

Properties of Starch and Vital Gluten Isolated from Wheat Flour by Three Different Wet-Milling Methods

Abdulvahit SAYASLAN^{1*}

Paul A. SEIB²

Okkyung Kim CHUNG³

¹Department of Food Technology, Vocational School, Karamanoğlu Mehmetbey University, Karaman, TURKEY

²Department of Grain Science and Industry, Kansas State University, Manhattan, KS, USA (Retired)

³USDA/ARS, Grain Marketing and Production Research Center, Manhattan, KS, USA (Retired)

*Corresponding Author

e-mail: sayaslan@kmu.edu.tr

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Abstract

Wheat is extensively used for food, feed, seed and wet-milling. Wheat starch and vital wheat gluten isolated by wet-milling of wheat flour are utilized in numerous food and nonfood applications. Five commercial wet-milling processes, namely Martin, Batter, Alfa-Laval/Raisio, Hydrocyclone and High-Pressure Disintegration (HD), have been employed to co-produce wheat starch and vital gluten. Martin and Batter are the traditional processes, whereas Alfa-Laval/Raisio, Hydrocyclone and HD are the modern-day processes. In the traditional processes, separation of starch and gluten starts with a stiff dough or batter with optimally developed gluten and proceeds with kneading and/or screening to separate starch granules from gluten mass. In the modern-day processes, however, separation starts with a sheared flour-water or dough-water dispersion with partially developed gluten and proceeds with centrifugation to separate starch granules from gluten network. The purpose of this study was to compare the pasting properties of starches and breadmaking qualities of vital glutes isolated from RBS-98 wheat flour by three different laboratory wet-milling methods, namely highly sheared flour-water dispersion (HS-FWD), moderately sheared dough-water dispersion (MS-DWD) and dough-washing (Martin), which respectively represent the commercial wet-milling processes of HD, Hydrocyclone and Martin. Three wet-milling methods were found to be somewhat comparable in many respects, yet they differed in certain wet-milling parameters. Damaged starch levels and RVA pasting properties of A-starch fractions isolated by all three wet-milling methods were quite similar, indicating that starch was neither mechanically damaged nor physico-chemically altered during shear treatment of HS-FWD or MS-DWD methods. Similarly, breadmaking qualities of vital glutes isolated by all wet-milling methods did not fluctuate extensively, suggesting that gluten proteins were not damaged nor altered during shear treatment of HS-FWD or MS-DWD methods. In other words, wet-milling methods used for the isolation of starch and vital gluten are of almost no or limited influence on the properties of starch and vital gluten.

Keywords: Wheat, flour, wet-milling, method, starch, pasting, vital gluten, quality

INTRODUCTION

Wheat and wheat products are widely used for food, feed, seed and wet-milling to co-produce starch and vital gluten. Wheat starch and vital wheat gluten are utilized in numerous food and nonfood applications. The wet-separation of gluten and starch from wheat flour is based on the differences in their density and particle size. In the presence of water and such mechanical energy as mixing, kneading or shearing, gluten proteins of flour tend to aggregate and form particles that are larger in size but less dense than starch granules [1-2].

Five commercial wet-milling processes, namely Martin (dough-washing), Batter, Alfa-Laval/Raisio, Hydrocyclone and High-Pressure Disintegration (HD), have been employed to co-produce wheat starch and vital wheat gluten, all of which start with flour rather than wheat kernel. Martin and Batter are considered traditional processes, whereas the other three relatively new processes, i.e., Alfa-Laval/Raisio, Hydrocyclone and HD, are considered modern-day processes [1, 3-4]. In the traditional processes, separation of starch and

gluten starts with a stiff dough or batter with optimally developed gluten and proceeds with kneading and/or screening to separate starch granules from gluten mass. In the modern-day processes, however, separation starts with a sheared flour-water or dough-water dispersion with partially developed gluten and proceeds with centrifugation to separate starch granules from gluten network [1, 4-5].

