# IMPROVING THE FUNCTIONALITY AND BIOACTIVITY OF WHEAT BRAN

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# Dedication

This dissertation is dedicated to my two sons, Joshua Carter (J.C.) Petrofsky and Mason Alexander Petrofsky. Boys, as you have noticed, I have been working toward earning my Ph.D. for your entire lives. It has been much longer in fact. You may have wondered why I continued to work on this year after year. That's because earning my Ph.D. has always been my dream and long ago as a young man, I decided that I would never give up on my dreams.

Go confidently in the direction of your dreams. Live the life you've imagined.

- Henry David Thoreau

Don't let the fear of the time it will take to accomplish something stand in the way of your doing it. The time will pass anyway; we might just as well put that passing time to the best possible use.

- Earl Nightingale

Let me tell you the secret that has led me to my goal. My strength lies solely in my tenacity.

- Louis Pasteur

Now, J.C. and Mason, let's dream some new dreams together.

# Abstract

Wheat bran, including the aleurone layer, contains the vast majority of phenolic antioxidants found in the wheat kernel. Unfortunately, about 80% of phenolic acids in wheat bran are structurally bound and insoluble. These bound phenolics are neither bioactive nor bioavailable during digestion. Additionally, wheat bran contains 43% total dietary fiber, but only 3% soluble dietary fiber. Insoluble fiber is less functional than soluble fiber which has been shown to lower cholesterol and regulate blood sugar.

We hypothesized that processing could improve the functionality of wheat bran and bioavailability of phytochemicals in the bran. Specifically, we aimed to maximize the physical properties of viscosity and water hydration capacity in wheat bran, while also maximizing the release of bound phenolic antioxidants from the bran.

Wheat bran processing included physical treatments of dry grinding, high shear mixing, high pressure homogenization (HPH), and alkali chemical treatments with different concentration, time, and temperature. Sample analysis included particle size, viscosity, water hydration capacity (WHC), water extractable material (Wa-Ex), free phenolics, and visual imaging by scanning electron microscopy (SEM).

Prescreening results showed that while HPH helped reduce particle size of bran regardless of treatment, only alkali chemical treatments released the vast majority of bound phenolics. Alkali treatments also contributed to viscosity increase, with interaction of variables of alkali concentration, treatment time, and temperature. Variables for optimization studies included bran grind, alkali concentration, reaction

time, and reaction temperature, while process treatments that remained constant were high shear mixing after chemical pretreatment and HPH conditions.

Two factorial designs were conducted to optimize viscosity and WHC of bran while maximizing release of bound phenolics. The second factorial design was an augmentation to the first and data was combined for statistical analysis. Viscosity maximum was reached using 0.5mm screen size in bran dry grinding and chemical treatment conditions of 60°C soak temp, 24 hour soak time, 0.1N NaOH concentration. WHC maximum was reached using 0.5mm screen size in bran dry grinding and chemical treatment conditions of 48°C soak temp, 20 hour soak time, 0.7N NaOH concentration.

Overall, process optimization was successful and produced wheat bran with a 500% increase in viscosity, 200% increase in WHC, 500% increase in soluble fiber, and a 300 fold increase in free and soluble bound phenolic antioxidants. Visual confirmation by SEM validated analysis results and showed the optimized bran had a very open and porous structure due to the chemical weakening of the alkali treatment and high shear pulverization of the HPH treatment.

The optimized viscosity process was scaled up to produce a large quantity of samples for further research in this collaborative study. Work to separate or concentrate the soluble fraction of processed bran utilized centrifugation to produce additional samples of more soluble and more insoluble processed bran fractions.

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# Background

## *Whole Grains and Health*

Although 6 to 11 servings of grain products are recommended daily with a minimum of three of those servings as whole grain (Welsh et al., 1994), less than 10% of Americans are consuming 3 servings of whole grain foods per day. In fact, the average intake is less than 1 serving of whole grains per day (Marquart et al., 2007). Low consumption of whole grains is surprising when their health benefits are considered. Although consumers have more choices of whole grain foods lately, they often choose refined grain products due to appearance, taste, texture, preferences (Marquart et al., 2007).

Consumption of whole grain foods has been linked to reduced risk of chronic diseases (Marquart et al., 2007) including coronary heart disease (Steffen et al., 2003), cancer (Jacobs et al., 1998), diabetes (Fung et al., 2002), and obesity (Koh-Banerjee et al., 2004). Based on epidemiological and clinical data, the US Food and Drug Administration also allows several label claims for whole grain foods and grains containing fiber that reduce the risk of coronary heart disease and some cancers (United States Food and Drug Administration, 2008).

