IMPROVING DIETARY FIBER QUALITY AND APPLICATION PROPERTIES OF WHEAT BRAN

A THESIS

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1 Introduction

Although USDA MYPyramid food guidelines suggest three or more servings of whole grain products daily, which means in general half of the grains should come from whole grain, less than 10% of the U.S. population consumes three servings daily. (Marquart, Jones, Cohen, & Poutanen, 2007) (USDA, 2009) The solution of this problem can be studied in two ways. On one hand, if the nutritional value of whole grain ingredients can be somehow concentrated, the daily servings needed for each person can be less. On the other hand, if the sensory experience of the whole grain products can be more enjoyable, consumers would choose to eat more whole grain every day.

In whole grain wheat bakery products, wheat bran fractures, which are rich in dietary fiber- the key whole grain ingredient, serve as the “indicator” and carrier of whole grain concept. Wheat bran can be modified in the two ways above.

From physiological perspective, the solubility of dietary fiber (DF) in wheat bran can be a target to apply important improvement. The DF in cereal brans is categorized into soluble and insoluble fractions. The two types of DF fractions have very different functioning mechanisms and effects. Although both fractions are concluded to be very beneficial human health, the functions of soluble DF are even more favored than insoluble DF. However, the soluble DF fraction content is usually less than 10% of total DF, whereas insoluble fraction is greater than 90%. It is especially true for wheat bran.

A physic-chemical property of wheat bran that is closely associated with DF solubility is the viscosity of soluble DF. Studies showed that only viscous soluble DF

intrigues positive physiological responses. Literature shows that increase in soluble DF portion and viscosity could be achieved by mechanical milling and chemical modifications. If the wheat bran used in bakery industry can have enhanced soluble DF content with high viscosity, the bakery products' physiological functions can be viewed as ideally "concentrated".

In the sensory quality point of view, the mixing of wheat bran into flour often causes unpalatable coarse texture and relatively hardness of whole grain bread. Despite the various health effects consumers have been aware of, the unpalatibility caused by wheat bran is the major barrier to consumers' acceptance of whole grain products. Improving the palatability is essential to increase the intake of whole grain products among consumers.

Many studies have been conducted to analyze and solve the sensory drawback of whole grain baking products. The studies generally suggested that processing approaches which decrease the particle size and increases water retention capacity (WRC) can help reduce the undesired grittiness and hardness.

2 Objectives

Proper processing methods to improve the nutritional value as well as sensory characteristics of wheat bran would be of great interest for food manufacturers.

In the study of this thesis, the author tried to design a process or a combination of multiple processes, using alkali, high shear mixer, high pressure homogenizer and xylanase, to achieve the following objectives for bran from a white soft winter wheat:

1. To enhance nutritional value by increasing soluble DF content without compromising total DF content.
2. To increase the viscosity and water hydration capacity of bran to benefit both the nutrition and baking properties
3. To reduce particle size of wheat bran fractures to the fineness that minimizes texture change.
4. To increase surface area of bran fractures to possibly increase cholesterol binding capacity.

3 Background

3.1 Wheat Bran

Wheat bran comprises about 14.5% of the kernel weight, but contains about 70% of the DF of the whole wheat seed.(Fincher & Stone, 1986; Stone, 1996) It is a by-product of the roller milling of white wheat flour. As the health benefits of whole grain becomes aware, wheat bran is included in final flour during milling as an indicator of whole grain products. Wheat bran is one of the most important dietary fiber sources used in the bakery industry (Vermaa, Huclb, & Chibbar, 2009). Besides used as source of DF in whole grain products, wheat bran is sold as animal feed material. (Betsha S. & Melaku S., 2009)Wheat bran is not a single layer of cells, but several layers outside the starchy wheat endosperm. These layers consist of different types of cells, which have walls with varying chemical compositions. (Harris, Chavan, & Ferguson, 2005)

3.2 Dietary Fiber in Wheat Bran as Key Nutrients

DF is arguably the key nutrient in whole grain food in terms of physiological effects. Although cereal brans are often referred as DF, it is not the whole of the bran, but only some compounds in the bran cell walls which are the constituents of DF. Two types of tissue formed by wheat bran cell walls are the components of wheat DF: parenchymatous tissue, and lignified tissue. In the parenchymatous tissue, the main constituent groups of DF polymers are hemicelluloses (e.g. arabinoxylans and β-glucans), cellulose, proteins, and phenolic esters. In the lignified tissue, the main constituent groups of DF polymers are hemicelluloses (e.g. glucuronoarabinoxylans), cellulose, lignin and phenolic esters, and proteins. The portion of lignified tissue is greater. (Selvendran, 1984)

The establishment of a clear and well-rounded definition of DF has experienced discussion by people from academic community, industry and government since mid-twentieth century. The latest version of DF definition was published in 2001 by the AACC. The definition covers the chemical composition and physiological function and nutritional. It also considers the limitation of DF analytical methods. The definition is:

"Dietary fiber is the edible parts of the plant and analogous carbohydrate that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the human large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fiber promotes beneficial physiological effects, such as, laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation." (the American Association Of Cereal Chemists, 2001)

Wheat bran is most used in whole grain baking products as a source of dietary fiber. The addition of wheat bran imparts the health benefit of dietary fiber (DF) to baking products. However, wheat-based whole grain baking products usually contain less soluble DF per serving than cereal products made from oat. This is because wheat bran has smaller portion of soluble DF in total DF than oat. Soluble DF, especially viscous soluble DF has been viewed as superior in physiological effect than insoluble DF. The content of soluble DF is often labeled in the Nutrition List on packaging of products of high soluble DF as a sales promoting factor. Increasing soluble DF content in wheat bran can be a remarkable improvement in both health effect and profitability of wheat-based whole grain products.

3.3 Physiological functions of dietary fiber

In the mouth and stomach, (Trowell, Burkitt, & Heaton, 1985) dietary fiber prolongs the time of chewing and dilutes the energy concentration of the food. This also results in an extended time of digestion, which in turn influences the amount of food consumed, and causes a reduced intake of calories. Dietary fiber that increases the viscosity of the digestion mass delays the speed of excretion, which leads to a prolonged feeling of satiety, and therefore reduces further consumption of food. In wheat bran, pectin is a major soluble DF constituent. It has positive effect on the dumping syndrome, i.e. uncontrolled emptying of the stomach (Behall & Reiser, 1986).

3.4 Physicochemical properties of DF and Wheat Bran Matrix

The physicochemical properties of DF cannot be separately talked about without mentioning the food matrix where DF polymers locate and the interaction of DF with other food ingredients. The mechanisms of these interactions base on the DF molecules' chemical properties, macromolecular structures, the number of charges and polar groups on the surface of the polymeric chains. Wheat bran's surface features are the structural basis of many of DF's properties, such as water retention capacity, gel formation ability, cation binding ability, sterol binding ability and microbiological usability. These properties explain the molecular-level mechanisms of DF's physiological responses.

The most-studied physicochemical characteristics of DF or DF sources such as bran are solubility, viscosity, water retention capacity (WRC), and particle size. They affect both DF's physiological effects and its baking performance.

3.5 DF Solubility

The solubility of DF is determined by its types of linkages, degrees of branching and amount of substitution of the main polysaccharide chains. Insoluble DF is a cluster of numerous polysaccharide chains through many inter-chain linkages. Soluble DF has much lower molecular weight and degree of branching than insoluble DF. Soluble DF constituents include polysaccharides such as pectins, gums, psyllium, and β-glucans. They can be extracted from the matrix with water, but mostly cannot dissolve in ethanol.

Most soluble DF constituents produce highly viscous aqueous solution, or form gel. Besides the interaction with water, soluble DF can be charged, polarized or depolarized, hydrophilic or hydrophobic. These various properties lead to a range of reactions with other food ingredients such as cations and cholesterol. These interactions and reactions impair soluble DF its special physiological effects which are very different from insoluble DF. (Endress & Fischer, 2001)

3.6 DF Viscosity

The nature of viscosity of DF is the ability of DF polysaccharides to physically react within DF molecules or with other molecules to thicken or form gel.(Morris, Cutler, Ross-Murphy, Rees, & Price, 1981) Viscosity is more of a property of soluble DF's instead of insoluble DF's. Viscous soluble DF thickens when mixed with water. Although insoluble fiber contributes to the measured viscosity value, viscosity of insoluble DF doesn't show significant correlation with insoluble DF's physiological effects. (Dikeman & Fahey, 2006)

As is concluded by many researchers, high viscosity is critical to soluble DF in terms of the physiological function responses in human subjects, animals, and animal-

alternative in-vitro models. Viscous soluble DF has been associated with health benefits such as attenuation of blood glucose and cholesterol concentrations, prolonged gastric emptying, and slower transit time through the small intestine. (Malkki, 2001)

Vuksan et al. (Vuksan et al., 2006)indicated that it is viscosity rather than quantity that determines dietary fiber's lipid lowering effects. They studied the role of viscosity on serum lipid profile using a typical North American diet containing wheat bran to represent low viscosity fiber, psyllium for medium viscosity fiber, and a viscous fiber blend for high viscosity fiber. Only the high viscosity fiber blend significantly improves blood lipid profile in healthy subjects.

A study conducted by Davidson et al. (M. H. Davidson et al., 1998) cast doubt to the physiological function of low viscosity soluble fiber. They gave hypercholesterolemic men and women dietary fiber supplement containing pectin and gum arabic (4:1 ratio) through apple juice. The high density lipoprotein cholesterol level and serum lipid responses did not change significantly between the control group and treatment groups. The study suggests that soluble fiber in the low viscosity state may not bear significant physiological function, and that the vehicle or concentration of soluble fiber may be essential for its functionality.

However, Wolf et al. (Wolf et al., 2003) increased the viscosity of soluble fiber in vivo by incorporating guar gum with an amylase. Their results showed that this enzyme-induced viscous dietary fiber could stabilize blood glucose levels in human subjects. Comparing the two studies above, it can be concluded that a high viscosity matrix may be critical for soluble dietary fiber's functionality in blood sugar attenuation.

3.7 DF Water Retention Capacity

The water retention capacity (WRC) of the processed wheat bran is very important to its application in bakery products. If the bran is to be incorporated into bread flour, the bran will be responsible for a remarkable part of the flour's dough-making quality, especially the texture of final products. WRC is one of the determinants for the visco-elastic characteristics in dough.