In Martin process, gluten proteins are strongly aggregated during dough mixing to form a matrix of gluten threads that enmeshes other dough components. In Batter process, gluten proteins, as in Martin process, are strongly aggregated in the mixing step due to warmer temperature of water [1-2, 5-9]. In contrast to highly aggregated gluten particles formed in the mixing step of the traditional wet-processes of Martin and Batter, modern-day processes start with an aqueous dispersion of flour components at ~30% solids that is formed by gently blending either a highly sheared flour-water dispersion (HS-FWD) or a moderately sheared dough-water dispersion (MS-DWD). Alfa-Laval/Raisio and HD processes 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. The HS-FWD is produced by high-shear mixing of flour and water by two ways; either by a rapidly moving mixer-homogenizer blade that acts on slowly moving flour-water mixture or by rapidly moving a flour-water mixture through a small stationary aperture. In contrast to Alfa-Laval/Raisio and HD processes, Hydrocyclone process begins with a MS-DWD that possesses intermediate-sized gluten particles. The MS-DWD is formed by mixing a slack or stiff dough, followed by subjecting it to a forced-convection mixing with additional water in a holding vessel. Moderate fluid shear is required in the vessel to disperse dough components and to prevent sedimentation of gluten particles and starch granules. In addition, moderate shear forces in the holding tank together with excess water probably decrease the size of gluten particles originally formed during dough mixing [1-2, 5].

Wheat starch is commonly 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 [1, 10-11]. Wheat starch also is converted to starch hydrolysis products, including sweeteners, especially in Europe and Australia [12]. Being a valuable co-product of commercial wheat starch production, vital wheat gluten is widely used in bakery products to improve product quality by means of increasing flour protein content and/or quality [13-18]. Vital wheat gluten is also used in the production of breakfast cereals and snacks; pizza, meat and cheese analogs; breading and batter mixes; and in specific meat, fish and poultry products [19].

The purpose of this study was to compare the pasting properties of starches and breadmaking qualities of vital glutes isolated from a standard bread wheat flour (RBS-98) by laboratory wet-milling methods of HS-FWD, MS-DWD and Martin.

MATERIALS AND METHODS

Material

A standard bread flour (RBS-98) with 11.8% protein (14% mb), obtained from USDA/ARS Grain Marketing and Production Research Center, Manhattan, KS - USA, was used in three wet-milling methods employed in this study.

General Methods

Moisture contents of flour and wet-milling fractions were determined by the American Association of Cereal Chemists (AACC) method 44-15A [20]. Protein contents (Nx5.7) of flour, wet-milling fractions and flour-starch-vital gluten blends prepared for baking tests were measured by combustion method using an FP Protein/

Nitrogen Analyzer (Leco Corp., St. Joseph, MI). Total and damaged starch contents of wet-milling fractions were determined by the AACC methods 76-13 and 76-31, respectively [20], using assay kits from Megazyme International Ireland Ltd, Wicklow, Ireland. Mixogram of RBS-98 flour was acquired by the AACC method 54-40A [20] using a 10-g mixograph (National Manufacturing Co., Lincoln, NE). Scanning electron micrographs (SEMs) of freeze-dried samples of HS-FWD, MS-DWD and flour-water dough for Martin were taken with a Hitachi S-3500N scanning electron microscope (Hitachi Science Systems, Ltd., Tokyo, Japan). The samples for SEMs were prepared as described below in the wet-milling of RBS-98 flour by HS-FWD, MS-DWD and Martin methods. Approximately 5-10 g of samples from each of three wet-milling methods were removed and placed in a centrifuge bottle, and the contents rapidly frozen (~2 min) by immersing the bottle in dry ice-acetone. The frozen samples were then freeze-dried. SEMs were taken after sprinkling a small crushed sample onto double-sided adhesive tape pressed atop a specimen stub. Following coating with gold-palladium, the samples were viewed with the scanning electron microscope.