Whole grains or foods made from them contain all the essential parts and naturally-occurring nutrients of the entire grain seed (Whole Grains Council, 2004). The approximate composition of whole wheat is 80% endosperm, 15% bran, and 5% germ. The vast majority of phytonutrients are contained in the bran and germ, not the starchy

endosperm. Phytonutrients (also called phytochemicals) are defined as biologically active compounds of plant origin that when ingested provide certain functional benefits beyond basic nutrition. Grains contain many thousands of unique phytonutrient structures, but the most important groups can be classified as phenolics, carotenoids, vitamin E compounds, lignans, $\beta$-glucan, and inulin (Liu, 2007).

While increasing consumption of whole grains is one important approach to improve health, another is to increase the health benefits of grains by improving phytonutrient bioavailability. Bioavailability can be defined as the degree and rate at which a substance (such as a phytonutrient) is absorbed into a living system or is made available at the site of physiological activity (Merriam-Webster Online Dictionary, 2008). Another commonly used term, bioaccessibility, is the amount of an ingested nutrient that is available for absorption in the gut after digestion (Hedrén et al., 2002). So not only is it important to free the phytonutrient from the food and ingredient matrix to make it bioaccessible for absorption, but also for it to be absorbed and available to react at the site of action.

## *Phytonutrient Bioavailability*

Although some grains such as popcorn, brown rice, or puffed wheat cereal are eaten whole, most are processed to some degree by grinding or milling. Liyana et al. (2006) demonstrated that sequential removal of the bran layers by abrasion reduced the antioxidant capacity of the remaining grain. The bran and aleurone layers of wheat contain high concentrations of phenolics that act as antioxidants by free radical

scavenging and/or metal chelation (Liyana-Pathirana et al., 2006; Liyana-Pathirana and Shahidi 2006). Because the majority of phenolic antioxidants in bran are insoluble and bound to cell wall material (Adom and Liu, 2002), their content has been underestimated in the literature. Additionally, the vast proportion of hydroxycinnamic acids present in the diet is ester-linked to cell wall polymers and cannot be absorbed through the gastrointestinal tract wall in this form (Andreasen et al., 2001). Although release of insoluble bound phenolic antioxidants from bran occurs in the intestine through microbial fermentation and enzymatic reactions of xylanases and esterases, primarily only the free and some of the soluble conjugate phenolics are absorbed into the blood through the intestinal lumen to become bioavailable (Kroon et al., 1997).

Many factors affect food nutrient "availability" for absorption in the gut including processing conditions, the chemical state of the nutrient, release from the food matrix, possible interactions with other food components, presence of suppressors or cofactors, formation of stable compounds that are slowly metabolized, and the food or ingredient microstructure (Parada and Aguilera, 2007). When administered to rats by perfusion at different concentrations (10-50μmol/L), free ferulic acid absorption was directly proportional to the perfused concentration suggesting passive diffusion or facilitated transport not at saturation. About 6% of the ferulic acid was absorbed into the bloodstream and 50% was distributed to peripheral tissues as conjugated derivatives (Adam et al., 2002). When fed to rats as wheat bran instead of free form, plasma concentration of ferulic acid was only 83.5% lower. More than 38% of the ingested

ferulic acid from bran was excreted in the fecal matter (Adam et al., 2002) indicating the bran microstructure greatly affected the bioavailability of the ferulic acid in bran.

## *Wheat Kernel Morphology*

Also called the wheat berry, the kernel contains three distinct parts that are separated during the milling process to produce flour: Bran, endosperm, and germ (Montana Wheat & Barley Committee, 2006). The bran comprises about 14.5% of the kernels weight and contains many layers that protect the grain from harm (insects, bacteria, molds, and severe weather). Bran is included in whole wheat flour or often sold separately (mostly for animal feed). The endosperm, about 83% of the kernels weight, contains primarily starch and storage protein and provides food for the growing seed. It is the source of refined white flour. The germ, about 2.5% of the kernels weight, contains the plant embryo or seed and is often separated from flour in milling because the fat content limits flour's shelf-life (North Dakota Wheat Commission, 2007). **Figure 1** depicts the three components with added detail to show the different layers of the bran.

## Bran Layer Identification

Some additional background information on bran layers is necessary prior to reviewing data of phenolic content in specific bran layers. **Figure 2** shows the evolution of bran layers progressing from anthesis to the mature kernel (Wheat: The Big Picture 2007).