Bran is responsible for a significant part for whole grain flour's WRC. Different fractions of wheat represent a broad range of WRC. In wheat flour, starch in its intact granule form can absorb 0.39g to 0.87g water per gram of dry weight (Rasper & De Man, 1980), starch broken by processing can absorb from 2g to 4.3g water per gram of dry weight (Bushuk & Hilnka, 1964; Bushuk, 1966), while pentosans, which are major constituents of wheat bran, can absorb between 5g and 15g water per gram of dry weight. (Jelaca & Hlynka, 1971) Wheat bran has greater WRC than other bran that are also good dietary fiber sources such as oat bran and corn bran, which makes wheat bran the best source of fiber supplement for whole grain bread-baking.

Berton et al.(Berton, Scher, Villieras, & Hardy, 2002) tested WRC of soft wheat flours from each stages of flour milling. The WRC was obtained with three testing methods. The results showed that the milling process enhanced the flours' WRC despite of differences of measuring methods. And as the milling process intensified, the final flours tripled the WRC of the flours from the beginning of milling. He also found that the pentosan amount was well correlated with the WRC, although the correlations also depended on testing methods. He concluded that when using a certain testing method,

higher WRC of flour would be generated when using a more intense degree of milling, which indicated that flour of a smaller particle size may yield higher WRC.

The WRC of flours processed in various ways and their related baking performance have been studied by many researchers. (Merritt & Stamberg, 1941; Miller, 1968; Sollars, 1972; Yamazaky, 1953; Yamazaky, 1955)

Reducing particle size is an effective method to increase WRC. The hydration capacity varies when different processing conditions are used. Maskan (Maskan, 2002) studied the WRC of three wheat products: firik, dovme and wheat grains, of which firik is immature wheat spike that are roasted, sun dried and cracked; and dovme is wrought wheat. Firik was the smallest in particle size, and the only one that was processed. In his results firik showed significantly higher WRC than the other two. His gave a hint that processing that may degrade the original matrix might increase WRC of wheat products. His study agrees with a previous study by Tang et al. (Tang, Sokhansanj, & Sosulski, 1994)who reported that the WRC is positively related to the size of grain due to the increased surface area for water absorption.

Freeze-drying or air-drying wouldn't affect the WRC. Sollars (Sollars, 1973) studied the WRC of reconstituted wheat flour. One of his results was that the freeze-drying and air-drying did not generate significant difference in WRC.

Soluble fractions don't have effect on WRC of flour. The same research done by Sollars (Sollars, 1973) showed that the WRC of flour soluble fraction did not show apparent WRC, although the addition of soluble pentosans increased the dough water absorption in the extensograph and baking performance. (Jelaca & Hlynka, 1972) The

reason might be that in flour the soluble fractions resolve in the water and do not attach to the insoluble matrix any more.

3.8 Wheat Bran Processing Methods

3.8.1 Mechanical Treatments

3.8.1.1 High Shear Mixing

The high shear mixer used in this study is a batch type. It uses a high speed rotor to create flow and shear. In the working process of a batch type high shear mixer, fluid is sheared when the velocity of the fluid at the outside diameter of the rotor is higher than the velocity at the centre of the rotor. The components, usually immiscible liquids or powder, get dispersed, homogenized, reduced in particle size, or emulsified.

In this study, the high shear mixer dispersed the water-soaked or alkali-soaked bran evenly into water, reduced bran particle size slightly, and created viscous and evenly-mixed slurry.

3.8.1.2 High Pressure Homogenization

The high pressure homogenization was performed by an M-110Y microfluidizer® processor from Microfludics. Homogenization is accomplished by forcing the slurry at high pressure through small holes in processing chamber. Process pressure used was 23,000 psi, the highest possible pressure of the microfluidizer. Two stages of homogenization were achieved by using a 200 µm chamber at the first stage and a 100 µm chamber at the second stage.

The fluid must be mixed evenly and reduced to a certain particle size before feeding to homogenization

3.8.2 Alkaline Treatment

Hemicellulose and cellulose are two major cell-wall constituents in wheat bran. Hemicellulose and cellulose degrade under alkaline conditions. Alkaline treatment is used in this study to improve wheat bran DF's solubility and the health promoting functions of other characteristics mentioned above.

A significant reduction in molecular weight is observed when cellulose is boiled with dilute sodium hydroxide, even with the careful exclusion of oxygen (Fargher & Higginbotham, 1924).

Davidson (G. F. Davidson, 1938) suggested that the molecular weight reduction was caused by the detachment of short-chain material from the reducing ends of the cellulosic molecules. The predominant mechanism (Colbran & Davidson, 1961)of cellulose degradation was shown to be the formation of the 3-deoxy-2-C-(hydroxymethyl)-erythro- and threo-pentonic acids (D-glucoisosaccharinic acids) as in the alkaline degradation of 4-O-methyl-D-glucose. The dissolution of cellulose molecules from reducing ends does not continue without an end. Stabilization of the reaction is achieved by a competing reaction (Machell & Richards, 1957) (Machell & Richards, 1960a) . During the degradation of a 4-O-substituted glucose at the reducing end of a cellulose molecule, b-hydroxycarbonyl elimination can occur. At the same time, b-alkoxycarbonyl elimination occurs at a higher frequency. The balance of these two reactions results in the formation of terminal 4-O-substituted 3- deoxy-D-arabino- and ribo-hexonic acid units, such as substituted D-glucometasaccharinic acids. Besides the 3-

deoxyhexonic acid units, 16 other stablizing acid terminal units have been detected in alkali-boiled hydrocellulose.(Johansson & & Samuelson, 1974)(Johansson & Samuelson, 1975)(Johansson & Samuelson, 1978)

Hemicelluloses are of much lower molecular weight than cellulose; some are branched and, as they are not crystalline, do not present the same barriers to accessibility as does most of the cellulose. Water soluble arabinoxylan is a major constituent of soluble DF. The basic repeating unit is an anhydroxylopyranose molecule linked by a b-(1, 4)-bond. The alkaline degradation of arabinoxylan leads to the production of the 3- deoxy-2-C-(hydroxymethyl)-tetronic acids (xyloisosaccharinic acid) as the major product (Niemela, Alén, & Sjöström, 1985). The most significant differences between arabinoxylan and cellulose are the presence of branching and the variety of other carbohydrate species as side groups, for example, 4-O-methylglucuronic acid. (Knill & Kennedy, 2003)

The mechanism of alkaline treatment indicates that insoluble DF could be degraded to soluble DF. And as a result the amount of soluble DF would increase. But the amount of insoluble DF might not decrease significantly, because the alkaline treatment also causes lignin to degrade to insoluble DF.

4 Sample Preparation

4.1 Materials

Wheat bran: King Wheat Bran, bran from a soft white winter wheat provided by King Milling Company (Lowel, MI)

Xylanase BX/AN (Enzyme Development Corp., Combination of 2 xylanases, produced from *Trichoderma longibrachiatum, A. niger*)

Sodium Hydroxide (Fisher Chemicals, Pittsburgh, PA, USA)

Sodium acetate buffer: To make 1 Liter of buffer 0.6805g of sodium acetate (Sigma-Aldrich, S1429, Saint Louis, MO, USA) was dissolved in 1000 mL of sterilized deionized water, and then 0.30025 mL of acetic acid was added to adjust the pH to 5.23.

Magnetic stirrers and stirring bars

Convection oven: 103±2^{o}C

Balance: 0.1 mg accuracy

Rotor beater mill: (Retsch GmbH, Model SR 300, Haan, Germany)

High shear mixer: Ultra-Turrax T25 Basic high shear mixer (IKA-Works, Wilmington, NC, USA)

High pressure homogenizer: M-110Y Laboratory Microfluidizer Processor (Microfluidics, Newton, MA, USA) equipped with 200µm and 100µm chambers.

Incubator: Innova 4300 incubator shaker (New Brunswick Scientific, Edison, NJ, USA)

Freeze dryer: VirTis Freezemobile 25EL freeze-dryer (Gardiner, NY, USA).

Evaporator: Home-made evaporator

RVA: Rapid Visco Analyzer (RVA, Newport Scientific, Springfiled, IL, USA).

GC-MS (Agilent 7890-5975C, Santa Clara, CA) with a flame ionization detector and a DB-5-MS capillary column

UV-visible spectrometer

Shaking water bath: 95-100^{o}C, 60^{o}C

Centrifuge: 50ml, 13800g for 20 min

4.2 Methods

Untreated Wheat Bran—Abbreviated as "Control"

Wheat bran was ground to no greater than 0.5 mm in diameter using the rotor beater mill. Moisture content of duplicate samples was measured by AACC Moisture method 44-15A.

High Shear Mixer Treated Bran—Abbreviated as "Mixer-C"

Wheat bran was added to distilled water to make a liquid mixture that contained 5% dry matter. The slurry was incubated at 60 °C and 160 rpm for 24 hrs using the incubator, and diluted with distilled water to 2%. The diluted slurry was high-shear mixed at the highest throughput for 5 min. The viscosity of the slurry was measured using RVA, and was freeze-dried.

High Pressure Homogenized Bran—Abbreviated as "HPH-C"

Wheat bran was added to distilled water to make a liquid mixture that contained 5% dry matter. The slurry was incubated at 60 °C and 160 rpm for 24 hrs using the incubator, and diluted with distilled water to 2%. The diluted slurry was high-pressure homogenized by being fed to the microfluidizer at 2.3k psi first through the 200 µm

chamber, then the 100 µm chamber. The collected slurry was fed back to microfluidizer to be processed three times in total. Then the homogenized slurry was freeze-dried.

Alkaline, Mixer, and HPH Treated Bran—Abbreviated as "AMH"

Wheat bran was added to NaOH water solution to make a slurry that contained 5% dry matter. The slurry was incubated at 60 °C and 160 rpm for 24 hrs using the incubator, and diluted with distilled water to 2%. The diluted slurry was high shear mixed as was Mix C, and then high-pressure homogenized by being fed to the microfluidizer at 2.3k psi first through the 200 µm chamber, then the 100 µm chamber. The collected slurry was fed back to the microfluidizer to be process three times in total. Then the homogenized slurry was freeze-dried. As various concentration of the NaOH solution was used for optimization purpose, the wheat bran sample treated with 0.05 N NaOH was abbreviated as "AMH-0.05"; then samples treated with 0.075N, 0.1N, 0.2N, 0.3N, 0.4N, 0.5N NaOH were in turns named "AMH-0.075", "AMH-0.1", "AMH-0.2", "AMH-0.3", "AMH-0.4", and "AMH-0.5". An MH-C was made by incubating bran with distilled water before mechanical treatments as described above.