Wet-Milling Methods

RBS-98 wheat flour was wet-milled to obtain starch, vital gluten and other fractions by three wet-milling methods (HS-FWD, MS-DWD, Martin). HS-FWD method was originally developed and described in detail by Sayaslan [4]. In brief, HS-FWD method included; (i) high-shear mixing of RBS-98 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 sheared dispersion into supernatant, protein-rich and starch phases, (iii) aging of the protein-rich phase, and finally (iv) isolation and purification of five wet-milling fractions (vital gluten, A-starch, B-starch, fiber, water-solubles). The process conditions of HS-FWD method were as follows; water-flour ratio 1.7, homogenizer speed 6,000 rpm, water temperature 35°C, gluten aging temperature and time 40°C and 20 min, Glutomatic washing time of the aged gluten 2.0 min. Except for wet gluten, all wet-milling fractions were dried at room temperature. Dried A-starch fractions with moisture contents of 10-14% were ground with a mortar and pestle and used for the measurement of pasting properties. Wet gluten fractions were freeze-dried after partly freezing and cutting wet mass into ~1 cm³ pieces (Flexi-Dry/MP, TMS Systems, Inc., Stone Ridge, NY). Freeze-dried glutes, which typically contained <5% moisture, were ground in a Thomas-Wiley intermediate mill (Thomas Scientific Co., Philadelphia, PA) to pass through a 420- μ m opening sieve.

MS-DWD method was a slight modification of the procedure of Czuchajowska and Pomeranz [21-22] by Sayaslan [4]. 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 mixing time (4.0 min). The dough pieces were then combined and placed in a 300-mL capacity bowl of a Waring blender containing 115 mL of water at 15°C. After 30 min of resting, the dough-water mixture was dispersed in the Waring blender (1.0 min, full speed) to form a MS-DWD. The dispersion was then transferred to a 300-mL centrifuge bottle and centrifuged at 2,500 x g for 15 min. Vital gluten, A-starch, B-starch, fiber and water-solubles fractions were isolated, purified and dried as in the HS-FWD method with the exception that protein-rich phase was washed in the Glutomatic system without a maturation scheme.

Martin (dough-washing) method, described thoroughly by Sayaslan [4], started with a dough prepared in the same manner as in the MS-DWD. The dough was first covered with a wet cloth and rested at room temperature for 1 h. The rested dough was then hand-washed over a screen under a stream of water (1 mL/min, 25°C, total water usage 750 mL). Vital gluten, A-starch, B-starch, fiber and water-solubles fractions were isolated, purified and dried similarly as in the HS-FWD method with minor adjustments when necessary.

Pasting Property of Starch

Pasting characteristics of A-starches isolated by three wet-milling methods 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. 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.

Breadmaking Quality of Vital Gluten

Breadmaking qualities of the standard flour (RBS-98) and the vital gluten isolated from RBS-98 flour by three wet-milling methods (called "test gluten" hereafter) were evaluated by so-called starch-stress bake test procedure [23-24]. For this baking test, flour portion of the formula was a blend of standard bread flour (RBS-98), commercial wheat starch and test gluten. To prepare the blends, standard RBS-98 bread flour with 11.8% protein (14% mb) was first blended with commercial wheat starch at a ratio of 1:1 to reduce protein level of the flour from 11.8% to ~6% (14% mb). Then, test gluten was added to flour-starch mixture to raise its protein content back to ~12% (14% mb). Pup-loaf breads were baked by the AACC Method 10-10B [20], a straight-dough procedure with 90 min fermentation. Loaf volumes were measured using a rapeseed displacement apparatus, while crumb grain scores assessed by a trained panel.

Statistical Analysis

All trials were conducted in two replications with at least duplicate measurements and their means were compared by the LSD (least significant difference) multiple comparison test at $\alpha = 0.05$ level in one-way analysis of variance (ANOVA) using the Statistical Analysis System Software (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Properties of Standard Flour and SEM of Dispersions and Dough

RBS-98 standard wheat flour has average properties of straight-grade flours milled from hard winter wheats (Figure 1). Hard winter wheats are selected by breeders based on their flour-milling and breadmaking properties. A small percentage of hard wheat flour is also wet-milled to produce vital gluten and starch [16, 25]. As shown in Figure 1, the mixogram and pup-loaf volume (885 cc) of RBS-98 flour are typical of a bread wheat flour for adequate quality characteristics.