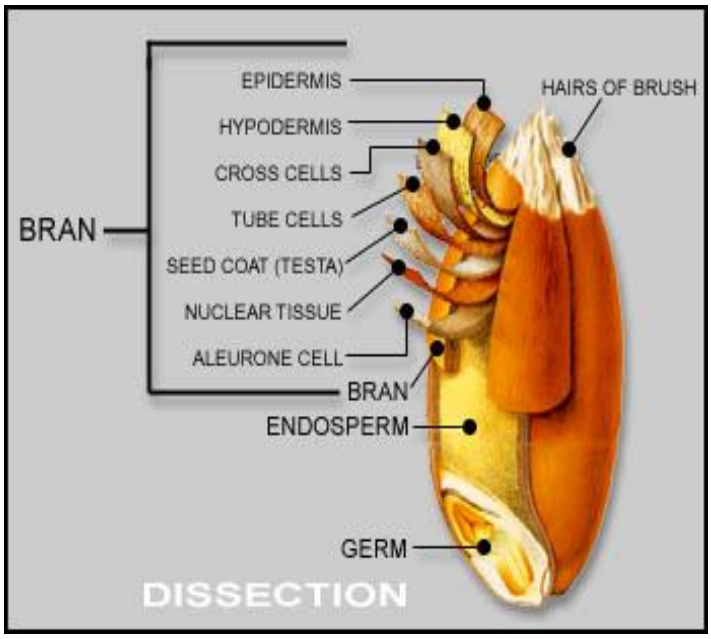


**Figure 1. Wheat Kernel Dissection** (The New Zealand Institute for Crop & Food Research Limited, 2002)

**Pericarp (outside)**
**1A** - cuticle of Outer Epidermis
**1** - Outer Epidermis
**2** - Hypodermis
**3** - Parenchyma (thin-walled)
**4** - Intermediate cells
**5** - Cross cells
**6** becomes **7** - Inner Epidermis/Tube cells

**Closer to the Embryo sac (inside)**
**8A** and **8B** - Outer Integument
**9A** and **9B** -Inner Integument
**10** - Nucellar epidermis
**11** - Nucellus
**12** - Aleurone
**13** - Starchy endosperm

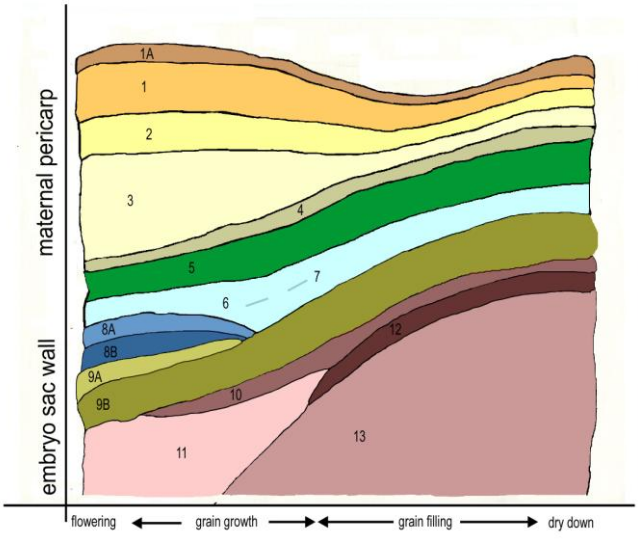


**Figure 2. Cell Layers Inside a Grain** (Wheat: The Big Picture, 2007)

Bran layers (including aleurone) in mature kernels have been grouped during tissue dissection along natural demarcations by Parker et al (2005) into four groups: Beeswing bran, or **BWB**, consists of the outer pericarp layers including brush hairs (Layers 1-44); **Cross cells** (Layer 5); **Testa +NE** consisting of the pigmented testa and the nucellar epidermis (NE, hyaline layer) (Layer 7-10); and the single layer of **aleurone** cells (Layer 12). Another view of layer separation is shown in **Figure 3** (Parker et al., 2005).