Alkaline, Mixer, HPH, and Xylanase Treated Bran—Abbreviated as "AMHE"

Wheat bran was added to NaOH water solution to make a slurry that contained 5% dry matter. The slurry was incubated at 60 °C and 160 rpm for 24 hrs using the incubator, and diluted with distilled water to 2%. The diluted slurry was high shear mixed as was Mix C, and then high-pressure homogenized by being fed to the microfluidizer at 23k psi first through the 200 µm chamber, then the 100 µm chamber. The collected slurry was fed back to the microfluidizer to be process three times in total. Then the homogenized slurry was freeze-dried. The freeze-dried sample was re-diluted with sodium acetate

buffer to contain 10% dry matter in a sterilized beaker, followed by adding BX/AN Xylanase powder to make a 0.3% enzyme solution (0.144g enzyme powder/ 480 ml buffer). The mixture was incubated at 60°C and 160 rpm for 24 hrs, and then freeze-dried.

Evaporated-dried AMH-0.1 Bran—Abbreviated as "AMHEv"

Instead of totally freeze-dried, the slurry of AMH-0.1 bran was evaporated until having 40% water left, then freeze-dried.

Statistics Analysis

Each analysis was conducted in duplicate. Data were subjected to analysis of variance (ANOVA). Means were separated using Fisher's protected least significant difference (LSD) test at P = 0.05.

5 SEM Study of Surface Features

Scanning Electron Microscope (SEM) enables us to directly observe the modification on wheat bran surface by processes in this study. The expected possible changes include signs of dissolution, degradation, cleavages, holes, roughness, and smoothness. Some of the explanations might be breakages of inter- and intra-molecular linkages to various extents by physical or chemical forces, or enzymatically. The modifications of surface features could cause changes in WRC, viscosity, particle size distribution. Studying the SEM images can acquire an intensive and fundamental understanding of other changes.

5.1 Materials and Methods

Five samples observed with a Hitachi S3500N variable pressure scanning electron microscope. The samples are Control, Mix-C, HPH-C, AHM, and AHME. Samples were freeze dried or oven dried, then sputter coated.

Three levels of magnification: 80, 800, and 2000 times of the original object were used.

5.2 Results and Discussion

Sample images were compared at the same magnification level.

Fig. 1 80× SEM image of Control

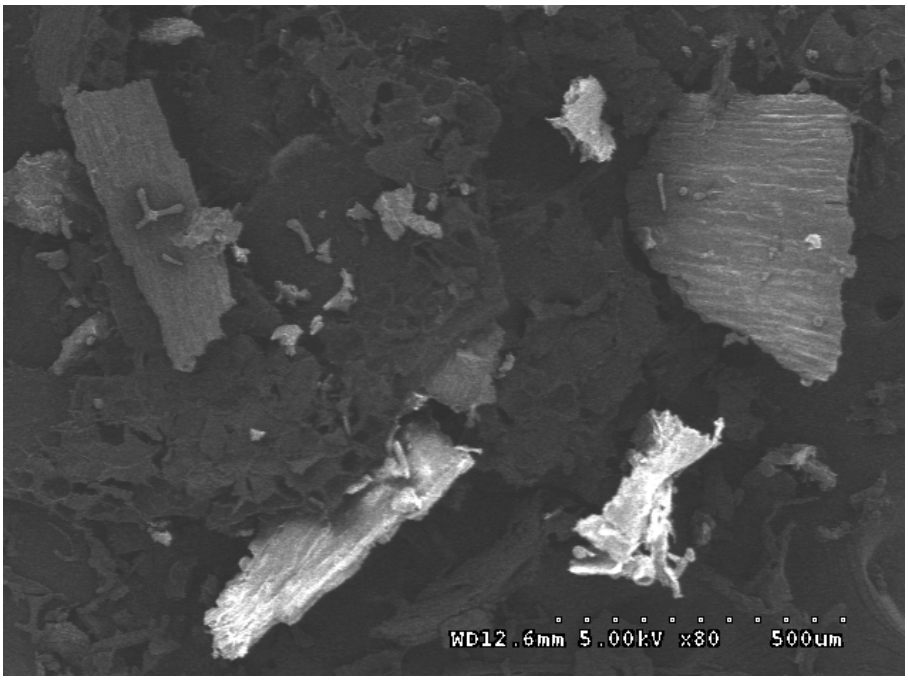

Fig. 2 80× SEM image of Mixer-C

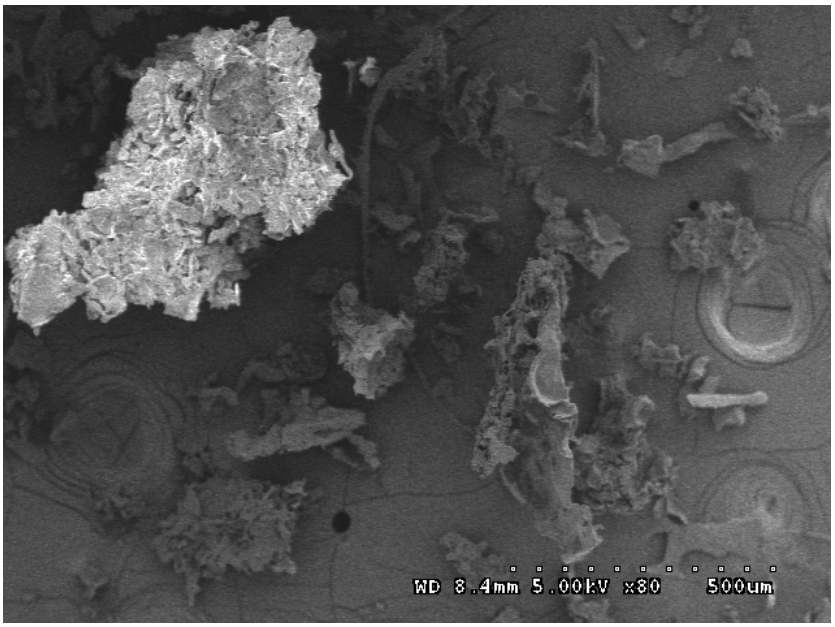

Fig. 3 80× SEM image of HPH-C

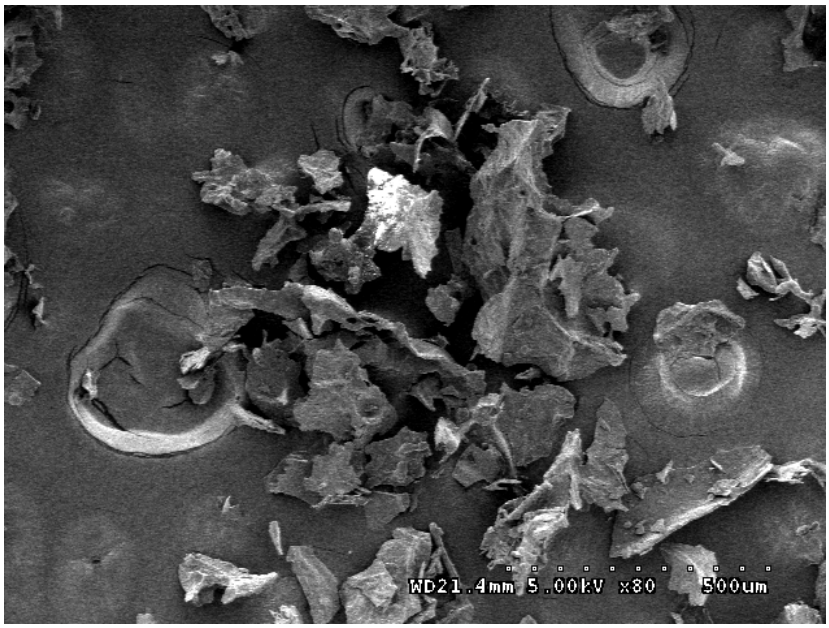

Fig. 4 80× SEM image of AMH-0.1

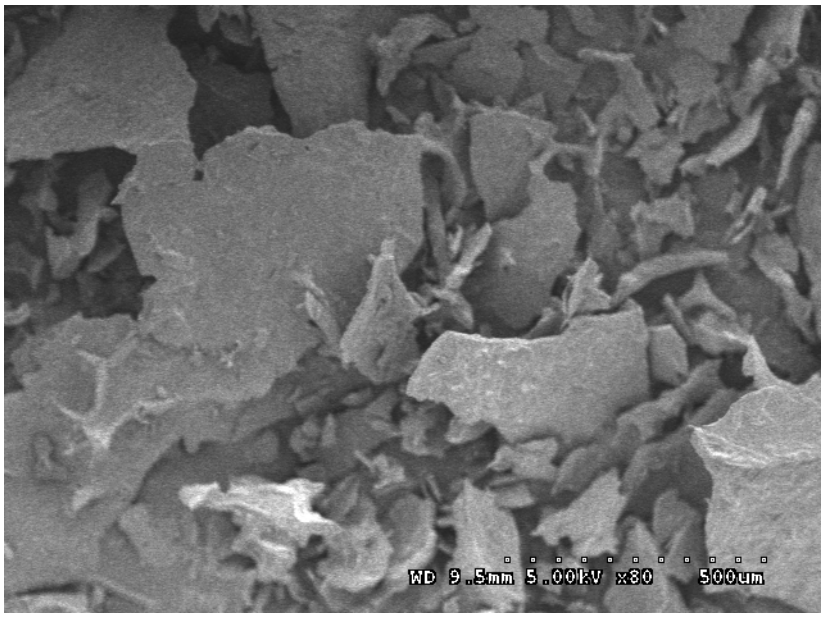

Fig. 5 80× SEM image of AMHE

Fig. 1, 2, 3, 4, and 5 are 80× SEM images in the sequence of Control, Mixer-C, HPH-C, AHM, and AHME. Control in Fig. 1 appeared as large, intact pieces with relatively fuzzy edges. In Fig.2, the particle size of the high shear mixed wheat bran dropped to 1/10 to 1/5 of untreated bran. The edge of each piece was clearer and less angular than Control. The sharp cutting of high shear mixer might be the reason of clean and regular edges. The existence of attached granules indicated no sigh of degradation. In Fig. 3, the particle size of HPH bran was reduced drastically. Tiny pieces of bran clustered to large and high rough granules. The edge of bran was fuzzy, thus did not show sign of degradation. In Fig 4, the particle size of AMH bran was even smaller than HPH-C in Fig. 3, which indicated the use of sodium hydroxide might increase or deepen cleavages. The edge of wheat bran pieces were clean and free of almost all attachments, which suggested cutting of links by alkaline degradation and dissolution of the end of polymer chains. The relatively tiny pieces of bran did not cluster as HPH-C did. Interestingly, in Fig.

5, AMHE bran appeared larger in particle size than AMH in Fig. 4. The surface was very smooth with clean and round edges. It showed that the AX/BN xylanase dissolved the loose end of bran polymer chain with higher efficiency than alkaline. And some linkages between sides of “folding” that was generated by mechanical forces could also be degraded by enzyme, which split the clustered pieces of bran, and made it look bigger.