SEMs of optimally sheared or mixed HS-FWD, MS-DWD and flour-water dough are shown in Figure 2. In the HS-FWD, where flour and water were extremely sheared without forming a dough, gluten network was observed to be an assembly of interconnected thin gluten threads with almost totally freed starch granules from gluten matrix. In the case of flour-water dough that was mixed at optimum water absorption and mix time, gluten proteins were aggregated to form a continuous sheet-like structure that tightly enmeshed starch granules as the dispersed phase. When that dough was rapidly mixed in water with moderate shear in a blender to form a MS-DWD, the sheet-like network observed in the dough was transformed to thick thread-shaped particles of gluten network with most starch granules freed of gluten. It is evident that gluten matrices to be used for each wet-milling method are entirely different from one another

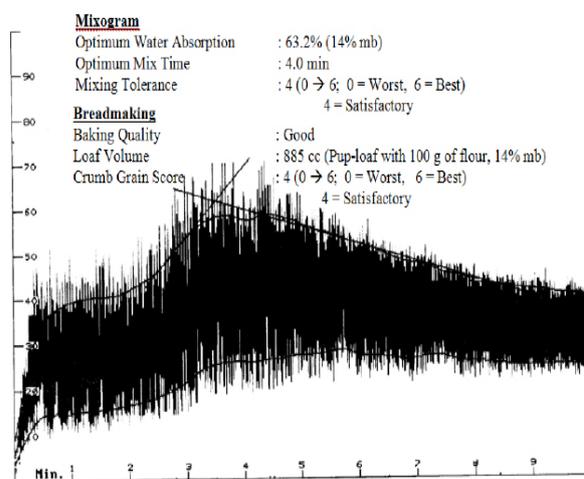


Figure 1. Mixogram and breadmaking quality data of standard RBS-98 flour

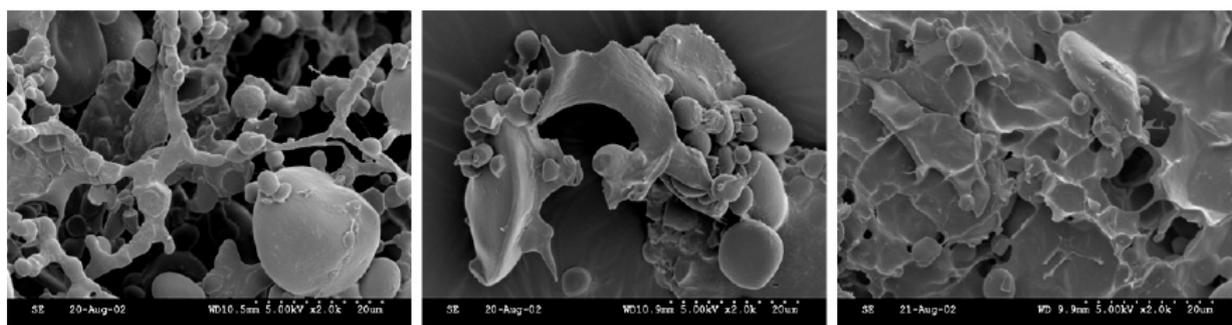


Figure 2. Scanning electron micrographs (SEMs) of optimally sheared or mixed (a) HS-FWD, (b) MS-DWD and (c) flour-water dough for Martin

due to the different types of mechanical energy input (shearing, mixing plus shearing or only mixing) applied during their preparation.

Comparison of Three Wet-Milling Methods

Distribution of flour solids in five fractions of three wet-milling methods, namely HS-FWD, MS-DWD and Martin, are presented in Table 1. In terms of the recoveries of total solids, all three methods were quite comparable. However, Martin method gave the highest recoveries of solids in vital gluten and A-starch fractions. HS-FWD and MS-DWD methods gave similar recoveries of solids in their gluten fractions, but the recovery of solids was lower in A-starch fraction of MS-DWD method. In the high-shear processes, levels of flour solids in water-solubles fractions were slightly elevated. The drawbacks of Martin process, which include the need of strong gluten-aggregation traits and utilization of excess amount of water during wet-processing [4], may be balanced by high recoveries of protein and starch and reduced levels of water-solubles.

