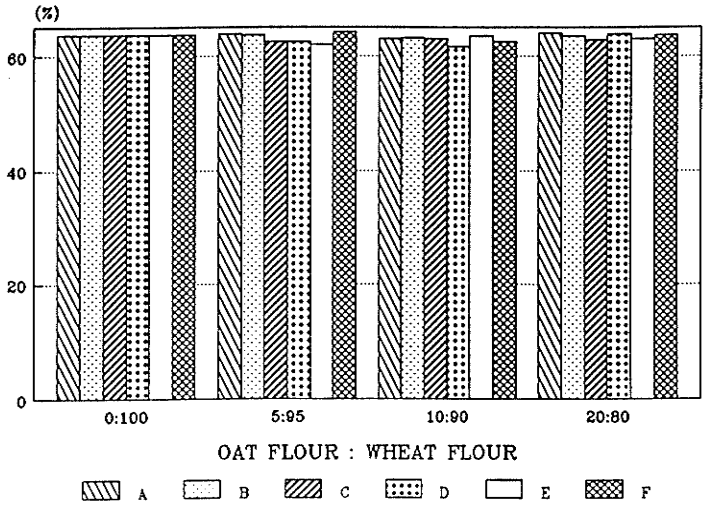


FIGURE 25. Gluten moisture content (%) of oat flour dilutions.

gluten moisture content of the wheat flour. Therefor, variations in gluten content of composite flours and their dilutions arise from variations in gluten dry matter, not variations in the moisture holding capacity of the gluten.

5. Fat Binding Capacity

Oat flours vary in their ability to bind canola oil (FIGURE 26). In contrast to the study of commercially prepared groats (Chang and Sosulski, 1985), which indicated that the bran fraction of oat flour had the highest fat binding capacity (FBC); the sample with the least bran, Flour D, is highest in FBC. Also in contrast to the results of that study, FBC is directly related to Hunterlab L values: lighter coloured flours are also higher in FBC.

These apparent contradictions may be resolved by the fact that different oils are being compared. Chang and Sosulski used corn oil, an oil with oleic:linoleic acids approximately 2:1. Analyses for this thesis used canola oil, an oil containing oleic and linoleic acids in the opposite proportions to corn oil (1:2).

FBC of the composite flours of Flour D are not related to dilution levels. Dilutions of other flours were not examined for FBC.

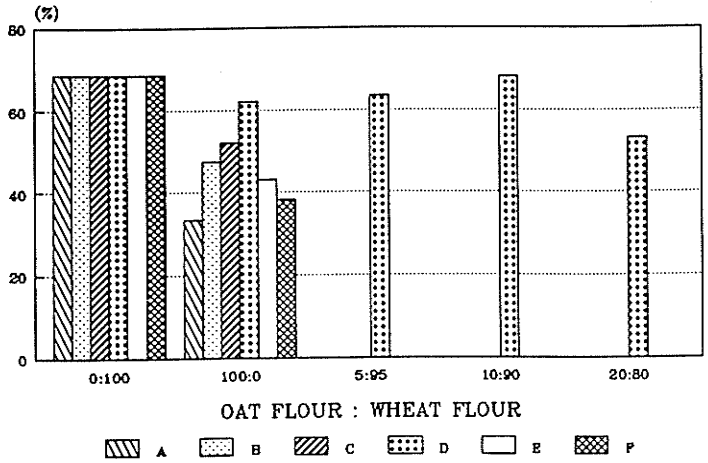


FIGURE 26. Fat binding capacity (%) of some oat flour dilutions.

6. Zeleny Sedimentation Volume

Zeleny sedimentation volumes are very low for oat flours; Flour D, the oat flour lowest in fibre, has the lowest volume (FIGURE 27). This property of Flour D is not evident in the wheat composites, again indicating the existence of oat-wheat interactions. Increasing oat flour dilution levels result in decreasing ZEL. Flour A displays a slightly higher volume for all dilutions than the other flours.

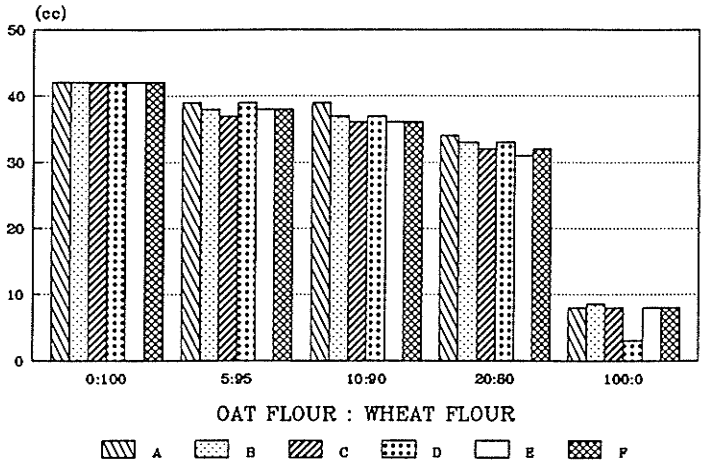


FIGURE 27. Zeleny sedimentation volume (cc) of oat flour dilutions.

7. Breadbaking

a) Appearance. Pup loaf crumb and crust structures are displayed in FIGURES 28, 29 and 30. Increasing levels of oat flour result in smaller loaves of darker, denser crumb structure. Breads baked from flours from each company differ in crust colour at all dilution levels: Flour B is darker than Flour A; Flour D darker than Flour C; and Flour F darker than Flour E. Upper crusts of 5% composites are darker than upper crusts of 20% composites.