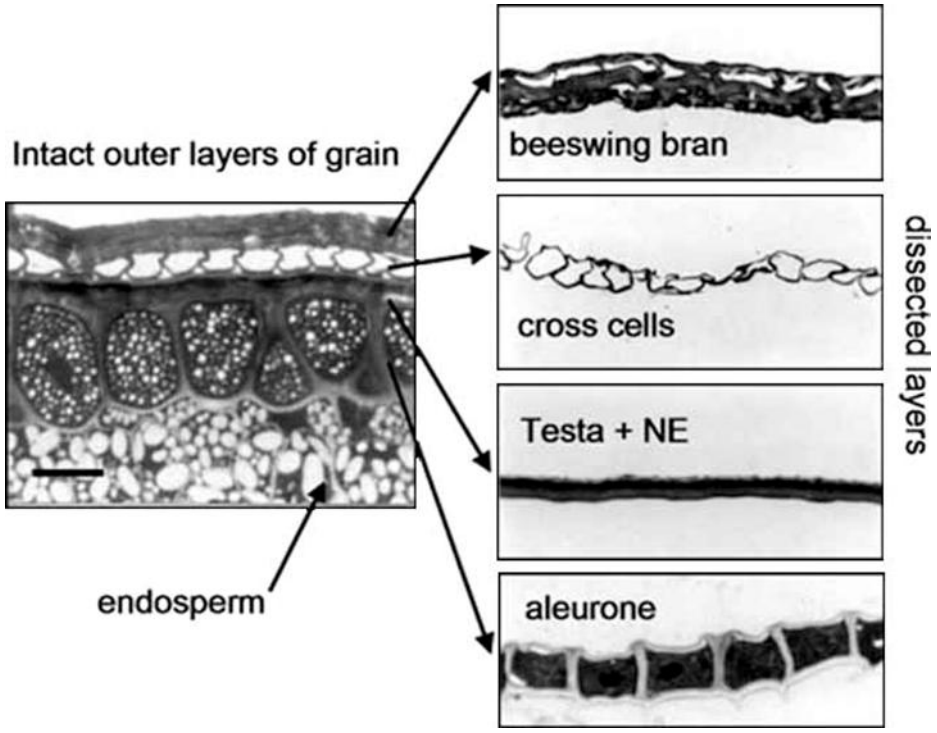


**Figure 3. Bran Layer Separation Micrograph** (Parker et al., 2005)

## *Wheat Phenolics and Measurement*

Whole grains contain a host of phytonutrients—plant components that offer health benefits—phytates, phytoestrogens, lignans, phenols, antioxidants (Slavin et al., 1999). The bran and germ supply the majority of biologically active components found in the grain (Fulcher and Rooney-Duke, 2002). They contain amino acids—protein building blocks, higher levels of B vitamins (thiamin, riboflavin, niacin, pantothenic acid) and certain minerals (calcium, magnesium, phosphorus, potassium, sodium and iron) while the endosperm is mainly starch. Wheat bran and germ also contribute a more concentrated source of antioxidants to whole grain products than refined breads and cereals and many common fruits and vegetables (Miller et al., 2002).

Wheat bran is composed mainly of polysaccharides, including arabinoxylans, xyloglucans and cellulose, but also contains significant amounts of phenolic acids, lignin and some proteins. Phenolic acids present in the cell wall are thought to play an important part in the cross-linking of polysaccharides with other cell-wall components, including lignin through ester and ether bonds, and also in the cross-linking of polysaccharide chains (Parker et al., 2005).

Phenolic acids such as ferulic acid are found mainly in wheat aleurone and bran layers (the aleurone layer is actually the protective outer layer of cells in the endosperm, but it adheres to the bran and is removed with the bran during milling). Although the antioxidant activity of phenolic acids is well known, their health benefits are less clear. Wattenberg (1985) suggested an anti-carcinogenic mechanism of phenolic compounds suggesting caffeic and ferulic acids prevented the formation of carcinogens from

precursor compounds and blocked the reaction of carcinogens with critical cellular macromolecules.

## Extraction of Free, Esterified, and Insoluble Bound Phenolics in Bran

Fruits & vegetables are often cited as excellent sources of antioxidants whereas, grains tend not to be mentioned due to relatively low levels of antioxidant activity reported in the literature. However this is because most studies have reported the phenolic levels of grains using various aqueous solutions of methanol, ethanol and acetone to extract soluble phenolics (Liyana-Pathirana et al., 2006; Zhou and Yu, 2004). Recent work has shown that grains have vastly greater levels of phenolics than previously thought due to methodology improvement that measures phenolic acids in all their forms: free, esterified, and insoluble-bound. Adom and Liu (2002) have shown that the major portion of phytochemicals in the grains is present in the bound form (85% in corn, 75% in oats and wheat, and 62% in rice).

Soluski et al. ( 1982), Naczk and Shahidi (1989), and Liyana-Pathirana and Shahidi (2006) evaluated free, esterified, and bound phenolics in commercially available soft and hard wheats that were fractionated into whole wheat, refined flour, and bran. Their results shown in **Table 1** expressed as ferulic acid equivalents indicate that the bran fraction not only contains the vast majority of phenolic compounds, but the majority (>80%) are in bound form. Ferulic is the major phenolic acid in wheat bran and constitutes around 85% of the total phenolics in all its forms (Kim et al., 2006). The significant differences in phenolic content between hard and soft varieties are not

unusual. Differences in phenolic content and distribution have been reported to be influenced by genetic, environmental, and preparation differences (Klepacka and Fornal, 2006).