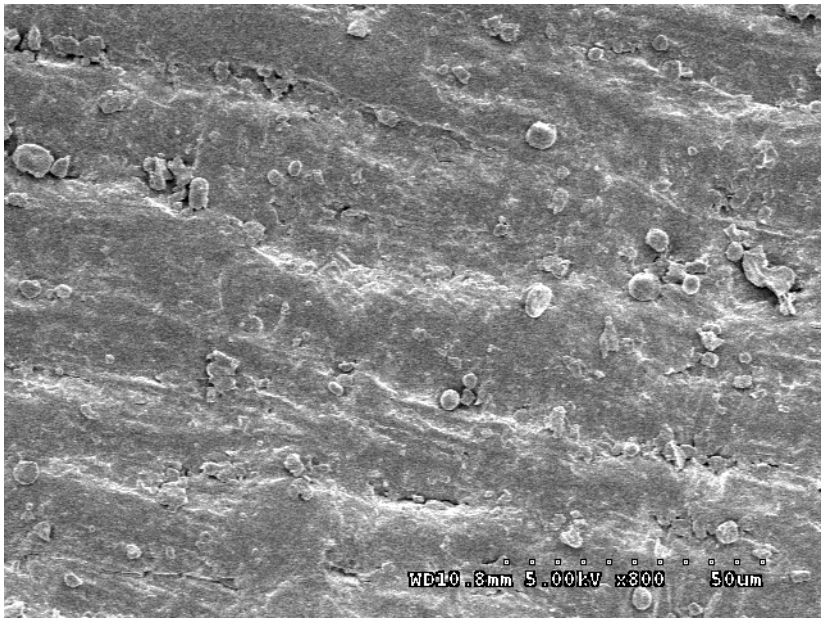

Fig. 6 800× SEM image of Control

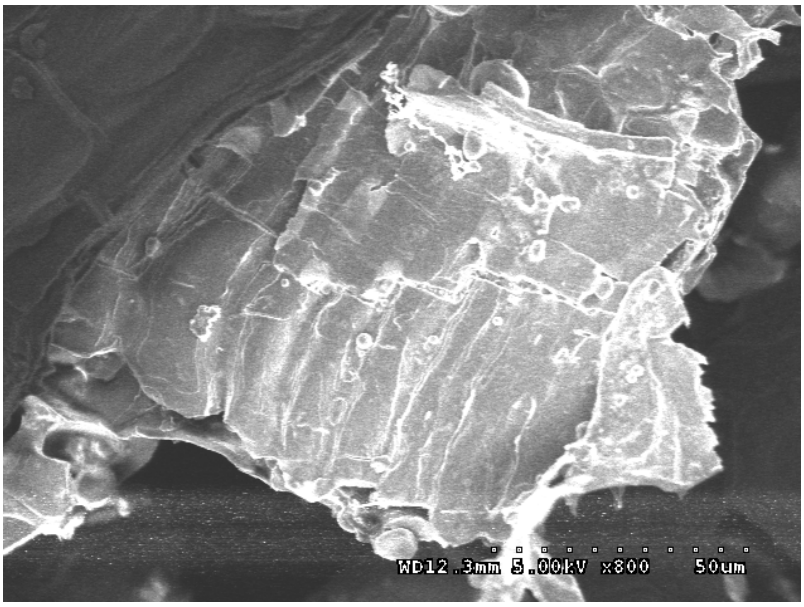

Fig. 7 800× SEM image of Mix-C

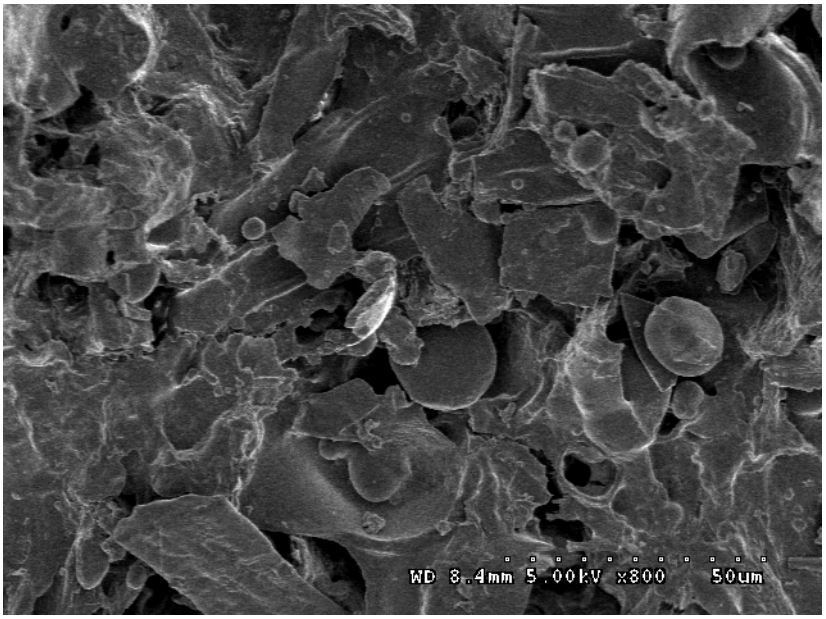

Fig. 8 800× SEM image of HPH-C

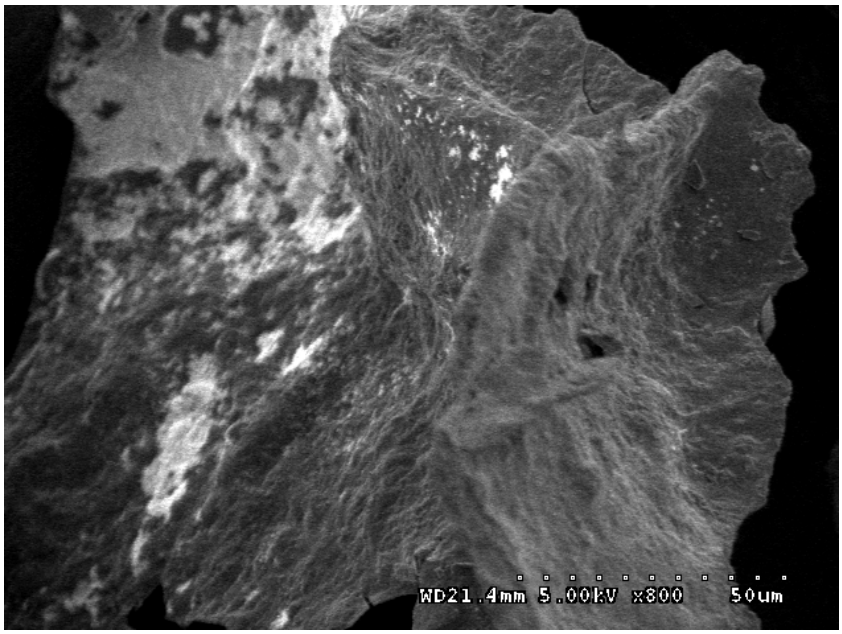

Fig. 9 800× SEM image of AMH-0.1

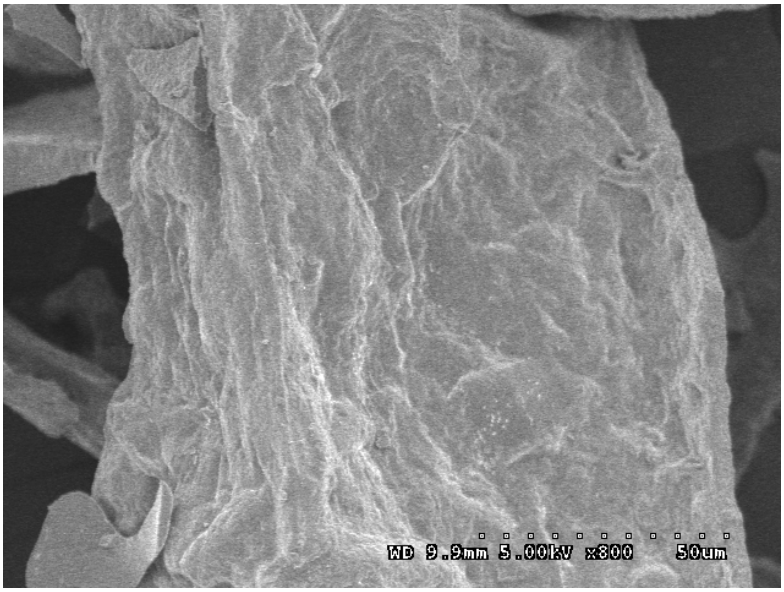

Fig. 10 800× SEM image of AMHE

Fig. 6, 7, 8, 9, 10 are 800× SEM images in the sequence of Control, Mix-C, HPH-C, AHM, and AHME. The 800-time magnification enabled the observation of length and depth of cleavages on bran surface. In Fig. 6, control bran appeared with very few incontinuous cleavages along the natural veins. In Fig. 7, Mix-C bran had long, continuous and deep cleavages all over bran fragments, exposing layers in bran texture. In Fig. 8, HPH-C bran did not show many cleavages, probably because that bran fragments already broke along the cleavages into smaller fragments. In Fig. 9, AMH bran showed a cleavage condition similar to HPH-C bran, but with a unique feature: holes penetrating shallow or deep into the smooth bran surface. The holes reflected degradation caused by the alkali treatment. In Fig. 10, interestingly, although AMHE went through all the processing units that AMH had, AMHE bran did not show deep holes or as many distinct cleavages as AMH did.