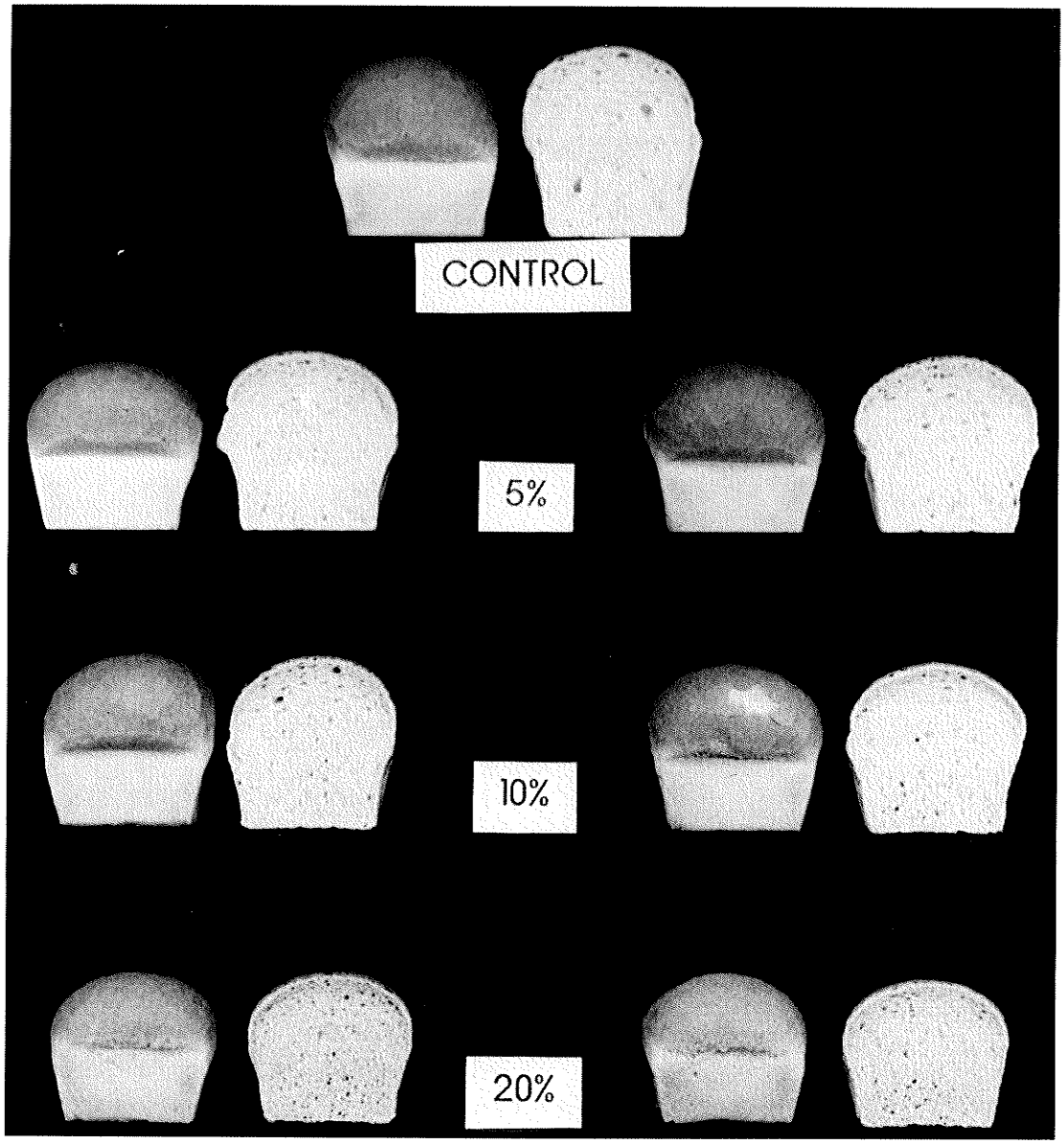


FIGURE 28. Photographs of experimental loaves showing external and internal characteristics from wheat and composites of Flours A and B.

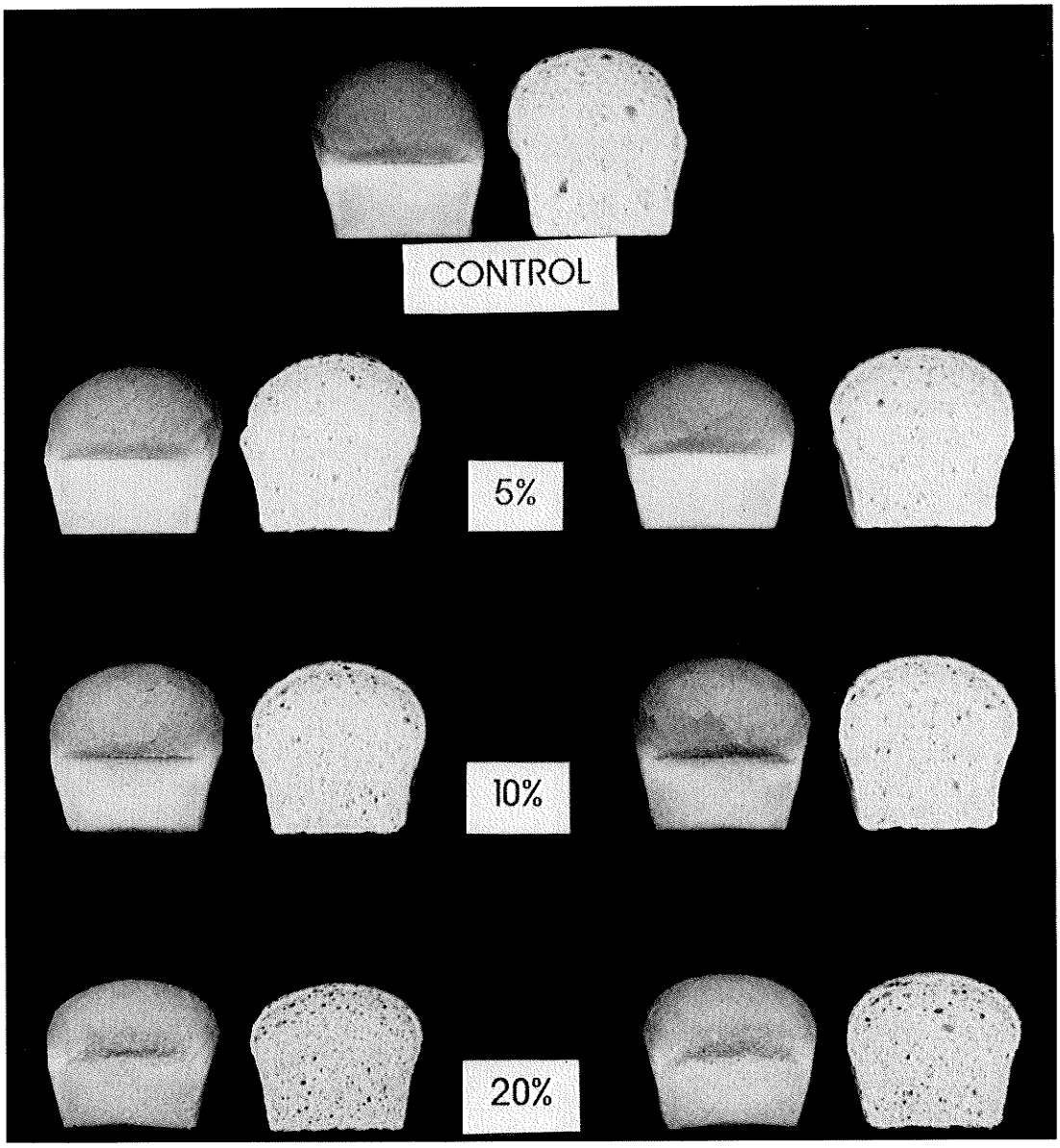


FIGURE 29. Photographs of experimental loaves showing external and internal characteristics from wheat and composites of Flours C and D.

