BENCH-SCALE WET-MILLING OF WHEAT FLOUR: DEVELOPMENT OF A TEST TO FRACTIONATE A HIGHLY SHEARED FLOUR-WATER DISPERSION AND ITS COMPARISON WITH FRACTIONATION BY THE DOUGH-WASHING TEST

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ABSTRACT

A bench-scale wet-milling test for wheat flour was devised to mimic the industrial processes that begin with a highly sheared flour-water dispersion (HS-FWD). The test was developed on a Regional Baking Standard (RBS-98) flour, which is a flour of 76 hard winter wheat composites. The HS-FWD wet-milling test included: i) high-shear mixing of the flour (65.0 g, db) and water (100 mL, 35°C) with a homogenizer to form a HS-FWD; ii) centrifugal fractionation (2,500 x *g* / 15 min) of the HS-FWD into supernatant, protein-rich, and starch phases; iii) aging of the protein-rich phase; and iv) isolation and purification of five fractions (the gluten, A-starch, B-starch, fiber, and water-solubles). Response surface methodology was used to optimize the high-shear mixing and gluten-aging steps. Regression models showed that water-flour (db) ratios of 1.6-1.8, homogenizer speeds of 5,000-7,000 rpm, and added-water temperature of 30-35°C in the high-shear mixing step gave high recoveries (80-84%) of the A-starch fraction with 0.20-0.30% protein. The highest recovery (~78%) of flour protein in the gluten fraction with ~83% (db) protein (Nx5.7) was achieved when the protein-rich phase was aged in added water (100 mL) at 30-40°C for 15-25 min without agitation. At the optimal process conditions (water-flour ratio: 1.7, homogenizer speed: 6,000 rpm, water temperature: 35 °C, gluten aging temperature and time: 40°C and 20 min, the Glutomatic washing time of the aged gluten: 2.0 min), 80.3% of flour starch was recovered in the A-starch fraction with 0.24% protein, 78.8% of flour protein in the gluten fraction with 82.9% protein, and 98.8% of flour solids in the five wet-milling fractions. The recoveries of starch and protein by the HS-FWD wet-milling test were 7-10% lower as compared to the Martin dough-washing method. The Rapid Visco-Analyzer (RVA) pasting curves of

the A-starch fractions and the breadmaking qualities of the gluten fractions isolated by the two tests were comparable. Flours with weak compared to strong dough-mixing characteristics, including flours of hard wheats, a soft wheat, and waxy wheats, generally gave lower recoveries of the A-starch fractions with 0.20-0.30% protein. The missing A-starch either contaminated the gluten fraction and lowered its protein content, or increased the B-starch fraction.

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LIST OF ABBREVIATIONS

AACC	American Association of Cereal Chemists
ANOVA	Analysis of variance
CRA	Corn Refiners Association
CV	Coefficient of variation
db	Dry basis
FAO	Food and Agriculture Organization (of United Nations)
HD	High-Pressure Disintegration (process)
HMW-GS	High-molecular weight glutenin subunits
HWWQL	Hard Winter Wheat Quality Laboratory (of USDA/ARS/GMPRC)
HS-FWD	Highly sheared flour-water dispersion
ICC	International Association for Cereal Science and Technology
IPP	Insoluble polymeric protein
IWGA	International Wheat Gluten Association
LMW-GS	Low-molecular weight glutenin subunits
LSD	Least significant difference
mb	Moisture basis
MC	Moisture content
MMT	Million metric ton(s)
MS-DWD	Moderately sheared dough-water dispersion
RBS-98	Regional Baking Standard (flour from 1998 crop year)
RSM	Response surface methodology
RVA	Rapid Visco-Analyzer
SEM	Scanning electron microscopy/micrograph
USDA ARS/GMPRC	United States Department of Agriculture/ Agricultural Research Service/ Grain Marketing and Production Research Center
USDA/NASS	United States Department of Agriculture/ National Agricultural Statistics Service
W/F	Water-flour (ratio)
wb	Wet basis

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DEDICATION

I wholeheartedly dedicate this work to two most important persons in my life. First is my beloved mother, ***Nazmiye Sayaslan***, who passed away in the middle of this work with an unprecedented wish to see its completion and homecoming of the author. May the Almighty God have mercy on her soul and grant her paradise. Next is my beloved father, ***Ibrahim Sayaslan***. Together, they sacrificed a lot while molding me into the person I am today. I cannot thank them enough for their absolute love and sacrifices throughout my life, and above all for their constant prayers for my success.

INTRODUCTION

Wheat is the leading cereal grain produced in the world, and is used for food (67%), feed (20%), and seed (7%). Oleson (1994) reported that 6% of wheat is consumed in the wet-processing of wheat flour to co-produce wheat starch and vital wheat gluten. Wheat starch and gluten are used in foods, and also in animal feeds and industrial adhesives.

The wet-separation of the gluten proteins and starch from wheat flour is based on their water insolubility, density, and particle size. The gluten proteins in wheat endosperm tend to aggregate and form particles that are larger in size but less dense than starch granules. Aggregation of the molecular strands of the gluten proteins in the presence of water is accelerated and increased by the concentration and composition of the proteins, and by the temperature of the aggregation medium between approximately 15 to 50°C. When the gluten proteins of flour aggregate into a cohesive mass, they exclude most of the starch granules, but bind much of the flour lipids.

Five commercial wet-milling processes have been employed to co-produce wheat starch and vital wheat gluten, all of which start with flour rather than wheat kernels (Seib 1994, Maningat and Bassi 1999). Until the mid 1970s wheat starch was produced either by the Martin (dough-washing) process, which was invented in 1835 and was the dominant process (Anderson 1967, 1974, Fellers 1973, Barr 1989, Zwitserloot 1989a), or by the Batter process developed in 1944 (Anderson 1967, 1974, Fellers 1973). Then, the Alfa-Laval/Raisio (Kerkkonen et al 1976, Maijala 1976, Dahlberg 1978) and the

Hydrocyclone (Verberne and Zwitserloot 1978, Verberne et al 1979) processes were commercialized in the mid- and late 1970s. Finally in 1984, the High-Pressure Disintegration (HD) process was introduced (Zwitserloot 1989a, 1989b, Witt 1997).

Among the recent processes, the Hydrocyclone and Raisio are commonly used in North America, and the HD process is popular in Europe (Maningat and Bassi 1999). The advantages of the relatively new processes include reduced consumption (<4 parts water / part flour) of water, reduced processing time, and decreased sensitivity of yields of starch and gluten to the rate of aggregation and to the strength of the gluten during processing (Zwitserloot 1989a, 1989b). The Martin process continues to be used in many countries to recover investment costs. However, the Batter process appears to have been abandoned.

Commercial Wet-Processes for Wheat Flour

The Martin and Batter processes may be considered the traditional methods to wet-process wheat flour (Fig. 1) (Meuser et al 1989). In the Martin process, flour (1 part) and water (~0.6 part) are mixed to a stiff dough. The dough is rested briefly and then subjected to kneading in a trough under a water spray (Anderson 1967, 1974, Fellers 1973, Seib 1994). During dough mixing, the gluten proteins are strongly aggregated to form a matrix of gluten threads that enmeshes other dough components. According to Robertson and Cao (1998a, 1998b), the gluten threads have lengths in the centimeter to meter range. Apparently, a gluten matrix is created during mixing of the dough because the concentration of gluten is moderately high, and the temperature of the dough is warm. The dough is kneaded under a water spray, and the water breaks the bonding forces between the starch granules and the gluten threads. The starch is released into the liquid phase and simultaneously the gluten is concentrated in the residual solid phase. Robertson et al (2000) showed that a Glutomatic dough-kneading/washing instrument gave the best separation of starch from gluten when a flour was mixed to optimum to form a mechanically strong dough.

In the Batter process, a smooth flowable batter is prepared by mixing flour (1 part) and warm water (0.7-1.8 parts, 50-55°C). After resting, two parts (flour basis) of cold water are added and the slurry is mixed in a cutting pump to form small curds of gluten. The gluten is collected on a vibrating or gyrating screen under a water spray, and the throughs are centrifuged to collect the starch. As in the Martin process, the gluten proteins in the Batter process are strongly aggregated in the mixing step because of the warm temperature of the water (Anderson 1967, 1974, Fellers 1973, Knight and Olson 1984, Seib 1994). However, the length of the gluten threads appears to be in the millimeter to centimeter range (Fellers 1973, Robertson and Cao 1998a, 1998b).

In contrast to the highly aggregated gluten particles formed in the mixing step of the traditional wet-processes for wheat flour, modern-day processes start with an aqueous dispersion of flour components at ~30% solids that is formed by gently blending a highly sheared flour-water dispersion with additional water. That dispersion, which then undergoes fractionation in the process, may be termed a highly sheared flour-water dispersion (HS-FWD). The Raisio and HD processes (Fig. 2) both begin with a HS-FWD which apparently possesses thin and relatively short threads of gluten that are dispersed in water among free starch granules. A HS-FWD is produced by high-shear mixing of flour and water in one of two ways, either by a very rapidly moving mixer blade that acts on the slowly moving flour-water mixture or by very rapidly moving a flour-water mixture through a small stationary aperture.

Intermediate-sized gluten particles appear to be formed in the mixing steps of the Hydrocyclone process (Fig. 2). A moderately sheared dough-water dispersion (MS-DWD) is the initial mixture subjected to fractionation equipment in that process. After mixing a slack to stiff dough made from flour and water, the dough and approximately two-fold water by weight of flour at room temperature are subjected to forced-convection mixing in a holding vessel. Moderate fluid shear is required in the vessel to disperse the components in the dough and to prevent sedimentation of the gluten particles and the starch granules. In addition, the moderate shear forces in the holding tank together with excess water probably decrease the size of the gluten particles originally formed in dough mixing.

The shear forces used in the HS-FWD processes (Raisio and HD processes) accomplish two important objectives. First, the indigenous association between the starch granules and the protein matrix in a flour particle is replaced by an association with water. Second, the high-shear forces cause the hydrated gluten molecules to collide and slightly aggregate (Zwitserloot 1989b, Meuser 1994, Witt 1997, Bergthaller et al 1998). But the high fluid-shear forces restrict the size of the gluten aggregates to thin thread-shaped particles having lengths in the micrometer to millimeter range (Robertson and Cao 1998a, 1998b). Apparently, when the shearing of the flour-water mixture is stopped and the sheared batter is diluted to ~30% solids, the gluten threads interact to produce what has been termed a gluten "gel" network impregnated with starch granules (Eliasson and Larsson 1993).

The aggregation of the gluten proteins in a HS-FWD process must be controlled carefully because in the next step of the process, separation of the dispersed components in a fluid shear depends on their behavior in a two- or three-phase decanter centrifuge (Meuser 1994, Bergthaller et al 1998). According to Meuser (1994), the elasticity of the gluten "gel" (matrix) is a crucial factor to the success of the centrifugal separation. Meuser (1994) proposed that during centrifugation the individual starch granules cause stretching of a micro-zone of the gluten matrix until it fails, thereby allowing the granule to pass through the "gel" and to sediment. Upon the passage of a granule, the micro-zone of the gluten matrix rebuilds through new connections between gluten threads, which reestablishes the weak elasticity of the network. For an efficient separation of the starch granules from the gluten "gel", the elasticity of the gluten matrix during centrifugation must be weak enough to allow the passage of the starch granules in many micro-zones, yet large enough not to allow collapse of the matrix on the macro-scale. In a batch centrifugation of a HS-FWD, approximately 80% of the starch passes through the "gel" matrix, and the "gel" then appears to partially collapse to form the protein-rich phase.

In both the Raisio and HD processes, the flour-water mixture is first sheared to form a pumpable fluid or batter. As pointed out before the high fluid-shear forces are generated differently. Then, the highly sheared batters are diluted to a HS-FWD, and in both processes, decanter-type centrifuges are employed in the initial fractionation step (Maijala 1976, Witt 1997, Lindhauer and Bergthaller 2002). In the Raisio process, a flour-water slurry is homogenized by high-shear mixing (800-1,200 rpm) at 30-50°C with a pin mill (Kerkkonen et al 1976, Maijala 1976), whereas in the HD process, flour and water are gently blended and the resulting slurry is sheared at 30-40°C by pumping under high pressure (50-150 bar) through a homogenizer valve, which is a small aperture inside a housing (Meuser et al 1989, Zwitserloot 1989a, Witt 1997, Bergthaller et al 1998).

The Hydrocyclone process begins with a flour-water dough, and the dough is dispersed under moderate shear with water to give the MS-DWD having 20-30% solids. The shearing of the dough releases gluten threads, which are collected in a hydrocyclone instead of a decanter centrifuge at the initial stage of separating gluten from starch (Barr 1989, Maningat and Bassi 1999). Instead of a dough-shearing approach, others (Verberne and Zwitserloot 1978, Verberne et al 1979) report that a flour-water batter (water-flour ratio of ~1.5) at 30-40°C can be directly fed to a hydrocyclone wherein the gluten threads spontaneously aggregate into curd-like pieces in the light phase while the starch is concentrated in the dense phase. It seems that this original approach has been modified to include a dough-mixing and -shearing step to produce intermediate-sized gluten particles that may improve separation and gluten recovery in a hydrocyclone (Barr 1989, Maningat and Bassi 1999).

In summary, modern-day wet-processing of wheat flour begins with two approaches to producing a metastable dispersion (~30% solids) of gluten particles (threads) and starch granules in water. Either a dough is produced, rested, and dispersively mixed in additional water to form a MS-DWD, or flour and water are intensely sheared to give a highly sheared batter, which is then gently diluted with water to produce a HS-FWD. The separation of the components in a MS-DWD is initiated by a hydrocyclone, which removes a protein-rich light phase from the dense phase containing the starch. This process is called the Hydrocyclone method. In the case of the HS-FWD processes, centrifuges (two- or three-phase) are employed first to separate at least one-half of the starch fraction from the dispersion. The two- or three-phase centrifuge also yields a protein-rich phase, while the three-phase centrifuge produces an additional phase, which is the lightest phase containing the water-solubles/particulates. In these various processes, the protein-rich phase, which may be aged (matured) optionally for a period of time, is treated to aggregate the gluten into larger particles and release the low-grade B-starch. The gluten particles are collected by sieving and the B-starch by centrifugation. The gluten, prime starch (A-starch), and low-grade starch (B-starch) streams are eventually dried. A low level of fine fiber fraction (mainly endosperm cell walls) is also collected when purifying the starch.

Laboratory Tests for Wet-Milling Quality of Wheat Flour

Small-scale laboratory tests that mimic the industrial wet-milling of corn (maize) and grain sorghum are available starting with 100 g of grain (Eckhoff et al 1996, Singh and

Eckhoff 1996, Xie and Seib 2000). Laboratory tests to assess the wet-milling quality of wheat flour are available for the Martin (Schafer 1975, Godon et al 1983) and Batter processes (Weegels et al 1988, Hamer et al 1989). No small-scale laboratory test has been established to determine the quality of wheat flour for wet-milling by HS-FWD and Hydrocyclone processes.

Bergthaller et al (1998) and Lindhauer and Bergthaller (2002) reported a bench-scale test to simulate the HS-FWD process. Flour (100 g on a wet basis, wb) and water (87 mL, 35°C) were mixed in a blender (Braun kitchen mixer, specifications not reported) for two short periods of time (2 x 20 s). Then, the mixture was dispersed with additional water (~100 mL) at 35°C in the blender (4 x 2-3 s) to aggregate gluten curds from the mixture at ~33% solids concentration. The mixture was poured over a screen with 200-µm openings, and the overs were hand-washed with water (2-3 x 100 mL) while the throughs were collected. The combined throughs were resieved on a screen with 50-µm openings, and the overs washed with water and added to the other overs to give the gluten fraction. The throughs were centrifuged to collect the sedimented starch fraction. It seems that this test method differs from the commercial HS-FWD processes. The authors reported that the flour-water mixture was mixed first at high speed to aggregate the gluten proteins and not to form a highly sheared batter. Then, in a second high-shear mixing, the starch granules were released from the gluten particles. In addition, the sheared mixture was separated by sieving as is the procedure practiced in a Batter process. It appears this bench-scale test resembles a Batter but not a HS-FWD process (Anderson 1967, 1974, Fellers 1973, Weegels et al 1988, Hamer et al 1989).

The goal of this research was to design a bench-top HS-FWD wet-milling test to determine the suitability of wheat flours for wet-processing by a high-shear fluid process. The laboratory test that mimics modern-day wet-milling of wheat flours should start with a high-shear mixing of flour in water. Then, the sheared dispersion should be subjected to centrifugation to effect the first separation of flour components. Finally, the phases separated by centrifugation should be treated by conventional approaches to isolate and purify the following fractions: prime or A-starch, vital wheat gluten, secondary or B-starch, fiber, and solubles.

LITERATURE REVIEW

Production and Utilization of Wheat

Wheat is the leading cereal grain produced in the world, with a production of nearly 600 million metric tons (MMT) in 2000 according to the U.S. Department of Agriculture, National Agricultural Statistics Service (USDA/NASS 2001). It is used worldwide for food (67%), feed (20%), and seed (7%). The wet-milling of wheat flour to produce starch and vital gluten as the major co-products has been reported to account for 6% of total production (Oleson 1994).

Common or bread wheats (*Triticum aestivum*) with hard or soft endosperm texture constitute about 95% of the world production, whereas durum wheat (*Triticum turgidum* var. durum) accounts for ~5%. Durum and hard wheats contain more protein (12-16%) than soft wheats (8-10%). Hard wheat flours are used mostly for producing yeast-leavened breads and rolls as they form strong viscoelastic doughs when mixed with water. Soft wheat flours, however, give weak doughs and are thus preferred for cakes, cookies, and crackers. Being the hardest in endosperm texture among wheats, durum wheat is milled to semolina instead of flour and used in pasta, spaghetti, and couscous production because of its strong gluten elasticity and desirable yellow color. For Asian noodles and steam and flat breads, hard or soft wheat flours with weak- to medium-strength gluten are suitable (Moss 1973, Oleson 1994, Seib 1997).

Hard and soft wheat flours with a high protein content (>11%) are preferred in wet-milling to co-produce vital wheat gluten and wheat starch. However, European wet-millers today prefer low-protein wheat to maximize production of subsidized wheat starch. In the USA, the wet-processing industry uses flours mostly milled from hard wheats (Maningat and Bassi 1999), whereas flours milled from soft wheats are preferred in Europe (Lindhauer 1997, W. Bergthaller 2001 – *personal communication*, Lindhauer and Bergthaller 2002).

Besides the commonly grown wild-type (normal, non-waxy) wheats with ~28% amylose and partial waxy wheats with 20-27% amylose in their starch, wheats that contain starch with <1% amylose, called waxy wheats (Seib 2000), have been recently developed by several groups of researchers (Nakamura et al 1995, Chibbar et al 1997, Kiribuchi-Otobe et al 1997, Yasui et al 1997, Graybosch 1998, C.F. Konzak 1998 - *personal communication*, E. Souza 2001 – *personal communication*, Morris and Konzak 2001, Grant et al 2001). At least one variety of spring waxy wheat, named Leona, was released for cultivation by Dr. Edward Souza of the University of Idaho, Aberdeen, ID. Waxy wheats are likely to be used in wet-milling to produce waxy wheat starch plus vital gluten. In more recent times, production of a wheat with an elevated amylose level (~37%) in its starch has been reported (Yamamori et al 2000, Morita et al 2002). Specialty wheats will likely be wet-processed in the future *(see below "Development of Waxy and High-Amylose Wheats").*

Composition of Wheat Flour

The wheat kernel is composed of three distinct anatomical layers, namely, germ or embryo (2-3% by weight of kernel), bran (14-16%), and endosperm (81-84%) (Ziegler and Greer 1978). Both hard and soft endosperm wheats are dry-milled to flour, whereas durum wheat with extra hard endosperm is dry-milled to semolina.

After cleaning and tempering, wheat is passed through a series of corrugated rolls to open the kernels. The coarsely ground wheat is sifted to separate the endosperm tissue from the bran and germ fractions. The separated endosperm is then crushed on a series of smooth-surfaced rolls to reduce the particle size and then sifted for further purification from bran particles. Thus, straight-grade wheat flour is predominantly endosperm tissue (Ziegler and Greer 1978, Bass 1988, Posner 2000). Depending on the wheat endosperm hardness and severity of the milling process, dry-milling of wheat mechanically damages some starch in flour, which significantly influences the end-use of a wheat flour (Ziegler and Greer 1978, Pomeranz 1988, Bunn 2001).

Typical straight-grade wheat flour (dry basis, db) is composed of starch (75-85%), protein (7-15%), nonstarch polysaccharides (3-4%), lipids (2-3%), and minerals or ash (~0.5%) (Lineback and Rasper 1988, Pomeranz 1988, Chung 1989, Hoseney 1994). Starch is the major component of wheat flour and occurs as water-insoluble and dense granules of microscopic (1-40 μm) size. Wheat starch consists of two types of granules (bimodal size-distribution); the large (>10 μm) disk-shaped A-granules (~70% by weight of starch) and the small (<10 μm) mostly spherical B-granules. Isolated and purified wheat starch is composed mainly of amylose and amylopectin polymers (total of ~99%), plus low levels of internal lipids (~1%) and proteins (0.1-0.2%) (Morrison 1988, Seib 1994, 1997, Baldwin 2001). Wild-type wheat starch contains ~28% amylose, while partial waxy wheat starch contains 20-27% amylose. The amylose content of waxy wheat starch is <1% (Seib 2000, Yamamori and Quynh 2000). The level of lipids in wheat starch varies from ~1% in wild-type wheats (Morrison 1988) to 0.1-0.2% in waxy wheats (Yasui et al 1996, Reddy and Seib 2000, Lumdubwong and Seib 2001), and they are primarily (~90%) lysophospholipids (Seib 1997). In wet-processing of wheat flour, most of the large A-starch granules are recovered with a high purity (<0.3% protein) in the A-starch fraction, whereas the majority of the small B-starch and damaged starch granules along with some insoluble-pentosans are concentrated in B-starch fraction.

Wheat flour proteins are traditionally classified by their solubility (Osborne classification) into albumins (water-soluble), globulins (dilute-salt soluble), gliadins (70-90%-alcohol soluble), and glutenins (dilute-acid or dilute-alkali soluble). The albumins and globulins constitute 15-20% of total flour proteins. The water-insoluble storage proteins of glutenins and gliadins account for 80-85% of total proteins in wheat flour but with varying ratios by genotype. Differences in the glutenins and gliadins in wheat significantly influence its end-use quality (Pomeranz 1988, Hoseney 1994, Lookhart and Bean 2000).

Glutenins are polymeric proteins with molecular weights from 5 to 20 million Da. The glutenins are composed of either high-molecular weight glutenin subunits (HMW-GS) or low-molecular weight glutenin subunits (LMW-GS). The molecular weights of HMW-GS range from 80 to 130 kDa, whereas those of LMW-GS vary from 30 to 60 kDa. On the other hand, gliadins are monomeric proteins composed of 30 or more α-, β-, γ-, and ω-gliadins, which are grouped by their electrophoretic mobility. Their molecular weights range from 30 to 60 kDa (Bietz et al 1973, Pomeranz 1988, Hoseney 1994, Lookhart and Bean 2000, Shukla 2001).

When flour is admixed with water and mechanical energy is input by mixing, it is the storage proteins that form a unique viscoelastic and cohesive mass (gluten) that is a prerequisite for the functionality of wheat flour in many foods according to the International Wheat Gluten Association (IWGA 1981). During wet-milling of wheat flour, the storage proteins are concentrated to ~80% protein (Nx5.7) as the vital gluten fraction. Almost all the globulins and up to half of the albumins of flour may also remain in the vital gluten fraction isolated by dough-washing (Pence et al 1956).

Wheat flour nonstarch polysaccharides are mainly endosperm cell wall materials, including 2-3% (by weight of flour) pentosans or hemicelluloses (Hashimoto et al 1987, Lineback and Rasper 1988, Courtin and Delcour 2002) and 0.5-1% (1-3), (1-4)-β-D-glucan or simply mixed β-glucan (Beresford and Stone 1983). The majority of mixed β-glucan and 20 to 40% of flour pentosans are water-soluble, while the remaining pentosans are water-insoluble. In wheat flours, a small level (0.2-0.3%) of crude fiber, i.e., cellulose, is also present as an apparent contamination of bran introduced during dry-milling of wheat (Hoseney 1994). In wet-processing, water-soluble pentosans and mixed β-glucan end up mostly in the water-solubles/particulates fraction, while the water-insoluble pentosans are recovered primarily in the B-starch fraction and to a less extent in the vital gluten fraction.

Wheat flour lipids are grouped into starch lipids (~1% of flour) and nonstarch lipids (~2%) based on their location (Chung 1997). Nonstarch lipids are further classified by their solubility into free lipids, which are extracted with nonpolar solvents, and bound lipids that are extracted with polar solvents after the removal of free lipids (Chung et al 1978, Morrison 1978, Chung 1997, Chung and Ohm 2000). In wheat flour wet-processing, in addition to lipids already bound to the flour proteins, most of the free nonstarch lipids of flour also become bound to gluten proteins during dough-mixing and gluten washing and thus concentrated at 5-10% (by dry weight of gluten) in the vital gluten fraction (Chung and Tsen 1975, Chung 1986).

Wheat Flour Specifications for Wet-Processing

Wheat starch and vital wheat gluten are currently manufactured only from wheat flour. Wheat kernels have been considered as a raw material in several processes to produce vital gluten and starch because starting with kernels eliminates the expense of dry-milling. Moreover, dry-milling of flour prior to wet-processing results in some loss of starch and protein, and it damages starch that further lowers its recovery. However, none of the whole-wheat processes have been commercialized mainly due to the poor color of starch and gluten. In addition, the gluten contains excessive bran, ash, and lipid, and is of inferior quality (Kempf and Rohrmann 1989, Zwitserloot 1989a, Maningat and Bassi 1999).

For wet-milling, flour is primarily dry-milled from hard wheats in North America and from soft wheats in Europe (Lindhauer 1997, Maningat and Bassi 1999, W. Bergthaller 2001 – *personal communication*, Lindhauer and Bergthaller 2002). It was reported (Maningat and Bassi 1999) that a small proportion of durum or soft wheat flour could be added to a hard wheat flour with no adverse effect on the separation, yield, and quality of co-products starch and gluten. Straight-grade and high-extraction coarse flours are used in wet-milling, and they are obtained, respectively, by a conventional dry-milling and by a short-flow dry-milling of wheat. Flour is not bleached nor malted nor enriched, and probably it has a high protein content (>11%), low starch damage, low ash, and low α-amylase activity (Maningat and Bassi 1999, Lindhauer and Bergthaller 2002). The starch damage can be minimized in a flour by choosing a short-flow milling process to produce a coarse flour and/or by starting with a soft wheat instead of a hard wheat. However, protein contents of soft wheats are often lower than those of hard wheats, which limits the utilization of soft wheats in wet-processing. Also, inferior bolting quality of soft wheat flours during their dry-milling is another drawback.

When necessary, sodium chloride and hemicellulases (pentosanases or xylanases) may be used in wet-milling, respectively, to adjust water hardness and to break down pentosans to reduce the viscosity of the separation medium (Hamer et al 1989, Weegels et al 1992, Christophersen et al 1997).

Behavior of Wheat Flour When Wetted with Water

Structural changes that occur during wetting of flour particles with water are of critical importance in wet-processing of wheat flour. Bernardin and Kasarda (1973a, 1973b) demonstrated that when a wheat flour particle or thin section (50 μm) of endosperm tissue was wetted with a drop of water on a microscope slide, protein microfibrils were observed to form. A single flour particle was completely wetted within 0.05 s and reached its maximum volume (≥ 20-fold increase) in 5 s. The protein fibrils appeared to be pulled by osmotic forces from inside the endosperm cells through breaks in the cell walls and on into the liquid phase. The hydrated protein fibrils varied in diameter from ~2 μm down to a lower limit of ~10 nm, and were of indeterminant length. The fibrils exhibited elastic deformation characteristic of gluten proteins. Also, starch granules were observed to adhere to the surface of the hydrated protein fibrils in the static system on the microscope stage (Fig. 3). The hydrated protein microfibrils were proposed to form the continuous phase of dough when a wetted flour was mixed to aggregate these microfibrils. The α-gliadin (Kasarda et al 1967) and the glutenin fraction (Tu and Tsen 1978) isolated from wheat flours also appeared to be fibrillar when wetted.

Significance of Wheat Flour Components in Wet-Processing

The elastic and cohesive wheat proteins, the viscous pentosans and mixed β-glucan, and the small B-starch and damaged starch granules differentiate wet-milling of wheat flour significantly from that of corn. Wet-milling of corn begins with steeping of the kernels in dilute sulfurous acid, but that of wheat begins with flour mixed with water (Seib 1994). The protein co-product from wet-milling of corn, called corn gluten, is hydrophobic and absorbs ~1 part of water. It is dried and used in animal feed. The protein co-product isolated from wheat flour is unique in that it absorbs ~2 parts of water (Grace 1988) and forms a viscoelastic cohesive mass called vital wheat gluten, or simply gluten. Vital wheat gluten has been highly valued (~$600-1,600/metric tons) commodity (Sugden 1997, Holcomb 1999).

The formation of protein fibrils when wheat flour is wet with water and their subsequent aggregation into a cohesive mass in excess water are crucial to the separation of wheat gluten from flour (Meuser 1994). The ability of excess water to break the starch-protein bond present in a wheat flour particle is thus a key to the release of pure starch granules from wheat flour. Therefore, wheat starch production is often discussed together with wheat gluten production technology (Maningat and Bassi 1999).

Researchers (Weegels et al 1988, Hamer et al 1989, Meuser at al 1989, Zwitserloot 1989a, Meuser 1994, Roels et al 1998a, 1998b, Wang et al 2002) have reported differences in the rate and strength of gluten aggregation during wet-processing of wheat flours. The reasons for the various rates of the gluten aggregation in flour-water doughs and slurries are not fully understood. HMW-GS have been shown in the last 10 years to add strength (elasticity) to a wheat flour dough (Schropp and Wieser 1996, Veraverbeke et al 1998, Southan and MacRitchie 1999, Wooding et al 1999, Uthayakumaran et al 2000, Antes and Wieser 2001, Singh and MacRitchie 2001). The aggregation rate of gluten proteins in a Batter process appears to be accelerated by an increasing level of glutenin proteins (Roels et al 1998a, 1998b) and to be decelerated somewhat by the level of water-insoluble pentosans (Weegels et al 1991). However, the wet-processing quality of a wheat flour in a HS-FWD process and its relationship to the aggregation traits of flour gluten proteins remain mostly unknown.