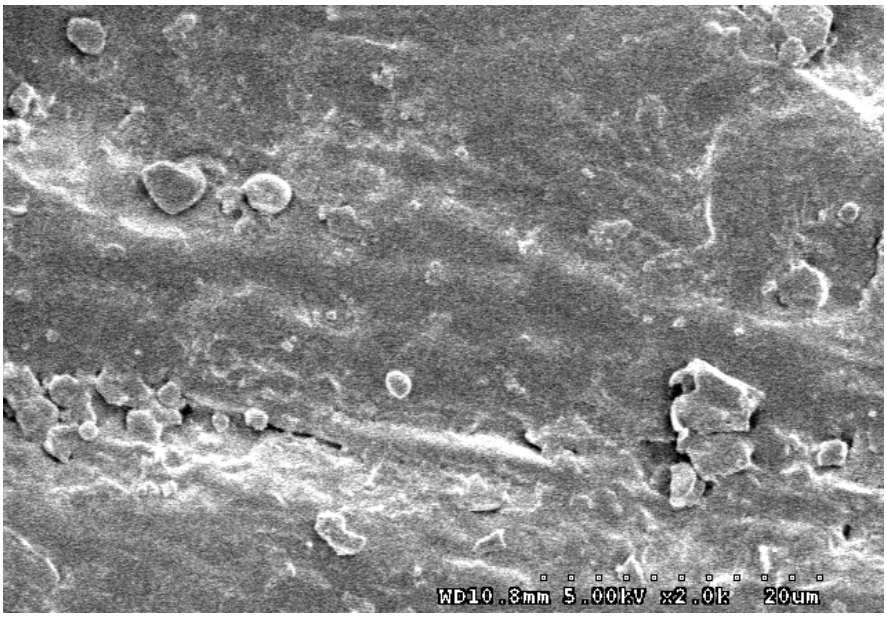

Fig. 11 2000× SEM image of Control

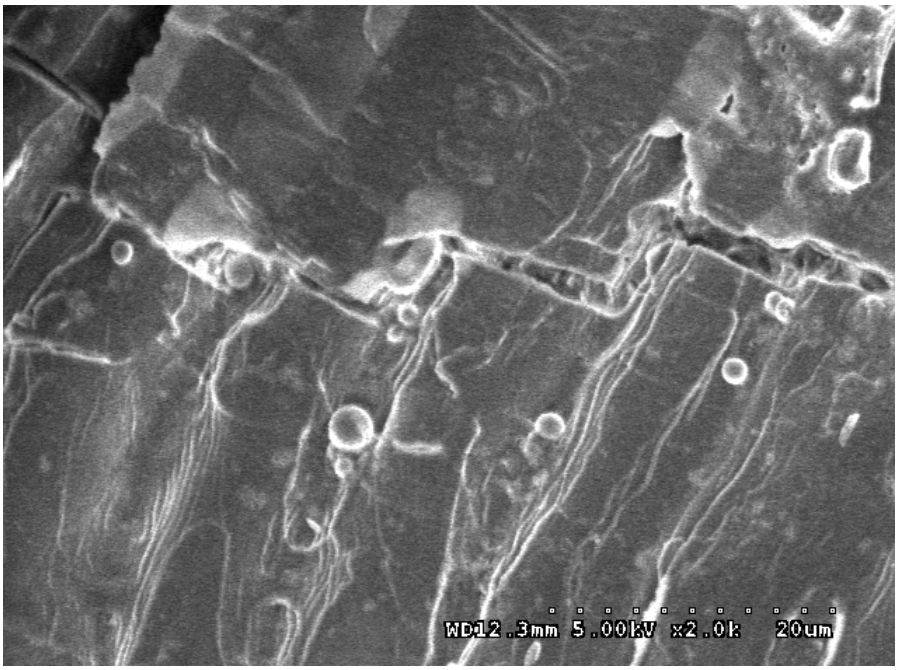

Fig. 12 2000× SEM image of Mix-C

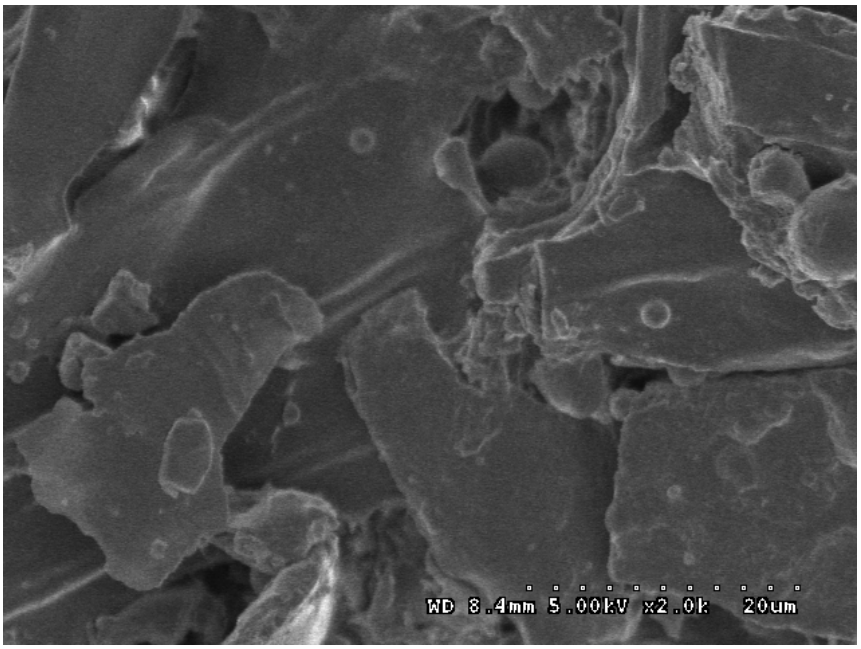

Fig. 13 2000× SEM image of HPH-C

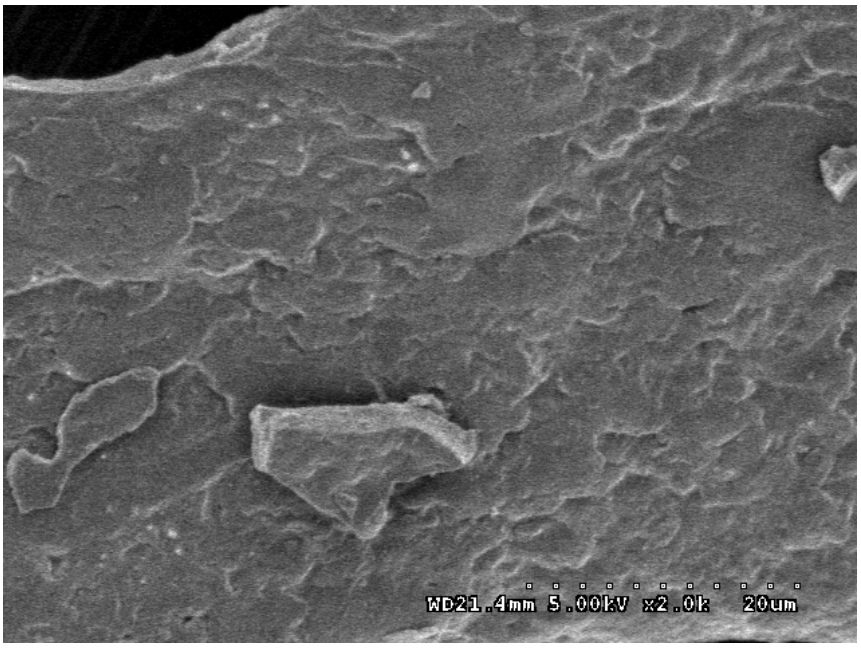

Fig. 14 2000× SEM image of AMH-0.1

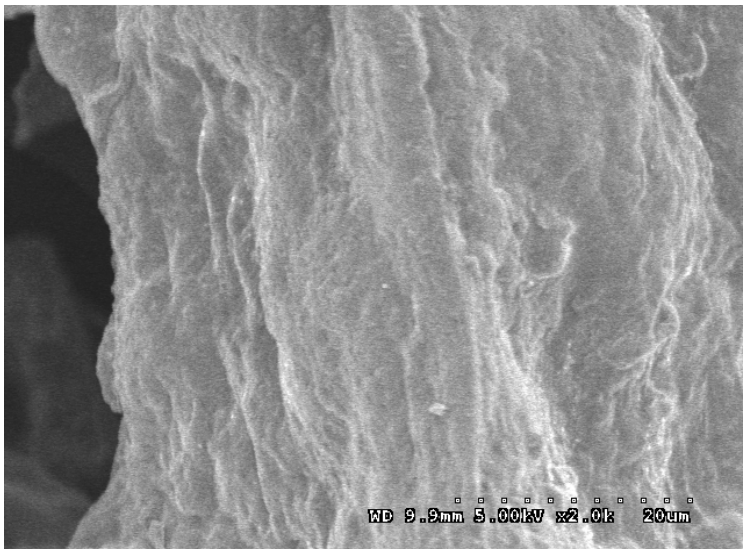

Fig. 15 2000× SEM image of AMHE

Fig. 11, 12, 13, 14, and 15 are 2000× SEM images in the sequence of Control, Mix-C, HPH-C, AHM, and AHME. The 2000-time magnification enabled the observation of pores and degree of flatness of bran surface.

In Fig. 11, the surface of control bran was flat, smooth, free of pores resulted degradation, and had sporadic attached starch pellets. In Fig. 12, the high shear mixing engraved intensive cracks in Mix-C bran, left a very coarse and multi-layered cutting edge. Ripple-like strips appeared on the surface along naturally-happened veins, indicating minor squeezing and folding forces from high shear mixing. In Fig. 13, high pressure homogenization gave bran very smooth surface texture, some highly coarse cutting rims, and some very abrupt cutting edges. In Fig. 14, the surface of AMH bran was far less smooth or flat than Control or mechanically treated bran. The “peeled-looked” surface was densely covered by scallop-shaped and multi-layered signs of alkaline corruption. Tiny pores were found all over the layers. In Fig. 15, the AMHE bran also had a “peeled” look, but a pronounced difference from Fig. 14 was that after

enzyme treatment, most of the layers and pores on the AMH bran disappeared, although the back frame of layers were still there. The enzyme dissolved surface was relatively round and smooth.

5.3 Conclusion

The 80-time magnified images of control and processed wheat bran provided a clue of how processes physically cut and folded bran fragments. Both Mixer-C and HPH-C were incubated in distilled water before proceeding to mechanical particle size reduction, but the incubation did not leave any sign of degradation. High shear mixing left multi-layered and multi-angular cutting edges to bran fragments though did not create folding or clustering macrostructures. High pressure homogenization produced bran fragment with even particle size much smaller than high shear mixing product. The difference is the bran edges after high pressure homogenization were relatively cleanly cut, and hundreds of tiny pieces of bran fragment agglomerated into balls. There were several special features about AMH bran. Alkaline treatment caused smoothness on the bran surface, and fuzziness at the edges, which were signs of degradation. Individual bran fragment was imparted folding structures. Although particle size of AMH bran was even slightly smaller than HPH bran, bran pieces scattered evenly in the microscope image without apparent clustering. AMHE bran showed similar scatterness and sign of degradation to AMH bran, but the folding structures seemed to be “flattened” by enzyme treatment.