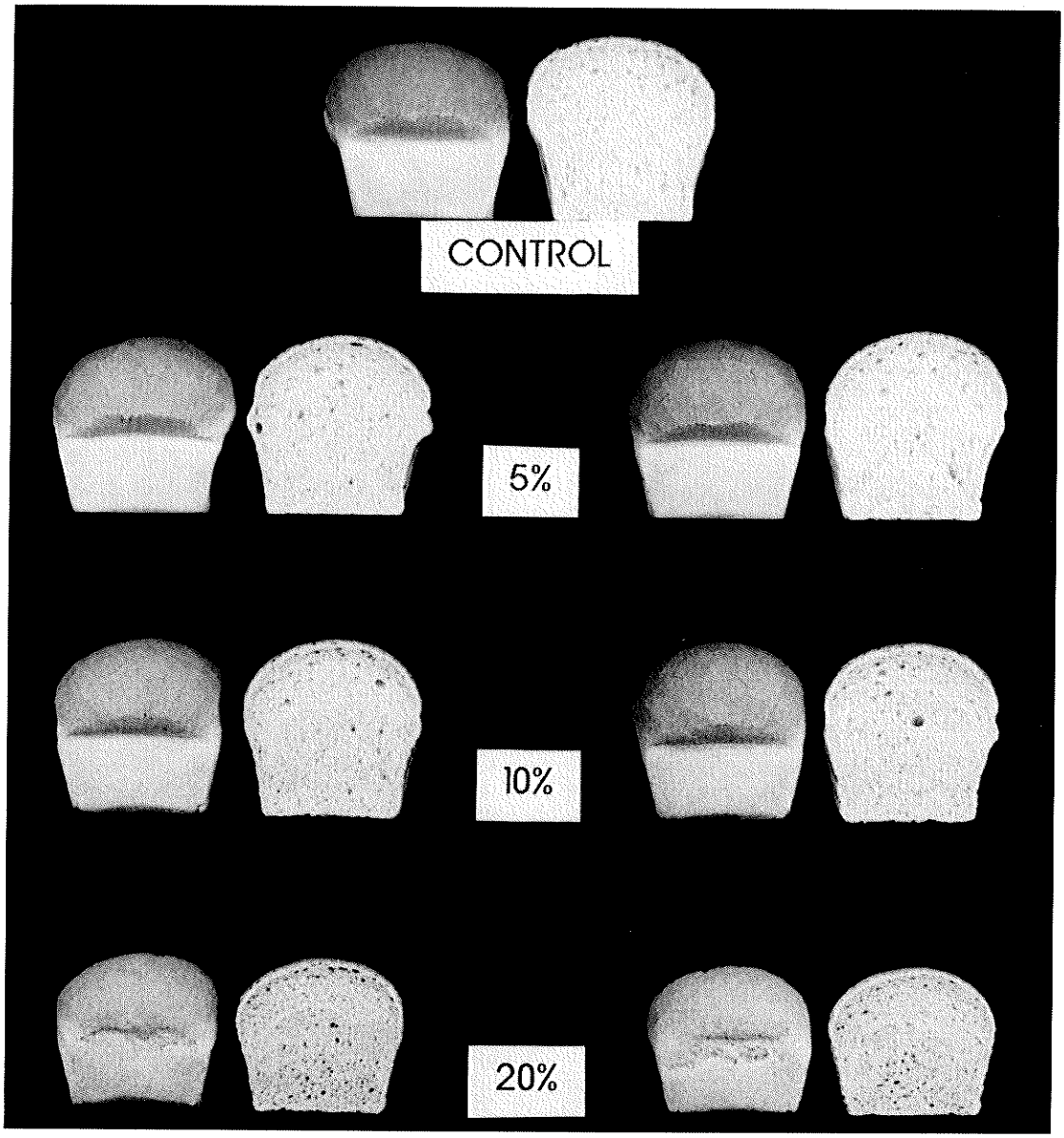


FIGURE 30. Photographs of experimental loaves showing external and internal characteristics from wheat and composites of Flours E and F.

b) Loaf volume. As indicated by the photographs in FIGURES 28, 29 and 30, increasing levels of oat flour decrease loaf volumes (LV) (FIGURE 31). These decreases are not related to oat flour chemistry (protein or fibre). Composites of Flour D, the oat flour lowest in fibre, produce the smallest loaves at the 5% and 10% dilution levels, and are similar to those of Flour C at the 20% level. Only a weak company effect is noted in that flours from Company CD differ in their response compared with the other two companies and loaves baked from composites of Flours C and D are the smallest at each dilution level. In addition, loaves baked from composites of Flour A have the largest LV at each dilution level.

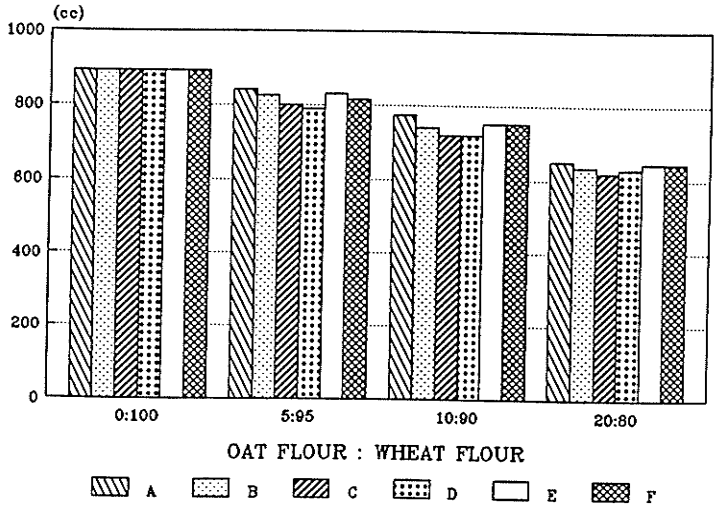


FIGURE 31. Loaf volume (cc) of pup loaves baked from wheat and oat-wheat composite flours.

c) Loaf firmness. The Baker Compressimeter indicates crumb firmness, as a force, only up to a value of 30 g. Based upon the linear relationship observed between force and compression depth of bread slices from 10-30 g, firmness values greater than 30 g were linearly extrapolated from two compression readings which resulted in force values of less than 30 g. Because of the possibility of nonlinearity of force and firmness at some point (Baker and Ponte, 1987), this calculated value may overestimate firmness.