As shown in Table 1, HS-FWD and MS-DWD methods gave similar recoveries (78-79%) of protein in their gluten fractions with similar purities (~83% protein). However, Martin method, which involved minimal shear during processing, gave a higher recovery (~87%) of protein in gluten fraction containing ~84% protein. The increase in protein recovery from ~78 to 87% in e gluten fraction of Martin method is most likely caused by the

fact that much of the water-soluble proteins in RBS-98 flour became associated with vital gluten fraction. It was reported by Pence and co-workers [26] that almost all globulin and up to one-half of albumin proteins may be held in gluten fraction by means of physical entrapments and disulfide bonding. On the other hand, reduced recovery of protein in HS-FWD and MS-DWD methods may be explained by the high-shear forces employed in those processes during preparation of sheared dispersions prior to centrifugation, which possibly caused soluble-proteins to be dispersed or some gluten particles to become smaller in size and lost to B-starch fraction. A-starch fractions obtained by all three methods had similar and acceptable level of purity (<0.3% protein).

Pasting Property of Starch

Damaged starch contents of A-starch fractions isolated by three wet-milling methods ranged from 1.6 to 2.1% (db), which is comparable to damaged starch level (2.0%) of a commercial starch (data not provided). RVA pasting properties of all A-starch fractions were quite similar (Figure 3), indicating that starch granules were neither mechanically damaged nor physico-chemically altered during the high-shear mixing of flour-water or moderate-shear mixing of dough-water dispersions in HS-FWD or MS-DWD methods, respectively. In other words, wet-milling methods used for the isolation of starch are of almost no influence on starch properties.

Table 1. Recovery of flour solids from RBS-98 flour by HS-FWD, MS-DWD and Martin (dough-washing) wet-milling methods^{a,b}

Wet-milling method	Vital gluten fraction				A-starch fraction				B-starch fraction (Tailings)	Fiber fraction	Water-solubles fraction	Total solids recovery in five wet-milling fractions (% of flour solids)
	Solids recovery	Protein recovery	Purity		Solids recovery	Starch recovery	Purity		Solids recovery	Solids recovery	Solids recovery	
			Protein content	Starch content			Starch content	Protein content				
	(% of flour solids)	(% of flour protein)	(%, Nx5.7)	(%)	(% of flour solids)	(% of flour starch)	(%)	(%, Nx5.7)	(% of flour solids)	(% of flour solids)	(% of flour solids)	
HS-FWD	12.9 ^b	78.8 ^b	82.9 ^a	11.0 ^b	63.1 ^b	80.3 ^b	95.8 ^a	0.24 ^a	13.2 ^b	0.9 ^b	8.7 ^a	98.8 ^a
MS-DWD	13.1 ^b	78.0 ^b	83.5 ^a	15.5 ^a	56.4 ^c	71.8 ^c	94.3 ^a	0.20 ^b	20.6 ^a	0.9 ^b	7.2 ^b	98.3 ^a
Martin	14.3 ^a	86.7 ^a	84.4 ^a	7.1 ^c	70.3 ^a	89.4 ^a	94.2 ^a	0.20 ^b	6.4 ^c	1.2 ^a	6.2 ^c	98.4 ^a

^aAll percentages are on dry-solids basis

^bDifferent letters in the same column indicate significant difference (P<0.05)

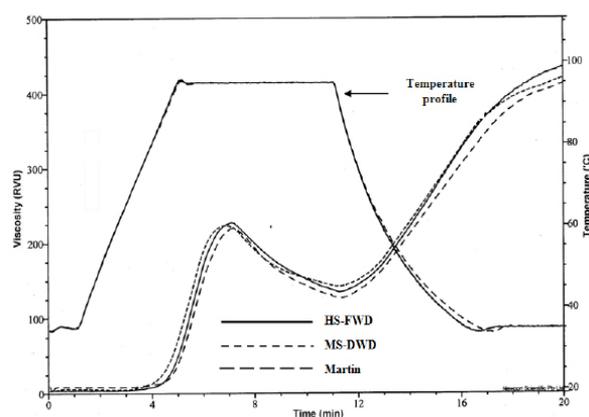


Figure 3. Rapid Visco-Analyzer (RVA) pasting curves of A-starch fractions isolated from RBS-98 flour by HS-FWD, MS-DWD and Martin (dough-washing) methods