In contrast to 0.5-1% of water-soluble pentosans in wheat flours (Hashimoto et al 1987, Lineback and Rasper 1988), there are almost no soluble-pentosans in corn. Water-soluble pentosans and swollen water-insoluble pentosans in wheat flours are reported to bind or hold 5- to 10-fold water by their mass (Bushuk 1966, Courtin and Delcour 2002) and to increase the viscosity of the liquid phase during wet-milling. This in turn causes a reduction in the sedimentation rate of small B-starch granules in a centrifugal field, translating to a reduced starch yield (Witt 1997). Additionally, water-insoluble pentosans have been claimed to sterically hinder gluten aggregation and reduce gluten yield and purity (Weegels et al 1991, Hamer et al 2000, Lichtendock et al 2000, Wang et al 2002). Adding pentosanases to wheat flour doughs was found to reduce the water-holding capacity of doughs, which indicates molecular degradation of pentosans (Courtin et al 1999, Gama et al 2000, Lichtendock et al 2000). Hydrolysis of pentosans explained the increased yields of gluten and starch (Weegels et al 1992). Pentosanases were shown to reduce the molecular weights of water-insoluble and water-soluble pentosans, which reduced the viscosity of the centrifugation medium and effected a better separation of fractions (Hamer et al 1989, Christophersen et al 1997, Hamer et al 2000). The modern-day wet-milling processes for wheat flour were designed, in fact, to remove the soluble-pentosans early in the processing, and thereby to avoid the viscosity build-up when separating starch and gluten (Witt 1997).

In brief, the disaggregation of starch from the indigenous protein matrix in a flour, the aggregation of dispersed wheat gluten proteins into a cohesive mass, the viscosity brought about by the pentosans and mixed β-glucan in flour-water mixture, and the presence of small plus damaged starch granules complicate the recoveries of pure gluten and starch from wheat flours. Those challenges have indeed stimulated researchers to design many processes for wet-milling of wheat and its flour.

Production and Uses of Wheat Starch

Starch is a renewable biopolymer isolated in high tonnage by various wet-milling processes from corn (maize) (Watson 1984, May 1987, Singh 1999), wheat (Knight 1965, Anderson 1967, 1974, Fellers 1973, Knight and Olson 1984, Cornell and Hoveling 1998, Maningat and Bassi 1999), potato (Mitch 1984, Bergthaller et al 1999), tapioca (Corbishley and Miller 1984, Rakshit 1999), and minor sources (Juliano 1984, Hahn

1999). Commercial prime A-starch isolated from wheat flour is composed on a dry basis primarily of starch (~99%) with low levels of lipids (~1%) and proteins (<0.3%).

Corn is the most important industrial source of starch worldwide followed by wheat, potato, and tapioca. Of the total starch production of about 36 million metric tons (MMT) in the world according to Corn Refiners Association (CRA 1997), approximately 81-83% is isolated from corn, 7-8% from wheat, 6% from potato, 4% from tapioca, and 1% from minor starch sources (CRA 1997, Witt 1997, Baere 1999). Although worldwide wheat starch production remains behind the production of corn, its production has doubled or tripled in the last two or three decades, especially in Germany and other European countries (Lindhauer 1997, Witt 1997). In the European Union countries, wheat starch comprises 27-30% of total starch production (Witt 1997, Baere 1999). In the USA, however, wheat starch production represents only ~2% of total starch production of 20-25 MMT (Maningat and Bassi 1999).

Wheat starch is used to produce modified starch, such as acid-thinned, bleached, oxidized, cross-linked, substituted, cross-linked/substituted, and other doubly modified starches, all of which find uses in food and nonfood applications (Maningat and Seib 1997). Wheat starch also is converted to starch hydrolysis products, such as sweeteners (Schenck and Hebeda 1992), especially in Europe and Australia.

Production and Uses of Vital Wheat Gluten

Vital wheat gluten is a valuable co-product of a wet-milling process that yields wheat starch (Grace 1988, Maningat et al 1994, Bergthaller 1997, Maningat and Bassi 1999). By the Codex Standard of the Food and Agriculture Organization (FAO), wheat gluten (db) must contain a minimum of 80% (Nx6.25) protein, which is ~73% when calculated by the Nx5.7 conversion factor used for wheat and wheat products. The moisture, ash, free lipids, and crude fiber contents of wheat gluten must not exceed 10.0, 2.0, 2.0, and 1.5%, respectively (FAO 2001). Typically, commercial wheat glutens have 5-10% moisture and analyze for (db) 73-82% protein (Nx5.7), 3-20% carbohydrates (primarily starch), 5-8% total lipids (1-2% free lipids), 0.5-1.5% ash, and ~1% pentosans (Wadhawan 1988, Maningat et al 1994).

The major portion of the gluten in the world is produced and consumed in North America, the European Union, and Australia (Wadhawan 1988). It is an important protein supplement in processed cereal products, causing one of the major expansions in grain processing worldwide. In parallel to recent increases in wheat starch production, world production of vital wheat gluten has increased from about 0.24 MMT in 1986 (Bergthaller 1997) to about 0.62 MMT in 1999 (Maningat and Bassi 1999). The demand for frozen-dough products and specialty baked goods such as bagels, hearth breads, and multi-grain breads has increased consumption of vital wheat gluten in the USA (Magnuson 1985, Holcomb 1999). Vital wheat gluten is used in breakfast cereals and snacks, meat and cheese analogs, breading and batter mixes, pizza, and in meat, fish, and poultry products (IWGA 1981, Bergthaller 1997). Wheat gluten also has gained increasing importance as an industrial protein, and is converted to wheat protein isolate, hydrolyzed wheat protein, and texturized or deamidated wheat gluten (Maningat et al 1994, Bergthaller 1997).

Industrial Processes Used for Wet-Milling of Wheat Flour to Co-Produce Vital Wheat Gluten and Wheat Starch

Kempf and Rohrmann (1989) listed a total of fifteen wet-milling processes starting with wheat kernels or wheat flour. Of those 15 processes, five have been used industrially, and all starting with flour. Currently, the Martin, Alfa-Laval/Raisio and Hydrocyclone processes are used in North America, and the HD process is popular in Europe. The Batter process, used between the 1940s and 1960s, appears to have been discontinued (Wadhawan 1988, Seib 1994, Maningat and Bassi 1999). In contrast, corn starch and its co-products are isolated in modern facilities essentially by a single process (Seib 1994), although new processes utilizing enzymes and ground corn in steeping, or ethanol as the processing medium, are being investigated (Singh and Johnston 2001, Johnston and Singh 2001). A new wet-milling process for wheat flour could be industrially accepted if it improves the efficiency of separation, increases product yield, improves product quality, or reduces fresh water consumption (Maningat and Bassi 1999).

Industrial manufacture of wheat starch and gluten involves physical separation of starch granules and gluten particles formed in a neutral aqueous system (Knight 1965). The wet-separation of the storage proteins (gluten) and starch from wheat flour depends on their water insolubility, density, and particle size. The storage proteins tend to aggregate into particles that are larger but less dense than starch granules. Aggregation of the storage proteins in the presence of water is accelerated by the concentration and composition of the proteins, and by a warm temperature (15-50°C) of the aggregation medium. All wet-milling processes for wheat flour coincide after the first stage where the gluten particles are first separated from the starch granules. Gluten is further aggregated to exclude starch and other impurities, and washed, dewatered, and flash-dried or spray-dried. Wheat starch is purified from contaminating protein by centrifugation and counter-current washing with fresh water, and then spray-dried.

The most recent technological advance in wheat flour wet-milling can be attributed to two fundamental steps: i) preparation of a pumpable batter of disintegrated flour particles without first mixing a stiff dough and ii) centrifugal removal of A-starch fraction from a water dispersion of the sheared batter by a decanter-centrifuge (Alfa-Laval/Raisio and HD processes) or by a hydrocyclone (Hydrocyclone process). The Martin and Batter processes, which were invented ~170 and ~60 years ago, respectively, may be considered the conventional processes. The modern-day wet-processes for wheat flour are the Alfa-Laval/Raisio, Hydrocyclone, and HD processes (Meuser et al 1989).

According to Robertson and Cao (1998a, 1998b), wet-milling methods may be differentiated by the size of the aggregated protein particles in a mixture of flour and water initially subjected to fractionation and by the mode of separating starch from gluten. In the Martin process, gluten strands in a mechanically strong dough are centimeters to a meter in length, and the dough is subjected to fractionation. The separation of starch and gluten from the dough is achieved by forcing excess water within the strong matrix as the dough is kneaded. The continuous kneading and spraying exposes occluded starch. In that manner, water gradually displaces the starch granules from the protein matrix into the liquid phase, resulting in shrinkage of the dough eventually into the gluten phase. The Batter process produces millimeter-long gluten strands, which coalesce into nodules of gluten when the batter is mixed with added water at a warm temperature. The nodules are isolated on the top of a vibrating screen. In a modern-day HS-FWD process, however, micrometer- to millimeter-sized proteins coalesce into a fine-meshed matrix within the dispersion. The separation of starch from protein is then achieved by a centrifugal force on the dispersion in a decanter-type centrifuge. In the Hydrocyclone process, the MS-DWD contains gluten threads with 1-10 centimeter in length (Verberne and Zwitserloot 1978) with the starch granules, and the gluten threads are concentrated in the overflow of the hydrocyclone. The concentrated protein stream is processed further to cause quick aggregation of gluten into large particles that can be collected by sieving.

In this dissertation, the literature review on wet-milling of wheat/flour is limited to the commercially used wet-milling processes plus a few processes that appears promising to co-produce starch and vital gluten in aqueous systems. Of the laboratory- or pilot-scale wet-milling methods for flour, only those used to test the wet-milling quality of wheat flours were reviewed.

1. Martin (Dough-Washing) Process

The manufacture of wheat starch and gluten dates back probably to ancient Egypt and Greece. The "dough-washing" process, originally reported by the Italian chemist Beccari in 1745 and further developed by Martin and proposed in Paris in 1853 (Anderson 1967, Fellers et al 1969, Knight and Olson 1984), is the oldest wet-milling process to isolate wheat starch and vital wheat gluten (Maningat and Bassi 1999). Early methods of starch production from wheat kernels instead of flour that started with various steeping or fermentation techniques, such as the Alsatin, Halle, and Alsace processes, were known (Kempf and Rohrmann 1989), but the Martin process was the only commercial process for the production of wheat starch and vital gluten until the 20th century. Even up to the 1970s, the Martin process remained the most popular wet-milling process for wheat flour (Anderson 1974, Knight and Olson 1984). Over time, the traditional Martin process was improved and modified to reduce the fresh water consumption from as much as 15 parts / part flour to as low as 5-7 parts / part flour (Zwitserloot 1989a). Fresh water conservation was achieved through recycling of process water or by developing new equipment for efficient separation of starch and gluten (Maningat and Bassi 1999).

As shown in Fig. 4, the modern Martin process consists of five basic steps: mixing white flour (1 part) and water (~0.6 part) into a stiff dough, washing out the starch and solubles from the dough/gluten matrix, drying the gluten, purifying the starch, and drying the starch and other fractions (Knight and Olson 1984, Wadhawan 1988, Witt 1997).

In this process, the stiff dough is allowed to rest briefly, which is thought to allow the gluten proteins to fully hydrate prior to washing. The starch is then washed out of the dough using a variety of techniques. The old methods relied on kneading the dough between roller bars under a spray of water. In a more recent design, the dough is mixed vigorously with water in a trough to give a rubbery mass of gluten particles in a milky suspension of starch. The mixture is then pumped inside a rotating hollow reel, equipped with a 40-mesh stainless steel screen. The reel is long and shaped like a truncated cone. The starch slurry passes through the screen whereas the gluten remains on top. Water is sprayed onto and through the screen to wash the starch off the gluten. The gluten is then transferred to a mixer and kneaded with excess water to extract the remaining starch, dewatered, shredded, and flash-dried to give vital wheat gluten. Alternatively, the wet gluten may be mixed with dilute ammonium hydroxide and the mixture spray-dried. A succession of steps including sieving, centrifuging, and passing through hydrocyclones with counter-current flow of water are employed to purify the starch slurry. Approximately 75-85% of the starch in flour is recovered as the prime A-starch fraction with <0.3% protein and 5-10% as the second-grade B-starch fraction with 1-4% protein. About 80-85% of flour protein is recovered in the vital gluten fraction with a protein (Nx5.7, db) content of 75-80% (Knight 1965, Fellers 1973). The Martin process was reviewed in detail by Knight (1965), Knight and Olson (1984), Wadhawan (1988), Barr (1989), Kempf and Rohrmann (1989), Witt (1997), and Maningat and Bassi (1999).

2. Batter Process

The Batter process was developed independently in 1944 by Hilbert and co-workers in the USA and by Shewfelt and Adams in Canada (Seib 1994). The Batter process was used by several plants in the 1940s and early 1950s in North America and Australia (Anderson 1967, 1974). In this process (Fig. 5), a smooth but thick flowable batter at 40-45°C is prepared by mixing flour (1 part) and warm water (0.7-1.8 parts, 50-55°C), where the level of water added depends on the class of wheat and its protein level. Hard wheat flours require more water than soft wheat flours, probably because of the extra water absorbed by their increased level of proteins and pentosans. The batter is aged for about 30 min in order for the gluten to hydrate and begin to aggregate, and then cold water equal to two times the weight of the flour is added. After vigorous mixing with a cutting pump, the gluten aggregates into a curd-like suspension, and the starch is washed away from the gluten curds and recovered on gyrating screens. The starch is purified and dried essentially as in the Martin process (Anderson et al 1958, 1960, Knight and Olson 1984, Seib 1994). Total starch recovery is about 90% (Anderson 1967).

The factors affecting the Batter process were further investigated by Anderson and co-workers (Anderson et al 1958, 1960, Anderson 1967). It was concluded that the flour-water ratio had the greatest effect on gluten recovery, and that a thick batter gave an increased yield of the gluten fraction but with a lower protein content. It was also found that a reduction in the speed of the cutting pump improved gluten purity. The batter consistency was affected by the flour-water ratio, wheat variety, and protein content. A straight-grade flour with 85% extraction rate was found to be the most cost effective raw material to produce vital wheat gluten. A typical vital gluten fraction isolated by the Batter process contained ~80% protein (db), and 75-85% of flour protein was recovered in the gluten fraction (Knight 1965, Anderson 1967).

3. Alfa-Laval/Raisio Process

The Alfa-Laval/Raisio process, as well as the HD process, begins with high-shearing of wheat flour in water to form a flowable batter. The batter is then blended with water to give a HS-FWD having ~30% solids (Fig. 6). The afore-discussed Batter process also begins with mixing flour and sufficient water to form a thick flowable batter, which is then diluted with water to ~20% solids. However, in the Batter process aggregation of gluten is done in the presence of all starch and pentosans in the diluted warm (40-45°C) batter, and aggregation may begin during the batter mixing and aging steps because they are done at warm temperatures of 40-45°C. In the Raisio process, precipitation of gluten particles does not occur for some time in the thick batter nor in the diluted dispersion. For that reason, at least one-half of the starch in the flour is sedimented in almost pure form when the HS-FWD is subjected to a centrifugal field. The protein-enriched phase from the centrifuge is then held at a warm temperature (30-50°C) to cause rapid aggregation of gluten, which remains vital in spite of the high-shear mixing of flour and water. Those findings were published by Fellers et al (1969) and Johnston and Fellers (1971) when they re-examined the ancient Fesca process that had been used to produce wheat starch.

The Alfa-Laval/Raisio process, developed and commercialized in Raisio, Finland in 1976, appears to be based on the work of Fellers and colleagues.

The Alfa-Laval/Raisio plant in Finland is a wheat dry-mill integrated with a wet-mill designed for the modified Fesca process. The dry-mill is of short-flow design and provides flour of ~80% extraction rate. In the wet-process (Fig. 6), flour (1 part) and water (1.2-2 parts) are combined to give a highly sheared batter that is homogenized by a special disk-type disintegrator, which is a pin mill rotating at 800-1,200 rpm. The high-shear mixing forms a uniform mixture (30-50°C) of starch granules in a network of gluten fibrils. Water-dilution of the highly sheared batter to ~30% solids gives a HS-FWD that is subjected to centrifugation. The two-stage decanter centrifuge gives a starch-cake fraction and a protein-concentrate fraction. The starch-cake fraction is resuspended in water and further purified by traditional techniques, including fine-fiber removal and washing the starch. Prime-starch recovery is 75-80% with <0.3% protein, whereas second-grade starch recovery ranges from 10 to 15% with 2-5% protein. The protein-rich phase is aged (matured) to complete the aggregation of gluten molecules to give gluten particles. The aging is done by resting the protein-rich phase at 30-50°C for 10 to 90 min without either mixing or diluting with water. Then, it is diluted with water and subjected to a pin-mill treatment at 400-900 rpm for 2 x 30 s. The gluten particles are purified on vibrating screens, washed, dewatered, and dried. Over 80% of flour protein is recovered in the vital gluten fraction, and the protein content of the fraction is about 80% (db). (Kerkkonen et al 1976, Maijala 1976, Dahlberg 1978, Seib 1994, Maningat and Bassi

1999). Wadhawan (1988) reported that one plant in the USA and another plant in the UK were built based on the principle of the Alfa-Laval/Raisio process.

4. Hydrocyclone Process

The Hydrocyclone process is among the new wet-milling processes for wheat flour. It was developed by the KSH Company of Holland in the late 1970s (Verberne and Zwitserloot 1978, Verberne et al 1979, Zwitserloot 1989a), and was reported in 1989 to be the most commonly used process around the world (Barr 1989). The advantages of the Hydrocyclone process are its low cost, absence of moving parts in equipment, a wide range of operating conditions, and low water usage (Maningat and Bassi 1999). Another possible advantage is that the protein "strength" of the flour seems less important than in the Martin and Batter processes. The water consumption is 4-5 parts / part flour in the Hydrocyclone process, compared to 5-7 parts / part flour in the improved Martin and Batter processes (Zwitserloot 1989a).

The process (Fig. 7) begins by mixing a flour-water dough followed by holding 10-30 min and then dispersing the dough by modest fluid shear in ~3 parts water. The MS-DWD, which has 20-30% solids, is pumped through filters to remove large particles. The filtered dispersion is then directly pumped into a series of hydrocyclones (McIntyre 1982, Knight and Olson 1984, Barr 1989, Maningat and Bassi 1999). Any zone in a vessel containing the MS-DWD where fluid motion is low ("dead zone") will result in local precipitation of gluten particles.

It has been reported that a batter may be mixed at high speed directly from flour (1 part) and water (1.5 parts), diluted and filtered, and then pumped into the multistage hydrocyclone system (Verberne and Zwitserloot 1978, Verberne et al 1979).

Due to the increased gluten concentration in the light stream in the hydrocyclones plus alignment of gluten threads in the shear field cause gluten to aggregate spontaneously in the hydrocyclone to form small curds or thick threads (Verberne and Zwitserloot 1978). Gluten threads with a hydrated density of ~1.1 g/cc and hydrated starch at 1.4 g/cc are collected, respectively, in the overflow and underflow streams. Often a four-stage hydrocyclone is used to separate the gluten from the starch followed by an eight-stage hydrocyclone to wash the starch in a countercurrent fashion to remove residual protein. After sieving the starch slurry through 75- and 50-µm opening sieves to remove fiber, a three-stage hydrocyclone is used to concentrate the starch slurry to ~40% solids followed by flash-drying to give A-starch (Maningat and Bassi 1999). The protein-rich phase may be aged as in the Raisio process and then treated to isolate the vital gluten and B-starch fractions.

5. High-Pressure Disintegration (HD) Process

The most recent industrial method to separate wheat starch and vital wheat gluten from wheat flour is the HD process. This process was developed by the Technical University of Berlin in collaboration with Westfalia Separator AG of Germany (Zwitserloot 1989a). This process has been labeled with different names including the Decanter-Based Weipro process (Kempf and Rohrmann 1989, Zwitserloot 1989a), Westfalia Separator 3-Phase-Decanter process (Witt 1997), Flottweg Tricanter process (Maningat and Bassi 1999), and the Barr and Murphy process (Svonja 1997). The HD process was developed originally for extracting starch from potato, and later modified for extracting starch from corn (Meuser et al 1985, Zwitserloot 1989a). In 1984, the technology was tailored to wheat flour wet-milling by three plants in Germany (Witt 1997), and since then it has become the most popular process in the EU for wet-milling of wheat flour (Maningat and Bassi 1999). This process has been reviewed by several authors (Bergthaller et al 1998, Meuser et al 1989, Meuser 1994, Seib 1994, Witt 1997, Zwitserloot 1989a, 1989b).

As with the other modern processes that yield starch and vital gluten, flour is the starting material in the HD process as shown in Fig. 8. Flour (1 part, wb) and water (0.85-0.95 part) are rapidly mixed into a lump-free slurry at ~47% solids concentration and 30-40°C, and the slurry is pumped through a high-pressure homogenizer. The slurry under high-pressure is forced at high velocity through a valve which creates high-shear forces that i) dislodge the starch from the endogenous gluten matrix present in flour and ii) disperse the starch granules and gluten fibrils throughout the continuous liquid phase (Zwitserloot 1989b). A similar highly sheared flour-water batter is created to start the Raisio process. However, in the Raisio process the shear is created by rapidly moving pins through a relatively slow moving slurry, whereas in the HS process a rapidly moving slurry is pumped through a stationary valve.

The sheared dispersion in the HD process is next diluted to ~30% solids to give a HS-FWD, which is pumped into a specially designed 3-phase decanter-centrifuge. The long axis of the conical-shaped bowl is displaced horizontally, and the end of the bowl for the heavy phase is fitted with a screw-type conveyor. The centrifuge is capable of separating the sheared dispersion into three phases. The most dense phase is termed the A-starch, which is almost pure except for low levels of protein (<1%) and fine fiber. The A-starch phase is further purified by counter-current washing with water in hydrocyclones to reduce the protein level to as low as 0.2%. The intermediate phase from the decanter centrifuge contains principally the gluten plus B-starch and some fine fiber. Gluten is aggregated and purified from B-starch and fine fiber by rotary screens and rotary gluten washers. Additionally, A-starch is recovered from its mixture with B-starch and fiber by a disk-bowl centrifuge that purifies the largest and most dense particles. Finally, the fiber is separated from the B-starch using rotary cone screens. Based on the data of Meuser et al (1989) and Witt (1997), the A-starch recovery is 80-85% (percent of starch in flour) with <0.3% protein, whereas second-grade B-starch recovery ranges from 8 to 12%. The least dense phase from the decanter centrifuge is considered the effluent stream and contains swollen insoluble pentosans, water-solubles, and fine gluten particles. The fine gluten particles are recovered by screening the light phase, and the remaining filtrate with pentosans is clarified using rotary cone screens and fed to the evaporator. The recovery of flour protein in the vital gluten fraction is 80-85% (Witt 1997).

The key advantage of the 3-phase decanter centrifuge in the HD process is that the viscous pentosans and water-solubles are removed early in the process. Eliminating the pentosans allows an effective separation of the starch and gluten, decreases fresh water required in starch purification, and reduces the effluent stream (Meuser 1994, Svonja 1997, Witt 1997). Water consumption in the HD process is reduced to below 3 parts / part flour. In comparison, fresh water consumption is reported to be 4-5 parts / part flour in the Hydrocyclone and Alfa-Laval/Raisio, and 5-7 parts / part flour in the modified Martin and Batter processes (Zwitserloot 1989a, Svonja 1997, Witt 1997). In addition, the A-starch yield is claimed to be increased by up to 10% compared to the Martin and Hydrocyclone processes (Zwitserloot 1989a).

Another advantage of the HD process is that it appears a "strong" protein flour is not required as starting material because the separation of starch and gluten is based on density difference. It has been stated that soft wheat flours with low starch damage are preferred for wet-milling in Europe (Lindhauer 1997, W. Bergthaller 2001 – *personal communication*, Lindhauer and Bergthaller 2002), but the weak protein in European wheats may aggregate at a slow rate in the HD process. Weak flours are known to impair wet-processing by the Martin and Batter processes because the dough strength is inadequate for the Martin process and because the rate of gluten aggregation in the stirred batter may be too slow for the Batter process (Knight 1965, Fellers 1973, Zwitserloot 1989a). European wheats are generally of the weak type, which may have led to the development of the HD process in Europe.

Other Potential Processes to Wet-Mill Wheat Flour or Wheat Kernels

1. Wet-Processing of Wheat Flour in Dilute Sodium Hydroxide or Ammonium Hydroxide

Dimler et al (1944) developed an alkaline process in which dilute aqueous sodium hydroxide was used to dissolve gluten from flour and separate insoluble starch granules by filtration or centrifugation. Flour (1 part) was mixed with 0.03 M sodium hydroxide solution (12 parts) to form a slurry at pH 10.6, which was maintained at 35°C or lower. The dispersion was tabled, filtered, or centrifuged to separate prime starch and low-grade starch, and then the pH of the dispersion was adjusted to 5.5-6.0 with sulfuric acid to precipitate non-vital gluten.

Phillips and Sallans (1966) of the National Research Council of Canada modified the alkaline process by replacing sodium hydroxide with ammonium hydroxide to preserve gluten vitality. Flour (1 part) was dispersed with 0.2 M ammonium hydroxide (4-5 parts) under intense mechanical shear. The starch was separated by centrifugation, and the supernatant was spray-dried to obtain vital wheat gluten. The gluten was reported to have good baking performance. A similar process was commercialized in Japan although a dark gray-colored gluten was produced (Schofield and Booth 1983). The ammonium hydroxide dispersion process is not popular because of the poor flavor and dark gray color of the gluten (Phillips and Sallans 1966).

2. Wet-Processing of Wheat Kernels in Dilute Hydrochloric Acid (Pillsbury Hydroprocessing)

Wheat kernels have been considered as a raw material in a number of wet-processes in order to eliminate expensive flour milling. Such a process also has the potential to increases yields of starch because of no loss in dry-milling and no loss due to starch damage. However, wet-processing of wheat kernels has not been commercialized due to poor starch whiteness and to excessive bran, ash, and fat in gluten as well as its poor color (Rodgers and Gidlow 1974, Zwitserloot 1989a, Maningat and Bassi 1999).

The Pillsbury Hydroprocessing method, referred to here as the hydrochloric acid process, was developed by Rodgers and Gidlow (1974) and further improved by Galle et al (1976). Wheat kernels (1 part) having 14-17% protein on a 14% moisture basis (mb) were steeped in aqueous hydrochloric acid solution having a pH of 0.8-2.5 (1-1.5 parts), which gave the mixture a pH of 2.4-3.4. After steeping 12-24 h at 37-40°C, the steeped and drained wheat gained moisture to 45-48% (wet basis, wb). After draining the steep liquor, the softened wheat was lightly macerated by a combination of crushing, squeezing, and rubbing actions with a Mono pump, which splits the bran and exposes the elastic-textured endosperm. The lightly macerated wheat was then dispersed in water (4-15% solids concentration, pH 2.4-3.4) using various shear-imparting mixing and pumping machines (i.e., centrifugal pump), which disintegrated the endosperm into a dilute suspension of fine particles while maintaining the bran and germ as coarse particles (>80% of bran and germ remains on 300-μm sieve). The endosperm particles and solubles were separated from the bran and germ on 700- to 1,200-μm screens at low pH (2.4-3.4). The pH of the endosperm slurry was then adjusted to 5.8-6.1 with aqueous sodium hydroxide to achieve gluten aggregation. Starch and vital gluten could be recovered as done in the Martin or Batter process (Figs. 4 and 5). The process was claimed to yield 78 to 80% starch (>95% recovery of starch in flour) with white color and substantially vital gluten. Recovery of flour proteins in the gluten fractions was not reported.

The Pillsbury Hydroprocessing method was not commercialized, perhaps because the process generates sodium chloride that must be disposed of.

3. Wet-Processing of Wheat Kernels in Water (Far-Mar-Co Process)

In the Far-Mar-Co process (Rao et al 1976, Rao 1979, Kempf and Rohrmann 1989), wheat kernels were tempered or steeped with water to 15-22% moisture (wb) and then pin-milled. The pin-milled whole wheat flour was sifted to separate the bran and germ from the endosperm fraction. The endosperm fraction and 65-75% water were mixed to form a dough-like structure, from which the starch was washed off by kneading under high-pressure water sprays leaving the wet gluten behind. The authors claimed low starch damage, high starch recovery, vitality of the gluten, and no use of chemical reagents. However, poor gluten quality, low starch whiteness, high cost of wet bran drying, and effluent disposal problems due to a high level of solubles resulted in an unacceptable process (Maningat and Bassi 1999).

4. Wet-Processing of Wheat Kernels in Water by Technical University of Berlin Process

Two wet-milling processes starting with whole wheat kernels were devised at the Technical University of Berlin by Meuser et al (1989) and Meuser (1994). In the first process, wheat kernels (soft endosperm) were steeped with tap water (amount not provided, probably in excess water) at 40°C for 3 to 6 h. After steeping, the steep water was decanted and the steeped wheat was milled in a corundum disk mill (Fryma, KMR-2) by adding water (10 parts / part wheat). The suspension was sieved through two series of screens (500- and 160-µm opening sieves) with further addition of water. The fiber fraction, which also contained the germ, was collected on the screens while the throughs from the endosperm were concentrated on a laboratory centrifuge. The overflow from the centrifuge was reused for the steeping of grain or for washing overs after sieving the suspension. In the sedimented phase, the agglomerated gluten atop the sedimented starch phase was collected over a 160-µm opening screen and washed with water. The overs (gluten) were freeze-dried, and the throughs sieved through a 63-µm screen to separate the fine fiber. Finally, the heavy starch phase was collected and dispersed with water (1 part / part solids). The dispersion was centrifuged; the top two layers, namely the solubles and B-starch, were combined and freeze-dried, while the A-starch was air-dried. The whole process required a total of ~90 min. A higher yield of A-starch based on dry wheat grain was reported in the German laboratory procedure compared to that in the commercial Alfa-Laval/Raisio processes (~58% *vs.* ~51-54%) when both were done on a ~80%-extraction wheat flour. Also, a lower yield of B-starch (~11% *vs.* ~14%) was obtained in the German procedure, but the gluten yield (8.4%) was lower than that in the Raisio process (9.9%). The authors predicted that the process would give similar starch and gluten yields and purities when upgraded to an industrial-scale process. The colors of the starch and gluten fractions were not reported.