Based upon determined values and extrapolated estimates, increasing dilutions of oat flour increase the firmness of bread (FIGURE 32). This increase is not directly related to oat flour chemistry (protein or fibre)

or composite loaf volume. Composites of Flour D, the oat flour lowest in fibre, produce the firmest slices at the 20% dilution level only. Breads baked from composites of Flour A are the softest breads at each dilution, and despite chemical similarities to Flour B, at the 20% dilution, Flours A are B are considerably different in loaf FIRM.

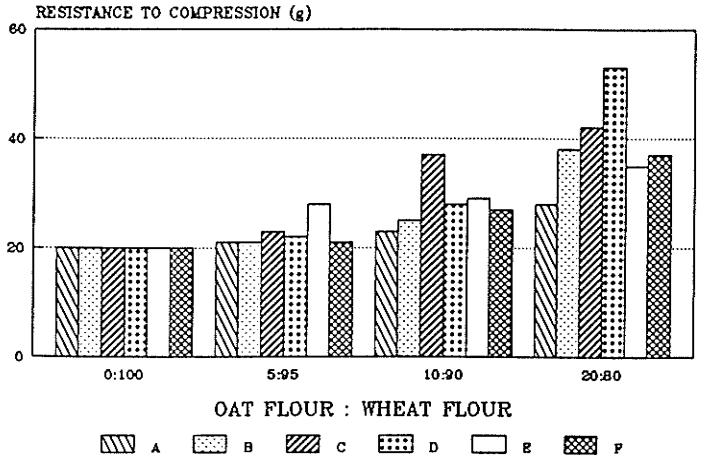


FIGURE 32. Resistance to compression (g) of pup loaf slices after three days storage.

E. STATISTICAL ANALYSES

1. *t*-Test.

Visual inspection of the analytical results indicates differences between oat flour composite functional properties. In order to obtain information regarding the statistical significance of these observed relationships, functional properties of the composite flours were analyzed with the *t*-test to test the null hypothesis: all oat flours function in a similar manner when combined with wheat.

Significant differences ($P<0.05$) of the functional properties between pairs of composite flours are identified in TABLE 16. Many differences between pairs of flours are demonstrated, but only few differences are identified between flours of each company: flours from Company AB or from Company CD differ significantly only in ZEL sedimentation, flours from

Company EF, differ only in farinograph ABS. It is to be expected that flours from Company AB or EF should be similar in their behavior, because of chemical similarities in flours from each company. However, it is unexpected to find that flours from Company CD, widely different chemically, are statistically very similar in their functional behavior with wheat. It is apparent that the chemical composition of oat flours does not provide sufficient information to predict the functional properties of oat-wheat composites. Based upon differences in functional behavior displayed by oat flours, the null hypothesis is therefor declared false, and it is accepted that oat flours differ in functional properties when combined with wheat.

TABLE 16. Oat Flour *t*-Test Comparing Functional Properties of Composite Flour Pairs

	AMYLOGRAPH			FARINOGRAPH			EXTENSIGRAPH			GLUTEN					
	PV	20MIN	SB	ABS	DDT	MTI	EXT	RES	AREA	WET	DRY	MOIST	ZEL	LV	FIRM
A-B													*		
A-C														*	
A-D											*		*	*	
A-E				**				*							**
A-F	*	*	*												
B-C															
B-D									*					*	
B-E			*											*	
B-F	*		*						*						
C-D													*		
C-E					*				**				*	*	
C-F	*				*									*	
D-E														**	
D-F	*	*					*	*	*					**	
E-F				*											

* P < 0.05
** P < 0.01

2. Paired *t*-Test.

Visual inspection of analyses, as well as the results of the *t*-test, indicate the presence of a company-based processing factor affecting oat-wheat composite flour functionality. The paired *t*-test was chosen to test the null hypothesis: company processing has no effect on oat flour functionality. Results for the paired *t*-tests are recorded in Table 17. Pairing Flours AB and Flours CD did not result in a significant difference (at the P=0.05 level) for amylograph PV. Reversing the pairs resulted in a significant difference at the 0.01 level. However, since both pairs of comparisons are not significant, it is concluded that the results are not due to company processing variables, but instead to individual flour differences. This conclusion is designed to decrease the probability of false acceptance (Type II error), but, it is at the expense of increasing the rate of false rejection (Type I Error).

Many significant differences are apparent between oat flour companies. These differences can generally be categorized in two ways. Composite flours from Company EF differ from those of Companies AB and CD in amylograph parameters and extensigraph RES. It is expected that this relationship is based upon the susceptibility of Flours A, B, C and D to wheat α-amylase. Composite flours from Company CD differ from those of Companies AB and EF in extensigraph AREA and LV. This relationship may arise from factors associated with differences in levels of protein

TABLE 17. Oat Flour Company Paired *t*-Test Comparing Functional Properties of Composite Flour Pairs

	AMYLOGRAPH			FARINOGRAPH			EXTENSIGRAPH			GLUTEN					
	PV	20MIN	SB	ABS	DDT	MTI	EXT	RES	AREA	WET	DRY	MOIST	ZEL	LV	FIRM
AB-CD									*			**	**	**	
BA-CD	**	*		*					*		*	*		**	*
CD-EF	**	**	**	*	*		*	*	**					**	
DC-EF	**	*	**	*	*		*	*	*					**	
EF-AB	**	**	**				*	*	*				*		*
FE-AB	**	*	**	*				*					**		

* P < 0.05
** P < 0.01

denaturation or decreased cysteine levels in Flours C and D.

Based upon differences in functional behavior displayed by flours produced by different companies, the null hypothesis is declared false, and it is accepted that company processing affects oat flour functionality.