The effects of mechanical processes, alkali treatment and enzyme degradation on forming or “flattening” cleavages could be studied in 800× SEM images. High shear

mixer cut deep and long cleavages all over the bran without breaking the bran along the cleavages. High pressure homogenization broke bran almost abruptly at one stroke, thus did not create many cleavages during the particle size reduction. Alkali seemed to penetrate holes over the bran surface. Enzyme appeared to smoothen the bran surface by partially dissolving the edges of cleavages or holes. In terms of surface area, high shear mixing, high pressure homogenization and alkali treatment all hugely increase bran surface area. It can also be estimated that the surface area enlargement due to the combination of three treatments would higher than any of the individual treatments, because each treatment increase surface area through different ways. Enzyme treatment on the other hand could have both effects, either decreasing surface area due to “flattening”, or increasing by degrading edges of bran. Whether the final effect of enzyme treatment is to increase or decrease surface area would need further research. Surface area is very important feature because it is closely associated with other characteristics such as water retention capacity, viscosity, and cholesterol binding capacity.

The chemical-mechanical combined treatment caused the highest degree of roughness on wheat bran surface by reserving the strengths of three individual treatments. The effects of high shear mixing and high pressure homogenization on extending bran surface area might not be comparable because the roughness they caused was not the same type. Although high pressure homogenization created more bran rims by breaking the bran more thoroughly and thinly, it had adverse effects. The hitting occurred in microfluidizer sometimes was too quick and powerful that it failed to

tear the bran layers apart and left breaking edges sharp. The strength about high shear mixer was that the cleavages it created usually exposed many layers in the bran, although the cleavages were not deep enough to make the bran crack into pieces. Alkali degraded the end of polymer chains, which deepened the exposed layers and created pores penetrating in the layers. Enzyme treatment, interestingly, flattened the bran surface by dissolving the rims of the layers and pores formed during previous treatments. Both mechanical treatments and alkali treatment added to the degree of roughness, and in varying ways respectively. Thus the combination of the mechanical and alkali treatments achieved the highest surface area. However, the enzyme treatment functioned adversely in terms of surface area, and potentially of water retention capacity and viscosity.

6 DF Solubility

6.1 Materials and Methods

6.1.1 Materials

Bran samples: Control, AMH-0.05, AMH-0.075, AMH-0.1, AMH-0.2, AMH-0.3, AMH-0.4, AMH-0.5.

Enzyme kit (Dietary Fiber Enzyme Kit, Megazyme Lt. Co. Wicklow, Ireland), including thermal-stable amylase, proteinase and amyloglucosidase

1-methylimidazole (Sigma-Aldrich, 67560, Milwaukee, WI, USA)

+/- 2-Octanol (Sigma-Aldrich, 74860, Milwaukee, WI, USA)

Sodium borohydride (Sigma-Aldrich, 71321, Milwaukee, WI, USA)

D-Allose -internal standard (Sigma-Aldrich, 285005, Milwaukee, WI, USA)

Acetic anhydride (Sigma-Aldrich, 33214, Milwaukee, WI, USA)

D-Glucuronic acid (Sigma-Aldrich, G5269, Milwaukee, WI, USA)

3, 5-Dimethylphenol (Sigma-Aldrich, 144134, Milwaukee, WI, USA)

Sulfuric acid: 12M and 18M (Fisher Chemicals, Pittsburgh, PA, USA)

Hydrochloride: 0.561N and 5% (Fisher Chemicals, Pittsburgh, PA, USA)

Sodium Hydroxide: 5% (Fisher Chemicals, Pittsburgh, PA, USA)

MES-TRIS blend buffer: 0.5M (Dissolve 19.52 g 2(N-morpholino) ethanesulfonic acid(MES) (Sigma-Aldrich, M8250, Milwaukee, WI, USA) and 14.2 g tris (Hydroxymethyl) aminomethane (TRIS) (Sigma-Aldrich, TI 503, Milwaukee, WI, USA) in 1.7 L deionized water. Adjust pH to 8.2 with 6.0 N sodium hydroxide).

Monosaccharide standards: Xylose, Galactose, Arabinose, Mannose, Glucose (Sigma-Aldrich, Milwaukee, WI, USA)

Enzyme purity test samples from Megazyme Lt. including citrus pectin, β- glucan (barley), wheat starch, casein, and high amylose starch.

6.1.2 Methods

1. Enzymatic treatment

1) Samples

Mark and weigh needed number of 50 mL centrifuge tubes with cap. Weigh duplicate 0.2500±0.0005g samples accurately into the centrifuge tubes. Add 10 ml MES-TRIS blend buffer solution to each centrifuge tube. Stir on vortex until sample is completely dispersed in solution.

2) Incubation with heat-stable α-amylase

Add 12.5 µL heat-stable α-amylase solution, while stirring at low speed. Place capped samples in shaking water bath at 95-100^{o}C, and incubate for 35 min with continuous agitation. Start timing once all tubes are in the hot water bath.

3) Incubation with Protease

Remove samples from hot water bath and cool to 60 ^{o}C. Scrape bran rings around tubes, and rinse side wall of tubes with 2.5 ml distilled water. Add 25 µL protease solution to each sample, put on the caps, and incubate in shaking water bath at 60± 1 ^{o}C with continuous agitation for 30 min. Start timing when temperature of water bath reaches 60 ^{o}C.

4) Incubation with amyloglucosidase

Remove samples from water bath, dispense 1.25 mL of 0.561 N HCl solution into samples while stirring. Check pH, which should be 4.1- 4.8. Adjust pH if necessary, with additional 0.5% NaOH solution or 5% HCl solution. Add 50 µL Amyloglucosidase solution while stirring on vortex. Place cap on and incubate in shaking water bath at 60 ^{o}C for 30 min with continuous agitation. Start timing when temperature of water bath reaches 60 ^{o}C.

2. Insoluble fiber and soluble fiber separation

Centrifuge the enzyme treated sample at 13800g for 20 min. Collect the supernatant to 150 ml tall form beaker with pipette. Dry the residue in 65-70^{o}C oven until appear dry.

3. Precipitation of soluble fiber

Add 60 ml 99% ethanol that is preheated to 60 C to the supernatant, mix, and let precipitate for 1 h at 4 C. Discard about 40 ml of upper supernatant, transfer the rest to 50 ml centrifuge tube, centrifuge, and discard the supernatant.

4. Wash of soluble and insoluble fiber

Wash pellet twice by suspending and re-centrifuging with 20 ml 80% ethanol, and then twice with 15 ml acetone. Before the last centrifuge, make sure as little material as possible remains on tube walls. Let residue dry completely overnight in 40C oven. Mix tube contents initially with round –melted glass rod when drying begins to avoid formation of compact residue. Keep glass rod in tube.

5. Acid hydrolysis of both fiber components

Add 2 ml 12 M sulfuric acid to dried residue, and immediately disperse it by vortex mixing or magnetic stirring. Leave at 35 C for 1 h, with mixing (occasional or continuous) to disperse cellulose. Rapidly add 22 ml water, cap tube, and mix, place in boiling water for 2h from reboiling and stir continuously; then cool to room temperature.

6. Filtration of Klason lignin

Run the hydrolizate through coarse fritted glass crucible, with filtrate into 25 ml volumetric flasks. Remove the volumetric flasks and make it 25 ml. Wash the residue twice with 95% ethanol and acetone. Dry the crucible and residue 1 h in desiccant, weigh. Put the crucible with residue in furnace at 500 C for 1 h. Cool crucible in desiccant for 1 h and weigh. The difference in weight is Klason lignin.

7. GC determination of Neutral Sugars

Collect 1 mL from both soluble and insoluble fiber cooled hydrolysate (total volume 25 ml), add 0.5 mL internal standard (1mg allose/mL in 50% saturated benzoic acid). Add 0.2 mL 12M $NH_3 \bullet H_2O$ and 0.005 mL octan-2-ol as surfactant, mix. Test the solution is alkaline, if not, add slightly more Ammonium hydroxide. Then add 0.1 mL 3M $NH_3 \bullet H_2O$ containing 100 mL sodium borohydride/ mL. Mix, leave for 1 h at 40 ^{o}C, add 0.1 mL glacial acetic acid, and mix. To 0.2 mL acidified solution, add 0.3 mL 1-methylimidazole and 2 mL acetic anhydride, and mix. After 10 min at room temperature, add 5 ml of water, mix, and when cooled add 1 ml of dichloromethane, agitate the contents vigorously on a vortex mixer and centrifuge for a few minutes to separate the mixture into two phases. Remove the upper phase by freezing at -80C and picking the ice out. Transfer lower phase to small vial for analysis by GC.

8. Determination of Uronic Acids

Put 1 mL of this solution in 20 ml test tube, and add 1.5 ml distilled water to dilute 2.5 times, stir. Mix 0.3 mL of this diluted solution with 0.3 mL of a solution containing 2 g sodium chloride and 3 g boric acid/100 mL in 50 mL centrifuge tube. Add 5 mL 18 M sulfuric acid slowly, and vortex mix. Place in water bath at 70 ^{o}C for 40min; then cool to room temperature in water.

When cool, add 0.2mL dimethylphenol solution (0.01 g in 100 mL glacial acetic acid), and vortex mix immediately. After 10-15min, read absorbance at 400 and 450 nm against water reference. Subtract reading at 400 nm from that at 450 nm. Plot difference in absorbance obtained for glucuronic acid standards over the range 0.025-0.125 mg/mL. Calculate sample concentrations, or read from graph.

9. Calculation of GC results

Due to losses of monosaccharides occurring during acid hydrolysis response factor is used to convert the experiment value to actual content. Response factor for individual sugars obtained from calibration run with a standard sugar mixture acid-hydrolyzed in parallel with test samples.

The amount of individual neutral sugars in g/ 100g sample weight is calculated by using the following formula:

AT× WI× 100× RF× 0.89/AI× WT

AT and AI are peak areas of the test sample and internal standard, respectively; WT and WI are the weight (mg) of the test sample and the internal standard, respectively; and RF is the response factor.