**Table 1. Free, Esterified, and Bound Phenolics Contents (μg FAE/g Defatted Material) of Whole Grains, Flour, and Bran of Hard and Soft Wheat[a]**

| Milling fraction | Free | Esterified | Bound | Total |
|---|---|---|---|---|
| HWF | 137 ± 4 a | 234 ± 13 a | 328 ± 14 a | 699 |
| SWF | 161 ± 6 b | 278 ± 12 b | 464 ± 16 b | 903 |
| HWW | 353 ± 16 a | 954 ± 34 a | 2149 ± 43 a | 3456 |
| SWW | 478 ± 12 b | 1196 ± 59 b | 2144 ± 52 b | 3818 |
| HWB | 846 ± 31 a | 1365 ± 63 a | 11303 ± 126 a | 13514 |
| SWB | 981 ± 47 b | 1432 ± 42 b | 12186 ± 149 b | 14599 |

[a] Values are means of three determinations ± standard deviations. Abbreviations: HWF, hard wheat flour; SWF, soft wheat flour; HWW, hard whole wheat; SWW, soft whole wheat; HWB, hard wheat bran; and SWB, soft wheat bran. In each column, pairs carrying different superscripts are significantly ($p < -0.05$) different from one another (Liyana-Pathirana and Shahidi, 2006).

Liyana-Pathirana and Shahidi's (2006) extraction methodology is based on extracting with methanol-acetone-water multiple times, then extracting free phenolics with diethyl ether. This was different from Adom and Liu (2005) who used 80% ethanol and Kim et al. (2006) who used methanol. There were also differences in the grinding, particle size, and whether the samples were defatted prior to extraction. Even so, results within a laboratory should strive to be consistent and allow comparisons to be across samples. Direct quantitative comparison of different methodologies carried out by different laboratories is only possible if the same (exact) sample was used by all. However, qualitative comparisons do show similar trends in the extracted phenolics.

The sample extract used to obtain free phenolics is then treated with acid or base to break the ester bonds attaching mainly ferulic acid (up to 95% of esterified phenolics) to sugars. Researchers have used 2M NaOH (Kim et al., 2006; Adom et al., 2005), 4M NaOH (Liyana-Pathirana and Shahidi, 2006), or multiple sequential levels from 0.1M to 4M NaOH (Parker et al., 2005) for different lengths of time, followed by neutralization or acidification with HCl. Acidic and alkaline hydrolyses are used to cleave the ester bond in separation and characterization of specific phenolic compounds. The hydrolysis conditions, acid or alkaline only, or in different sequence, can significantly affect the total yield and profile of phenolic acids (Kim et al., 2006). Kim et al. (2006) also found degradation of cinnamic acid derivatives, *p*-coumaric, caffeic and ferulic acids when heat was used during acid hydrolysis.

Insoluble bound phenolics in grains are freed using similar alkaline and acid hydrolysis except this time the extractions are done on the previously extracted material.

The goal is mainly to break ester bonds between ferulic acid and sugar structures (mainly arabinoxylan) and ether bonds between ferulic acid and lignin to free the phenolics for extraction. Because ferulic acid forms both of these types of bonds (Liyana-Pathirana and Shahidi, 2006) it may link xylan and lignin structures (Klepacka and Fornal, 2006) making it more difficult to extract. Conditions for hydrolysis were similar to the esterified hydrolysis for all three methods reviewed (Liyana-Pathirana and Shahidi, 2006; Adom and Liu, 2002; Kim et al., 2006). Results all agree that the vast majority of phenolic acids are in insoluble bound form concentrated in the bran material. In this case, particle size of starting material may have an increased impact on final hydrolysis results. Because ferulic acid is a structural part of insoluble cell wall material, a reduction of cell walls to smaller pieces would act to improve the extent of hydrolysis. Although it seems a simple test to evaluate the extent of the extraction of phenolics, the author is unaware of researchers evaluating the digested grain matter for residual phenolic material either by auto fluorescence under a microscope or by any other method.

Because of the complexity of digestion, it is difficult to quantify the bioavailability and health benefits of bound phenolics in whole grains and bran. It has been shown that bound phenolics have physiological activity, but this activity is often measured once freed from their matrix. While free phenolics and some soluble bound phenolics are absorbed in the small intestine, Hemery et al. (2010) demonstrated that reducing bran particle size by grinding correlated to an increase in in-vitro phenolic acid bioaccessibility from once bound phenolics. Although Yuan et al. (2005) reacted insoluble dietary fiber to extract soluble feruloylated oligosaccharides and administered

them to rat erythrocytes in-vitro, it would be impossible to conclude the bound phenolics would have the same effect in-vivo. In the large intestine, some bound phenolics are undoubtedly freed and may have beneficial antioxidant properties. For example, bacteria in the colon may release bound phytochemicals through fermentation to provide site-specific health benefits. This may partially explain a potential mechanism associated with grain consumption in the prevention of colon and other digestive cancers, along with breast and prostate cancer, as supported by epidemiological studies.