3. Regression Analysis

The linear correlation coefficients for functional properties of wheat and oat-wheat composite flours, obtained by means of regression analysis, are recorded in TABLE 18. Other significant relationships may be present, but because of a non-linear nature or experimental design will not be identified through this means.

Significant relationships ($P<0.05$) are depicted by means of an Ishikawa diagram (FIGURE 33). Although originally designed to look like a fish skeleton (Juran and Gryna, 1980), this Japanese problem-solving tool becomes triangular in shape with the inter-relationships of the functional properties of composite flours. In this diagram, it is the type of line joining the functional properties that is important, not the length. The type of line is an indication of positive or negative relationship as well as level of significance.

Amylograph characteristics are significantly related to each other. Of the farinograph measurements, only DDT is significantly related (linearly) to another functional property - amylograph PV. Extensigraph AREA and EXT are both significantly related to WET gluten content, ZEL sedimentation and LV. In addition, extensigraph AREA is directly related to DRY gluten and inversely related to loaf FIRM. ZEL sedimentation volume is highly related to LV. LV and FIRM are inversely related.

This type of diagram (Ishikawa) has application in both quality assurance and product development settings. Knowledge of significant relationships among flour functional properties leads to making educated decisions regarding the choice of testing procedure. This diagram is based solely upon functional properties of six oat flours combined with one wheat flour. It is recommended that a broader base of flours be tested to prepare an Ishikawa figure for industrial purposes.

TABLE 18. Correlation Coefficients Comparing Functional Properties of Oat Flour Dilutions 0:100, 5:95, 10:90 and 20:80

	AMYLOGRAPH			FARINOGRAPH			EXTENSIGRAPH			GLUTEN					
	PV	20MIN	SB	ABS	DDT	MTI	EXT	RES	AREA	WET	DRY	MOIST	ZEL	LV	FIRM
PV	1.00														
20MIN	* 0.82	1.00													
SB	* 0.80	* 0.78	1.00												
ABS	0.57	0.42	0.31	1.00											
DDT	* 0.81	0.64	0.70	-0.59	1.00										
MTI	0.53	0.30	0.28	0.45	0.47	1.00									
EXT	* -0.78	-0.46	-0.51	-0.72	-0.75	-0.39	1.00								
RES	0.50	0.41	0.56	0.58	0.54	-0.14	-0.66	1.00							
AREA	-0.66	-0.24	-0.29	-0.42	-0.59	-0.67	* 0.78	-0.07	1.00						
WET	-0.71	-0.40	-0.38	-0.63	-0.72	-0.67	* 0.78	-0.23	** 0.88	1.00					
DRY	-0.69	-0.44	-0.36	-0.63	-0.72	-0.68	0.73	-0.20	0.82	** 0.97	1.00				
MOIST	0.23	0.29	0.05	-0.31	0.30	0.34	-0.15	-0.01	-0.15	-0.33	-0.54	1.00			
ZEL	* -0.73	-0.31	-0.50	-0.67	-0.68	-0.58	** 0.88	-0.41	** 0.88	* 0.78	0.72	-0.11	1.00		
LV	-0.68	-0.23	-0.36	-0.62	-0.66	-0.58	** 0.89	-0.31	** 0.94	* 0.84	0.76	-0.08	** 0.94	1.00	
FIRM	0.62	0.23	0.42	0.22	0.61	0.52	-0.69	0.11	** -0.87	* -0.78	-0.71	-0.08	** -0.80	* -0.83	1.00