A second process starting with whole wheat kernels was developed also by Meuser (1994). First, tempered wheat kernels were ground into a whole wheat flour on an UltraRotor mill (impact mill). The process steps, which were demonstrated on whole-wheat and straight-grade flours, consisted mainly of suspending the flour in water at ~45% solids concentration, followed by removal of the bran and germ by wet-sieving. The endosperm suspension that passed through the screen was subjected to a high-pressure disintegration in a homogenizer. The sheared batter is diluted to ~25% solids concentration, and the HS-FWD centrifuged in a decanter-type centrifuge. Several steps in the process, including suspending the flour, sieving the flour suspension, homogenizing the endosperm particles to form a batter, and centrifugally separating the HS-FWD into two phases are of critical importance for the eventual aggregation of the gluten phase into particles that can be gathered on proper sieves. An aggregation sufficient to achieve separation, which is characterized by a certain elasticity of the gluten, can be attained by carefully adjusting the process variables, such as solids content and mechanical energy input during suspending, sieving, and homogenizing. Gluten aggregation into particles early in the process is detrimental to the separation of bran and germ, and should be avoided. The proposed process was reported to give 98-99% dry matter recovery as compared to an unidentified industrial process with 92% dry solids recovery from wheat flour. The recoveries and purities of the wet-milling fractions were not provided.

Laboratory Test Methods to Assess Wet-Milling Quality of Wheat Flour

1. Test Methods for Martin (Dough-Washing) Process

Schafer (1975) designed a pilot-scale process to test the wet-milling quality of wheat flours to be processed by the Martin process. The test includes mixing a stiff dough, resting the dough, and kneading the dough under water to wash away the starch from the dough/gluten mass in an extractor similarly designed to those used in the industrial Martin process. The test gave a gluten fraction, prime- and second-grade starch fractions, and water-solubles/particulates fraction. It was claimed that the test gave quantitative and qualitative results that predicted the industrial wet-milling quality of a flour by the Martin process.

Godon et al (1983) described a pilot-scale method (3.5 kg of flour) for the Martin process, which was aimed primarily at the separation and purification of starch and gluten starting with the intensive washing of a stiff dough with water in a small-scale extractor. Dry gluten yields varied from 11 to 13% (75-86% protein recovery) with 75-80% (db) protein contents. Recovery of starch based on starch in flour was 73-80% in the A-starch fraction. The authors showed that the test results for several flours were similar to the results obtained in the industrial Martin process. According to Hamer et al (1989), the pilot plant of Godon and co-workers was demonstrated to be useful in evaluating the wet-milling quality of different wheat varieties by the Martin process.

In addition to the pilot-scale test methods of Schafer (1975) and Godon et al (1983) for the Martin process, the official laboratory methods to wash wet gluten from a wheat flour dough according to the American Association of Cereal Chemists (AACC 2000) and the International Association for Cereal Science and Technology (ICC 1999) may be adapted with slight modifications/additions to assess the wet-milling quality of wheat flours. The approved Method 38-10 of the AACC requires 25 g of flour and involves hand-washing of a stiff dough-ball that is mixed manually with a spatula until almost all starch is washed away from the wet gluten. The starch milk then can be sieved and centrifuged to purify starch and obtain water-solubles/particulates. The separated fractions can be dried and quantified to estimate the wet-milling quality of a flour. Additionally, the Method 38-12 of the AACC and Methods 137/1 and 155 of the ICC, which specify the Glutomatic instrument (Perten Instruments North America, Inc., Reno, NV) to wash wet gluten from 10 g of flour, may be used to approximate wet-milling quality. It was found that some flours, for example, waxy wheat flours with poor mixing quality, fail to form a cohesive dough-ball upon mixing in the Glutomatic, which causes blinding of the screen during kneading of the dough under water (Sayaslan, Chung, and Seib – *unpublished*). It appears the gluten proteins in those flours have poor aggregation properties, and those flours would probably have poor wet-processing quality for the Martin and Batter processes.

2. Test Methods for Batter and Modified Batter Processes

The Batter process has been thoroughly studied at the pilot-scale level (Anderson et al 1958, 1960, Anderson 1967). The pilot-scale process was very similar in design to the industrial Batter process shown in Fig. 5. The process was tested with soft and hard wheat flours at 100 lb of flour in each batch. The water-flour ratios ranged from 0.7 to 1.8 depending on the flour used. Soft wheat flours required less water while more water was used for hard wheat flours. Recoveries of protein in the gluten fractions ranged from 75-80% based on the protein in the flour, and the gluten fractions contained ~80% protein (Nx5.7, db). Recoveries of starch in the total starch fractions without any purification were ~90%, and those starch fractions were contaminated with 3-5% protein.

A modified Batter process test was designed originally for 5 kg of flour and later downsized to 1.8 kg of flour (Weegels et al 1988, Hamer et al 1989), and was used to fractionate flour into starch, gluten, pentosans, and solubles. The test procedure appears to be a hybrid process wherein a MS-DWD is treated as in the Batter process to aggregate gluten particles. In this test procedure, a dough (0.7 part water / part flour) was prepared by mixing in a Hobart mixer and rested for 8 min. The dough was then added to water (5 parts) at 20°C and mixed 18 min in the Hobart mixer to form MS-DWD having ~14% solids. The dispersion was pumped to a gluten-coagulation tank with a Mono pump where water (40 parts / part flour) was added and the slurry mixed to bring about gluten precipitation. Next, the slurry was pumped over a vibrating stack of screens with decreasing size openings (400, 250, 125, 90, 50, 31 μm). Gluten particles were retained on the top two screens, bran particles on the 125- and 90-μm screens, and pentosans and fine fiber on the 50- and 31-μm screens. The solubles and starch slurry passed through the stack of screens, and a three-phase decanter separator made by Westfalia Separator AG (Oelde, Germany) was used to purify the starch. The procedure provided information on the yield and purity of starch and gluten fractions, and on the losses of protein and other soluble solids in the effluent. The ratio of the gluten retained on the top sieve to the total gluten recovered on the top two sieves was referred to as gluten agglomeration index. That index was considered a measure of the rate (or extent) of agglomeration of proteins in the diluted batter of various flours, and was proposed to distinguish the wet-milling quality of flours for a Batter process. The test method distinguished wet-milling differences between bakery wheat flours and feed-quality flours. Roels et al (1998a, 1998b) confirmed that the test is capable of distinguishing flours with varying gluten aggregation rates that were correlated positively with the level of glutenins and negatively with the level of pentosans in flours.

Bergthaller and co-workers (Bergthaller et al 1998, Lindhauer and Bergthaller 2002) at the Federal Center for Cereal, Potato and Lipid Research in Detmold, Germany reported a bench-scale method to mimic the industrial HD process. However, the test seems to mimic a Batter process. In the bench-scale test, flour (100 g, wb) and water (87 mL, 35°C) were mixed in a blender (Braun kitchen mixer) for two short periods of time (2 x 20 s). Immediately after mixing, water (100 mL at 35°C) was added and the combination was mixed again (4 x 2-3 s). The final solids concentration in the dilute "batter" was ~33%. Gluten aggregated into small pieces in the diluted batter and the gluten particles were collected by pouring the mixture over a sieve with 200-μm openings. The overs were hand-washed with water (2-3 x 100 mL), while the throughs were sieved over a 50-μm openings sieve to remove fine fiber. The starch in the throughs was collected and purified by centrifugation and washing with water. This test procedure differs from the HD and Raisio processes where a HS-FWD is subjected to centrifugation in order to obtain a protein-rich phase and >50% of starch in the sedimented starch phase. Instead, the authors reported that flour and water were mixed at high-speed (exact speed not reported) to aggregate gluten proteins, and that the aggregated gluten in admixture with starch was subjected to a second high-shear mixing to release the starch granules from the gluten matrix. Thus, gluten was aggregated into particles large enough for sieving in the presence of all flour components, which is the procedure practiced in the Batter process (Anderson 1967, 1974, Fellers 1973, Weegels et al 1988, Hamer et al 1989).

3. Test Methods for HS-FWD Processes

Bergthaller et al (1998) devised a pilot-scale test starting with a special mixing unit that was reported to be of a screw design and capable of continuously high-shear mixing a minimum of 20 kg of flour with water to give a metastable dispersion of particles in a flowable dispersion. It was stated that the special mixing apparatus produced the required shear forces at its fast-moving surfaces at a lower cost of purchase and maintenance compared to the high-pressure disintegrator unit used in the HD process. It will be recalled that in the HD process a flour-water slurry is pressurized to flow through a valve that creates high-shear forces which dislodges starch from gluten proteins in the flour. In the pilot-scale Detmold process, flour and water at ~47% solids content were mixed at 35°C and 1,300-1,800 rpm screw rotation speed to form a highly sheared batter. After diluting to ~33% solids content by adding water at 35°C, the resulting dispersion was passed through a 2-phase decanter centrifuge of pilot-scale size from Westfalia Separator AG (Oelde, Germany). The light phase from the centrifuge contained water-solubles, pentosans, gluten, and a low level of starch, whereas the heavy phase contained mainly starch with water-insoluble pentosans and a low level of gluten and fiber. The two phases were processes as shown in Fig. 6 to give two starch fractions (A-and B-starch), gluten, fiber, and water-solubles. The authors compared the wet-milling qualities of two flours milled from German wheats of feed- and elite-grades. They claimed that both the 100-g flour bench-scale test *(see above)* and the 20-kg pilot-scale test showed the difference in wet-milling qualities of the flours. The key criteria used to judge wet-milling quality were the recoveries of the A-starch and the gluten fractions and their purities.

Summary of Wet-Processing Methods for Wheat Flour

Wheat starch and vital wheat gluten are currently produced industrially through wet-milling of wheat flours principally by four processes; the Martin, Alfa-Laval/Raisio, Hydrocyclone, and the HD processes. The processes differ mainly i) in the forms of the flour-water mixtures presented to the fractionation equipment (centrifuge, hydrocyclone, or screen) and ii) the initial separation of starch and gluten fractions from flour. However, the processes essentially merge into one as the intermediate starch and gluten-particle streams are purified to give 99% pure starch and 80% protein vital gluten. Handling of a second-grade starch stream, flour water-solubles, and fibrous residues may differ at various locations. In the Martin process, a strong flour and water are mixed into a dough, and over 80% of the starch is displaced out of the continuous gluten matrix of the dough by its kneading under water. In the Hydrocyclone process, a slack or stiff dough is mixed from a strong or weak flour, and the dough is placed in extra water at 30-40°C and mixed by stirring to give a dispersion of ~20% solids concentration. Constant agitation of the 20% level of solids appears to produce elongated threads of gluten or tiny particles. Pumping the dough dispersion through a hydrocyclone gives the gluten-rich light phase and the starch-rich dense phase.

The Raisio and the HD processes both start with a strong or weak flour in 1 to 2 parts of water. After subjecting the mixture to high shear at 30-40°C generated by either high-speed mixing (Raisio) or by high-velocity pumping through a valve (HD), the highly sheared batter is diluted with water to give a metastable suspension of starch granules in a "mesh network" of gelled gluten fibrils or gluten threads. Centrifugation with either a two-phase (Raisio) or a three-phase (HD) centrifuge delivers a dense phase containing at least half of the starch in the flour contaminated with <1% protein. The two-phase centrifuge also delivers a light phase containing gluten, pentosans, and water-solubles, whereas the three-phase centrifuge delivers an intermediate phase containing mostly protein plus a light phase of water-solubles and pentosans. The protein-rich phase is next treated to induce rapid precipitation of gluten particles which are screened off a low-grade starch.

Pilot-scale and laboratory tests are available to assess the wet-milling quality of flours for the Martin and Batter processes. Yet, there are no laboratory tests reported for the HS-FWD (Alfa-Laval/Raisio and HD) and MS-DWD (Hydrocyclone) processes. It is important that laboratory procedures to evaluate the wet-milling quality of wheat flours by modern-day flour-water dispersion processes be available.

A wet-milling test developed for a HS-FWD process should be applicable with minor adjustments to both hard and soft wheat flours. In Europe soft wheats may be preferred for wet-milling by a HS-FWD process because of growing of high-yielding wheats, demand for wheat starch, and lower levels of damaged starch in soft wheat flours (Zwitserloot 1989a, Bergthaller 1997, Lindhauer 1997, Lindhauer and Bergthaller 2002). On the other hand, the rate of gluten aggregation in HS-FWD may be faster for hard than soft wheat flours because of their customarily higher levels of the HMW-GS fractions. At the same time, hard wheat flours may contain increased levels of pentosans (Lempereur et al 2001), which interfere with gluten aggregation (Weegels et al 1991, Hamer et al 2000, Lichtendock et al 2000, Wang et al 2002). The interference of gluten aggregation by pentosans is eliminated to some extent by use of a three-phase decanter centrifuge that removes the soluble pentosans in the light phase so that they do not contaminate the gluten in the middle phase (Svonja 1997, Witt 1997).

Development of Waxy and High-Amylose Wheats

Waxy wheats were first produced in Japan in 1994 by conventional plant breeding techniques (Nakamura et al 1995). Japanese investigators found that some hexaploid wheats ($2n= 6x= 42$) with three sets of chromosomes (AABBDD) were deficient in one or two of the three possible waxy proteins in their starch (Yamamori et al 1994). The waxy proteins, namely Wx-A1, Wx-B1, and Wx-D1, are respectively coded by the homologous genes located on the chromosome arms 7AS, 4AL, and 7DS (Nakamura et al 1995). Waxy protein is another name for granule-bound starch synthase I, the enzyme associated with amylose synthesis. Wild-type (nonwaxy) wheats contain all three waxy proteins, whereas waxy (amylose-free) wheats lack all of the waxy proteins in their starch. On the other hand, partial waxy wheats are deficient in one or two of the three possible waxy proteins in their starch (Seib 2000).

Hexaploid waxy wheat lines have been produced by genetically eliminating the three waxy proteins through crossbreeding techniques. "Bai Huo", a Chinese wheat cultivar that lacks the Wx-D1 protein, was crossed with "Kanto 107", a Japanese cultivar that lacks the Wx-A1 and Wx-B1 proteins. As expected, one in 64 of the F_2 progenies was a waxy wheat lacking all three waxy isozymes (Nakamura et al 1995). In more recent years, waxy wheats have been produced by conventional breeding in Canada (Chibbar et al 1997) and in the USA (Graybosch 1998, Morris and Konzak 2001, E. Souza 2001 - *personal communication*). In addition, mutagens have been used to produce waxy wheats, where partial waxy wheats, such as Kanto 107 in Japan (Yasui et al 1997, Kiribuchi-

Otobe et al 1997) and "Ike" in the USA (C.F. Konzak 1998 - *personal communication*) were treated with mutagens, such as the ethyl methanesulphonate. Tetraploid (durum) waxy wheat lines also were developed by cross-breeding (Grant et al 2001). In more recent times, production of a wheat with an elevated level (~37%) of amylose in its starch was reported (Yamamori et al 2000, Morita et al 2002). Because waxy wheats have been produced by conventional breeding, consumer products made from waxy wheats will not be required to be labeled as genetically modified organisms.

Physical properties of hexaploid waxy wheat starches (Yasui et al 1996, Hayakawa et al 1997, Fujita et al 1998, Sasaki et al 2000, Abdel-Aal et al 2002) and their chemical modifications (Reddy and Seib 2000) have been studied. Waxy wheat starch was found to contain 1.2-2.0% apparent amylose and 0.12-0.29% lipids (Yasui et al 1996), in contrast with 22-25% amylose and 0.8-1.2% lipid in normal wheat starch (Morrison et al 1984). As expected, waxy wheat starch showed the A-type x-ray pattern and had an increased level of crystallinity compared to wild types, thereby elevating the enthalpy of gelatinization (Hayakawa et al 1997, Fujita et al 1998). A tetraploid waxy wheat starch also showed similar gelatinization and pasting properties (Grant et al 2001). Hydroxypropylated and cross-linked hexaploid waxy wheat starches were shown to give thick pastes with improved freeze-thaw stability (Reddy and Seib 2000). Maltodextrins with dextrose equivalents of ~1 and ~10 were produced from waxy wheat starch because of low levels (<0.1%) of lipids in the starch (Lumdubwong and Seib 2001). Wet-milling characteristics of waxy wheat flours have not been investigated, and there are no reports on the breadmaking quality of the gluten proteins from waxy wheats.

OBJECTIVES

1. Obtain a hard-wheat flour representative of commercial hard wheats and design a laboratory wet-milling test for 65 g (db) of wheat flour that simulates a highly sheared flour-water dispersion (HS-FWD) process, such as the Alfa-Laval/Raisio and the High-Pressure Disintegration (HD) processes.

2. Determine separation efficiencies by the recoveries of total dry solids, starch, and gluten fractions and their purities.

3. Use Response Surface Methodology (RSM) to determine the optimum combination of water-flour (W/F) ratio, water temperature, and fluid shear rate (homogenizing speed) in the high-shear mixing step of the laboratory HS-FWD wet-milling test.

4. Determine the optimum combination of aging (maturation) temperature and time in the gluten-aging step of the laboratory HS-FWD wet-milling test to obtain the highest recovery of the gluten fraction with high-protein purity.

5. Compare the bench-scale HS-FWD wet-milling test with the traditional dough-washing (Martin) method.

6. Distinguish the differences in the wet-milling qualities of flours, dry-milled from various wild-type and waxy wheats with hard or soft endosperm, by the designed HS-FWD wet-milling test.

MATERIALS AND METHODS

Materials

A Regional Baking Standard (RBS-98) flour with 11.8% protein (14% mb), generously provided by Dr. Okkyung Kim Chung (USDA/ARS Hard Winter Wheat Quality Laboratory, HWWQL, Manhattan, KS), was used to devise the HS-FWD wet-milling test. The RBS-98 flour is dry-milled from a composite of four hard red winter wheat varieties ("Ogallala", "Karl 92", "7853", and "Jagger") harvested from ~19 Kansas Wheat Performance Trials locations in 1998.

Four hard red winter wheats, Karl 92, Jagger, "2137", and "TAM 110", which, respectively, have extra strong, strong, medium, and weak dough-mixing properties, were obtained from Dr. J. Martin (Hays Branch of State Agricultural Experiment Station, Kansas State University, Manhattan, KS). The Jagger, 2137, and TAM 110 wheats were harvested from two locations, Hays and Colby, KS in 2002, and were blended before milling. The Karl 92 wheat sample was harvested in 1997 from Hays, KS. A flour sample dry-milled from "Chisholm" wheat with a medium- to strong-mixing property was kindly provided by Dr. G. Lookhart (GMPRC, Manhattan, KS). A coarsely ground flour obtained by a short-flow dry-milling process was from Midwest Grain Products, Inc., Atchison, KS, through the courtesy of Dr. C.C. Maningat. A soft red winter (SRW) wheat flour (AT 91W) having 12.2% protein (14% mb) was kindly provided by Dr. C. Gaines (USDA/ARS Soft Wheat Quality Laboratory, Wooster, OH).

In addition to the wild-type or partial waxy hard and soft wheats, three waxy wheats with medium-hardness endosperms were included in the study. One red winter waxy wheat line (Ike Wx 4; HRWW / Wx 99-130) was provided by Dr. C.F. Konzak (Northwest Plant Breeding Co., Pullman, WA). The Ike Wx 4 line, referred to as "winter waxy" wheat in this dissertation, was produced by mutagenesis of the partial waxy wheat Ike, and was harvested in 2001. Two spring waxy wheat lines (99ID496 and 99ID525) were supplied by Dr. R.A. Graybosch (USDA/ARS, University of Nebraska, Lincoln, NE). The spring waxy lines were produced from crosses of hard spring wheats with a waxy wheat that had resulted from a cross of Kanto 107 and Bai Huo partial waxy wheats. The two waxy spring wheat lines were harvested in 2002 in Nebraska. The flours of these two waxy wheat lines were blended in equal portions before being wet-processed, and the blend is called "spring waxy" wheat. A sample of "Leona" with 10.8% protein (14% mb), a recently released soft spring waxy wheat variety, was provided by Dr. E. Souza (University of Idaho, Aberdeen, ID). All wheat samples were tempered at 15.5% moisture for 16 h and roller-milled to straight-grade flours on a Buhler experimental mill (M202, Buhler Co., Uzwil, Switzerland).

General Methods

Moisture, ash, and crude fat contents of flours and dried wet-milling fractions (A-starch, B-starch, and gluten) were determined, respectively, by approved Methods 44-15A, 08-01, and 30-25 of the AACC (AACC 2000). Protein (Nx5.7) contents of flours and their wet-milling fractions were measured by a combustion method on an FP Protein/Nitrogen

Analyzer (Leco Corp., St. Joseph, MI). Total starch and starch damage were assayed by AACC-approved Methods 76-13 and 76-31, respectively, using assay kits from Megazyme International Ireland Ltd, Wicklow, Ireland. Amylose contents of flours were measured using amylose/amylopectin assay kits from Megazyme International Ireland Ltd. Single kernel characteristics of wheat samples were determined on the Single Kernel Characterization System (SKCS 4100, Perten Instruments North America, Inc., Reno, NV) on 300 kernels. Particle-size distributions of flours were calculated as weight percent passing through 38-, 53-, 75-, 150-, and 250-μm opening screens using Alpine Jet Sieve (Augsburg, Germany).

Insoluble polymeric protein (IPP) content of flours was determined essentially by the method of Bean et al (1998). Flour (1.0 g, db) was mixed with 4.0 mL of 50% (v/v) aqueous 1-propanol and the mixture dispersed by hand with a spatula. The sample was mixed continually for 5 min on a vortex mixer, centrifuged at 8,160 x *g* for 5 min, and the supernatant discarded. This extraction procedure was carried out a total of three times. After the extractions, the pellet was freeze-dried and analyzed for protein. The IPP content was calculated as the percentage of total protein (Nx5.7) in a flour sample.

Pasting properties of starches were measured using a Rapid Visco Analyzer (RVA, Newport Scientific, Warriewood, NSW, Australia). Starch (3.0 g, 14% mb) and water (flour plus water; 28.0 g) were placed in an RVA canister and mounted on the RVA instrument. The temperature profile consisted of holding the sample for 1 min at 35°C, heating to 95°C over a 4-min period, holding at 95°C for 6 min, cooling to 35°C over a 5-min period, and holding at 35°C for 4 min.

Mixograms of flours were determined by the AACC method 54-40A using a 10-g mixograph (National Manufacturing Co., Lincoln, NE). The mixograms were interpreted as described by Finney and Shogren (1972).

Dry solid contents of the water-solubles/particulates fractions were determined by sampling an aliquot (20.0 mL) of the total fraction (1,800-2,000 mL) and oven-drying at 105°C for 3 h. Prior to drying at 105°C for 3 h, the excess water in a sample was evaporated by incubating the sample at 80-90°C overnight. Dry solids contents of the fiber fractions were determined by oven drying of whole fractions (~20 g) at 105°C for 3 h. Soluble proteins and water-soluble solids in a flour at 35°C were determined as follows. Flour (10.0 g, db) and water (100 mL at 35°C) were combined in a 250-mL stoppered centrifuge bottle, and the bottle was shaken at a medium speed for 10 min in a reciprocal water bath maintained at 35°C (Precision Instruments, Winchester, VA). The slurry was centrifuged at 2,500 x *g* for 15 min, and an aliquot of the supernatant was assayed for protein and dry solids. Water absorption of the isolated A-starches was determined as follows. Starch (2.0 g, db) and water (20 mL at 35°C) were combined in a 50-mL stoppered centrifuge bottle with a known weight, and the bottle was shaken vigorously by hand for 1 min. The slurry in the bottle was incubated at 35°C in the water bath without agitation, and then centrifuged at 2,500 x *g* for 15 min. The supernatant was decanted and the bottle containing the sedimented starch weighed to calculate the amount (g) of water absorbed per gram of dry starch.

Scanning electron micrographs (SEM's) of freeze-dried samples of RBS-98 flour-water dough, the rapidly mixed dough-water dispersion, and the HS-FWD were acquired with a Hitachi S-3500N (Hitachi Science Systems, Ltd., Tokyo, Japan) scanning electron microscope operated at 5 kV. The samples were prepared as described below in the wet-milling of the RBS-98 flour by the HS-FWD test, the rapidly mixed dough-water dispersion, and the Martin wet-milling methods. Approximately 5-10 g of a sample from each of the three wet-milling methods was removed and placed in a centrifuge bottle, and the contents rapidly frozen (~2 min) by immersing the bottle in dry ice-acetone. The frozen sample was then freeze-dried. The SEM's were taken after sprinkling a small crushed sample onto double-sided adhesive tape pressed atop a specimen stub. Following coating with gold-palladium, the sample was viewed with the scanning electron microscope.

Baking performance of the gluten fractions isolated by different wet-milling processes was evaluated by baking pup-loaf breads from blends prepared by a starch-stress bake-test formula (Miller and Hoseney 1996). For this baking test, the flour portion of the formula was a blend of the RBS-98 flour, commercial wheat starch (Midwest Grain Products, Inc., Atchison, KS), and laboratory-isolated test gluten. To prepare the blends, the RBS-98 flour with 11.8% protein (14% mb) was first blended with commercial wheat starch at a 1:1 ratio to reduce the protein level of the flour-starch blend to ~6%. The test gluten (8% of the weight of the blend) was then added to elevate the protein level of the final blend to 11-12% (14% mb). Pup-loaf bread was baked by the AACC approved Method 10-10B, a straight-dough procedure with 120 min fermentation, at the USDA/ARS HWWQL/GMPRC, Manhattan, KS. The protein content of each blend was determined and specific loaf volume per unit of protein was calculated by dividing the loaf volume by the protein content of the blend.

Development of Highly Sheared Flour-Water Dispersion (HS-FWD) Wet-Milling Test

1. Response Surface Study I

A three-way (3 independent variables with 3 levels each) response surface methodology (RSM) was used to design experiments to optimize the high-shear flour-water mixing step that produces the batter in the HS-FWD wet-milling test. The RSM design specified a total of 20 randomized experimental runs (10 treatment combinations x 2 replications) (Table I), which covered the 27 possible treatment combinations (Walker and Parkhurst 1984, C.E. Walker 2002 - *personal communication*). Three factors, namely water-flour ratio, water temperature, and shear rate, were selected as the independent variables. Each variable consisted of three levels: water-flour weight (db) ratios of 1.4, 1.7, and 2.0; water temperatures of 20, 27, and 34°C; and shear rates of 2,500, 5,000, and 7,500 rpm. The shearing (homogenization) time was fixed at 2.0 min. Responses measured were the batter temperature after homogenization, recovery of flour starch in the A-starch fraction, and recovery of flour proteins in the gluten fraction. Recoveries of starch and protein were calculated based on their levels in the RBS-98 flour. The collected data were analyzed by RSM-Plus© Enhanced Response Surface Methodology Analyzer and General Equation Modeler (AEW Consulting, Lincoln, NE) software to fit to a second-order (quadratic) multiple regression model for each response ($Y = A + BX_1 + CX_2 + DX_3 + EX_1X_2 + FX_1X_3 + GX_2X_3 + HX_1^2 + IX_2^2 + JX_3^2$). Full models were used to develop surface and contour plots and thus all models included an intercept term, three first-order terms, three second-order terms, and three two-way interaction terms. The RSM-Plus© software provided the coefficient of determination (R^2), coefficient of multiple correlation, and the standard error of estimate for models to reflect the goodness of the fit of the data to the models.

2. Wet-Milling Test Starting with HS-FWD

Flour (65.0 g, db) and water (amount and temperature as per experimental design) were measured. Approximately one-half of the water was placed in a 250-mL clear wide-mouth centrifuge bottle (Cat. No. 05-432A, Fisher Scientific, Chicago, IL) and then the flour followed by the remaining water were added. In that manner, formation of lumps of flour on the bottom of the centrifuge bottle was prevented. The flour and water were gently blended with a spatula for 5-10 s, and the slurry was subjected to high-shear mixing with a homogenizer (Model Pro 350, Pro Scientific Inc., Monroe, CT) equipped with a 37-mm diameter rotor-stator generator at speeds of 2,500, 5,000, or 7,500 rpm for 2.0 min. The slurry in the centrifuge bottle was exposed to the shearing action of the rotor-stator mixer by manually moving the bottle vertically at about 30 cycles per minute. The temperature of the highly-sheared batter was recorded upon completion of shearing. The rotor-stator generator was rinsed by a water stream (~20 mL, 25°C) from a wash bottle, and the washings were added to the bottle containing the dispersion. After the solids concentration of the HS-FWD was adjusted to ~27% solids by adding water (~40 mL at 25°C), the centrifuge bottle was capped and the contents mixed by inverting the bottle manually 10 times. In the experiment where the HS-FWD was rested, the hand-mixing of the batter plus water was carried out at the end of the resting period.

The HS-FWD was then centrifuged at 2,500 x *g* for 15 min in a swinging-bucket rotor (Model JS 7.5) centrifuge (Model J2-21, Beckman Coulter, Inc., Palo Alto, CA). The top liquid phase was decanted and saved for combining with other liquid supernatants generated during the process. The top thin layer of sedimented semi-solids was a gel, probably comprised of small and damaged starch granules and insoluble-pentosans. The gel phase was not clearly visible but was readily differentiated by its soft texture as opposed to the strong elastic texture of the protein-rich phase. Immediately under the gel was the protein-rich phase, which constituted a thick layer, and was atop a thin layer of fiber. The gel and protein-rich layers were collected together by carefully scraping off with a spatula. The combined layers were briefly mixed, and then divided in two equal portions. The two portions were added each to a Glutomatic washing chamber fitted with a 250-μm opening screen. The Glutomatic instrument (Model 2200, Perten Instruments North America, Inc., Reno, NV) was prerun for 20 s before mounting the chambers because the dough-mixing step normally used to isolate gluten from flour was not needed. The instrument was put on hold at the starting time of the gluten-washing step, and the chambers containing the gluten-concentrate phase were mounted. The gluten-isolation step was then initiated and continued at a preset water stream of 50-60 mL / min for 5.0 min. The wet gluten portions retained inside the two chambers were combined together with the gluten recovered on top of two sieves (425- and 250-μm openings) after the vibratory wet-sieving of the gluten washings and a slurry of the sedimented A-starch *(see below)*. The combined wet gluten materials were placed in a thick-walled centrifuge bottle, expanded to several volumes under low pressure, and the expanded gluten then dried on a freeze-dryer (Flexi-Dry/MP, TMS Systems, Inc., Stone Ridge, NY). The dried gluten fraction was ground on a Thomas Wiley Mill (Cat. No. 08-338, Fisher Scientific, Chicago, IL) to pass through a 420-μm opening sieve. The dried gluten fraction was analyzed for moisture and protein contents.