## *Processing Technologies*

### Physical Processing

Physical processing can be as simple as grinding and may include dry methods such as ball milling or wet methods such as high pressure homogenization. Separation of wheat bran into its layer components has been tested. Harris (2005) fed wheat bran, pericarp, or aleurone fractions to rats and examined the recovered material from the feces by multiple microscopy techniques. Most of the bran material passed through digestion intact with only the aleurone cells showing some degradation, primarily by bacterial enzymes. Smaller particles of bran are hypothesized to allow freer access to enzyme target sites (Ferguson and Harris, 1997). Carole et al. (Antoine et al., 2004) demonstrated an increase in water extractable p-coumaric acid from wheat bran (4.3 times) and aleurone (3.4 times) samples after increasing ball milling times to achieve progressively smaller particle size, however even the increased free p-coumaric acid released after ball

milling for 16 minutes was only 6 percent of the total. Chau et al. (2006) tested the effects of ball milling, jet milling, and high pressure homogenization (HPH) on insoluble star fruit fiber. Although all reduced particle size dramatically, only HPH processing produced dramatic increases in water holding capacity, swelling capacity, oil-holding capacity, and fiber solubility compared to the other methods.

The bioavailability of bran nutrients varies greatly depending on bran particle size and technological treatments (Caprez and Fairweather-Tait, 1982; Yu and Kies, 1993). Yu and Kies (1993) processed maize bran to different sized particles including finely ground, wet-milled maize bran; coarsely ground, wet-milled maize bran; finely ground, dry-milled maize bran; or coarsely ground, dry-milled maize bran, and fed nine human subjects controlled diets containing unsupplemented bread or bread supplemented with milled bran of different particle sizes. They found that in general, better apparent bioavailability was achieved with the finely ground, dry-milled maize bran than with the other test bran. The increased bioavailability may be attributed to damaged cells and increased surface area in the finely milled samples. However, there is still a substantial amount of nutrients within even the finely milled particles, which could be released if the matrix structure can be further opened up or expanded to a highly porous medium.

## Chemical Processing

Treatment of bran with acid or alkali can effectively break covalent bonds including ester linkages between phenolic acids and arabinoxylan chains. Kim et al. (2006) investigated the effect of hydrolysis and extraction conditions on the yield and profile of

phenolic acids. After extraction with 80% methanol to remove free and soluble conjugate phenolics, the insoluble material was hydrolyzed by acid then alkali or alkali then acid. Alkali hydrolysable phenolic acids comprised more than 96% of the total freed by hydrolysis. Ferulic acid comprised approximately 90% of the total phenolic acids liberated by alkaline hydrolysis but was not freed by acid hydrolysis. It appears likely that their hot acid hydrolysis degraded the ferulic acid such that very little was recovered when followed by alkaline hydrolysis.

Although the free and soluble conjugate phenolic fractions demonstrate strong antioxidant activity, the bound fraction represents a greater potential if released. Liyana-Pathirana and Shahidi (2006) utilized alkaline hydrolysis to liberate bound phenolics from wheat bran and several tests for antioxidant activity were performed indicating significantly greater contribution by the hydrolyzed bound fraction than free or esterified fractions. Total Antioxidant capacity (TAC) of the bound fraction was 89.6% and 88.6% of the total for hard and soft wheat bran respectively.

When free and soluble conjugate phenolics are released through chemical treatments, their bioavailability and antioxidant activity increases. Ohta et al. (1997) showed differences in antioxidant activity of free ferulic acid (FA), feruloyl arabinose (FAA), and feruloyl arabinoxylane (FAXn) in a LDL oxidation system. Antioxidant activity was FAA > FAXn > FA and the LDL oxidation inhibiting activity of FAA was also dose dependent. FAA administered orally to rats was found to exist as the conjugated form of FAA (25%) and FA (75%) in the circulation system. So in addition

to free ferulic acid, some soluble conjugated phenolics are also absorbed and bioavailable.

## Thermal Processing

Thermal processing of grains can take the form of a final processing step such as retort cooking (canning), baking or extrusion cooking/puffing or as an intermediate step to prepare an ingredient for further processing including steam explosion or extrusion cooking.

Dewanto et al. (2002) utilized a retort canning process to cook sweet corn at different temperatures and times. When compared to an unheated control, free and soluble conjugate ferulic acid content increased significantly in heat treated samples (100°C, 115°C, 121°C ($p<0.01$), while bound ferulic acid decreased significantly ($p<0.05$). A similar increase in free and soluble conjugate ferulic acid ($p<0.01$) and decrease in bound ferulic acid ($p<0.05$) was seen for corn retorted at 115°C for 10, 25, or 50 minutes vs. the unheated control. Antioxidant activities also increased significantly ($p<0.01$). Interestingly, the amount of liberated free phenolics was very low compared to the soluble conjugate fraction indicating the increased antioxidant activity was due mostly to conjugated glycosides with ferulic acid esterified to neutral sugar chains (Dewanto et al., 2002).