* $P < 0.05$

** $P < 0.01$

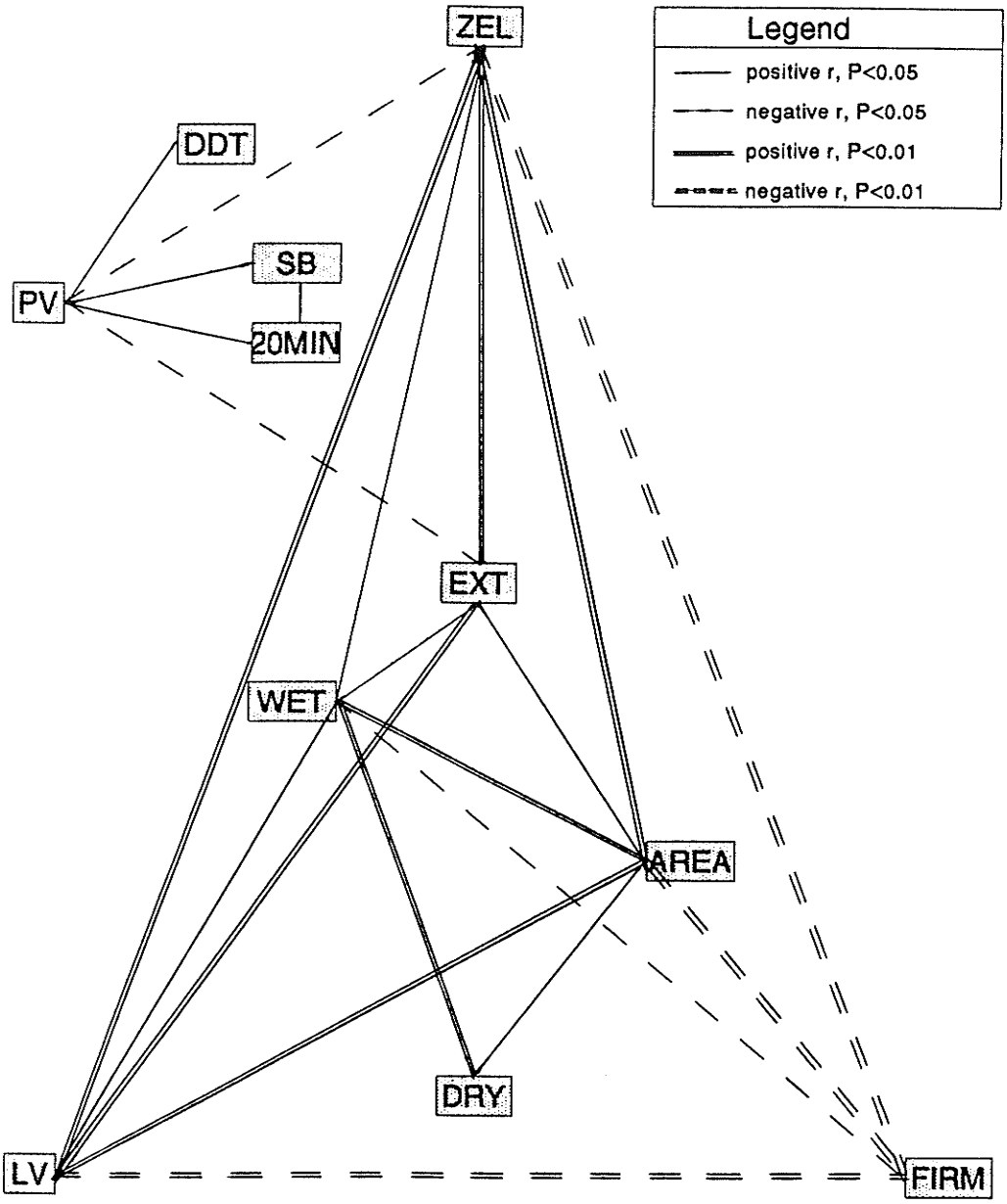


FIGURE 33. Ishikawa diagram showing significant relationships of linear correlation coefficients.

The Ishikawa diagram is also useful in making general conclusions regarding the relationship of functional properties to breadbaking characteristics (LV and FIRM). With the exception of extensigraph EXT, factors directly related to LV are inversely related to FIRM, and so only LV will be discussed. According to this diagram, linear predictors of LV are ZEL sedimentation, WET gluten content, and extensigraph AREA and EXT.

In addition, amylograph PV, through its inverse relationships with EXT and ZEL, and farinograph DDT, through its relationship with PV, are also linear indicators of LV.

Visual examination of the data has indicated the presence of interactions between oat and wheat flours. Regression analysis, comparing the effects of dilution (with or without the wheat analysis considered as a control) to oat-wheat composite functional properties were analyzed in order to examine the null hypothesis: there is no interaction between oat and wheat flours. The results are listed in TABLE 19. Large differences between values, differing only in the presence of a wheat control, are attributed to the interaction between oat and wheat flours. These interactions are most noticeable in extensigraph RES and amylograph 20-MIN. Differences are also noted in amylograph PV and SB, farinograph DDT and gluten MOIST. A small difference is also found in loaf FIRM and farinograph ABS.

As a results of these findings, the null hypothesis is declared false, and it is concluded that a chemical interaction between oat and wheat flours affects the functional behavior of the composite flours.

TABLE 19. Correlation Coefficients Comparing Functional Properties of Composite Flours (5:95, 10:90, 20:80), With or Without Wheat Flour as a Control (100:0), to the Effects of Dilution

	AMYLOGRAPH			FARINOGRAPH			EXTENSIGRAPH			GLUTEN					
	PV	20MIN	SB	ABS	DDT	MTI	EXT	RES	AREA	WET	DRY	MOIST	ZEL	LV	FIRM
WITH WHEAT	0.40	-0.30	-0.01	** 0.78	0.57	0.68	** -0.93	0.42	** 0.95	** -0.87	** -0.78	-0.04	** -0.96	** -0.98	** 0.91
WITHOUT WHEAT	** 0.87	0.74	0.56	0.69	* 0.77	0.67	** -0.90	** -0.93	** 0.93	** -0.92	** -0.88	0.29	** -0.93	** -0.97	* 0.79

* $P < 0.05$

** $P < 0.01$

V. SUMMARY

A. OAT FLOUR

1. Chemical Analyses

Flours A and B, representing Company AB were similar in chemical composition, as were Flours E and F, from Company EF. Flours C and D, produced by Company CD differed chemically, with Flour D the lowest of the six flours in ash and all fibre components (indications of essentially complete bran removal).

Wide variations were found in levels of protein (12.6-15.0%, N x 5.5), ash (1.2-1.9%) and fibre (TDF, 5.6-14.1%; NDF, 2.9-9.0%; SDF, 2.5-5.1%; ß-glucan, 1.8-4.0%). Little variation was observed in lipid content of the oat flours (TL, 9.9-10.7%; FL, 7.4-8.1%; BL, 2.1-2.9%), or fatty acid composition (palmitic:oleic:linoleic = 18:40:35). Starch levels (62.0-71.4%) were related to protein and fibre levels. Only Flour A exhibited signs of starch damage (17.2 FU); Flour B was not analyzed in this test. All oat flours were similar in amino acid composition, with the exception of flours from Company CD which contained lower levels of cysteine. Oat flours exhibited lipid levels that were unique for each flour, apparently unrelated to company of origin or other chemical components. Osborne fractionation and electrophoretic results (band intensity) indicated flours varied, on a company of origin basis, in levels of protein denaturation. Extent of protein denaturation was inversely related to prolamin levels. Flours from Company CD were the least denatured and contained highest levels of prolamin. However, in addition to the effects of company processing, bran removal from Flour D also affected the level of prolamin.

Flours C, E and F contained the highest levels of protein, ash and

fibre. Examination of the fibre analyses indicated the various fibre components of oat flour (NDF, SDF, ß-glucan) are fundamentally linked in their association to each other as well as to ash. High protein levels in the oat flours studied were associated with high levels of fibre and ash.

2. Enzyme Analyses

Oat flours exhibited enzyme inactivation, in comparison to a sample of ground, unsteamed groats. Enzyme activity was not related to protein denaturation, as demonstrated by electrophoretic results.

3. Colour Analysis

Oat flours differed in colour. Flours highest in ash, protein and fibre content, were also the darkest - both in flour and slurry form. The flour lowest in ash content, was brightest as flour or slurry.

4. Functional Analyses

a. Effects of bran removal. Flour D, lowest in fibre and ash (indicators of essentially complete bran removal), was also the lowest in farinograph ABS and ZEL sedimentation volume; and highest in FBC. Flour D was also the flour lowest in bound lipid and polyphenol content; factors which may also contribute to the properties listed.

b. Effects of high protein. Of the oat flours examined, Flours C, E and F contained the highest levels of protein. Farinograph curves, with their longer DDT and smaller MTI, indicated that high protein oat flours were also stronger (in dough mixing) than lower protein flours. However, since high protein was also associated with high ash and fibre levels, the high protein level may not be entirely responsible for the indicated results.

c. Effects of protein denaturation. Amylograph 20-MIN viscosity, an indicator of shear-thinning behavior, was inversely related to protein denaturation of oat flours. That is, flours exhibiting the greatest signs of protein denaturation also exhibited the greatest signs of shear-thinning. It is possible that it is not the denatured protein, but associated processing-related factors that are responsible for this behavior.

d. Effects of company processing. Differences in company processing, not related to protein denaturation, resulted in unique company properties of amylograph PV and farinograph ABS.