10. Calculation of soluble and insoluble fiber

Soluble fiber= neutral sugars + uronic acids in the supernatant of first centrifugation

Insoluble fiber= Klason lignin+ neutral sugars+ uronic acids in the residue of first centrifugation

6.2 Results and Discussion

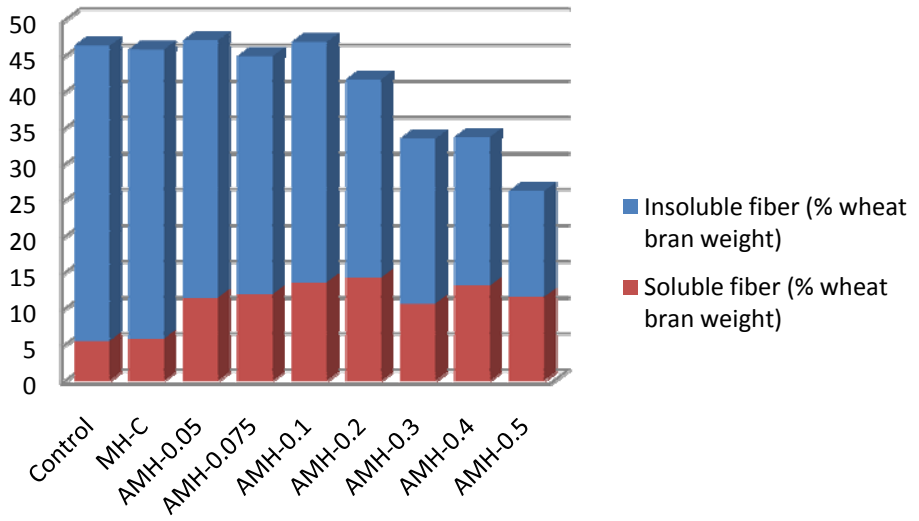

Fig. 16 Effect of processing on fiber solubility. Percentage of sample weight vs. types of sample (explanation of abbreviation shown on page 15-17)

Fig.16 showed that the impact of alkali solution concentration had clear tendencies on both the solubility and the total yield of wheat bran DF. On total DF yield, although soaking bran in distilled water resulted in a minor loss of total DF, soaking in alkali solution at low concentration of 0.05N, 0.075N and 0.1N gave marginally higher fiber yield than control bran. The yields at these three concentration levels were around

47%, while difference among levels was not significant. However, DF yield decreased quickly and linearly from 47% to 26% when NaOH concentration rose from 0.1N to 0.5N.

On fiber solubility, the portion of soluble increased drastically when NaOH solution is used for soaking. Before NaOH is used, the soluble fiber in control bran weighed 12% in total fiber. The value was 13% in water-soaked bran. When 0.05N NaOH to 0.5N was used, soluble fiber portion in total fiber increased from 24% to 44%. However, the decreased total fiber yield compromises the soluble fiber yield to different extents. Among all concentration levels, 0.2N NaOH treated bran gave the highest 15% soluble fiber yield and an 42% total fiber yield out of bran material, and 0.1N NaOH treatment gave the second highest 14% soluble fiber yield and the highest 47% total fiber yield.

The increase in fiber solubility could be explained by the alkali's effect on solubilization of bran matrix. (Vermaa et al., 2009)Theoretically, alkali solution saponifies bound acetic acid and phenolic acids, and may cleave linkages between lignin and other cell wall constituents. It may also loosen and open the inter- or intra-molecular bonds in or between hemicelluloses, lignin, protein and silica, which lead to dissolving and swelling of cell walls. The increase in total fiber at low NaOH concentration may be due to the degradation of non-fiber substance to fiber. And the total fiber loss at high NaOH concentration may be because of the over-solubilization of fiber constituents. The effect of water soaking on the slight increase in soluble fiber and loss in total fiber may be because of the solubilization and partial degradation of fiber constituents by water-activated endogenous-enzymes in wheat bran.

6.3 Conclusion

The data shows that alkali has double effects on wheat bran. NaOH from 0.05N-0.5N can significantly increase the fiber solubility. The effect is positively correlated to NaOH concentration. However, NaOH from 0.2N-0.5N also causes considerable loss of total fiber, which indirectly compromises the soluble fiber yield. The loss increases with the concentration of NaOH. This indicates that a middle concentration might be an optimal choice for production of both soluble fiber and total fiber. Considering the cost of NaOH in processing, 0.1N might be the optimal concentration in manufacturing in this study.

7 Viscosity

The change of viscosity during processing is crucial to DF quality, because soluble DF is only physiologically effective when viscosity is high. Since original wheat bran soluble DF was proved effective, if the viscosity was raised during processing, the resulted soluble fiber would be even healthier.

7.1 Materials and Methods

The nine samples tested for viscosity were Control, MH-C, AMH-0.1, AMH-0.2, AMH-0.2, AMH-0.4, AMH-0.5, AMHE, soluble DF from control bran (SDF-O), and soluble DF from AMH-0.1.

Viscosity was measured on the RVA.

1. As-is samples from intermediate processing steps at 2% dry matter were stirred at 160rpm at 37°C until viscosity stabilized (usually after 2-3 minutes) and a 3 minute average viscosity was taken.

2. Dried samples were used at 6% dry matter suspensions, stirred at 160 rpm 37°C, and a three minute average viscosity was taken in the stable area from minutes 5 to 8.

7.2 Results and Discussion

Results were shown in Fig. 17.

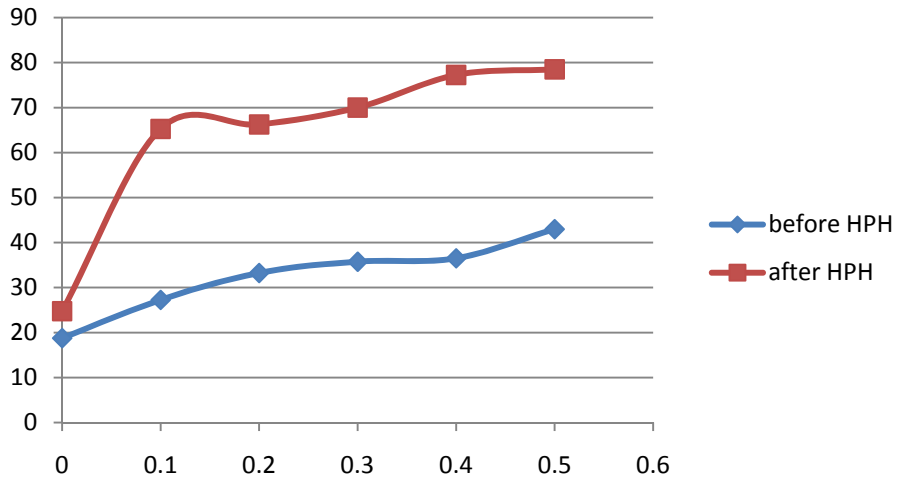

Fig. 17

Effect of processing on viscosity. Viscosity value (cp) vs. concentration of alkali (N)

The overall trend was that viscosity was positively correlated with alkali concentration. The usage of sodium hydroxide at 0.1N immediately raised the viscosity of Control by 165%. Then from 0.1N to 0.3N, viscosity increased slowly. From 0.3N to 0.4N, viscosity showed a small hop from 180% to 212% increase. From 0.4N to 0.5N, viscosity reached a plateau, which was 215% higher than Control. Although 0.4N and 0.5N treatments achieved significantly higher viscosity than 0.1N-0.3N, this advantage did not seem to be able to balance the loss of soluble DF at these two high concentrations. Since the yield of soluble DF is of higher priority, where 0.1N is favorable, the 180% viscosity increase at 0.1N would be counted into the quality of product DF.

7.3 Conclusion

Viscosity of soluble DF extracted from AMH-0.1 and Control showed directly the effects of processing. The 2% solution of soluble DF from both brans did not appear very viscous. But when the viscosity value of pure water was subtracted, the difference was

obvious. The relative-to-water viscosity of Control soluble DF was 1 cp, while processed soluble DF was 7.5 cp. Thus it could be concluded that the AMH combined processing can very effectively increase soluble DF along and soluble DF in bran matrix.

Another noteworthy fact was the difference between viscosity test results before and after high pressure homogenization. Two viscosity values were obtained for each sample, one after high shear mixer and the other after high pressure homogenization. The differences of these two values for all AMH samples did not vary a lot. The value range of the differences was from 33 cp to 40 cp, which was about half the total value range of 65 cp to 78 cp. The large portion of the increase indicated that high pressure homogenization was very effective on viscosity. The stable viscosity increase from that of high-shear mixed samples indicated that the difference of viscosity among samples might mainly be due to alkaline concentration instead of mechanical treatments. The reason why viscosity was not taken before high shear mixer was that slurries before high shear mixer was not even, thus unstable reading could result.

In the comparison of with and without enzyme treatment, viscosity of enzyme treated AMH sample was slightly (3%) lower than AMH bran. The result agreed with prediction based on SEM observation.

8 Water Retention Capacity

8.1 Materials and Methods

The standard AACC Method 88-04 (1983) for water binding capacity by centrifuge method was adopted with a smaller sample size. Processed bran (0.5 g, dry basis) was weighed into a tared 50 ml centrifuge tube. Distilled water (20ml) was added. The processed bran was dispersed with a glass pod and shaken vigorously. The centrifuge tube was capped and the mixture was allowed to rest for 1 hour. The mixture was then centrifuged for 10 min at 1,000 × g before the supernatant was decanted. The centrifuge tube was drained for 10 min by leaning downward at a 45-degree angle. The centrifuge tube inner and outside walls were wiped dry before weighed. The water retention capacity was calculated as the gain in weight (g) per gram of dry weight of the processed bran. Duplicate or triplicate was made to acquire a less than 0.5% standard deviation.

Two groups of samples were tested for WRC. The first group, which reflected the difference in effect of treatment types, included Control, Mix-C, HPH-C, AMH, AMHE, and AMHEv. The second group, which reflected the difference in effect of alkali concentration in AMH treatments, included AMH-0.05, AMH-0.075, AMH-0.1, AMH-0.2, AMH-0.3, AMH-0.4, AMH-0.5.

8.2 Results and Discussion

8.2.1 Effects of treatment types on WRC

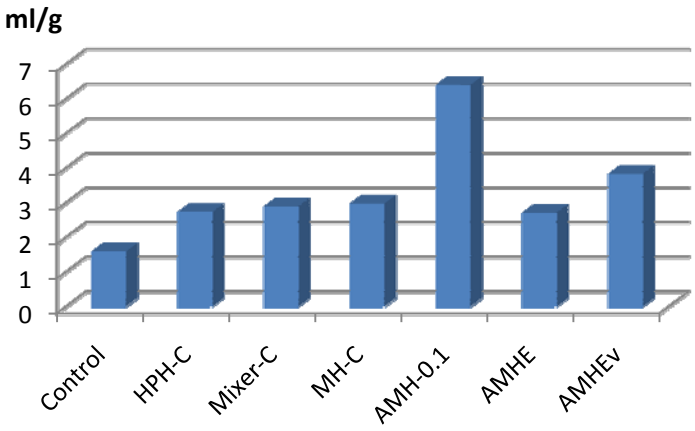

Fig. 18 Effect of processing types on WRC,

Water (ml) absorbed per gram of sample vs. sample type (explanation of abbreviation shown on page 15-17)

Individual treatments used in the study all increased wheat bran WRC by around 100%. High pressure homogenization and high shear mixing had very similar impact on WRC. However, the combination of two treatments did not show significant difference than individual treatments along. Alkali and mechanical treatments together reached 500% increase. Nevertheless, enzyme treatment after AMH showed negative effect. It decreased the WRC from 6 ml/g to 2.3 ml/g, which was about the same level as HPH-C or Mix-C. The down-stream processing: evaporation had adverse impact on WRC by decreasing the WRC of freeze-dried AMH bran by almost a half.