The thin layer of fiber layer plus the bottom layer of dense starch were mixed uniformly in 3 x 25 mL of water by vigorous hand-shaking. The slurry was subjected to wet-sieving at full amplitude (3.0 mm) on a vibrating sieve-shaker (Model Fritsch A-3, Gilson Company, Inc., Lewis Center, OH) equipped with a stack of three 8-in diameter screens. The openings on the screens starting from top to bottom were 425, 250, and 63 μm (U.S. Standard Sieve No. 40, 60, and 230, respectively). The 425- and 250-μm opening screens retained mostly small gluten particles, which were combined with the gluten mass generated by the Glutomatic washing. The 63-μm opening screen retained mainly the fine fiber and insoluble pentosans, which were called "fiber". After adding the slurry to the top screen, tap water was sprayed on the top screen at a rate of ~700 mL / min during the first 30 s of wet-sieving. The rinse water was turned off and sieving continued another 90 s. The throughs were collected and transferred to two 250-mL bottles and centrifuged (2,500 x *g* for 10 min). The supernatants in the bottles (#1) were decanted and added to the water-solubles/particulates. The sediments in the bottles were slurried in water (75 mL each), and then combined in one bottle. After centrifuging, the supernatant (#2) was removed and combined with the water-solubles/particulates fraction. The top thin layer (~1 mm thick) of the sedimented solids, often called "tailings", was carefully removed with a spatula and combined with the throughs or washings generated by the Glutomatic washing of the gluten. The sedimented starch was dried (6-10% moisture content, MC) in a forced-air convection oven (35-40°C) and ground on a laboratory mill (Model A-10, Tekmar-Dohrmann, Mason, OH) for 15 s to produce the "A-starch" fraction, which was assayed for protein and moisture contents.

The throughs from the Glutomatic separation were subjected to vibratory wet-sieving. The overs on the top two screens became part of the gluten fraction, whereas those on the bottom screen were part of the fiber fraction. The throughs from the vibrating sieves were transferred to two 250-mL bottles and centrifuged. The supernatants in the bottles (#3) were decanted and combined with the water-solubles/particulates fraction, while the sediments in the bottles were slurried in a total of 200 mL of water and combined in one centrifuge bottle. After centrifuging, the supernatant (#4) was combined with the water-solubles/particulates fraction, while the sediment was oven-dried (35-40°C), ground, and analyzed for moisture and protein contents. This fraction was called "B-starch". An aliquot (20.0 mL) of the total water-solubles/particulates fraction, which contained all the supernatants and totaled 1,800-2,000 mL, was heated in an oven at 80-90°C overnight to evaporate water and then dried at 105°C for 3 h. The residual dry solids was the water-solubles/particulates fraction.

The residue that remained on the 63-μm opening sieve after sieving the fractions was collected and dried at 105°C for 3 h. That residue was fiber fraction.

3. Aging of Protein-Rich Phase

After centrifugal fractionation of a HS-FWD and decanting the top liquid phase, the gel layer plus the protein-rich layer atop the fiber and sedimented starch layers were transferred to a 250-mL beaker with 100 mL of water at 25, 35, or 45°C per the experimental design *(see below)*. The beaker was placed in a water bath that was maintained at 25, 35, or 45°C and the mixture rested without agitation (1, 25, or 45 min as per experimental design). Upon completion of the aging of the gluten, the contents of the beaker were poured on the screens (425-, 250-, and 63-μm openings) and the throughs were saved to combine with the washings generated by gluten washing in the Glutomatic. The overs (gluten aggregates) on the 425- and 250-μm opening sieves were collected and divided into two equal portions and washed in the Glutomatic as previously described, except for modifications to two washing parameters. The washing period was reduced to 2.0 min from 5.0 min, and an 88-μm opening sieve was used instead of the 250-μm opening sieve. Prolonged washing on the large-opening sieve was found to elevate losses of proteins through the screen, which became part of the fiber and B-starch fractions. The wet glutens retained on the two washing chambers of the Glutomatic were combined, freeze-dried, and ground as described above.

4. Response Surface Study II

For the optimization of the gluten-aging step of the HS-FWD wet-milling test, a two-way (2 independent variables with 3 levels each) RSM design was followed (Table II) (C.E. Walker 2002 - *personal communication*). Two factors, namely, temperature of water added to the protein-rich phase in the beaker and aging time, were selected as the independent variables. Each variable consisted of three levels; water temperatures of 25, 35, or 45°C and aging times of 1, 20, or 45 min. The amount of water added to the protein-rich phase in the beaker was fixed at 100 mL. The recovery of flour proteins in the gluten fraction was measured as the response. The collected data were again analyzed by the RSM-Plus© software to fit to a quadratic multiple regression model ($Y = A + BX_1 + CX_2 + DX_1X_2 + EX_1^2 + FX_2^2$).

Wet-Milling Test on a Rapidly Mixed Dough-Water Dispersion

The method of Czuchajowska and Pomeranz (1993, 1995) was followed with slight modification. RBS-98 flour (65.0 g, db) was divided into two equal portions and mixed in two consecutive batches in a 35-g mixograph (National Manufacturing Co., Lincoln, NE) at optimum water absorption (63.2%, 14% mb) and optimum mixing time (4.0 min). The doughs were combined and placed in a 300-mL capacity bowl (Cat. No. 14-509-18C) of a Waring blender (Cat. No. 14-509-7N, both bowl and blender from Fisher Scientific, Chicago, IL) containing 115 mL of water at 15°C. After 30-min resting, the dough and water were mixed rapidly in the Waring blender for 1.0 min at full speed. The dough dispersion was transferred to a 300-mL centrifuge bottle and centrifuged at 2,500 x *g* for 15 min. The vital gluten, A-starch, B-starch, water-solubles/particulates, and fiber fractions were isolated and purified as in the HS-FWD wet-milling test. The protein-rich phase collected upon centrifugation of the dough-dispersion was washed in the Glutomatic without a maturation scheme.

Wet-Milling by Martin (Dough-Washing) Method; Control Method

Starting with 65.0 g (db) of flour, a dough was prepared in the same manner as in the rapidly mixed dough-water dispersion method. The dough was then covered with a wet cloth and rested at room temperature for 1 h. The rested dough-ball was hand-washed under a stream of water at 25°C (~1 mL / min) with a total water usage of 750 mL. The wet gluten was freeze-dried and ground as described in the HS-FWD test. The starch milk was wet-sieved on a 63-µm opening sieve to collect the fiber, and throughs were centrifuged (2,500 x *g* 10 min) in six 250-mL bottles. The supernatants (#1) were decanted and saved, and the sediments were slurried with water (150 mL total) in one bottle and centrifuged. After decanting the supernatant (#2) and combining with #1, the upper pigmented layer (4-5 mm) was scraped off with a spatula and saved, while the sediment was slurried with 150 mL of water and centrifuged. The supernatant (#3) was combined with the others, and the combined supernatant was sampled for assay of water-solubles/particulates and for protein. The upper pigmented layer (~1 mm) was scraped off and combined with the previous one to give B-starch, while the sediment was recovered as the A-starch fraction. Both starch fractions were dried and ground as described previously.

Statistical Analyses

All analyses were carried out at least in two replications, and the means were compared by the LSD (least significant difference) multiple comparison test at α = 0.05 level in one-way analysis of variance (ANOVA), using the Statistical Analysis System Software, V. 6.12 (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

The RBS-98 flour has average properties of straight-grade flours milled from hard winter wheats in the region. The mixogram and pup-loaf volume (885 cc) given in Fig. 9 are typical of a strong bread-wheat flour containing 11.8% protein (14% mb).

Hard winter wheats are selected by breeders based on their flour-milling and breadmaking quality. In the USA, a small percentage of hard-wheat flour is also wet-milled to produce vital wheat gluten and starch (Witt 1997, Maningat and Bassi 1999). Wet-milling of wheat flour may increase in the future because wheat is likely to be transformed into valued materials by gene transfers. Waxy and high-amylose wheats are examples of specialty wheats that are currently in various stages of breeding. Transgenic wheats may be wet-processed to isolate or concentrate wheat starches or other valued substances.

In a HS-FWD process on various flours, there appear to be three sensitive steps that affect the recoveries and purities of the gluten and A-starch fractions, namely the high-shear mixing of flour and water to give a flour-water dispersion, the aging of the protein-rich phase obtained upon centrifugal fractionation of a HS-FWD, and the washing of the gluten particles to remove contaminants.

In this study, the high-shear mixing step and the aging of the protein-rich phase were investigated in detail, whereas the washing of the gluten particles was not studied thoroughly.

Purification of the protein-rich phase to produce the gluten fraction was assigned early in the project to the Glutomatic instrument so that the work might be repeated in other laboratories. The Glutomatic instrument was designed to exert a kneading force on a dough that is held on a sieve, and to wash the starch away from the cohesive gluten. In the HS-FWD test, the glutomatic performed adequately to purify the gluten from the protein-rich phase of a strong dough, but not a weak one. Kneading the protein-rich phase of a weak flour caused the slack protein-rich material to "blind" the sieve. In the commercial HS-FWD wet-processes, the protein-rich phase is usually agitated in excess water to produce particles of gluten and release starch (Kerkkonen et al 1976, Maijala 1976). In our laboratory HS-FWD test, additional processing of the protein-rich phase besides the Glutomatic washing appears to be warranted. One approach would be cutting/mixing of the protein-rich phase at low speed (~200-500 rpm) in additional water. A blade-shaped agitator with cutting/mixing action should produce gluten in small but filterable particles and simultaneously release the tiny starch granules into the liquid phase. The mixture would then be wet-sieved to collect the gluten particles from the starch milk.

In the HS-FWD wet-milling test, the high-shear mixing step, wherein the flour and water are mixed at high speed, must break the cohesive force(s) between starch and protein matrix in the endosperm and release individual particles (gluten threads and starch granules) into the aqueous medium. When the RBS-98 flour and water were mixed (27% solids) in a centrifuge bottle by vigorous shaking by hand instead of a high-shear mixing, the hand-mixed batter gave no visible separation of flour components upon centrifugation

(Fig. 10). However, when the flour-water mixture was homogenized to a batter at 6,000 rpm for 2.0 min, and the highly sheared batter blended with water to 27% solids in the HS-FWD, a clear separation of starch and protein-rich phases, plus a supernatant liquid phase, was achieved by centrifugation.

A flour-water mixture could also be high-shear mixed in a Waring blender to produce a dispersion that would separate upon centrifugation. However, the "dead zone" present near the shaft of the mixer blades of the blender promoted aggregation of the gluten fibrils to large particles, and those particles adhered to the shaft of the blades. Those adhering gluten particles were difficult to recover. Furthermore, visible gluten particles tended to break free of the shaft and contaminate the starch granules in the centrifugation step, which deteriorated the efficiency of separation.

Scanning electron micrographs of an optimally mixed flour-water dough, a rapidly mixed dough-water dispersion, and a HS-FWD are shown, respectively, in Figs. 11, 12, and 13. In the dough that was mixed at optimum water absorption and mix time, the gluten was aggregated to form a continuous sheet-like structure that tightly enmeshed the starch granules as the dispersed phase (Fig. 11). When that dough was rapidly mixed with additional water in a blender to form a dough-water dispersion at ~27% solids, the sheet-like network appeared transformed to thick thread-shaped particles of gluten with many of the starch granules mostly freed of gluten (Fig. 12). Those gluten threads were observed to be irregularly shaped and sized. Some of the gluten threads that are large in size, such as observed at the bottom of Fig. 12, contained occluded starch granules.

Under a centrifugal field, those large-sized particles would sediment together with starch and cause inefficient separation of protein and starch.

In the case of a HS-FWD (Fig. 13), where the flour and water were sheared directly without forming a dough, the gluten network was observed to be an assembly of interconnected gluten threads (Fig. 13). When the dispersion was rested those threads formed a weak "gel" enmeshing many free starch granules. During the centrifugal separation step, the fine network of the gluten threads apparently allowed more than 50% of the starch granules in the dispersion to pass through the "gel" at which point the "gel" collapsed due to the loss of solids to form the protein-rich phase atop the sedimented starch.

Modeling of High-Shear Mixing Step of HS-FWD Wet-Milling Test; Recoveries of Starch and Protein

The variables in the first RSM study were water-flour ratio, water temperature, and homogenizer speed, whereas the responses were the recoveries and purities of the gluten and A-starch fractions. The following variables were held constant: the flour (RBS-98), shear-mix time (2.0 min), and purification and isolation protocols to obtain the five fractions (Fig. 14). The regression equations (Table III) had coefficients of determination (R^2) of 0.98 for temperature of the sheared batter, 0.95 for A-starch recovery, and 0.65 for protein recovery. The marginal goodness of fit of the regression equation for protein recovery is due to the narrow range (66 to 72%) of protein recoveries. It was found that

~14% of the RBS-98 flour proteins were soluble in water at 35°C. Therefore, the maximum protein recovery in the gluten fraction from the RBS-98 flour would be ~86%.

The temperature of the highly sheared flour-water batter immediately after shearing varied from 26 to 43°C (Fig. 15). The temperature of the sheared batter increased with increasing water temperature and shear rate, but the water-flour ratio (33 to 42% solids, db) had little influence on the temperature of the sheared batter when the added water temperatures were 20 and 27°C (Fig. 15).

The contour plots obtained for the recoveries of starch and protein in the A-starch and gluten fractions, respectively, are shown in Figs. 16 and 17. After purification (Fig. 14), starch fractions contained 0.20-0.30% protein, while gluten fractions contained 87-93% (db) protein (Nx5.7). The much higher protein content of the gluten fractions in the bench-scale test is worth noting compared to that (73-82%, db) in commercial wheat glutens (Wadhawan 1988, Maningat et al 1994, Miller and Hoseney 1996). On the other hand, the purities of the A-starch fractions were the same.

After centrifugation of the HS-FWD, the sedimented starch "as is" contained 0.35-0.40% protein contamination, which was readily reduced to 0.20-0.30% protein contamination by washing with water. Fig. 16 shows that recovery of the purified A-starch fraction depended mostly upon shear rate up to ~6,000 rpm, above which starch recovery reached a plateau. Increasing water temperature from 27 to 34°C decreased recovery of A-starch by ~6%, perhaps because of added swelling of starch granules and an increase in their buoyancy, or because of excessive strengthening of the gluten "gel" (*see below*).

Fig. 17 shows that the protein recovery in a gluten fraction increased with a rise in the temperature of the water added to flour in the high-shear mixing step, possibly as a result of increased aggregation of the gluten proteins during mixing. According to Meuser (1994), the elasticity of the gluten "gel" in the HS-FWD is a crucial factor to the success of the centrifugal separation. During centrifugation the individual starch granules are thought to stretch a micro-zone in the "gel" until it fails, which allows the granule to pass through the "gel" and to sediment. Upon the passage of a granule, the micro-zone of the gluten matrix forms new connections between gluten threads and reestablishes the elasticity of the network. For an efficient separation of more than 50% by weight of starch granules from the gluten proteins, the elasticity of the gluten matrix during centrifugation must be low enough to allow the passage of the starch granules in many micro-zones, yet large enough to resist collapse of the matrix on the macro-scale. Increased aggregation of gluten proteins during the high-shear mixing would increase protein recovery in the wet-process, and at the same time decrease starch recovery because the thicker gluten threads would interfere with passage of the starch granules through the "gel" network.

The level of protein (87-93%, Nx5.7, db) in the purified dry gluten fractions varied more than the level of starch in the purified A-starch fractions (contaminated with 0.20-0.30% protein). The gluten protein is known to be contaminated mainly by wheat starch and lipids (Maningat et al 1994, Miller and Hoseney 1996), and much less so by endosperm cell walls. It is assumed much of the cell wall material accompanied the starch streams in Fig. 14, and was eventually separated in the fiber fraction by screening of a starch stream.

As shown in Fig. 17, the recovery of flour proteins in the gluten fraction was low, ranging from 66 to 72%, as opposed to the theoretical maximum of ~86% of flour protein. In the RSM experiment to model the high-shear mixing of flour and water on the RBS-98 flour, the protein-rich phase collected after centrifugation of the HS-FWD was not aged. Aging might have given more complete aggregation of the proteins in the gluten fraction. Even more importantly, the protein-rich phase was processed in the Glutomatic over a screen with relatively large openings (250 μm), and the collection and washing of the gluten was carried out for a 5.0-min period. Because of the extended washing for 5 min of gluten with water at 25°C in the Glutomatic, the wet gluten became cool and was crumbly instead of being elastic and extensible. Therefore, the large screen openings allowed some gluten particles to be lost. It was found that a 2.0-min processing of the protein-rich fraction in the Glutomatic with a sieve having 88-μm openings increased gluten recovery by ~5%, and the gluten fraction contained 82-85% protein (db). A sieve with 88-μm openings is specified by official methods in determining the wet gluten and gluten index in the Glutomatic. In all other experiments in this thesis requiring processing of a protein-rich phase in the Glutomatic, a total process time of 2.0 min and a screen of 88-μm openings were used.

Modeling of Gluten-Aging Step in HS-FWD Wet-Milling Test

The highest (~83%) recovery of starch in the A-starch fraction was obtained (Fig. 16) when a HS-FWD was prepared from a highly sheared batter at a W/F ratio of 1.7, shear rate of 6,000 rpm, and water temperature of 25°C. Under those conditions, 72% of flour proteins were recovered in the gluten fraction by immediately processing the protein-rich phase in the Glutomatic for 2.0 min over a 88-μm openings screen. However, protein recovery in the gluten fractions in commercial high-shear processes has been reported to range from 75 to 85% of flour protein, often >80% (Maijala 1976, Dahlberg 1978, Witt 1997). In order to increase protein recovery in the laboratory test, the protein-rich phase was subjected to aging, a step that was reported in the industrial high-shear Raisio process (Kerkkonen et al 1976, Maijala 1976, Dahlberg 1978).

The optimum conditions of aging were investigated following a two-way RSM design (Table II). At the high-shear mixing conditions that gave the highest (~83%) recovery of the A-starch fraction, an RSM model (R^2 = 0.97) was developed for the time and temperatures used to age the protein-rich phase (Fig. 18). When the protein-rich phase was aged in added water at 30 to 45°C for 10 to 30 min without agitation, ~75% recovery of flour proteins was achieved in the gluten fraction as opposed to 72% recovery without aging (Fig. 18). At those aging conditions, the wet-gluten fraction was observed to be a cohesive and elastic mass, whereas it became too extensible at prolonged holding (>30 min) of the protein-rich phase in water at temperatures >40°C. Also, even a slight agitation of the protein-rich phase during aging promoted disaggregation of the gluten and caused lower protein recovery *(data not shown)*. Perhaps the addition of extra water (100 mL) to the protein-rich phase (~60 g, wb), which was done to control temperature, negatively impacted the gain in gluten recovery. The RSM modeling of the high-shear mixing of flour and water to a highly sheared batter (Fig. 17) indicated that increasing the temperature of water added at the high-shear mixing step from 20 to 34°C improved protein recovery with a slight reduction in starch recovery in the A-starch fraction (Fig. 16).

After setting the aging conditions of 40°C and 20 min for the protein-rich phase, a series of experiments was conducted to determine whether increasing the water temperature from 25 to 40°C in the high-shear mixing step (Fig. 17) would influence recovery of flour protein in the gluten fraction. As the water temperature was increased from 25 to 35°C, the protein recovery increased from ~75 to ~79% (Fig. 19). Further increasing the water temperature to 40°C did not change protein recovery. The recovery of the A-starch fraction was slightly reduced (83 *vs.* 80%) as the water temperature increased from 25 to 35°C. The HS-FWD test in Fig. 14 incorporated these parameters to increase recovery of protein in the gluten fraction.

Aging of HS-FWD Prior to Centrifugation

Witt (1997) showed in an industrial HD process that a 15-min aging of a high-pressure homogenized (sheared) flour-water dispersion prior to fractionation in a three-phase decanter centrifugation gave the best separation of the A-starch and protein-rich phases.

In the laboratory HS-FWD wet-milling test at optimized high-shear mixing and gluten aging conditions, aging of the HS-FWD for 15 min as opposed to no-aging gave no statistically significant differences in the recoveries of the protein and A-starch fractions (Fig. 20).

Based on the above findings, the following process conditions for the HS-FWD wet-milling test were chosen as optimum: W/F ratio of 1.7, water temperature of 35°C, and homogenizer speed of 6,000 rpm for 2.0 min in the high-shear mixing step; gluten aging temperature and time of 35°C and 20 min, respectively, in 100 mL of water; and gluten-washing time of 2.0 min in the Glutomatic in two washing chambers fitted with 88-μm opening sieves. Although variations in the W/F ratio had no significant influence on the recoveries and purities of the fractions, a W/F ratio of 1.7 was favored based on the qualitative observations during the homogenization and centrifugal fractionation of the HS-FWD. At the lower W/F ratios, homogenization of the flour-water slurry appeared to be difficult because of the elevated viscosity of the mixture. At the elevated W/F ratios, however, the sheared batter appeared to become highly aerated during the turbulent mixing, which in turn yielded a thin layer of protein on top of the supernatant liquid phase upon centrifugation of the HS-FWD.

Repeatability of HS-WFD Wet-Milling Test

The HS-WFB wet-milling test at the optimized conditions for the high-shear mixing step and the protein-rich phase aging step showed a high repeatability (Table IV). A total of five replications were run at the optimum conditions, which were W/F ratio of 1.7, homogenizer speed of 6,000 rpm, and water temperature of 35°C in the high-shear mixing step, and 20-min of aging in 100 mL of water at 40°C without agitation in the aging of the protein-rich phase. The coefficients of variation (CV) for the recoveries of the five wet-milling fractions and their purities ranged from 0.7 to 12.1%. The CV values for the recovery of flour protein in the gluten fraction and the recovery of starch in the A-starch fraction, which are the two most important fractions in wet-milling of wheat flour, were 0.9 and 1.7%, respectively.

Comparison of Three Bench-Scale Wet-Milling Tests

The wet-milling data for the HS-FWD test were compared to those of the rapidly mixed dough-water dispersion method and the Martin dough-washing method (Tables V-VII), all three tests being done on the RBS-98 flour. The total solids recovered in the three wet-milling tests were all approximately 98% (Table V). The HS-FWD and rapidly mixed dough-water dispersion methods gave similar recoveries (78-79%) of protein in their gluten fractions with similar purities (83% protein) (Table VI). However, the Martin dough-washing method, which involved minimal shear during processing, gave a higher recovery (~87%) of protein in the gluten fraction containing ~84% protein. The increase in protein recovery from ~78 to 87% in the gluten fraction of the Martin process likely resulted because much of the ~14% water-soluble proteins in the RBS-98 flour became associated with the gluten fraction. On the other hand, the reduced recovery of protein in the HS-FWD and rapidly mixed dough-water dispersion methods may be explained by the high-shear forces employed in those processes during preparation of a sheared dispersion prior to centrifugation, which caused the soluble-proteins to be dispersed and possibly some of the gluten particles to become smaller in size and to be lost to the B-starch fraction. The insoluble polymeric protein (IPP) contents of the HS-FWD and the RBS-98 flour were comparable (40.9 *vs.* 42.2%), which indicates that the high-shear mixing of the flour and water did not cause a significant solubilization of the insoluble proteins. A higher level of protein (4.3%) was recovered in the B-starch fraction of the HS-FWD method than in the Martin (0.9%) and the rapidly mixed dough-water dispersion (2.4%) methods (Table VII). Also, ~30% more protein was recovered in the water-solubles/particulates fractions of the HS-FWD and rapidly mixed dough-water dispersion methods as opposed to that recovered in the Martin dough-washing method (14.8 *vs.* 9.5%) (Table VII). It was reported that almost all the globulins and up to one-half of the albumins may be held in the gluten fraction by means of physical entrapments and disulfide bonding (Pence et al 1956). Fane and Fell, as cited by Rausch (2002), reported in 1977 that 16% of total flour protein was not recovered in the gluten fraction isolated by the Martin process, which translates into a protein recovery of 84% in the gluten fraction.

In terms of the A-starch recovery, the Martin method was the best (~89%), followed by the HS-FWD (80%) and the rapidly mixed dough-water dispersion methods (72%) (Table VI). In the Martin method, the A-starch fraction was obtained by centrifugation of the starch milk that contained both the A- and B-starch fractions washed from a dough mass, and therefore some A-starch that would otherwise be recovered in the B-starch fractions of the HS-FWD and rapidly mixed dough-water dispersion methods was recovered in the A-starch fraction in the Martin method. The rapidly mixed dough-water dispersion method gave a low A-starch recovery (72%) compared to 80% for the HS-FWD wet-milling test in its A-starch fraction because the centrifugal separation of the highly sheared dough-water dispersion was not as sharp as in the HS-FWD test. Large aggregates of gluten with entrapped starch granules occur in a dough-water dispersion as shown in Fig. 12. The A-starch fractions obtained by all the three methods had similar purities (<0.3% protein). Recoveries of starch in the B-starch fractions were inversely associated with the recoveries of starch in the A-starch fractions (Tables VI and VII).

The distribution of flour solids in the wet-milling fractions of the three methods are given in Table V. The Martin method gave the highest recoveries of solids in the gluten and A-starch fractions. The HS-FWD and rapidly mixed dough-water dispersion methods gave similar recoveries of solids in their gluten fractions, but the recovery of solids was lower in the A-starch fraction of the rapidly mixed dough-water dispersion. In the high-shear processes, the levels of flour solids in the water-solubles/particulates fractions were slightly elevated (Table V).

The drawbacks of the Martin process, which are the need of strong gluten-aggregation traits for flour and the utilization of a large amount of water during wet-processing, may be balanced by i) the high recoveries of protein in the gluten fraction, ii) high recovery of starch in the A-starch fraction, and iii) a ~10% reduction in solubles and suspended solids in process water.

Pasting Properties of A-Starch Fractions and Breadmaking Qualities of Gluten Fractions Isolated by Three Bench-Scale Wet-Milling Tests

The damaged starch contents of the A-starch fractions isolated by the three wet-milling methods ranged from 1.6 to 2.1% (db), comparable to the damaged starch content (2.0%) of a commercial starch. The RVA pasting properties of all the A-starch fractions were quite similar (Fig. 21), which indicates that starch was not damaged by the high-shear mixing of flour and water or rapidly mixing a dough and water.

The breadmaking qualities of the gluten fractions isolated by the three methods were evaluated in a starch-stress bake test (Miller and Hoseney 1996). In this bake tests, approximately one-half of the protein in the RBS-98 flour was replaced by a test gluten fraction. As summarized in Table VIII, loaf volumes of the breads baked from the three test gluten fractions did not differ significantly after correcting the loaf volumes by the protein contents of the respective blends. In terms of the crumb grain of the loaves, the gluten fraction isolated by the Martin method gave an improved score of the crumb grain.

Assessment of Wet-Milling Qualities of Various Wheat Flours by HS-FWD Wet-Milling Test

1. Wet-Milling of Hard Wheat Flours

The HS-FWD wet-milling test (Fig. 14) was used to evaluate the wet-milling properties of hard wheat flours with varying dough-mixing characteristics. Five hard wheat flours dry-milled from Karl 92, Jagger, Chisholm, 2137, and TAM 110 wheats were included, which, in declining order (G. Lookhart 2002 – *personal communication*), have extra strong to weak dough-mixing properties. The mixograms of those flours are shown in Fig. 22. As compared to the control flour (RBS-98), Karl 92 had a much stronger dough-mixing property while Jagger had a similar dough-mixing profile. Flour samples of 2137 and TAM 110 showed relatively weak dough-mixing properties. Chisholm flour had an unusually long mixing time (~15 min) due to its low protein content, and was reported to give bread with a good loaf volume and crumb grain score (G. Lookhart 2002).

Fig. 23 is a photograph taken after the centrifugation of the HS-FWD of those flours prepared according to the optimized HS-FWD wet-milling test. The quantity of the A-starch sediment and the sharpness of its demarcation from the protein-rich phase in the various highly-sheared flour-water dispersions seem to correlate positively with the dough-mixing properties of the flours. Karl 92 and Chisholm flours gave the highest proportion of sedimented A-starch phase while 2137 and TAM 110 gave the lowest proportion. The HS-FWD of Jagger had intermediate behavior.

The demarcation plane between the starch and protein-rich phases were much clearer for the flours with strong dough-mixing characteristics. When the HS-FWD from the flours having strong dough-mixing characteristics were subjected to centrifugal force, the fine-meshed gluten "gel" in those flours probably collapsed at a later time after start of centrifugation. The extra time would allow more of the dense starch granules to pass through the fine network of gluten and to sediment. In the case of the flours with weak dough-mixing characteristics, the gluten "gel" in their HS-FWD apparently collapsed sooner in the centrifugal field, and further sedimentation of starch granules was prevented. The collapse of a weak gluten network may explain the less clearly defined plane between the starch and protein-rich phases where particles protrude into the starch phase (Fig. 23).

Table IX summarizes wet-processing data by the HS-FWD wet-milling test for the hard wheat flours. Recoveries of flour protein (79-82%) in the gluten fractions isolated from all the flours were comparable to that (79%) of the control flour, except for the reduced protein recovery (72%) from Chisholm flour. However, protein concentrations in the glutens (60-70%) isolated from the weak flours (2137 and TAM 110) were lower than those (82-90%) isolated from the flours with strong dough-mixing characteristics. The protein content of the gluten fraction isolated from Jagger flour was also low (73%) as compared to the control (79%) probably because the higher protein level (13.9%) in Jagger flour gave a larger quantity of the protein-rich phase in wet-processing. That larger bulk of protein-rich phase was subjected to the same level of washing (2 min) in the Glutomatic, which may have been inadequate to remove contaminating B-starch.