B. OAT-WHEAT FLOUR COMPOSITES

1. Oat-Wheat Chemical Interactions

Evidence for oat-wheat chemical interactions have been identified in five ways in this thesis. First, proximate compositions of composite flours indicated altered moisture levels, decreased protein levels and increased ash levels with respect to levels expected from dilution effects. Second, by comparing 100% oat flours with the composites, a synergistic effect was noted in amylograph SB (20% dilutions), farinograph DDT (10% and 20% dilutions) and WET gluten content (5%); and the composites displayed higher results than either the oat or wheat flour individually. Synergistic inhibition was demonstrated in amylograph PV. Third, by comparing the effects of dilution with or without the wheat as a control, extensigraph RES, amylograph properties, gluten MOIST, farinograph DDT and ABS, and loaf FIRM gave evidence of oat-wheat interactions. Fourth, the results of reduced and unreduced SDS-PAGE indicated protein changes in oat flour, unrelated to disulphide interactions, that resulted from association with wheat flour. Fifth, the effects of storage produced unexpected amylograph results and a change in gluten formation ability.

Three reasons may explain the oat-wheat interactions. First, as

indicated in Falling Number values, Flours A, B, C and D were more easily attacked by wheat amylases than Flours E and F. Second, SDS-PAGE results indicate changes occur in oat proteins, unrelated to protein disulphide formation, when oat flour is mixed with wheat flour. Third, because of the high lipid content of oats and the moisture content of wheat flour (12.5%), oxidative changes associated with oat lipids may result in an interaction between oat and wheat flours. However, no analyses were conducted to give support to this possibility.

2. Functional Analyses

a. Effects of bran removal. Reduced fibre content of one oat flour (Flour D), resulting from removal of oat bran, decreased farinograph ABS. Company-related processing effects affected functional properties of this flour to a greater extent than fibre levels, since Flour C, produced by the same company, with the highest fibre levels, generally behaved in a manner similar to Flour D. However, at the 20% dilution level, Flour D produced the firmest bread, indicating oat bran removal from oat flour may be detrimental to breadbaking functionality at high levels of oat flour dilution.

b. Effects of high oat flour protein. At the 20% oat flour dilution level, high protein levels were directly related to amylograph PV and 20-MIN viscosity. Also at the 20% level, there was an inverse relationship between high oat flour protein and ZEL sedimentation. Since ZEL is so closely related to LV (r=0.94**), it appears that high oat flour protein is a negative factor in bread baking at high levels (20%) of oat flour. However, because of the association of high levels of protein, ash and fibre in the oat flours examined, the high levels of oat protein may not be directly responsible for decreased LV.

c. Effects of oat flour protein denaturation. Osborne solubilities and PAGE analyses indicated the proteins in flours from Company EF are highly denatured, those from Company AB are less denatured and proteins from Company CD are the least denatured. Osborne solubilities indicated that

protein denaturation is mostly associated with the loss of globulin and prolamin fractions. As composites, flours from Company CD, with the highest prolamin solubilities, demonstrated the lowest farinograph ABS, the lowest extensigraph RES, the lowest LV and the highest FIRM at the 20% oat flour dilution. It appears that protein denaturation to decrease the prolamin composition results in beneficial effects in LV and FIRM. The associated increased farinograph ABS that accompanied protein denaturation may be responsible for these benefits.

d. Effects of company processing. As indicated by paired *t*-tests, composite flours from Company EF differed from the other oat flours in all amylograph properties as well as extensigraph RES. Composite flours from Company CD differed from the other oat flours in extensigraph AREA and LV. Company AB differed from Company CD in gluten MOIST and from Company EF in ZEL. Companies CD and EF differed in farinograph ABS and DDT as well as extensigraph EXT.

3. Breadbaking factors

Summarizing the conclusions obtained above from the functional analyses: bran removal from oat flour may be detrimental to breadbaking (FIRM) only at high levels of oat flour; high oat flour protein (ash, fibre) may contribute to decreased LV; factors associated with protein denaturation are beneficial to breadbaking characteristics; and processing conditions of Company CD were of more significance to breadbaking characteristics than levels of protein or bran.

According to the Ishikawa figure, functional properties directly contributing to LV are ZEL sedimentation, extensigraph AREA and EXT, and WET gluten. Amylograph PV and farinograph DDT are indirectly associated with LV. Bread firmness after three days storage is inversely related to LV, but may be produced by different flour properties than those responsible for LV.

Farinograph absorption is noticeably absent from this list of functional properties contributing to LV. Results of Oomah (1983) indicated LV increased with higher water absorption in commercial oat

flour composites. The oat flour with highest farinograph absorptions, as a flour or as composites, also exhibited highest LV and least FIRM at all dilutions (Flour A). It is possible that the one sample with bran removed, which exhibited different farinograph absorption properties, has skewed the results of the other five oat flours to produce insignificant correlation coefficients with respect to LV and FIRM.

VI. CONCLUSIONS

1. Oat flours differed in chemical composition and functional properties. Company processing effects overshadowed chemical influences (protein, fibre, ash) of farinograph absorption and amylograph viscosities on a statistical basis. Farinograph dough strength properties (DDT and MTI) of oat flours were directly related to levels of protein, fibre or ash. Bran removal, identified in one oat flour by low ash and fibre contents, decreased farinograph absorption and Zeleny sedimentation volume, and increased the fat binding capacity, in comparison to the other five oat flours. It is presumed that these company-related processing effects will also extend to the nutritional influences of oat flour.

2. Oat flour protein, ash and fibre levels exhibited a fundamental association, and together were related to colour analyses. Therefor, the use of ash or colour measurements may provide a screening device for protein or fibre levels in an industrial ingredient control setting requiring information for nutritional labelling.

3. Interactions between oat and wheat flours were related to storage, susceptibility to wheat amylases or non-disulphide protein association of the flours. Because of these interactions, functional properties of most oat-wheat composites were not directly related to the effects of dilution.