The WRC of freeze-dried AMH bran was the highest among individual or combined treatments used in this study. But the peak WRC from this combination was not simply the sum of WRC of three individual treatments. High shear mixing and high pressure homogenization generated a rise of 100%, respectively. But the two

treatments combined only gave slightly higher WRC than either of the two. When alkali treatment was applied prior to the two mechanical treatments, the result almost tripled that of two mechanical treatments together. In this sense, mechanical treatment had a limit in improving the WRC of wheat bran, and the way alkali treatment functioned was beyond what merely mechanical treatments were able to do. The application of AX/BN the xylanase, radically decreased the WRC enhanced by previous treatments. Although the value was still higher than Control, it dropped by half from the WRC value of freeze-dried AMH bran. In terms of WRC, enzyme treatment was not an ideal step to add after alkali and mechanical treatments.

8.2.2 Effects of alkali concentration

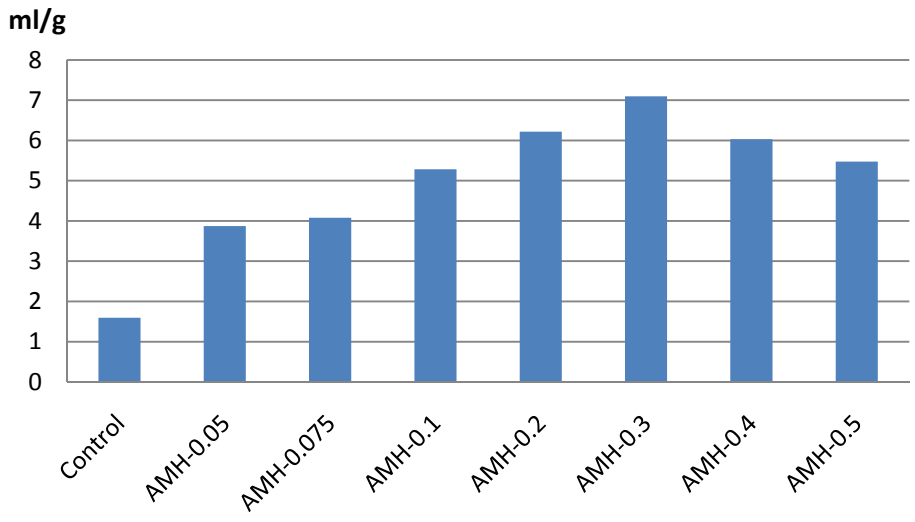

Fig. 19 Effect of alkali concentration on WRC

Water (ml) absorbed per gram of sample vs. sample type (explanation of abbreviation shown on page 15-17)

Effects of alkali concentration on WRC of wheat bran produced from AMH combined treatments was not a consistent trend. From 0N to 0.3N, WRC of bran climbed steadily along the increase of NaOH concentration. The application of as low as 0.05N NaOH immediately raised WRC by 140%. 0.075N NaOH brought a marginal increase from the 0.05N result. When NaOH concentration was raised to 0.1N, the result was 230% higher than Control, and 35% higher than the 0.05N treatment. When NaOH was raised to 0.2N, the result was 280% higher than Control. The increase stopped and peaked at 0.3N, when the rise from Control reached 350%. The WRC started to fall continuously at 0.4N and 0.5N, when the values were 275% and 240% higher than Control.

8.3 Conclusion

The results provided interesting topics for further study. The rise of WRC from high pressure homogenization and high shear mixing suggested that the reduction of particle size of bran might contribute to the increase of WRC. On the other hand, high pressure homogenization produced bran with much smaller particle size than did high shear mixer, but the WRC of bran from these two processes did not show significant difference. This fact indicated that the correlation between WRC and particle size was not linear. As showed in the SEM images, alkaline treatment might severely not affect particle size, but probably increased the surface area. Meanwhile, enzyme treatment may have reduced surface area of bran. The drastic increase of WRC of AMH bran and drop of WRC of AMHE bran suggested that WRC might be correlated to a higher extent with surface area rather than particle size.

The explanation of two-direction results in the second series of tests seemed to be associated with two factors that happened as alkali concentration increased: 1) the increase of surface area, and 2) the decrease of insoluble DF. The factors competed with each other in the way that WRC is positively correlated with both surface area and insoluble DF. The increase of the alkali concentration probably increased the intensity of degradation and dissolution of wheat bran surface. The alkali treatments dissolved the end of polymer chain at the surface of bran, and exposed reactive groups that could bind water molecules. The more reactive groups were exposed, the higher WRC would be. On the other hand, the amount of insoluble DF decreased had a consistent trend to decrease as NaOH concentration increased from 0.1N to 0.5N because of the switch of insoluble DF to soluble DF. Insoluble DF is the only DF portion that is responsible for WRC.

9 Color Measurements

9.1 Materials and Methods

9.1.1 Materials

Control, MH-C, AMH-0.1, AMH-0.2, AMH-0.3, AMH-0.4, and AMH-0.5 were tested.

9.1.2 Methods

A MiniScan XE Plus tristimulus colorimeter (Hunter Associate Laboratory Inc. Reston VA, USA) was used. The freeze-dried bran powders was evenly put on the bottom of the glass measuring cup and mounted to about 1 cm in depth. The L, a, b values of bran samples were taken in triplicate after calibrating the colorimeter with black glass and white porcelain board.

9.2 Results and Discussion

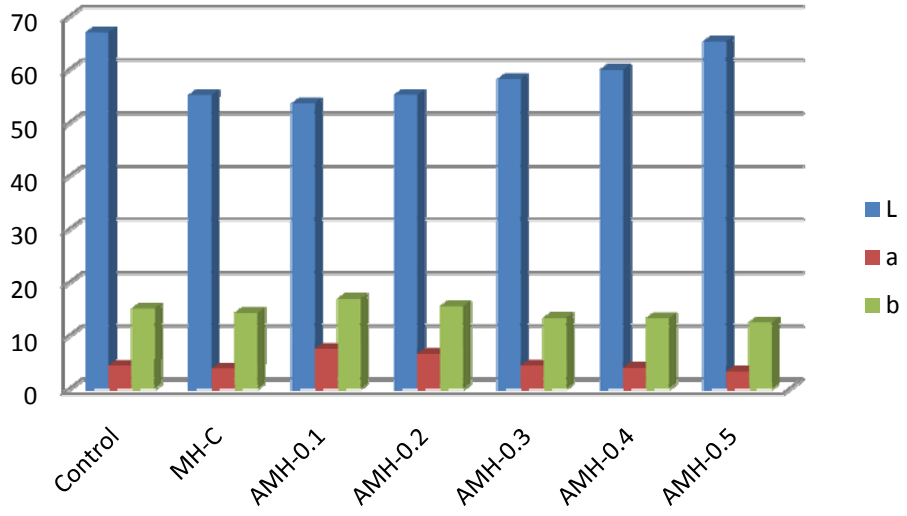

Fig. 20 Effect of alkali concentration on color change

Value of three stimuli vs. sample types (explanation of abbreviation shown on page 15-17)

The change of color resulted from AHM combined treatments were tested using a colorimeter. Control bran, MH-C, AMH-0.1, AMH-0.2, AMH-0.2, AMH-0.4, and AMH-0.5 were studied. Results were shown in Fig.. . In the color measurement system used in this study, L represents brightness; a represents red (+)/green (-); and b represents yellow (+)/ blue (-).

9.3 Conclusion

The trends for three color elements were different but reasonable. Control bran had the greatest brightness. After soaking in distilled water and two mechanical treatments, the brightness decreased and in turn, the bran appeared a little darker. When 0.1N NaOH was applied in soaking solution, the degree of darkness increased. But as the NaOH concentration was raised to 0.2, 0.3, 0.4, and 0.5N, the brightness recovered along with NaOH concentration. Thus 0.1N NaOH pretreatment produced the darkest color.

On the red/green axis, all the bran samples were on the red side. Water-soak pretreatment slightly decreased the redness. NaOH treatment at 0.1N increased the redness to an extent even higher than control. But as NaOH concentration increased, the redness decreased slowly. From 0.3N to 0.5N, the values of redness of processed bran were already lower than Control. In all, 0.1N NaOH pretreatment imparted the highest redness among the samples.

On the yellow/blue axis, all the bran samples were on the yellow side. The trend of change was the same as what happened to the red/green axis. Alkali processing

increased the yellow tint, when 0.1N NaOH produced the highest degree of yellowness among the samples.

The higher degree of dark, red, and yellow tints is considered as color mark of whole grain food products. Processes that enhance such tints would be preferred by manufacturers because the products will win consumers' recognition and favor. In this sense, alkali pretreatment is necessary, and the alkali concentration of 0.1N NaOH is an optimal condition.

10 Conclusion and Future Work

The experimental results suggested that singular or combined treatments discussed in this study are able to improve the nutritional and baking quality of wheat bran through significantly modifying dietary fiber solubility, viscosity, water retention capacity, particle size, and color.

Singular treatments worked in different ways, and had various degrees of impacts on wheat bran properties. Among the treatments, alkali incubation pretreatment played a crucial role in increasing dietary fiber solubility, bran surface area and viscosity. The concentration of sodium hydroxide was the most important parameter in the study. Mechanical treatments including high shear mixer and high pressure homogenization drastically bran decreased particle size, increased surface area, and twisted bran micro-structure. Enzyme treatment on the other hand unexpectedly did not show positive effect. It dissolved the rough ends of bran polymer produced by previous treatments, and unfolded the microstructures. Thus enzyme treatment compromised the retention of total dietary fiber and the increase of surface area.

Combined treatments of alkali, high shear mixer and high pressure homogenization achieved better results than any singular treatment. At the optimal sodium hydroxide of 0.1N which was determined based on dietary fiber yields and solubility, the combined treatment increased 200% of soluble dietary fiber when retaining 100% of total dietary fiber yield. The viscosity of soluble dietary fiber relative to water was increased more than 500%. An improvement in physiological function is expected to due to the viscous fiber increase. Meanwhile, the increase of water

retention capacity and decrease in particle size are expected to contribute to higher quality of bakery products using the processed bran. The darker color resulted from the processing may promote whole grain consumers' recognition.

Future studies in more specific correlations between treatments and physicochemical properties, and between changes of individual physicochemical properties will be helpful in further optimizing wheat bran processing procedures.

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