Breadmaking Quality of Vital Gluten

Breadmaking qualities of vital gluten fractions isolated by three methods were evaluated using a starch-stress bake test [23]. In this baking test, approximately one-half of the protein in RBS-98 flour was replaced by a test gluten. As summarized in Table 2, loaf volumes of breads baked from the test glutes isolated by three wet-milling methods did not differ significantly when loaf volumes were corrected by protein contents of their respective blends, suggesting that gluten proteins were not damaged nor altered extensively during shear treatment of HS-FWD or MS-DWD methods. In terms of crumb grain structure, however, breads containing vital gluten fraction isolated by Martin method had somewhat higher crumb grain scores, probably because much of the water-soluble solids, particularly water-soluble proteins, remained in vital gluten fraction of Martin method due to lack of shear force in that method as discussed above. In short, wet-milling methods used for the isolation of vital gluten are of limited effect on breadmaking quality of gluten.

CONCLUSIONS

In this study, pasting properties of starches and breadmaking qualities of vital glutes isolated from RBS-98 wheat flour by three different laboratory wet-milling methods, namely highly sheared flour-water dispersion (HS-FWD), moderately sheared dough-water dispersion (MS-DWD) and dough-washing (Martin), which respectively represent the commercial wet-milling processes of HD, Hydrocyclone and Martin, were investigated. Three wet-milling methods were found to be somewhat comparable in many respects, yet they differed in certain wet-milling parameters. Damaged starch levels and RVA pasting properties of A-starch fractions isolated by all three wet-milling methods were quite similar, indicating that starch was neither mechanically damaged nor physico-chemically altered during shear treatment of HS-FWD or MS-DWD methods. Similarly, breadmaking qualities of vital glutes isolated by all wet-milling methods did not vary extensively, suggesting that gluten proteins were not damaged nor altered during shear treatment of HS-FWD or MS-DWD methods. In conclusion, wet-milling methods used for the isolation of starch and vital gluten are of almost no or limited influence on the properties of starch and vital gluten.

REFERENCES

- [1] Sayaslan A. 2004. Wet-milling of wheat flour: Industrial processes and small-scale test methods. *LWT - Food Science and Technology*. 37:499-515.
- [2] Van Der Borgh A, Goesaert H, Veraverbeke WS, Delcour JA. 2005. Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. *Journal of Cereal Science*. 41:221-237.
- [3] Meuser F, Althoff F, Huster, H. 1989. Developments in the extraction of starch and gluten from wheat flour and wheat kernels. In: *Wheat Is Unique* (ed. Pomeranz Y), pp. 479-499. American Association of Cereal Chemists, St. Paul, MN.

Table 2. Breadmaking qualities of RBS-98 flour and blends containing vital glutes isolated from RBS-98 flour by HS-FWD, MS-DWD and Martin methods

Flour or blend ^a	Flour or blend protein content (%, Nx5.7, 14% mb)	Loaf volume (cc)	Loaf volume after correcting for protein content ^b (cc / g protein in flour or blend)	Crumb grain score ^b (0 → 6)
RBS-98 flour	11.8	885	74.9a	5.3a
HS-FWD	11.4	815	70.2a	3.8c
MS-DWD	11.1	753	67.8a	3.8c
Martin	11.7	800	69.7a	4.8b

^aBlend consisted of ~46/46/8 weight ratio of RBS-98 flour, commercial wheat starch and test gluten

^bDifferent letters in the same column indicate significant difference (P<0.05)