4. Oat flours differed in functional properties, with respect to each other, when combined with wheat. Differences were generally related to company processing variables including factors associated with protein denaturation and susceptibility to wheat amylase activity. These company processing effects generally overshadowed flour chemistry effects

(protein, fibre, ash). Examination of flours by differential scanning calorimetry (DSC) may identify factors responsible for company processing differences.

5. Functional properties of statistical significance directly related to breadbaking characteristics are Zeleny sedimentation, wet gluten, and extensigraph area and extensibility. In addition, amylograph peak viscosity, and farinograph dough development time and absorption are related to the breadbaking functionality of oat-wheat composite breads. Although levels of protein and bran may influence loaf volume and bread firmness, company processing conditions, possibly related to factors associated with protein denaturation, are more directly related to breadbaking characteristics. The oat flour highest in starch damage, amylograph peak viscosity, farinograph absorption and Zeleny sedimentation; and lowest in farinograph dough development time; produced the bread of highest loaf volume and least firmness at each of three dilution levels with wheat.

Chemical, colour, enzyme and functional analyses have been conducted on six commercial oat flours. Removal of bran from oat flour does not appear to unduly hinder breadbaking functionality, in comparison to other oat flours. Company processing effects are of greater significance than flour chemistry (protein, ash or fibre content) on functional properties of the flours, alone or as composites with wheat flour. It is therefor recommended that standard functional tests be devised for applications in the use of commercial oat flours in breadbaking. It is also recommended that further studies into potential applications of oat flour be based on functional properties, rather than flour chemistry (protein, ash or fibre).

VII. CONTRIBUTIONS TO KNOWLEDGE

1. A statistical method to evaluate the effects of dilution on oat flour functionality was presented and utilized.

2. A diagrammatic method (Ishikawa figure) expressing the interconnectedness of statistically significant correlation coefficients was presented.

3. A fundamental association of protein, ash and fibre (NDF, SDF and β-glucan) was described in whole oat flour.

4. A non-disulphide protein association between oat and wheat flours was identified.

5. A processing-related inhibition of some oat flours to wheat amylases was identified.

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VIII. APPENDIX

APPENDIX A. Moisture, ash, protein and falling number values of all oat–wheat dilutions.

SAMPLE	MOISTURE	ASH	PROTEIN	FALLING NUMBER
	%	%	%	sec
WHEAT	12.3	0.68	15.8	579
A–5	12.1	0.64	15.0	560
A–10	12.1	0.68	14.9	567
A–20	11.9	0.80	14.5	476
A–100	9.3	1.62	12.6	472
B–5	12.2	0.70	15.5	592
B–10	12.1	0.78	15.2	562
B–20	11.8	0.84	14.7	539
B–100	9.2	1.59	12.8	508
C–5	11.8	0.65	15.0	634
C–10	12.1	0.73	15.3	608
C–20	11.7	0.88	14.7	577
C–100	9.0	1.92	14.3	501
D–5	12.1	0.61	14.9	578
D–10	12.1	0.63	14.9	529
D–20	11.6	0.71	14.6	575
D–100	9.8	1.19	13.0	468
E–5	12.3	0.64	15.2	538
E–10	12.1	0.72	15.1	567
E–20	12.0	0.85	15.1	576
E–100	10.1	1.79	14.5	589
F–5	12.0	0.64	15.6	554
F–10	12.2	0.73	15.5	600
F–20	11.9	0.89	15.1	627
F–100	10.1	1.91	15.0	527

APPENDIX B. Technological data of all oat–wheat dilutions

	AMYLOGRAPH			FARINOGRAPH			EXTENSIGRAPH			GLUTEN						
	PV BU	20MIN BU	SB BU	ABS %	DDT min	MTI BU	EXT mm	RES BU	AREA sq cm	WET %	DRY %	MOIST %	FBC %	ZEL cc	LV cc	FIRM g
WHEAT	440	360	370	63.7	9.0	10	165	480	141	36.4	13.2	63.7	68.7	39	899	20
A-5%	390	280	330	64.9	7.5	20	170	460	137	36.8	13.3	63.9		39	840	21
A-10%	400	290	320	65.8	10.0	20	143	495	119	33.8	12.8	63.0		39	775	29
A-20%	450	310	330	68.4	9.0	20	106	560	94	31.2	11.2	64.1		34	650	28
A-100%	990	450	480	84.5	3.0	60							33.3	8.0		
B-5%	380	290	320	65.0	7.0	10	150	500	129	36.9	13.4	63.7		38	825	21
B-10%	400	290	340	65.6	8.5	20	147	485	124	35.3	13.0	63.2		37	740	25
B-20%	420	300	350	67.8	10.0	20	110	550	94	31.6	11.5	63.6		33	635	(38)
B-100%	1000	480	440	83.8	4.0	60							47.6	8.5		
C-5%	400	290	330	64.4	6.5	10	160	420	119	37.6	14.1	62.5		37	800	23
C-10%	410	290	340	64.1	10.5	0	135	525	114	34.9	12.9	63.0		36	720	(37)
C-20%	460	330	360	66.7	10.0	30	120	470	98	31.9	11.6	62.9		32	620	(42)
C-100%	860	560	430	69.9	7.0	40							52.2	8.0		
D-5%	(390)	(280)	(320)	63.9	6.5	10	155	460	133	39.2	14.7	62.5	63.6	39	790	22
D-10%	410	280	350	63.7	7.5	10	137	500	113	36.1	13.8	61.8	68.2	37	720	28
D-20%	450	300	350	63.4	10.0	30	115	430	79	31.0	11.2	63.9	53.4	33	630	(53)
D-100%	880	540	430	63.9	4.5	50							62.1	3.0		
E-5%	400	290	350	64.1	7.5	10	158	510	158	35.4	13.4	62.1		38	830	28
E-10%	420	300	360	65.2	9.5	20	135	540	135	34.9	12.7	63.6		36	750	29
E-20%	480	340	390	67.7	12.5	30	103	655	98	33.1	12.2	63.1		31	645	(35)
E-100%	780	410	350	75.6	6.0	30							43.1	8.0		
F-5%	410	300	350	64.6	8.5	10	150	505	132	36.4	13.0	64.9		38	815	21
F-10%	430	310	360	65.9	9.0	0	110	780	132	35.8	13.4	62.6		36	750	27
F-20%	480	340	380	68.1	12.5	20	98	690	100	32.5	11.8	63.7		32	645	(37)
F-100%	820	430	350	77.7	6.5	40							38.3	8.0		

Bracketed figures () are estimates. See text for explanations.