However, increasing the Glutomatic-washing time from 2.0 min to 3.0 and 4.0 min did not noticeably improve the purity of the gluten fraction. It is possible that the association between the starch granules and the proteins of the Jagger flour may be much stronger than that of the control flour.

All flours gave A-starch fractions with similar purities (0.22-0.24% protein), but the recoveries of starch in the A-starch fractions of 2137 and TAM 110 flours were much lower (64-67%) than other flours (76-89%). Recoveries of the B-starch followed an inverse trend to the recoveries of A-starch for all flours. Similar recoveries (>98.5%) of solids in the five wet-milling fractions were achieved from all flours.

The results indicate that aggregation characteristics of gluten proteins in a HS-FWD influence the fractionation of flour components in their wet-processing by a HS-FWD wet-milling process. Flours with strong dough-mixing characteristics appear to give strong porous gluten networks after high-shear mixing in water and dilution of the batter with water to ~27% solids. That dispersion maintains a porous gluten "gel" macrostructure for an extended period of time in a centrifugal field without collapse. Consequently, separation of the A-starch granules from the protein-rich phases occurs more efficiently. The more starch that is removed in the first centrifugation step, the easier it should be to isolate and purify the gluten fraction.

2. Wet-Milling of Coarsely Ground Wheat Flour

In industrial wet-processing of wheat flour, a short-flow dry-milling process to produce coarsely ground flour may be desirable to reduce mechanical damage to starch granules and reduce milling costs (Maningat and Bassi 1999). A flour sample produced by a short-flow dry-milling process (flour yield of 78.0% as compared to 63.5% for the RBS-98 flour) with coarse particles (Fig. 24) was wet-processed by the HS-FWD wet-milling test. The coarsely ground flour sample had a high level of bran contamination as indicated by its high levels of crude fiber (0.6%), ash (0.89%), and crude fat (1.4%) contents (db). Its damaged starch level was 6.8% (dry-flour basis), which was slightly higher compared to the laboratory-milled straight-grade RBS-98 flour with 5.4% damaged starch.

The coarsely ground flour sample gave a mixogram comparable to that of the control (Fig. 25). When the highly sheared dispersion of the coarse-ground flour was centrifuged, a thick layer of fiber/bran fraction was observed between the dense starch and the light protein-rich phases (Fig. 26). A reduced volume of starch was separated from the dispersion as compared to the control flour.

The coarse-ground flour gave significantly lower recoveries of flour protein in the gluten fraction and of starch in the A-starch fraction (Table X). Also, the purities of those fractions were significantly reduced. Because of the higher fiber/bran content in the coarse-ground flour, the fiber fraction from the coarse-ground flour was six times higher than from the control flour. Total recoveries of the flour solids in the five fractions were similar.

The reduction in recovery of flour protein from the coarsely ground flour may be caused at least in part by its relatively high content of nongluten proteins. As the flour extraction rate from wheat increases, proteins from the aleurone and bran layers of the kernels, which are nongluten proteins lacking the unique aggregation traits of gluten proteins, are counted as protein in the flour. Most of those proteins are probably water-soluble and in the wet-process they may become part of the water-solubles/particulates fraction rather than gluten fraction. Another possible explanation is that the high-shear mixing step was inadequate to completely disaggregate the large flour particles into free starch granules and gluten threads.

The reduction in the recovery of the A-starch fraction from the coarse flour was attributed to its high level of bran fiber. Bran particles were observed to be swollen and bulky in the HS-FWD, and the swollen bran particles likely interfered with the sedimentation of the starch granules during centrifugal fractionation. Some of the bran particles were large and at least one-half were collected on the 425-μm openings during wet-sieving of the first-sedimented A-starch. Also, some of the bran remained adhered to the protein-rich phase reducing protein purity. In an industrial HS-FWD process done on coarsely ground flour, the flour-water slurry should be sieved prior to high-shear mixing of that slurry, which was done in the pilot-scale HD wet-milling process of whole-wheat flour (Meuser 1994).

3. Wet-Milling of Soft Wheat Flour

A soft red winter wheat flour sample with 12.2% protein (14% mb) was wet-milled by the designed HS-FWD test. The mixogram (Fig. 27) of the soft wheat flour shows a mellow gluten strength in dough-mixing, typical of soft wheat flours. When the HS-FWD of the soft wheat flour (protein content: 12.2%, 14% mb) was centrifugally fractionated, separation of the phases was comparable to the control flour (protein content: 11.8%, 14% mb) (Fig. 28). The soft wheat flour gave similar wet-milling results to the control flour (Table XI), except for the reduced purity of the gluten fraction. The gluten fraction was probably contaminated with starch and insoluble pentosans, as evidenced by the reduced recoveries of the starch and water-solubles/particulates fractions. The wet gluten of the soft wheat was observed to stick to the Glutomatic sieve during washing. Instead of using water, sodium chloride solution (0.1M) was used in the Glutomatic washing of the protein-rich phase, yet the purity of the gluten fraction from the soft wheat flour did not change noticeably. Perhaps 0.1M sodium chloride should be used in the high-shear mixing step as well.

4. Wet-Milling of Waxy Wheat Flours

Over a three-year period, 1999 to 2001, fifteen waxy wheat lines with different endosperm-hardness indices were obtained from five different sources and the wet-milling properties of their flours were studied by the Martin and the rapidly mixed dough-water dispersion methods (A. Sayaslan, O.K. Chung, and P.A. Seib, *unpublished*). With the exception of two experimental lines, all other waxy wheat flours failed in the Martin process because of their weak gluten characteristics. Two waxy wheat lines gave flour-water doughs with relatively strong elastic properties. Thus the dough balls did not fall apart during the initial stage of dough-kneading in the Martin process. In contrast to the Martin process, all the waxy wheat flours could be wet-processed by the rapidly mixed dough-water dispersion method *(data not given)*. Upon centrifugation of the dough dispersion and sedimenting of approximately one-half of the starch, the proteins become concentrated in the protein-rich phase. Apparently, the elevated concentration of those proteins was sufficient to cause the gluten proteins to aggregate into particles of sufficient size by sieving.

Using the newly designed HS-FWD wet-milling test, a sample of Leona waxy wheat variety and two advanced waxy wheat lines, namely winter waxy and spring waxy wheat lines, were first dry-milled and then wet-milled. All three waxy wheats had medium-hardness endosperms (hardness indices of 42 to 49) as determined by the single kernel characterization system. The waxy wheats gave an average of 66.0% flour yield off the experimental Buhler mill as opposed to 69.6% from four hard winter wheats (Karl 92, Jagger, 2137, and TAM 110). Flaking of middlings has been reported when dry-milling waxy wheats (E. Souza 2001 – *personal communication*). The amylose contents of the starches were isolated from Leona, winter waxy, and spring waxy wheats were 6.7, 0.6, and 4.7%, respectively.

When the three waxy wheat flours and water were combined at a W/F ratio of 1.7, a level that was found suitable for flours from the RBS-98 and other hard and soft wheats (Fig. 16), the waxy wheat flours absorbed the water rapidly and formed a stiff batter. At this W/F ratio it was impossible to process the waxy wheat flours with the homogenizer to form a highly sheared flour-water dispersion. Abdel-Aal et al (2002) also reported elevated water absorption for a spring waxy wheat flour. Therefore, the W/F ratio was increased to 2.0 to give a HS-FWD with proper consistency as determined by the appearance and behavior of the batter during homogenization. The increased water absorption was thought at first to be caused by the waxy starch that might absorb more water. However, the isolated waxy and nonwaxy starches were found to absorb similar amounts of water (1.05 to 1.08 g water / g dry starch) at 35°C in a slurry and centrifugation test. Other flour components, such as pentosans, may be responsible for the increased water absorption in waxy wheats. It was previously found that waxy wheat flours contained an average of 20-40% more pentosans and 10-20% more crude fat while having 3-7% less starch than a wild-type wheat (Karl 92) flour and a partial waxy wheat (Ike) flour (A. Sayaslan, O.K. Chung, and P.A. Seib, *unpublished*). Pentosan contents of the waxy wheat flours used in this study were not determined, but they contained an average of 71.4% (db) starch in their flours compared to the average of 76.2% (db) for four hard wheat flours. The crude fat contents (1.5-1.7%, db) of Leona and spring waxy wheat flours were comparable to those (1.0-1.7%) of the wild-type and partial waxy wheat flours. However, the winter waxy wheat flour contained 2.4% crude fat. Also, crude fiber contents of the waxy wheat flours were slightly higher (0.34-0.36%, db) as compared to the nonwaxy wheat flours (0.20-0.27%). The lower percentage of starch in waxy wheat endosperm might be accounted for by an increase in pentosans.

The mixograms of the three waxy wheat flours are shown in Fig. 29. The winter waxy wheat flour gave a mixogram comparable to that of RBS-98 flour; however, the other two waxy wheat flours had very short mix times and poor mixing tolerances. The winter waxy wheat flour was in fact one of the two waxy lines among the fifteen waxy wheats that could be wet-processed by the Martin process.

When the HS-FWD using a W/F ratio of 2.0 were fractionated by centrifugation (Fig. 30), Leona waxy wheat variety gave the highest visible proportions of the sedimented starch and protein-rich phases, followed by winter waxy and spring waxy flours. Yet, the separation of the starch and protein-rich phases from the dispersion of Leona was not as good as with the control flour, probably because its weak gluten "gel" collapsed earlier during centrifugation than the control flour.

The wet-milling data for the waxy wheat flours are compared to the control flour in Table XII. Recoveries of protein from the waxy flours were lower than the control except for the spring waxy line. The purities of their gluten fractions also differed. The Glutomatic washing of the protein-rich phases from the HS-FWD of Leona and spring waxy flours were difficult because they were sticky and blinded the Glutomatic sieve, leading to reduced protein contents (67-71%) in their gluten fractions. On the other hand, the wet-gluten from the winter waxy flour was of medium cohesiveness during the Glutomatic washing, did not blind the sieve during washing, and gave a gluten fraction with a protein content of about 85%.

All waxy flours gave much lower recoveries (12-33% less) of the A-starch fraction, but their purities were comparable to the control flour (Table XII). Also different were the levels of solids recovered in the fiber (1-2% more) and water-solubles/particulates fractions (4% more) of the waxy flours, both of which were elevated compared to the RBS-98 flour. Recovery of total solids in the five wet-milling fractions of the waxy wheat flours did not differ significantly.

CONCLUSIONS

1. A bench-scale wet-milling test was devised to mimic the industrial wet-milling of wheat flours starting with a highly sheared flour-water dispersion (HS-FWD). The test is conducted on 65 g (db) of flour and five fractions are isolated, the gluten, A-starch, B-starch, fiber, and water-solubles/particulates. A homogenizer, vibrating wet-sieve, centrifuge, and gluten-washing instrument are required for the test.

2. The HS-FWD wet-milling test was optimized by the RSM, and indicates that three steps in the process are critical to the recoveries and purities of the gluten and starch fractions, the high-shear mixing step, the gluten-aging step, and the gluten-washing step.

3. The bench-scale HS-FWD test compared to the dough-washing (Martin) test indicates that weak flours can be wet-processed by the HS-FWD process but not by the Martin process. Strong-mixing flours are fractionated more readily by both processes, but the dough-washing process gives higher recoveries of the gluten and starch fractions and concomitant lower levels of water-solubles/particulates.

4. The HS-FWD wet-milling test can distinguish the wet-milling qualities of flours from hard and soft wheats, and waxy wheats, all having varying dough-mixing characteristics, and more importantly indicate when adjustments may be needed in a high-shear wet-process for wheat flour.

FUTURE WORK

The following work on the wet-milling test for the HS-FWD process is suggested.

1. Devise different gluten washing protocols depending on the quantity of the protein-rich phase separated by centrifugation and on the consistency (slack or highly elastic) of the gluten proteins during the purification step. One possibility is to use mild stirring with a cutter-blade to release the starch from the protein-rich phase

2. Extend the processing of the B-starch fraction to recover more starch with protein content of 0.20-0.30%.

3. Test additional samples of flour milled from hard and soft wheats. It is assumed that soft wheat flours with strong protein would be well suited for wet-processing.

4. Test the use of additives in the wet-milling test, such as pentosanases, sodium chloride, and ferulic acid, to facilitate the wet-fractionation of flours with poor wet-processing quality.

5. Investigate the effects of wheat kernel hardness and flour particle size on the wet-milling properties of wheat flours by a high-shear wet-process.

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Table I. Response Surface Methodology (RSM) to Model Temperature of Highly Sheared Flour-Water Batter, Recovery of Starch in A-Starch Fraction, and Recovery of Protein in Gluten Fraction with Three Variables in the High-Shear Mixing Step of the Highly Sheared Flour-Water Dispersion (HS-FWD) Wet-Milling Test

RUN #	RSM EXPERIMENTAL DESIGN			EXPERIMENTAL AND PREDICTED RESPONSES					
	Water-Flour (W/F) Ratio[a]	Homogenizer Speed (rpm)	Water Temp. (°C)	Highly Sheared Flour-Water Batter Temperature (°C)		Starch Recovery in A-Starch Fraction (% of flour starch)		Protein Recovery in Gluten Fraction (% of flour protein)	
				Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	1.4	7500	20	36.0	36.0	76.4	77.2	65.5	66.9
2	1.7	2500	20	27.5	27.5	68.8	70.1	66.7	66.7
3	2.0	5000	20	28.5	29.5	80.3	78.6	63.8	65.2
4	1.7	5000	27	32.7	33.1	76.5	79.6	65.2	66.3
5	1.7	5000	27	33.5	33.1	79.2	79.6	66.1	66.3
6	1.7	5000	27	34.0	33.1	81.1	79.6	67.5	66.3
7	2.0	7500	27	39.8	39.1	83.9	84.1	66.1	65.3
8	1.4	5000	34	38.0	38.3	73.1	72.6	69.5	69.6
9	1.7	7500	34	40.5	39.7	80.8	80.7	65.9	67.2
10	2.0	2500	34	34.0	34.4	60.4	56.4	69.4	68.4
11	1.4	7500	20	35.9	36.0	78.0	77.2	68.3	66.9
12	1.7	2500	20	27.5	27.5	71.4	70.1	66.6	66.7
13	2.0	5000	20	30.5	29.5	76.9	78.6	66.6	65.2
14	1.7	5000	27	32.5	33.1	80.0	79.6	66.1	66.3
15	1.7	5000	27	32.5	33.1	80.9	79.6	66.1	66.3
16	1.7	5000	27	33.2	33.1	80.0	79.6	66.7	66.3
17	2.0	7500	27	38.4	39.1	84.2	84.1	64.4	65.3
18	1.4	5000	34	38.5	38.3	72.1	72.6	69.7	69.6
19	1.7	7500	34	38.8	39.7	80.5	80.7	68.4	67.2
20	2.0	2500	34	34.8	34.4	52.3	56.4	67.3	68.4

[a] W/F ratio based on dry weight of flour, and batter solids varied from 33% (W/F: 2.0) to 42% (W/F:1.4).

Table II. Response Surface Methodology (RSM) to Model Protein Recovery upon Aging of Protein-Rich Phase in the Highly Sheared Flour-Water Dispersion (HS-FWD) Wet-Milling Test

RUN #	RSM EXPERIMENTAL DESIGN			EXPERIMENTAL AND PREDICTED RESPONSES	
	Aging Time (min)	Aging Temperature (°C)		Protein Recovery in Gluten Fraction (% of flour protein)	
				Experimental	Predicted
1	1	25		73.2	73.2
2	1	45		73.2	73.3
3	25	35		75.1	74.9
4	25	35		74.7	74.9
5	25	35		74.9	74.9
6	45	25		72.4	72.5
7	45	45		73.4	73.1
8	1	25		73.1	73.2
9	1	45		73.4	73.3
10	25	35		74.8	74.9
11	25	35		75.0	74.9
12	25	35		74.7	74.9
13	45	25		72.5	72.5
14	45	45		72.7	73.1

Table III. Regression Equations for Modeling of Temperature of Highly Sheared Flour-Water Batter, Recovery of Starch in A-Starch Fraction, and Recovery of Protein in Gluten Fraction When Varying Three Parameters in the High-Shear Mixing Step of the Highly Sheared Flour-Water Dispersion (HS-FWD) Wet-Milling Test

RESPONSE	RESPONSE SURFACE REGRESSION EQUATION[a]	COEFFICIENT OF DETERMINATION (R^2)	STANDARD ERROR OF ESTIMATE
	High-Shear Mixing Step (No Gluten Aging)		
Temperature of Highly Sheared Flour-Water Batter (°C)	$-31.46 + 14.86A - 0.0029B + 3.957C + 0.00070AB - 0.93AC - 0.00011BC + 0.85A^2 + 0.0000006B^2 - 0.025C^2$	0.98	0.82
Starch Recovery in A-Starch Fraction (% of flour starch)	$-30.88 + 46.89A + 0.0029B + 4.43C + 0.00017AB - 0.56AC + 0.00019BC - 9.33A^2 - 0.0000005B^2 - 0.089C^2$	0.95	2.41
Protein Recovery in Gluten Fraction (% of flour protein)	$61.90 - 9.90A - 0.00021B + 1.14C - 0.00089AB - 0.39AC - 0.000009BC + 6.18A^2 + 0.0000002B^2 - 0.0052C^2$	0.65	1.33

[a]The parameters were: A = water-flour (db) ratio, B = homogenizer speed (rpm), and C = water temperature (°C).

Table IV. Repeatability of the Highly Sheared Flour-Water Dispersion (HS-FWD) Wet-Milling Test at Optimized Conditions for the High-Shear Mixing Step and the Protein-Rich Phase Aging Step[a]

EXPERIMENTAL RUN	GLUTEN FRACTION		A-STARCH FRACTION		B-STARCH FRACTION	
Day	Protein Recovery (% of flour protein)	Protein Content (%, Nx5.7)	Starch Recovery (% of flour starch)	Protein Content (%, Nx5.7)	Starch Recovery (% of flour starch)	Protein Content (%, Nx5.7)
1	78.1	83.0	79.2	0.25	12.6	4.2
2	79.5	82.4	81.7	0.26	11.0	4.4
3	78.9	83.8	81.2	0.24	10.6	4.9
4	79.4	82.4	78.5	0.23	12.1	3.9
5	78.1	82.7	80.9	0.23	11.4	5.4
Mean	***78.8***	***82.9***	***80.3***	***0.24***	***11.5***	***4.6***
Standard Deviation	***0.7***	***0.6***	***1.4***	***0.02***	***0.8***	***0.6***
Coefficient of Variation (CV, %) (Relative Std. Dev.)	***0.9***	***0.7***	***1.7***	***6.4***	***6.7***	***12.1***

[a]All percentages on a dry-solids basis.

Table V. Recovery of Flour Solids in Five Fractions from the RBS-98 Flour by the Highly Sheared Flour-Water Dispersion (HS-FWD), Rapidly Mixed Dough-Water Dispersion, and Martin Wet-Milling Methods, All Done on 65 g (db) of RBS-98 Flour[a]

WET-MILLING METHOD	GLUTEN FRACTION (% of flour solids)	A-STARCH FRACTION (% of flour solids)	B-STARCH FRACTION (% of flour solids)	FIBER FRACTION (% of flour solids)	WATER-SOLUBLES + PARTICULATES FRACTION (% of flour solids)	TOTAL (% of flour solids)
HS-FWD	12.9b	63.1b	13.2b	0.9b	8.7a	98.8a
Rapidly Mixed Dough-Water Dispersion	13.1b	56.4c	20.6a	0.9b	7.2b	98.3a
MARTIN	14.3a	70.3a	6.4c	1.2a	6.2c	98.4a

[a]All percentages on a dry-solids basis, and different letters in the same column indicate significant difference ($P<0.05$).

Table VI. Comparison of Optimized Highly Sheared Flour-Water Dispersion (HS-FWD) Wet-Milling Test with Rapidly Mixed Dough-Water Dispersion and Martin Wet-Milling Methods, All Done on 65 g (db) of RBS-98 Flour[a]

WET-MILLING METHOD	GLUTEN FRACTION			A-STARCH FRACTION			B-STARCH FRACTION		
	Protein Recovery (% of flour protein)	Protein Content (%, Nx5.7)	Starch Content (%)	Starch Recovery (% of flour starch)	Starch[b] Content (%)	Protein Content (%, Nx5.7)	Starch Recovery (% of flour)	Starch Content (%)	Protein Content (% of flour protein)
HS-FWD	78.8b	82.9a	11.0b	80.3b	95.8a	0.24a	11.5b	87.6a	4.6a
Rapidly Mixed Dough-Water Dispersion	78.0b	83.5a	15.5a	71.8c	94.3a	0.20b	18.0a	87.3a	1.6c
MARTIN	86.7a	84.4a	7.1c	89.4a	94.2a	0.20b	5.2c	80.9b	2.0b

[a]All percentages on a dry-solids basis, and different letters in the same column indicate significant difference ($P<0.05$). [b]Starch content of a commercial wheat starch sample was determined to be 96.1% (db).

Table VII. Distribution of Starch and Protein of RBS-98 Flour in the Five Wet-Milling Fractions of the Highly Sheared Flour-Water Dispersion (HS-FWD), Rapidly Mixed Dough-Water Dispersion, and Martin Wet-Milling Methods[a]

WET-MILLING METHOD	COMPONENT	GLUTEN FRACTION (%)	A-STARCH FRACTION (%)	B-STARCH FRACTION (%)	FIBER[b] FRACTION (%)	WATER-[b] SOLUBLES + PARTICULATES FRACTION (%)	TOTAL RECOVERY (%)
HS-FWD	*Protein*	78.8	0.2	4.3	nd	14.8	98.1
Rapidly Mixed Dough-Water Dispersion		78.0	0.2	2.4	nd	14.6	95.2
MARTIN		86.7	0.2	0.9	nd	9.5	97.3
HS-FWD	*Starch*	1.4	80.3	11.6	nd	nd	93.3
Rapidly Mixed Dough-Water Dispersion		2.0	71.8	18.0	nd	nd	91.8
MARTIN		1.0	89.4	5.2	nd	nd	95.6

[a]All percentages on a dry-solids basis, [b]nd = Not determined.

Table VIII. Breadmaking Qualities of RBS-98 Flour and Blends Containing Glutens Isolated from RBS-98 Flour by the Highly Sheared Flour-Water Dispersion (HS-FWD), Rapidly Mixed Dough-Water Dispersion, and Martin Wet-Milling Methods

FLOUR OR BLEND[a]	FLOUR OR BLEND PROTEIN CONTENT (%, Nx5.7, 14% mb)	LOAF VOLUME (cc)	LOAF[b] VOLUME AFTER CORRECTING FOR PROTEIN CONTENT (cc / g protein in flour or blend)	CRUMB[b] GRAIN SCORE (0 → 6)
RBS-98 Flour	11.8	885	74.9**a**	5.3**a**
HS-FWD	11.4	815	70.2**a**	3.8**c**
Rapidly Mixed Dough-Water Dispersion	11.1	753	67.8**a**	3.8**c**
MARTIN	11.7	800	69.7**a**	4.8**b**

[a]Blends consisted of ~46/46/8 weight ratio of RBS-98 flour, commercial wheat starch, and test gluten.
[b]Different letters in the same column indicate significant difference ($P<0.05$).

Table IX. Wet-Milling of Control and Hard Wheat Flours with Different Dough-Mixing Properties by the Highly Sheared Flour-Water Dispersion (HS-FWD) Wet-Milling Test[a]

FLOUR	GLUTEN FRACTION		A-STARCH FRACTION		B-STARCH FRACTION		FIBER FRACTION	WATER-SOLUBLES + PARTICULATES FRACTION	TOTAL FLOUR SOLIDS RECOVERY IN FIVE FRACTIONS
	Protein Recovery (% of flour protein)	Protein Content (%, Nx5.7)	Starch Recovery (% of flour starch)	Protein Content (%, Nx5.7)	Fraction Recovery (% of flour)	Protein Content (%, Nx5.7)	Dry Solids Recovery (% of flour)	Dry Solids Recovery (% of flour)	(% of flour)
RBS-98 (CONTROL)	78.8c	82.9b	80.3bc	0.24a	13.2c	4.6abc	0.9c	8.7bc	98.8a
KARL 92	78.8c	86.9ab	82.5b	0.22a	11.8c	4.3bcd	0.7d	9.7ab	100.3a
JAGGER	82.2a	73.0c	76.8c	0.23a	13.9bc	4.7ab	0.7d	10.2ab	100.1a
CHISHOLM	71.8d	89.8a	89.0a	0.24a	12.6c	6.3a	1.4a	7.3c	99.6a
2137	81.3ab	70.0c	64.2d	0.23a	23.4a	2.6d	0.9c	8.7bc	98.5a
TAM 110	79.2bc	60.3d	66.6d	0.23a	18.1b	2.9cd	1.1b	10.5a	99.1a

[a]All percentages on a dry-solids basis, and different letters in the same column indicate significant difference ($P<0.05$).

Table X. Wet-Milling of Control and Coarsely Ground Wheat Flours by the Highly Sheared Flour-Water Dispersion (HS-FWD) Wet-Milling Test[a]

FLOUR	GLUTEN FRACTION		A-STARCH FRACTION		B-STARCH FRACTION		FIBER FRACTION	WATER-SOLUBLES + PARTICULATES FRACTION	TOTAL FLOUR SOLIDS RECOVERY IN FIVE FRACTIONS
	Protein Recovery (% of flour protein)	Protein Content (%, Nx5.7)	Starch Recovery (% of flour starch)	Protein Content (%, Nx5.7)	Fraction Recovery (% of flour)	Protein Content (%, Nx5.7)	Dry Solids Recovery (% of flour)	Dry Solids Recovery (% of flour)	(% of flour)
RBS-98 (CONTROL)	78.8a	82.9a	80.3a	0.24b	13.2b	4.6b	0.9b	8.7b	98.8a
COARSELY GROUND	61.0b	77.5b	74.9b	0.32a	17.7a	7.5a	5.4a	12.0a	99.6a

[a]All percentages on a dry-solids basis, and different letters in the same column indicate significant difference ($P<0.05$).

Table XI. Wet-Milling of Control and Soft Wheat Flours by the Highly Sheared Flour-Water Dispersion (HS-FWD) Wet-Milling Test[a]

FLOUR	GLUTEN FRACTION		A-STARCH FRACTION		B-STARCH FRACTION		FIBER FRACTION	WATER-SOLUBLES + PARTICULATES FRACTION	TOTAL FLOUR SOLIDS RECOVERY IN FIVE FRACTIONS
	Protein Recovery (% of flour protein)	Protein Content (%, Nx5.7)	Starch Recovery (% of flour starch)	Protein Content (%, Nx5.7)	Fraction Recovery (% of flour)	Protein Content (%, Nx5.7)	Dry Solids Recovery (% of flour)	Dry Solids Recovery (% of flour)	(% of flour)
RBS-98 (CONTROL)	78.8a	82.9a	80.3a	0.24a	13.2a	4.6a	0.9b	8.7a	98.8a
SOFT WHEAT	79.9a	65.3b	76.9a	0.27a	13.8a	3.2b	1.8a	5.9b	98.9a

[a]All percentages on a dry-solids basis, and different letters in the same column indicate significant difference ($P<0.05$).

Table XII. Wet-Milling of Control and Waxy Wheat Flours by the Highly Sheared Flour-Water Dispersion (HS-FWD) Wet-Milling Test[a]

FLOUR	GLUTEN FRACTION		A-STARCH FRACTION		B-STARCH FRACTION		FIBER FRACTION	WATER-SOLUBLES + PARTICULATES FRACTION	TOTAL FLOUR SOLIDS RECOVERY IN FIVE FRACTIONS
	Protein Recovery (% of flour protein)	Protein Content (%, Nx5.7)	Starch Recovery (% of flour starch)	Protein Content (%, Nx5.7)	Fraction Recovery (% of flour)	Protein Content (%, Nx5.7)	Dry Solids Recovery (% of flour)	Dry Solids Recovery (% of flour)	(% of flour)
RBS-98) (CONTROL)	78.8a	82.9a	80.3a	0.24a	13.2d	4.6a	0.9c	8.7b	98.8a
LEONA (WAXY)	75.5b	70.5b	56.5c	0.12b	28.5b	1.3c	1.8b	12.3a	98.2a
WINTER WAXY	73.3b	84.8a	68.4b	0.27a	22.7c	3.7b	3.0a	12.6a	99.1a
SPRING WAXY	79.5a	67.4c	47.4d	0.24a	32.3a	0.8d	1.7b	12.9a	99.2a

[a]All percentages on a dry-solids basis, and different letters in the same column indicate significant difference ($P<0.05$).