Hydrothermal processing, sometimes called steam explosion or flash explosion is accomplished by heating material with high pressure steam or heating a pressurized chamber containing the hydrated sample. When pressure is instantly released, moisture

flashes to steam and causes physical disruption of the fibers. This process has been used for years in the paper industry and more recently in preparation of fibers for conversion to ethanol. Schooneveld-Bergmans et al. (1998) explored the effects of dwell (5, 10, 15 min.) and temperature (180°C, 190°C, 200°C) of steam exploded destarched wheat bran on water extractable carbohydrate and ferulic acid content. They liberated 19% to 28% of the glucurono-arabinoxylans present in the parental bran. Ferulic acid remained bound to the arabinoxylans that were released but was extracted at up to 6 times the quantity of untreated bran.

In addition to modifying the phenolic content, extrusion processing also has important effects on soluble and insoluble dietary fiber which are important in increasing bile acid binding leading to lower cholesterol. Kahlon and Woodruff (2003) examined the effects on in vitro bile acid binding due to various cereal grains and concluded that only the high viscosity beta glucan soluble fibers in oats and barley contributed to increased bile acid binding whereas other soluble cereal dietary fibers did not proportionally increase binding. Additionally, insoluble dietary fiber was related to bile acid binding for rice bran, oat bran, and beta glucan enriched barley. Although other protein anionic, cationic, physical and chemical structure, composition, metabolites, or their interaction with active binding sites may contribute to bile acid binding effects as well. When testing extruded oat bran, Drzikova et al. (2005) noticed similar increased in vitro bile acid binding with increasing beta glucan content in digested oat based extrudates ($p<0.001$).

Testing bile acid binding in vitro has limitations and results must be confirmed in animal or human studies to demonstrate validity. For example, Kahlon et al. (2006a) did not show a strong correlation between dietary fiber solubility (or insolubility) of extruded wheat bran samples and in vitro bile acid binding though extrusion conditions significantly increased total and insoluble dietary fiber in the processed wheat bran. In another study, they fed similar extruded wheat bran products to hamsters in diets containing 10% total dietary fiber from the bran and measured significant in vivo reductions in total liver lipid and total liver cholesterol (23–35% and 28–31% respectively) (Kahlon et al., 2006b). Neutral sterol excretion was also significantly higher for the most highly processed bran samples vs. the unprocessed control.

## Biological Processing

Because phenolics are bound to arabinoxylan in wheat bran, reaction with xylanase enzymes produces two effects. First, the increase in extractable arabinoxylans modifies the physical characteristics of bran (Trogh et al., 2004). Second, the release of bound phenolics increases the bioavailability and antioxidant activity of the bran (Napolitano et al., 2006).

Yuan et al. (2006) optimized conditions for production of feruloylated oligosaccharides from wheat bran from wheat bran insoluble dietary fiber. They used 4.8g/L of a xylanase from *Bacillus subtillus* to react with bran (120g/L) in pH 5.2 acetate buffer at 42°C for 35 hours yielding 1.55 mM feruloyl oligosaccharides. Some free ferulic acid was also produced during the reaction possibly by feruloyl esterase in the

xylanase enzyme. The feruloyl oligosaccharides were shown to efficiently protect normal rat erythrocytes against hemolysis induced by free radicals under in vitro conditions showing their antioxidant potential (Yuan et al., 2005a)and strong free radical scavenging activity (Yuan et al. 2005b).

Benoit et al. (2006) tested the effectiveness of two feruloyl esterases from *Aspergillus niger* to release phenolics from agro-industrial by-products including autoclaved maize bran. Their FAEA enzyme released 40% of the ferulic acid indicating strong enzyme specific results versus only 8% release with the FAEB enzyme. In maize and wheat bran, arabinofuranose is esterified at O-5 by ferulic acid and the FAEA enzyme was mainly active on O-5 ester linkages.

Napolitano et al. (2006) tested various strains of *Trichoderma* species in their capacity to produce hydrolytic enzymes that solubilized insoluble dietary fiber in durum wheat fiber and barley spent grain. They found that their optimized conditions increased soluble dietary fiber in bran from 3.7% to 8.7%, increased free and soluble conjugate phenolic compounds from 0.9% to 3.4%, and water soluble antioxidant activity from 1.1% to 2.3%. A4-fold increase in the free and soluble conjugate ferulic acid content represented 40% of the total ferulic acid in the bran. It is expected that the increased free phenolics due to the enzyme treatments will result in increased phenolic compound bioavailability.