- [4] Sayaslan A. 2002. 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. PhD Dissertation. Kansas State University, Manhattan, KS.
- [5] Seib PA. 1994. Wheat starch: Isolation, structure and properties. *Oyo Toshitsu Kagaki*. 41:49-69.
- [6] Anderson RA. 1967. Manufacture of wheat starch. In: *Starch: Chemistry and Technology - Vol. II* (eds. Whistler RL, Paschall, EF), pp. 53-63. Academic Press, New York.
- [7] Anderson RA. 1974. Wet-processing of wheat flour. In: *Wheat: Production and Utilization* (ed. Inglett GE), pp. 355-365. AVI Publishing, Westport, CT.
- [8] Fellers DA. 1973. Fractionation of wheat into major components. In: *Industrial Uses of Cereals* (ed. Pomeranz Y), pp. 207-228. American Association of Cereal Chemists, St. Paul, MN.
- [9] Knight JW, Olson RM. 1984. Wheat starch: Production, modification, and uses. In: *Starch Chemistry and Technology - 2nd edition* (eds. Whistler RL, BeMiller JN, Paschall EF), pp. 491-506. Academic Press, New York.
- [10] Maningat CC, Seib PA. 1997. Update on wheat starch and its use. In: *Proceedings of International Wheat Quality Conference* (eds. Steele JL, Chung OK), pp. 261-284. Grain Industry Alliance, Manhattan, KS.
- [11] Sayaslan A, Seib PA, Chung OK. 2006. Wet-milling properties of waxy wheat flours by two laboratory methods. *Journal of Food Engineering*. 72:167-178.
- [12] Schenck FW, Hebeda RE. 1992. Starch hydrolysis products: An introduction and history. In: *Starch Hydrolysis Products* (eds. Schenck FW, Hebeda RE), pp. 1-21. VCH Publishers, New York.
- [13] Grace G. 1988. Preparation of vital wheat gluten. In: *Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs* (ed. Applewhite TH), pp. 112-115. American Oil Chemists Society, Champaign, IL.
- [14] Maningat CC, Bassi S, Hesser JM. 1994. Wheat Gluten in Food and Non-food Systems. *Technical Bulletin*, XVI-6. American Institute of Baking, Manhattan, KS.
- [15] Bergthaller WJ. 1997. New uses of wheat gluten and non-starch wheat components. In: *Proceedings of International Wheat Quality Conference* (ed. Steele JL, Chung OK), pp. 285-301. Grain Industry Alliance, Manhattan, KS.
- [16] Maningat CC, Bassi SD. 1999. Wheat starch production. In: *Proceedings of International Starch Technology Conference* (eds. Tumbleson M, Yang P, Eckhoff S), pp. 26-40. University of Illinois, Urbana, IL.
- [17] Magnuson KM. 1985. Uses and functionality of vital wheat gluten. *Cereal Foods World*. 30:179-181.
- [18] Holcomb RB. 1999. Overview of the Domestic Wheat Gluten Industry. OSU Extension Facts, WF-559. Oklahoma State University Cooperative Extension Service, Stillwater, OK.
- [19] IWGA. 1981. *Wheat Gluten - A Natural Protein for the Future - Today*. International Wheat Gluten Association, Prairie Village, KS.
- [20] AACC. 2000. *Approved Methods of the AACC - 10th edition*. American Association of Cereal Chemists, St. Paul, MN.
- [21] Czuchajowska Z, Pomeranz Y. 1993. Protein concentrate and prime starch from wheat flours. *Cereal Chemistry*. 70:701-706.
- [22] Czuchajowska Z, Pomeranz Y. 1995. Process for fractionating wheat flours to obtain protein concentrates and prime starch. U.S. Patent No. 5,439,526.
- [23] Miller RA, Hosney RC. 1996. Evaluating vital wheat gluten quality. *Cereal Foods World*. 41:412-416.
- [24] Sayaslan A, Chung OK, Seib PA. 2009. Dough-mixing characteristics of waxy wheat flours and breadmaking qualities of waxy wheat glutes. *International Journal of Natural and Engineering Sciences*. 3:81-88.
- [25] Witt W. 1997. Modern method of separating the components of wheat. In: *Proceedings of International Wheat Quality Conference* (eds. Steele JL, Chung OK), pp. 231-248. Grain Industry Alliance, Manhattan, KS.
- [26] Pence JW, Mecham DK, Olcott HS. 1956. Review of proteins in wheat flour. *Journal of Agricultural and Food Chemistry*. 4:712-715.