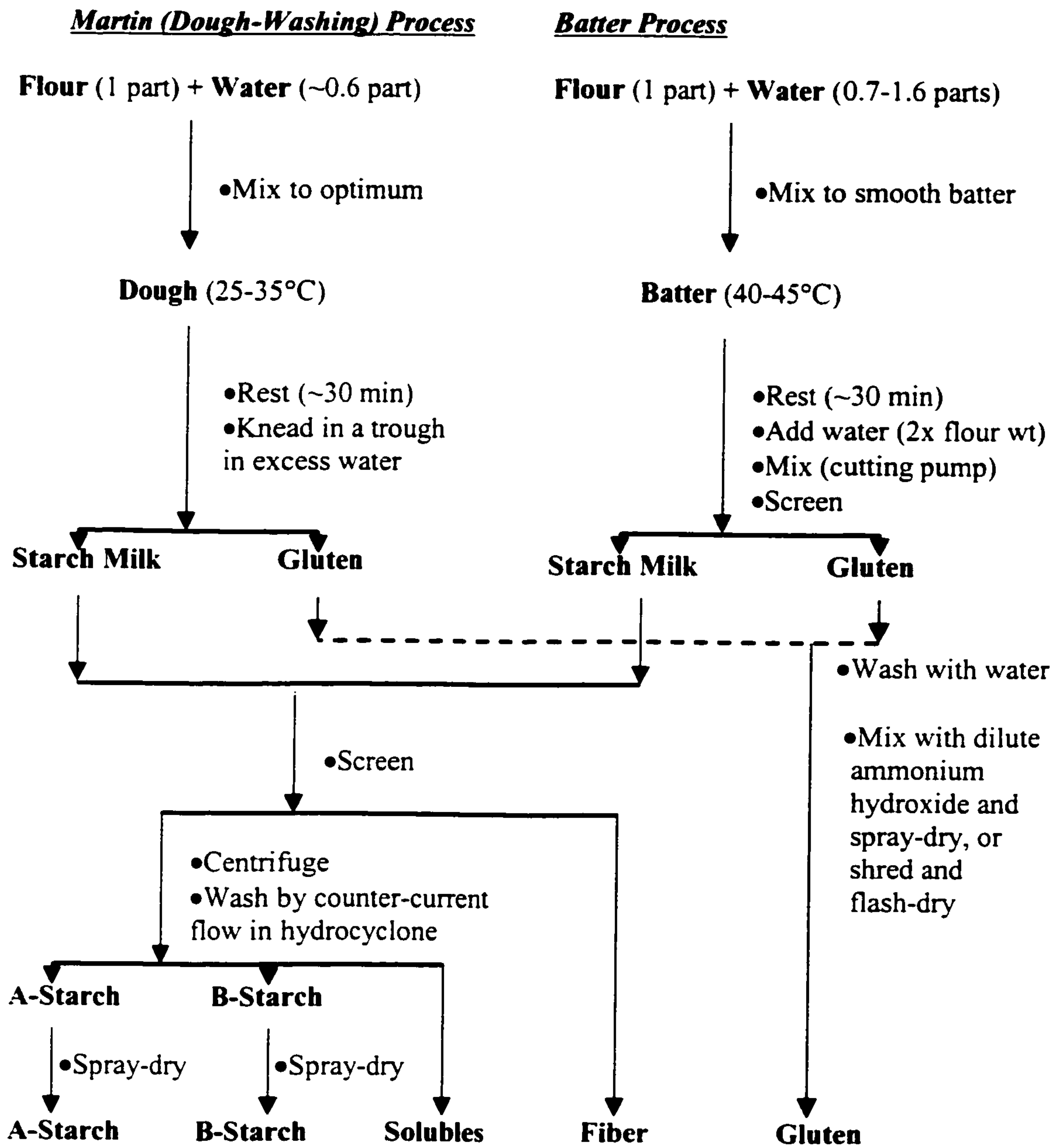


Fig. 1. Mixing and major separation steps in traditional wet-milling processes for wheat flour, i.e., the Martin and Batter processes.

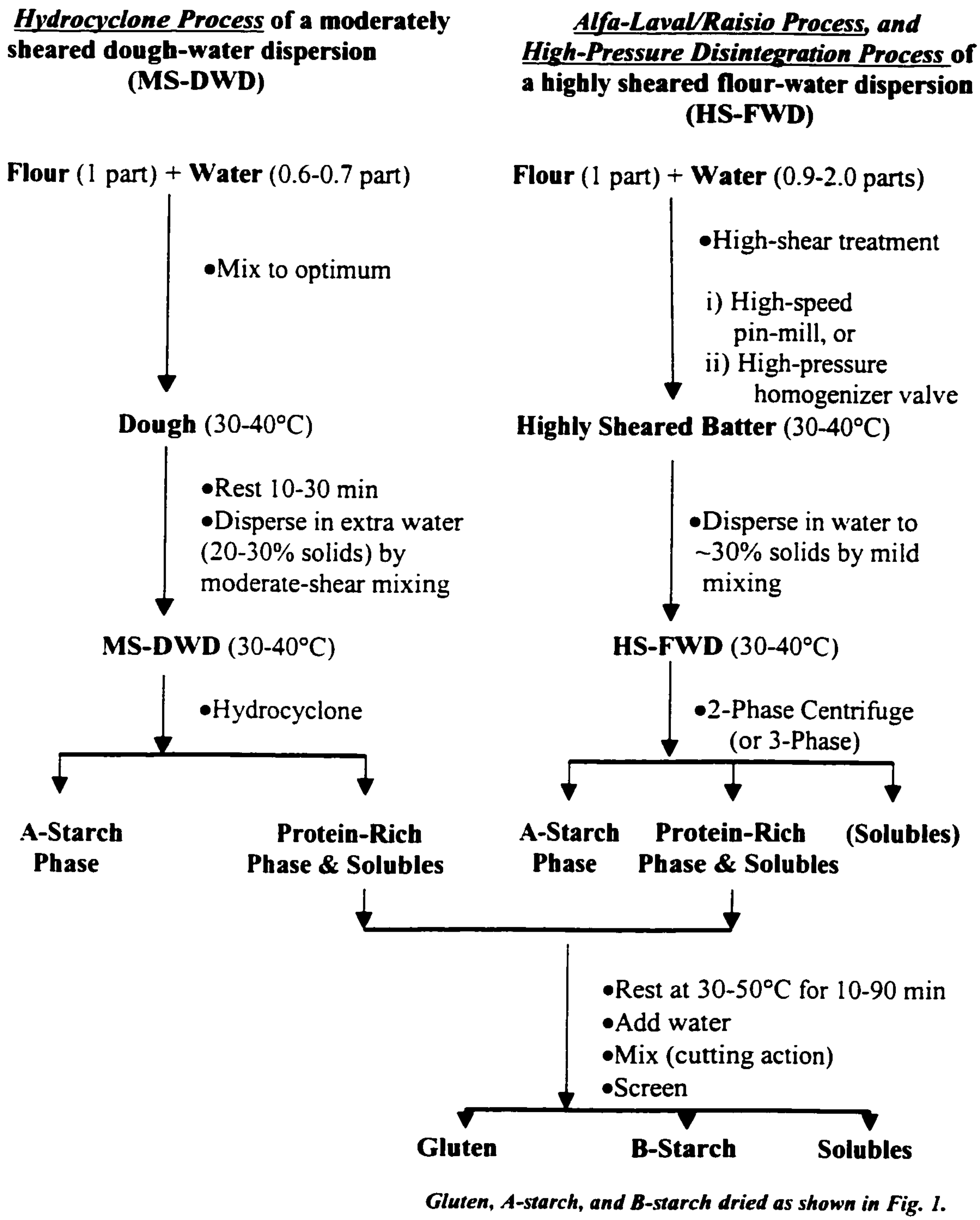


Fig. 2. Mixing and major separation steps in the modern-day Hydrocyclone process on a moderately sheared dough-water dispersion (MS-DWD), and in the Alfa-Laval/Raisio and High-Pressure Disintegration processes on a highly sheared flour-water dispersion (HS-FWD).

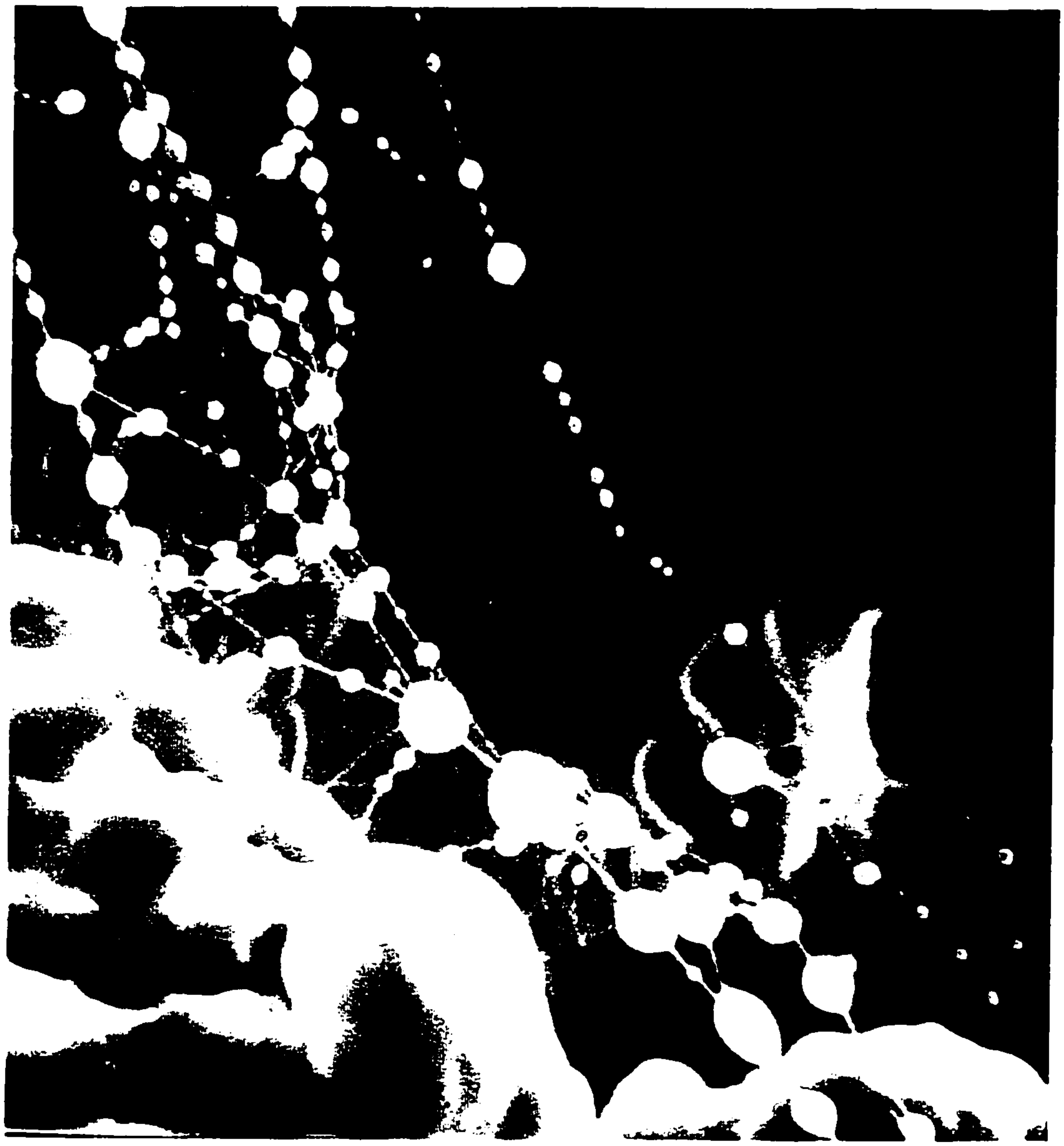


Fig. 3. Scanning electron micrograph of wheat flour-water interface of a flour particle wetted with water and freeze-dried.
Magnification ~1750X, (Source: C.R. Hoseney, *Unpublished*)

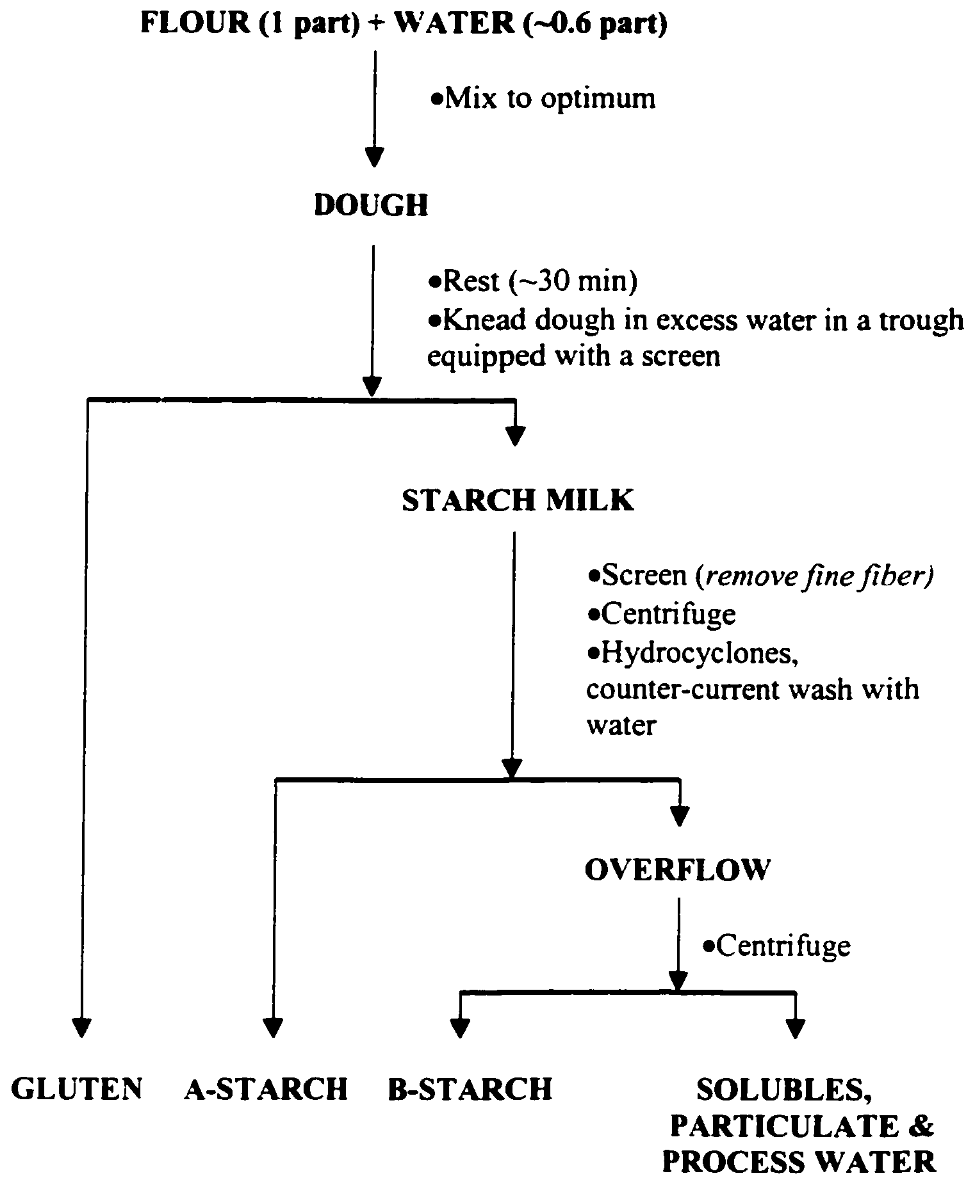


Fig. 4. Flow diagram for the Martin (dough-washing) process.
(Source: Seib 1994)

FLOUR (1 part) + WATER (0.7–1.6 parts, 50-55°C)

↓ •Mix to smooth batter

BATTER (40-45°C)

↓
•Rest (~30 min)
•Add water (2x flour weight)
•Mix (cutting pump) at warm temperature

GLUTEN CURDS & STARCH MILK

↓ •Screen

GLUTEN | **STARCH MILK**

↓

Refined as shown in Fig. 4.

Fig. 5. Flow diagram for the Batter process.
(Source: Seib 1994)

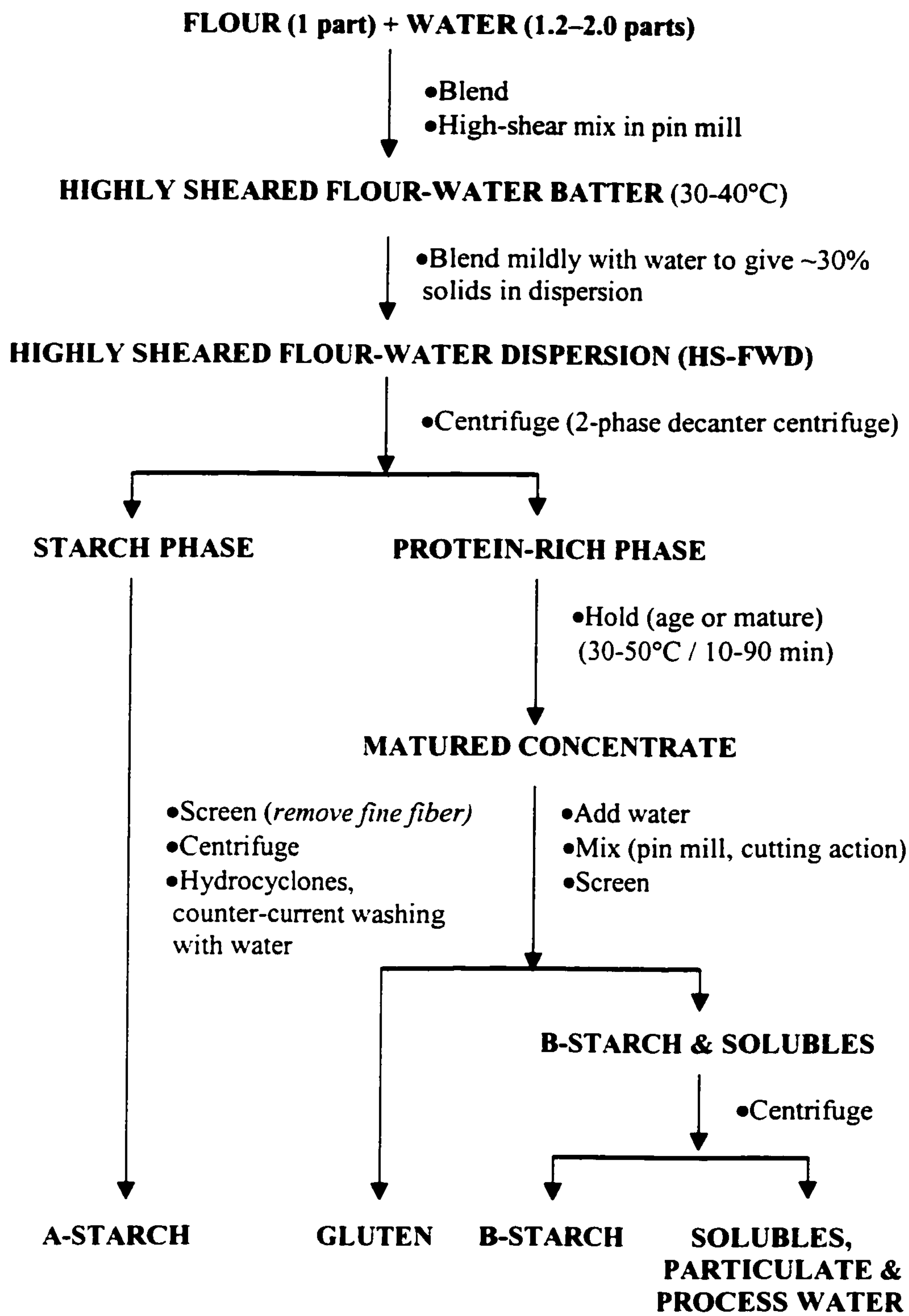


Fig. 6. Flow diagram for the Alfa-Laval/Raisio process on a highly sheared flour-water dispersion (HS-FWD).
(Source: Seib 1994)

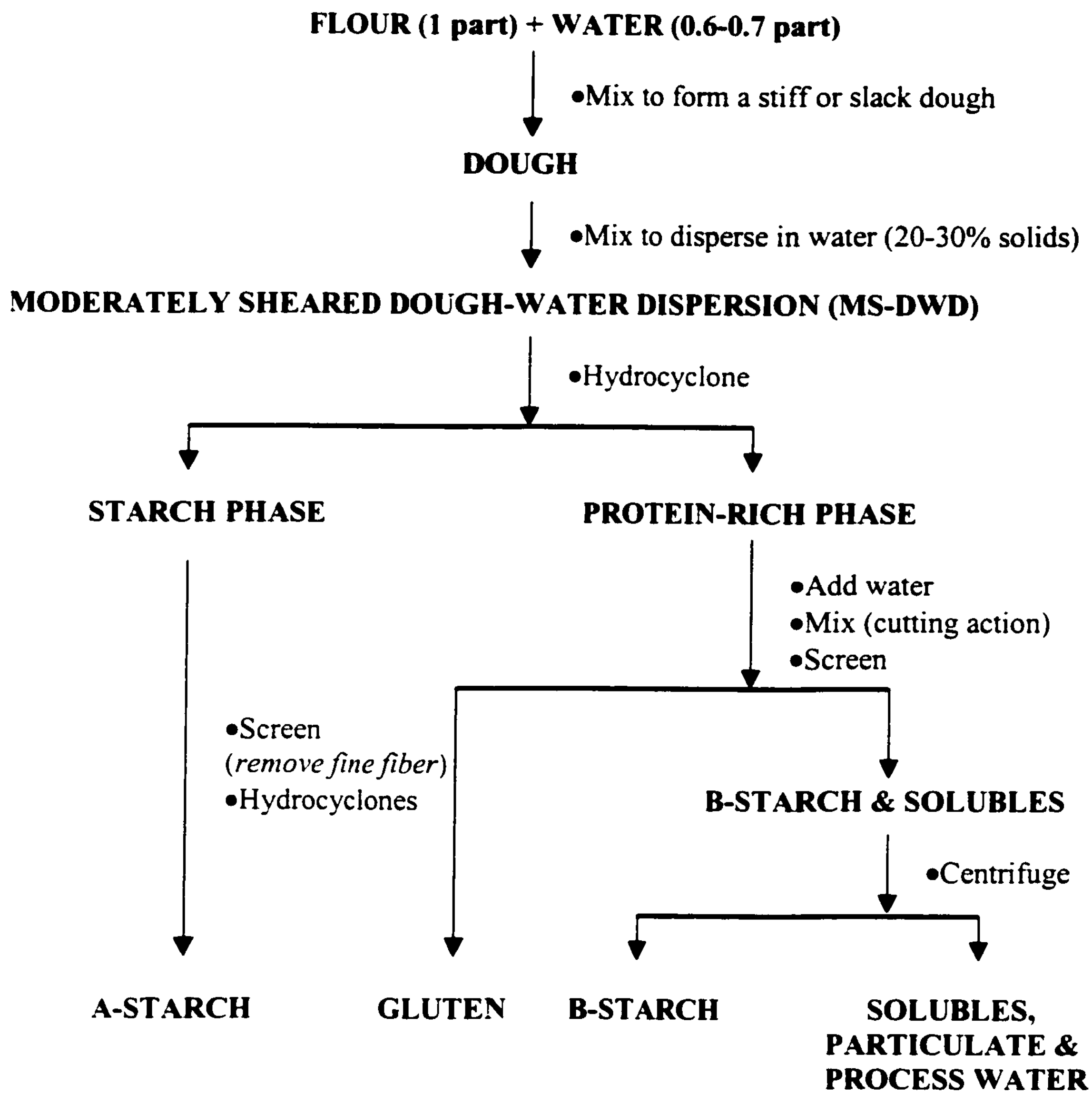


Fig. 7. Flow diagram for the Hydrocyclone process on a moderately sheared dough-water dispersion (MS-DWD).
(Source: P.A. Seib, *Unpublished*)

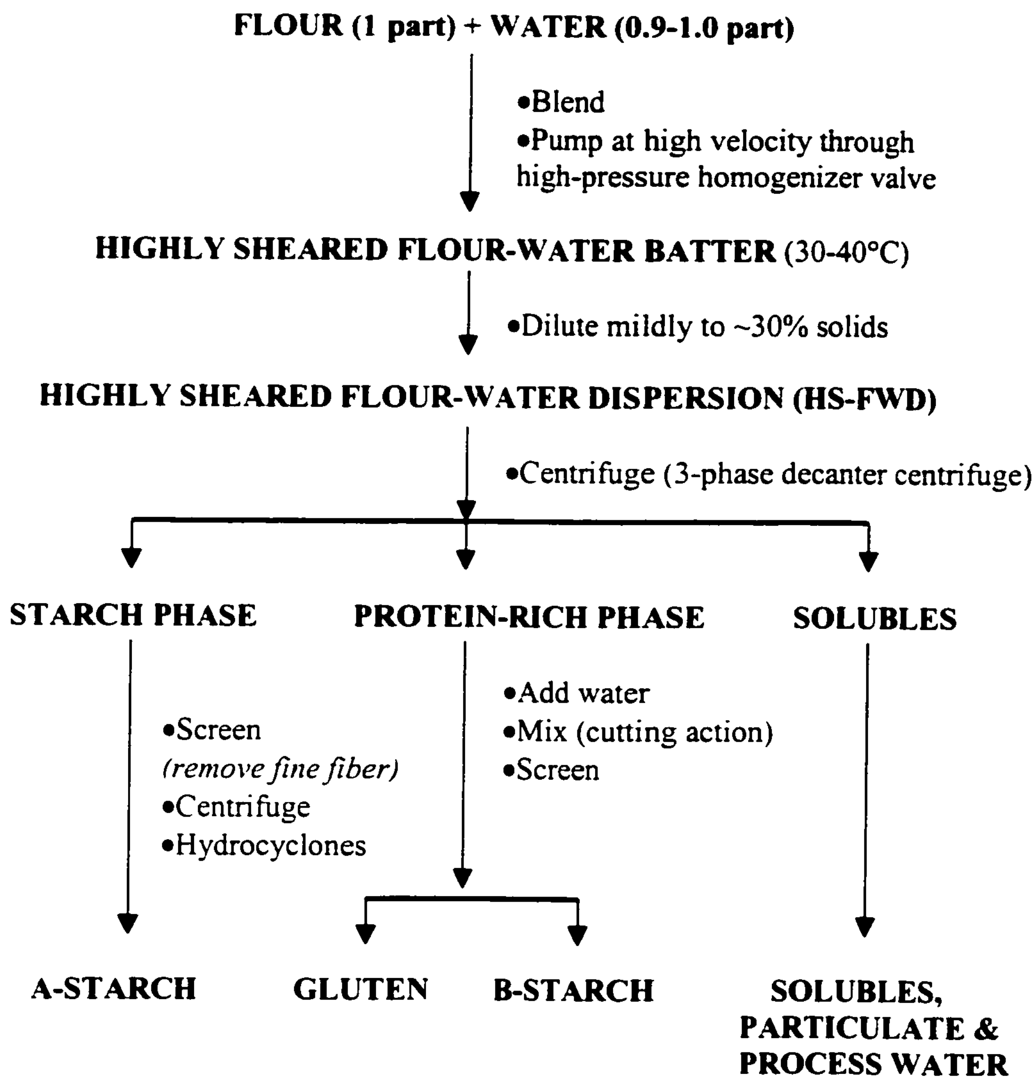
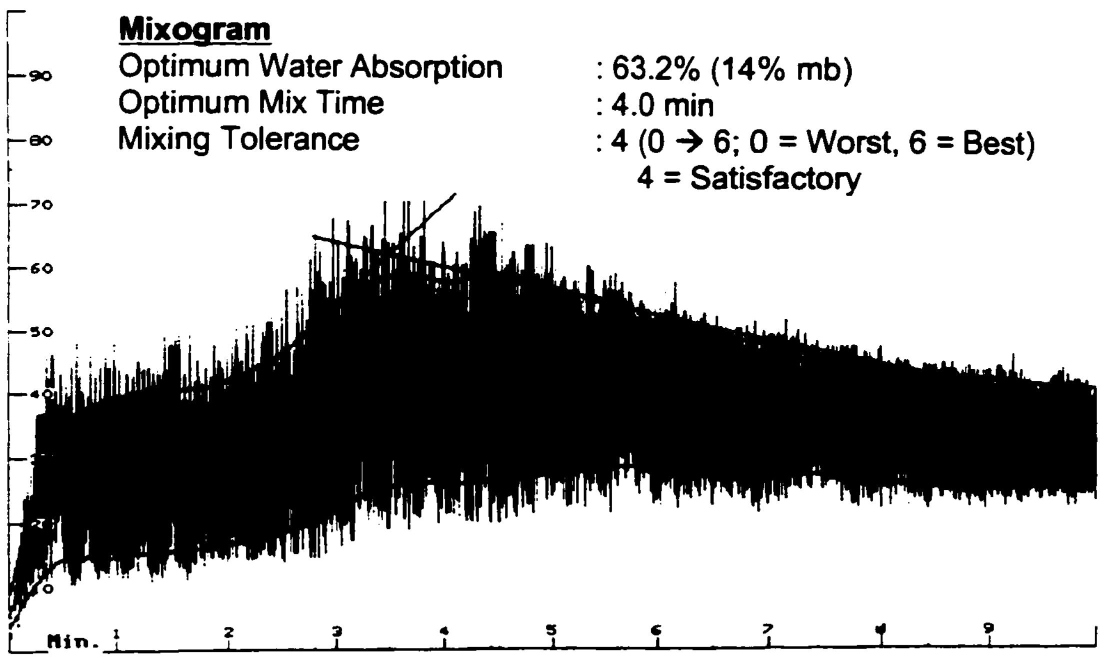


Fig. 8. Flow diagram for the High-Pressure Disintegration (HD) process on a highly sheared flour-water dispersion (HS-FWD).
(Source: Zwitserloot 1989a, Seib 1994, Witt 1997, Bergthaller et al 1998)

Mixogram

Optimum Water Absorption : 63.2% (14% mb)
Optimum Mix Time : 4.0 min
Mixing Tolerance : 4 (0 → 6; 0 = Worst, 6 = Best)
4 = Satisfactory



Breadmaking

Baking Quality : Good
Loaf Volume : 885 cc (Pup-Loaf with 100 g of Flour, 14% mb)
Crumb Grain Score : 4 (0 → 6; 0 = Worst, 6 = Best)
4 = Satisfactory

Fig. 9. Mixogram and breadmaking quality data of Regional Baking Standard (RBS-98) flour with 11.8% protein (14% mb), conducted at the USDA/ARS Hard Winter Wheat Quality Laboratory (HWWQL), Manhattan, KS.

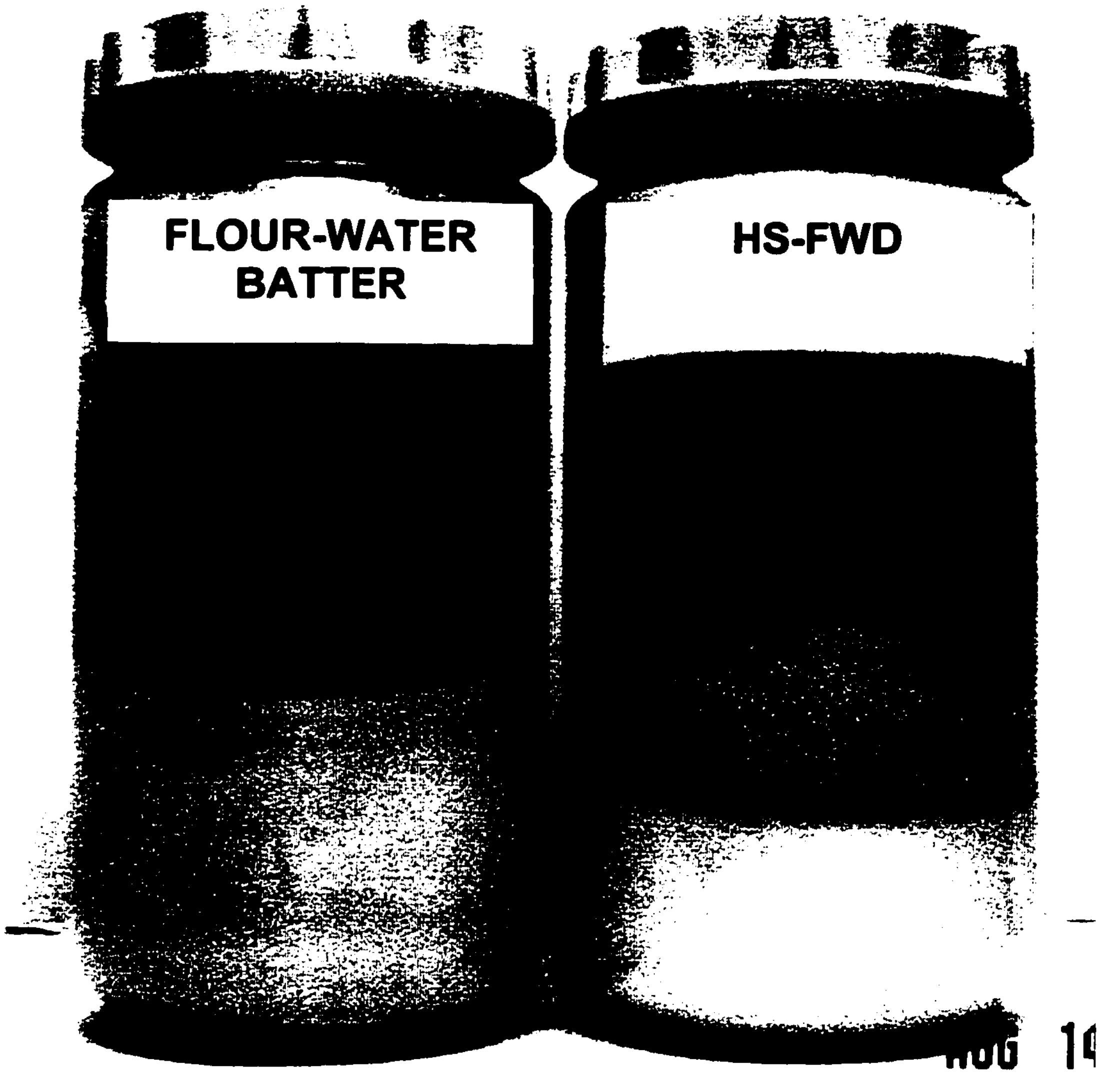


Fig. 10. Centrifugation (2,500 x *g* for 15 min) of a flour-water batter and a highly sheared flour-water dispersion (HS-FWD).

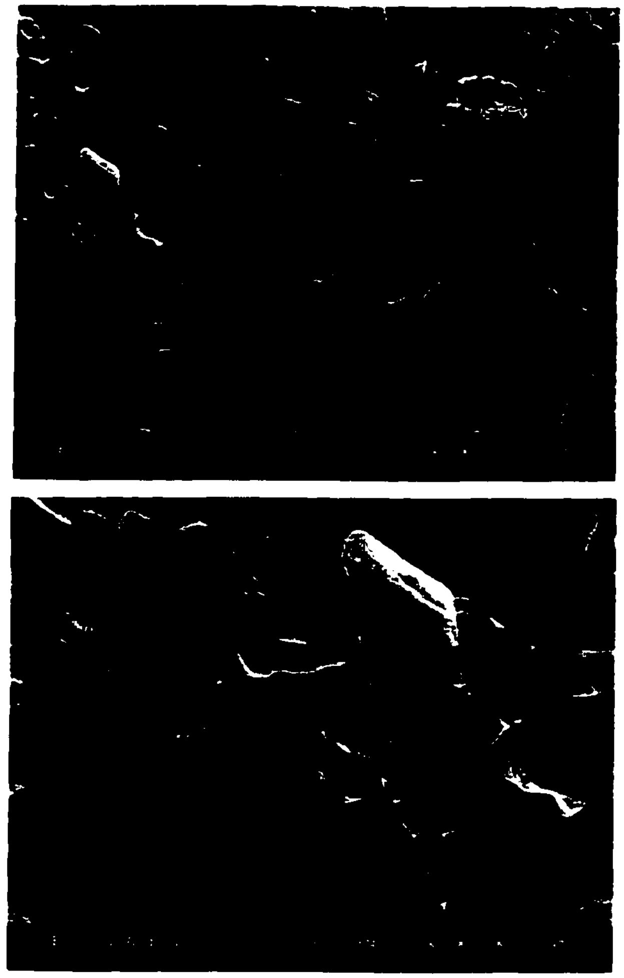


Fig. 11. Scanning electron micrograph of flour-water dough mixed to optimum and freeze-dried.

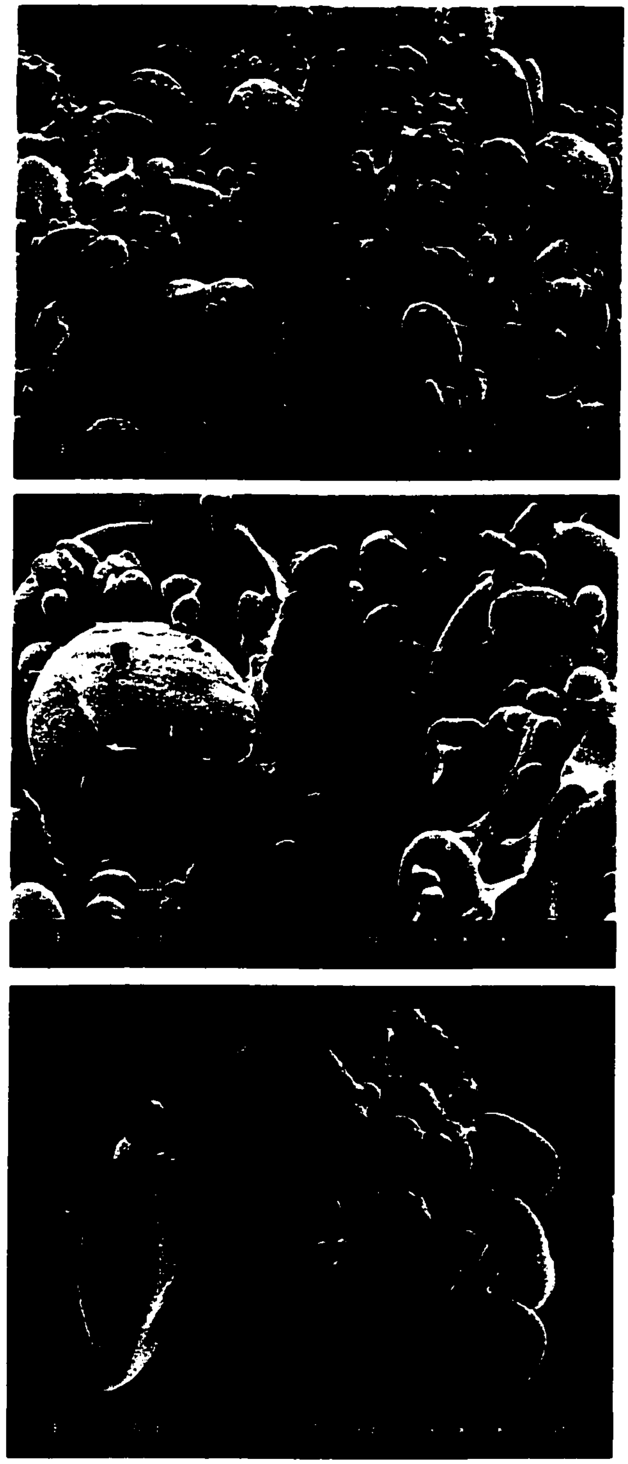


Fig. 12. Scanning electron micrograph of rapidly mixed dough-water dispersion at ~27% solids after freeze-drying.

Fig. 13. Scanning electron micrograph of highly sheared flour-water dispersion (HS-FWD) at ~27% solids after freeze-drying.

FLOUR (65.0 g, db) + **WATER** (100 mL, 35°C)

- Add flour to water (50 mL) in 250-mL wide-mouth centrifuge bottle and remaining water (50 mL)
- Blend 5-10 s with spatula

FLOUR-WATER SLURRY (~32°C)

- Shear mix with homogenizer (6,000 rpm / 2 min) head (37 mm in diameter) while moving centrifuge bottle vertically ~30 times / min
- Rinse homogenizer head with water stream (~20 mL at 25°C) and combine rinsing with homogenate
- Adjust solids concentration to ~27% by adding ~40 mL of water at 25°C
- Cap and mix bottle contents by inverting manually 10 times

HIGHLY SHEARED FLOUR-WATER DISPERSION (HS-FWD) (~36°C)

- Centrifuge in swinging bucket rotor at 2,500 x *g* for 15 min

BOTTOM **STARCH PHASE**	MIDDLE **PROTEIN-RICH PHASE**	TOP **WATER-SOLUBLES + PARTICULATES**
(~75 g, wb)	*(~60 g, wb)*	*(~100 mL)*
(~42 g, db)	*(~21 g, db)*	*(~2 g, db)*

(Continued on the next page)

Fig. 14. Flow diagram of the bench scale wet-milling test on a highly sheared flour-water dispersion (HS-FWD).

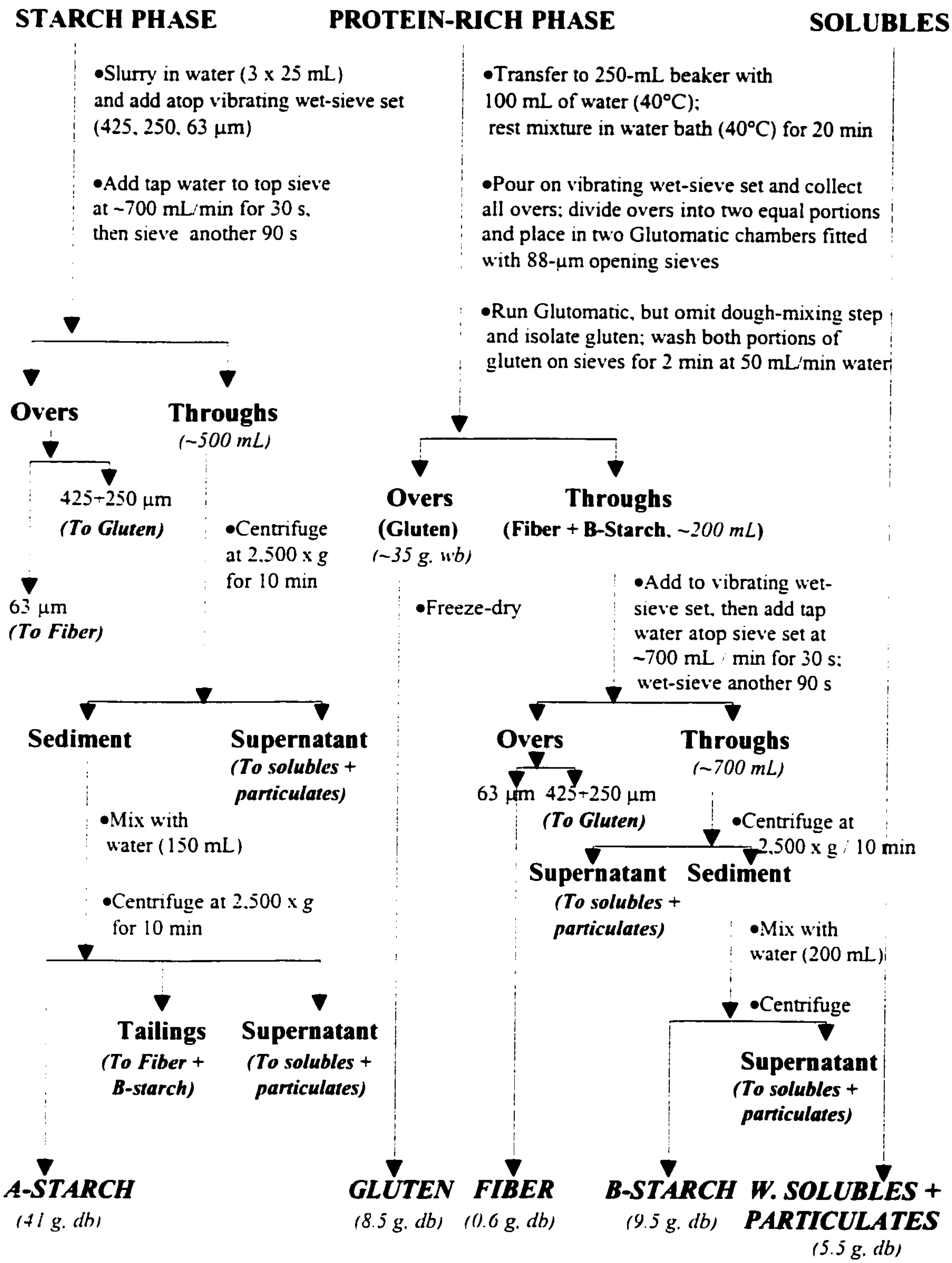


Fig. 14. *Continued.*

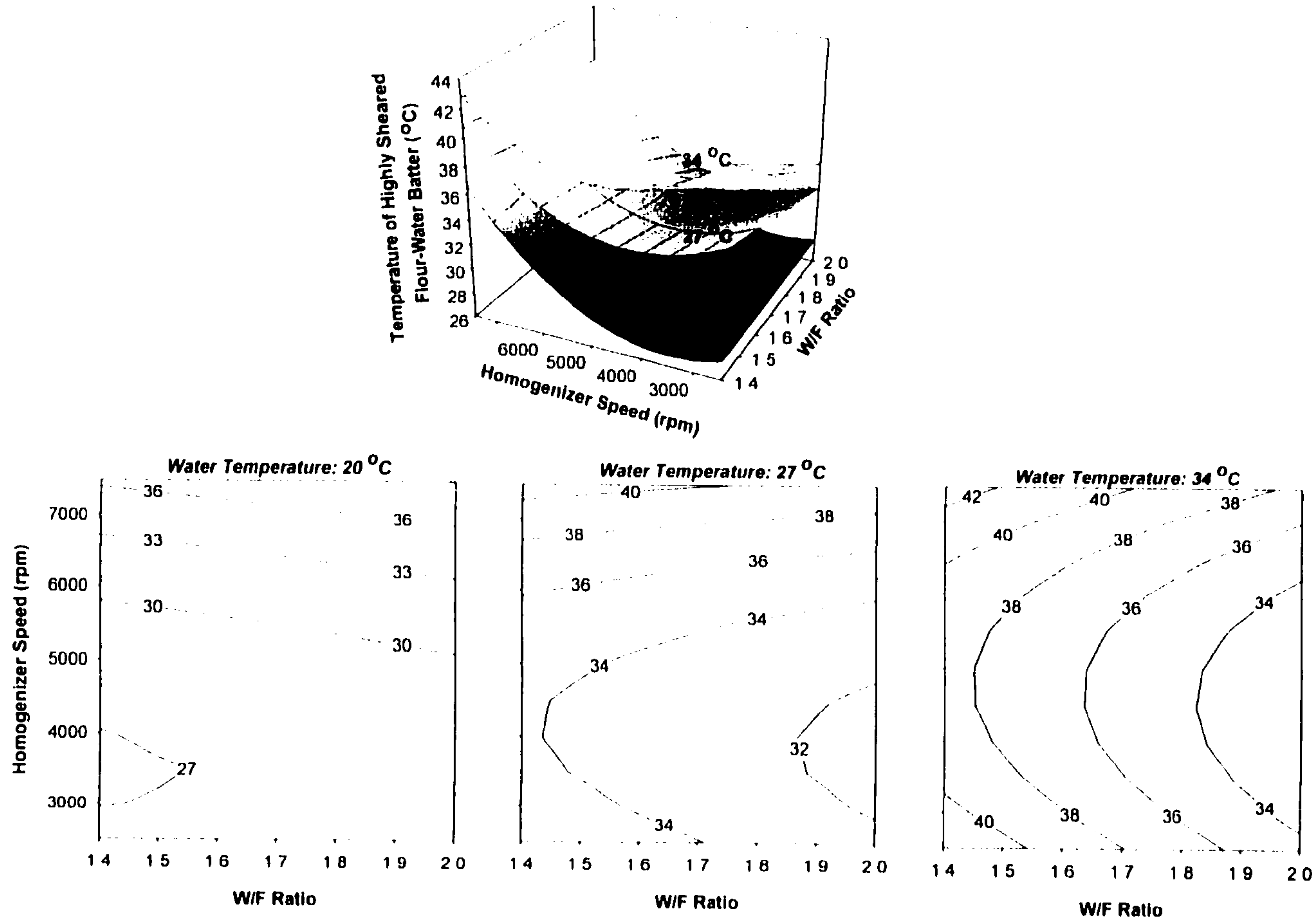


Fig. 15. Temperature of highly sheared flour-water batter determined in a modeling experiment of the high-shear mixing step. Surface (top) and contour plots (bottom) of temperature as a function of water-flour (W/F) ratio, shear rate (homogenizer speed), and water temperature ($R^2 = 0.98$).

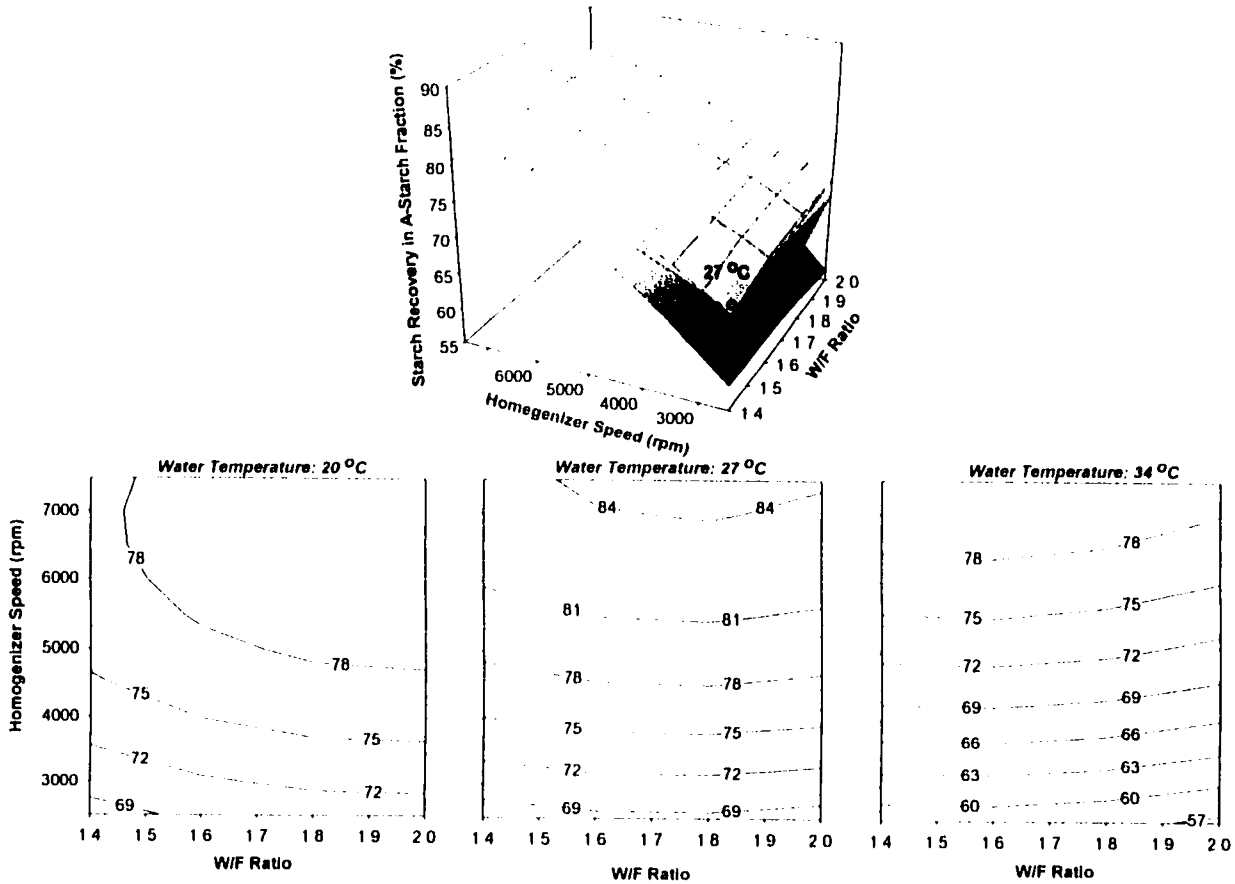


Fig. 16. Recovery of starch, based on starch in flour, in A-starch fraction determined in a modeling experiment of the high-shear mixing step. Surface (top) and contour plots (bottom) of starch recovery as a function of water-flour (W/F) ratio, shear rate (homogenizer speed), and water temperature ($R^2 = 0.95$). The highly sheared flour-water batter made from 65 g (db) flour was blended with water at 25°C to give a HS-FWD of ~27% solids, which was wet-processed to give A-starch fraction.

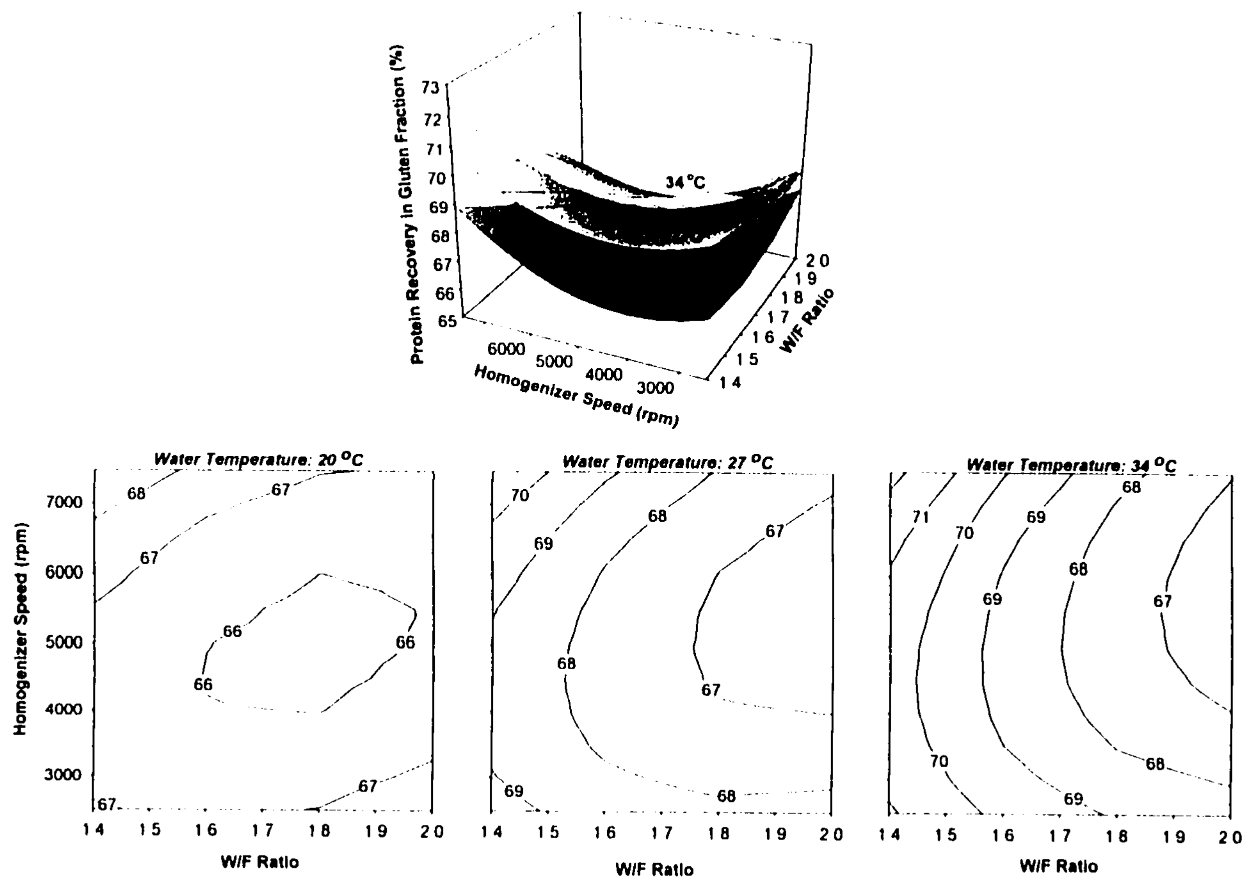


Fig. 17. Recovery of protein, based on protein in flour, in gluten fraction determined in a modeling experiment of the high-shear mixing step. Surface (top) and contour plots (bottom) of protein recovery as a function of water-flour (W/F) ratio, shear rate (homogenizer speed), and water temperature ($R^2 = 0.65$). The highly sheared flour-water batter made from 65 g (db) flour was blended with water at 25°C to give a HS-FWD of ~27% solids, which was wet-processed to give gluten fraction.

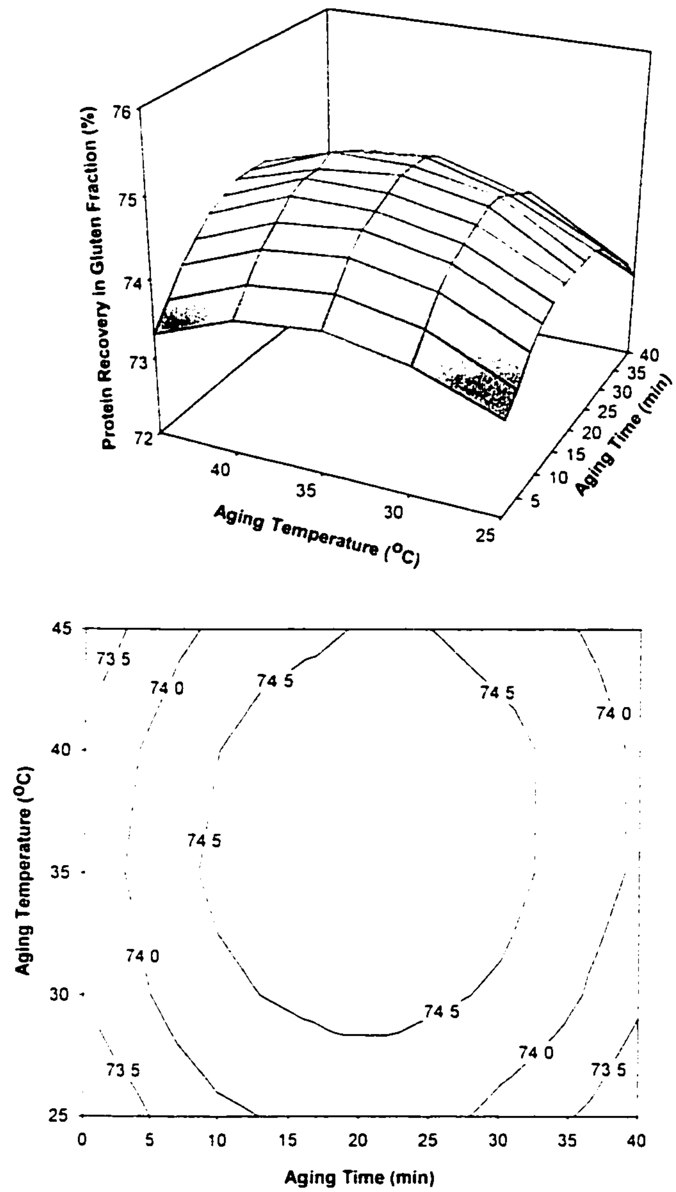


Fig. 18. Modeling of protein recovery in gluten fraction upon aging protein-rich phase at different time/temperature conditions. Surface (top) and contour plots (bottom) of protein recovery in gluten fraction (percent of protein in flour) as a function of time and temperature of aging protein-rich phase in 100 mL of added water without agitation. The regression equation for % recovery of protein = $66.26 + 0.097A + 0.42B + 0.00051AB - 0.0027A^2 - 0.0059B^2$, where A = aging time (min) and B = aging temperature (°C). The equation gave coefficient of determination (R^2) = 0.97 and standard error of estimate = 0.23.

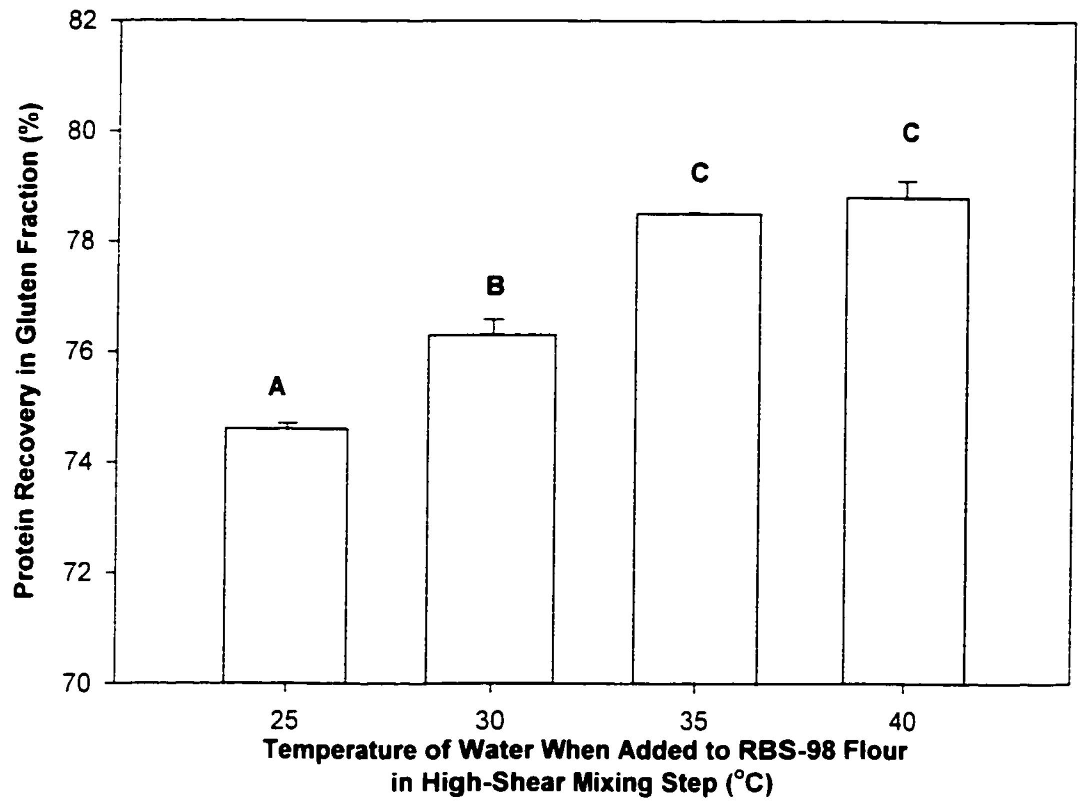


Fig. 19. Protein recovery in gluten fraction (percent of protein in flour) as affected by temperature of water added to flour in the high-shear mixing step of the highly sheared flour-water dispersion (HS-FWD) wet-milling test. The mixing parameters were W/F ratio of 1.7 and homogenizer speed of 6,000 rpm, and the aging parameters for the protein-rich phase were 40°C and 20 min. Recoveries with different letters indicate significant difference ($P<0.05$).

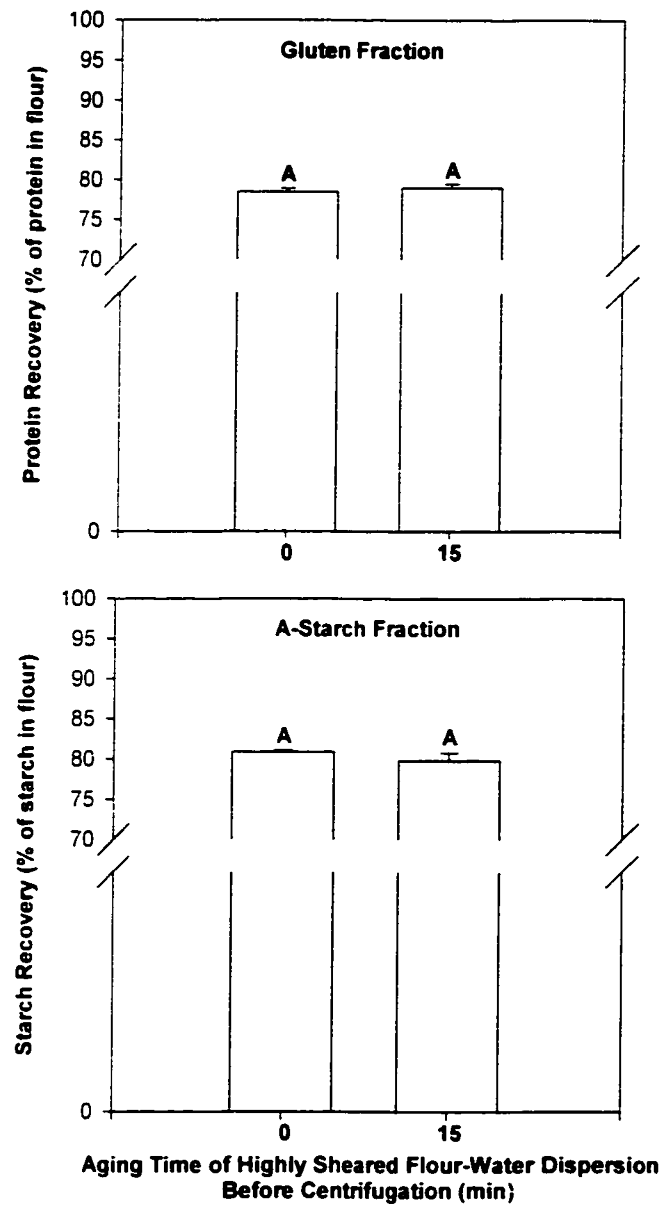


Fig. 20. Effect of aging highly sheared flour-water dispersion prior to centrifugation on recovery of protein in gluten fraction and recovery of starch in A-starch fraction at optimized high-shear mixing conditions (W/F ratio = 1.7, homogenizer speed = 6,000 rpm, water temperature = 35°C) and protein-rich phase aging conditions (40°C / 20 min). Recoveries with different letters indicate significant difference ($P<0.05$).

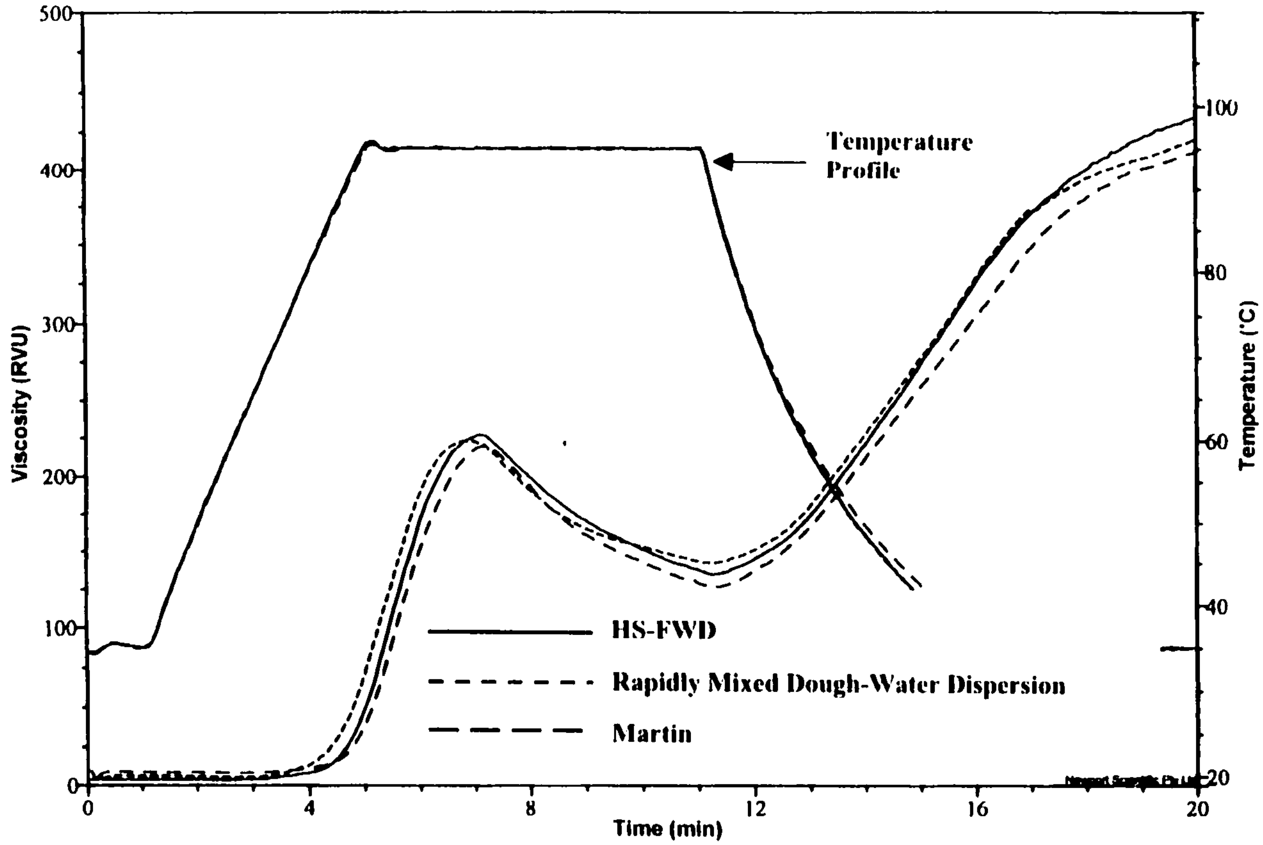


Fig. 21. Rapid Visco-Analyzer pasting curves of A-starch fractions isolated from the RBS-98 flour by the highly sheared flour-water dispersion (HS-FWD), Rapidly Mixed Dough-Water Dispersion, and Martin wet-milling methods.

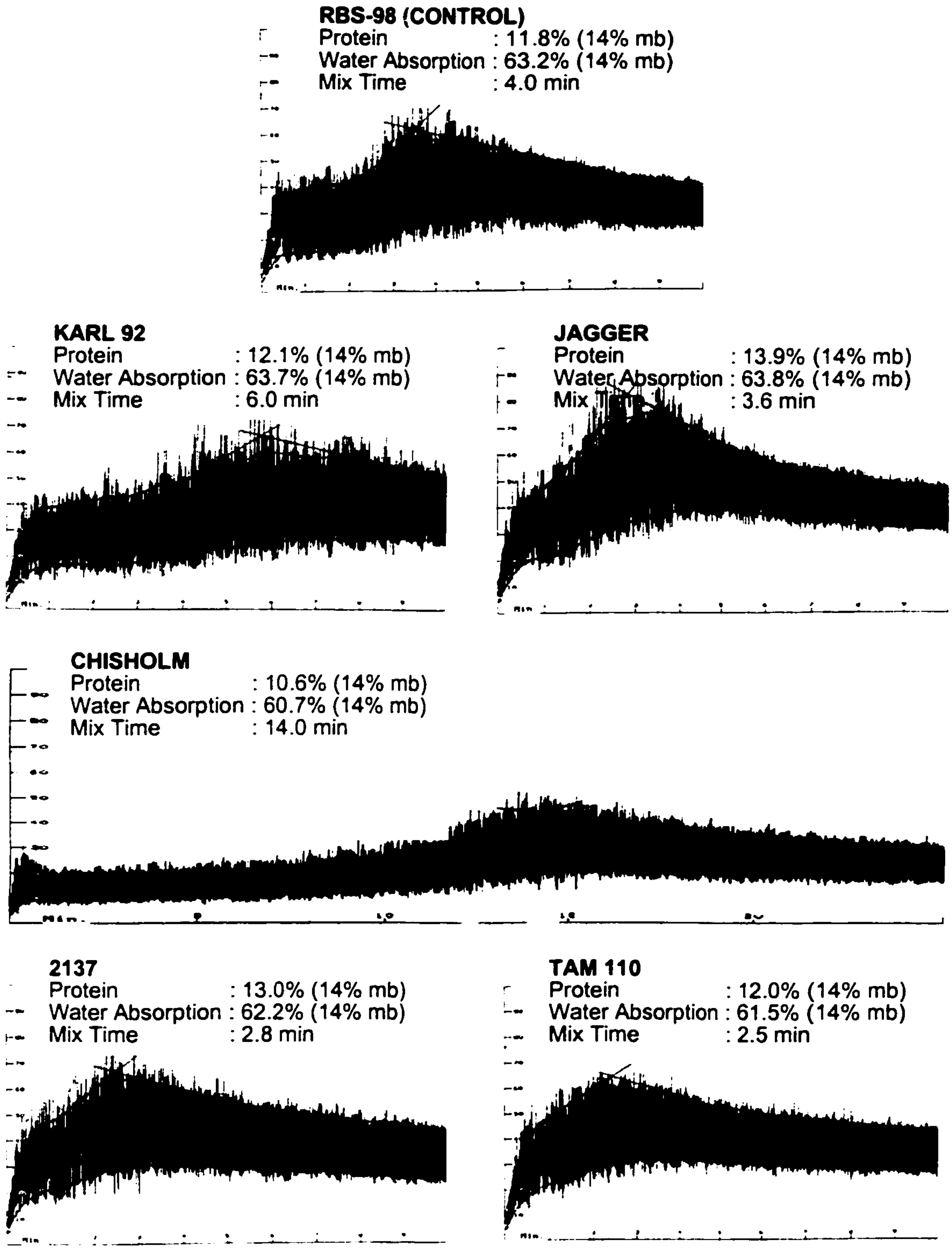


Fig. 22. Mixograms of control and five hard winter wheat flours at their optimum water absorption levels.

Fig. 23. Centrifugal fractionation of highly sheared flour-water dispersions (HS-FWD) of control and hard wheat flours.

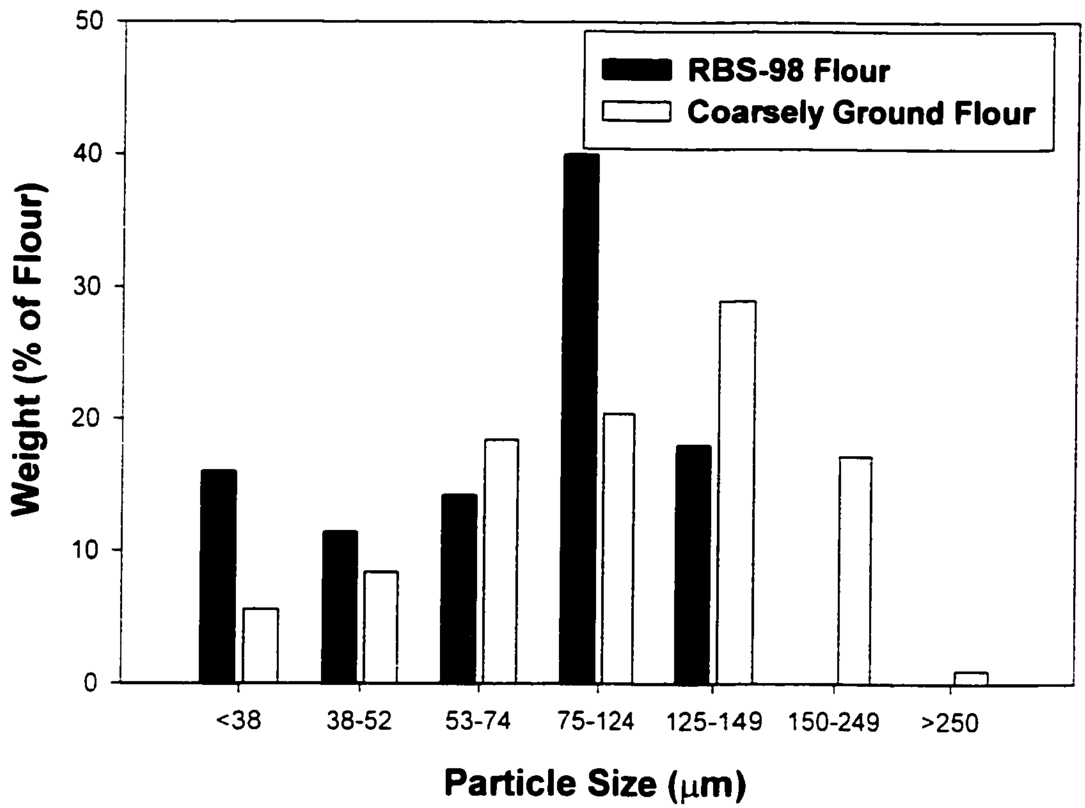
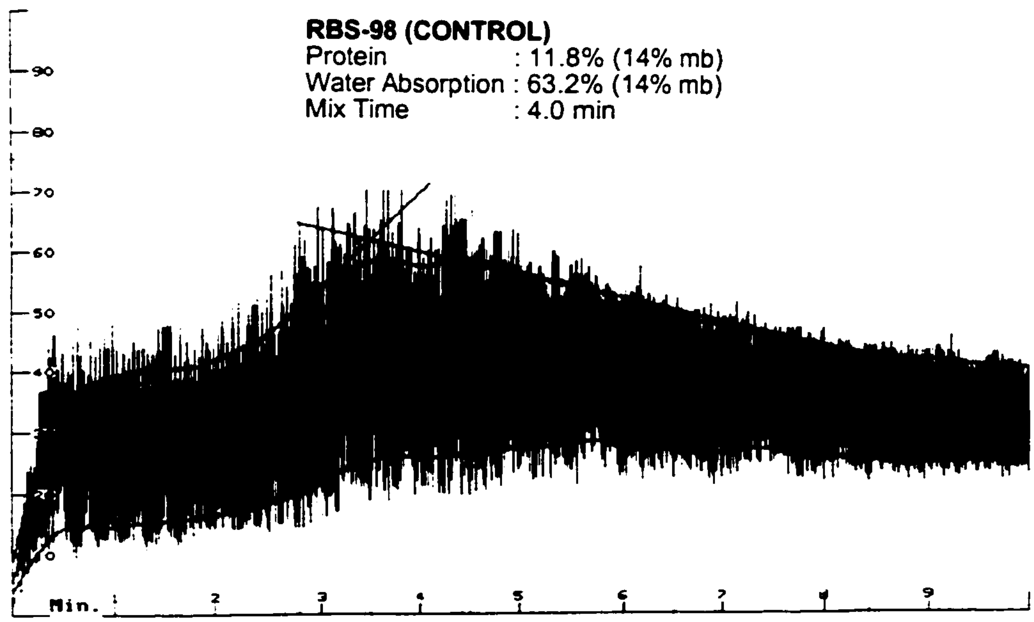


Fig. 24. Particle size distributions of control and coarsely ground flours.



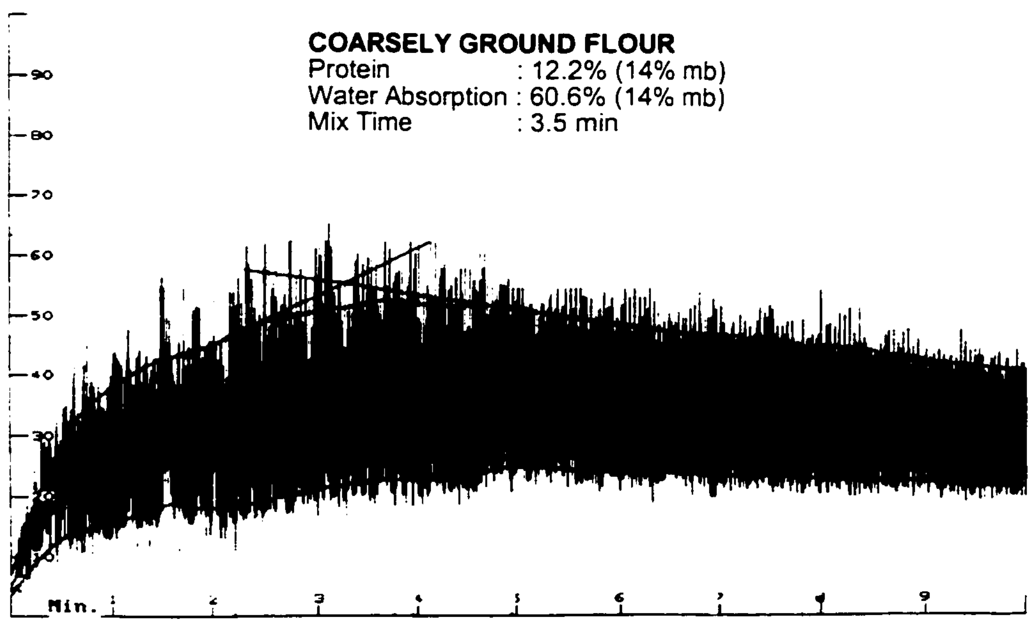
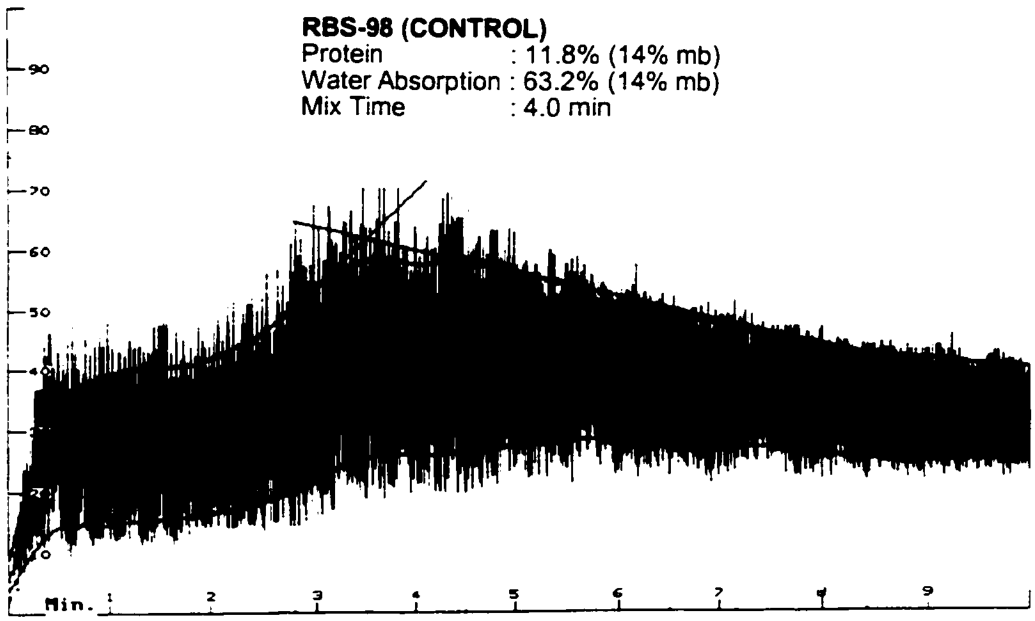


Fig. 25. Mixograms of control and coarsely ground wheat flours at their optimum water absorption levels.

Fig. 26. Centrifugal fractionation of highly sheared flour-water dispersions (HS-FWD) of control and coarsely ground wheat flours.



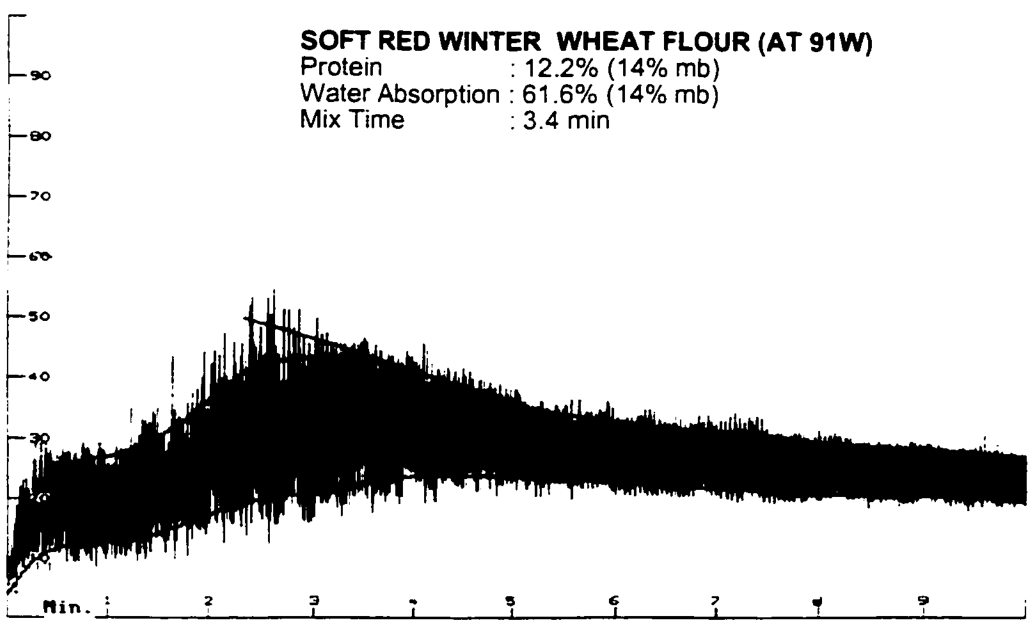


Fig. 27. Mixograms of control and a soft wheat (AT 91W) flours at their optimum water absorption levels.

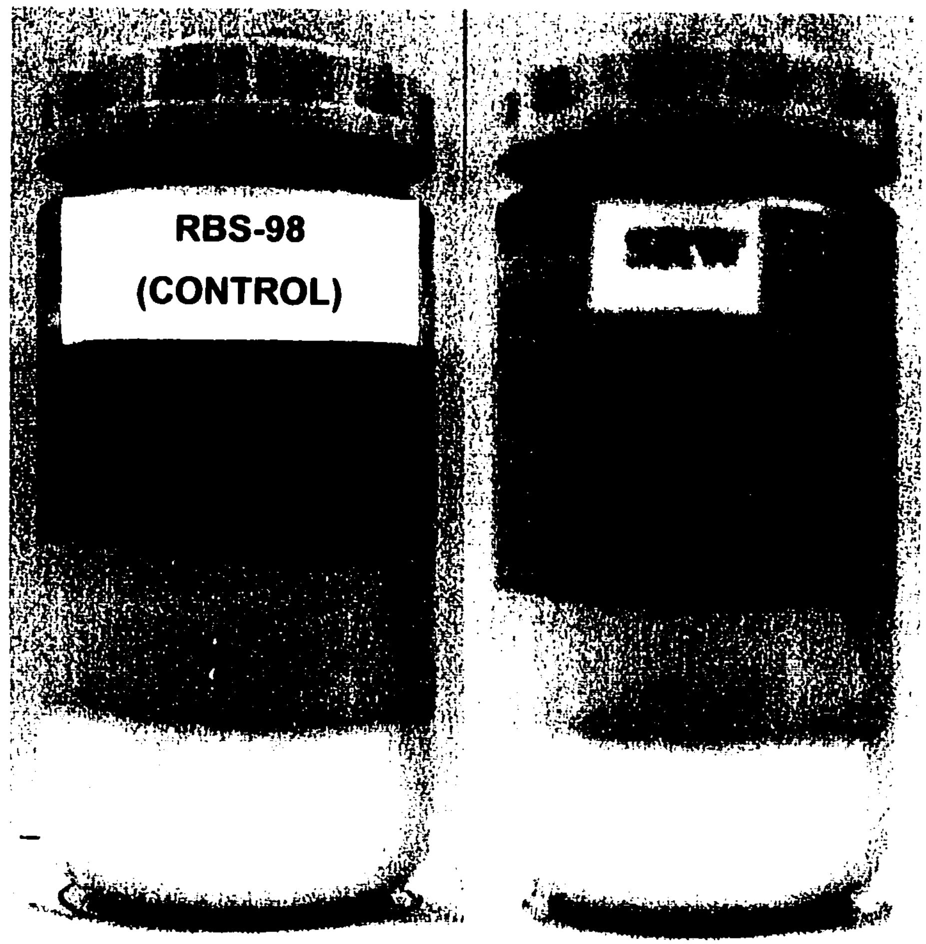


Fig. 28. Centrifugal fractionation of highly sheared flour-water dispersions (HS-FWD) of control and a soft red wheat (SRW) flours.

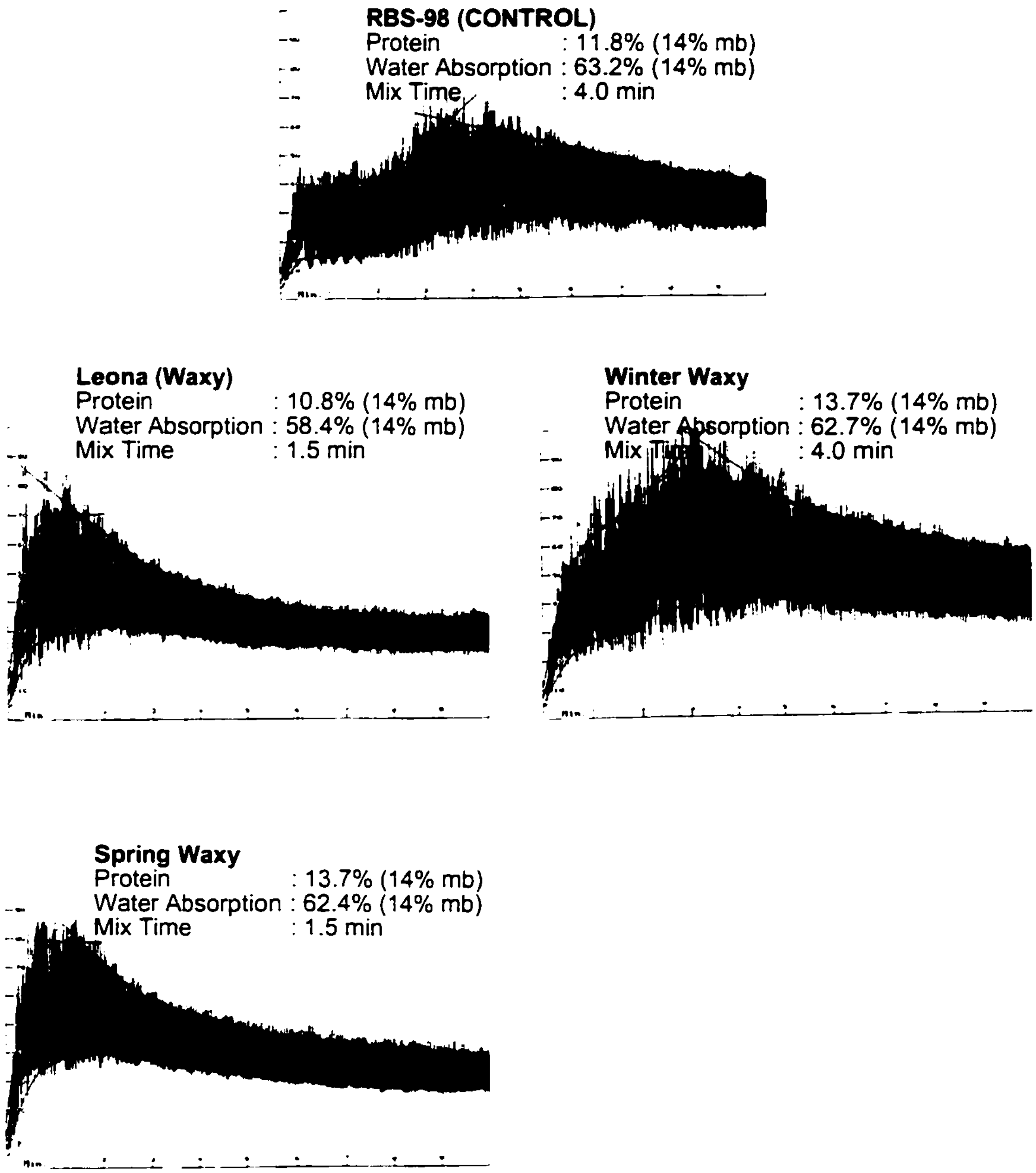


Fig. 29. Mixograms of control and waxy wheat flours at their optimum water absorption levels.

Fig. 30. Centrifugal fractionation of the highly sheared flour-water dispersions (HS-FWD) of control and waxy wheat flours. The batter of the control flour contained a water-flour (db) ratio of 1.7, whereas the batters of the waxy wheat flours were made at a water-flour ratio of 2.0. All batters were blended gently with water to give dispersions with ~27% flour solids.