Along with the importance of improved bioavailability of phytonutrients, ingredient functionality with respect to product quality is also a critical consideration. Trogh et al. (2004) utilized xylanase enzyme during bread making to convert water-unextractable

arabinoxylan (AX) to soluble AX and bread made with the xylanase enzymes had increased loaf volume. The results show that increasing levels of xylanase solubilized increasing amounts of arabinoxylan while also decreasing its average molecular weight. This was important because solubilization of AX increased viscosity in bread doughs at lower levels of enzyme while higher levels reduced viscosity due to reduction in AX molecular weight.

Enzymatic activity during sprouting of grains occurs both from endogenous grain enzymes as well as hydrolytic enzymes produced by indigenous microorganisms present in the outer layers of the kernel (Katina et al., 2007). When Katina et al. (2007) fermented germinated rye meal with *S. cerevisiae* yeast, free phenolic acid levels increased 10 fold vs. an increase of <25% for germination alone.

## Combined Processes

While enzyme treated maize bran released up to 30% of the total ferulic acid, steam explosion pretreatment (heat-up, holding at 190°C for 1 minute) followed by enzyme treatment (3% w/w protein/bran at 40°C for 24 h) increased the level of solubilized ferulic acid to 80% with about 2/3 as free ferulic acid (Saulnier et al., 2001). **Figure 4** shows the results of various combinations of steam explosion pretreatment and enzyme treatment. The steam explosion pretreatment needed to exceed 170°C to show effectiveness, however heating time was an important factor in their results as heat-up time in the reactor was 15 to 25 minutes to reach the target temperature. Steam explosion

pretreatment at pH = 4 was important for feruloylated oligosaccharide release as arabinose chains are sensitive to hydrolysis at low pH. Extending the steam explosion treatment to 160°C for 1 h also achieved 80% solubilization of ferulic acid as feruloylated oligosaccharides. Subsequent treatment with feruloyl esterases was then necessary to get ferulic acid in its free form (Saulnier et al., 2001).

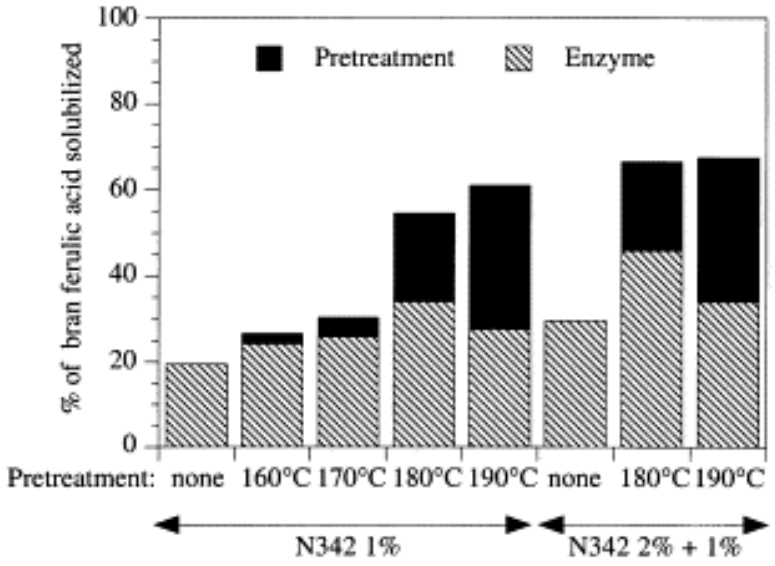


**Figure 4. Total Solubilization of Ferulic Acid from Maize Bran by a Combined Action of Flash-Explosion and Novozyme 342** (Saulnier et al., 2001).

Combining chemical and physical processes, Lundberg et al. (2003) developed a patented process to create highly refined cellulose (HRC) with high water holding capacity. The process utilized alkali pretreatment of cellulose followed by neutralization. The cellulose was then refined to break lignin and reduce fiber size. High pressure homogenization was then employed to produce the HRC. HRC had a water holding

capacity of 25 to 56g H2O per gram dry HRC. While creating HRC did not retain any solubilized parts of the cellulose, the process has many similarities to the process utilized to treat wheat bran in this research program including the pretreatment with alkali and subsequent high pressure homogenization treatment.

## *Relationship of Phenolics and Flavor in Whole Grain Wheat Products*

What are the reasons for low consumption of whole grains despite their known health benefits? Although more whole grain foods are becoming available to consumers, they often choose refined grain products due to appearance, taste, and texture preferences (Marquart et al., 2007). Lang and Walker (1990) found that untrained panelists could discriminate between the appearances of whole red vs. whole white wheat hamburger buns when tested under red light. When red or white wheat hamburger buns were tasted under fluorescent light, panelists scored them significantly different aligned with their preexisting preferences, but there was no significant winner overall. Bakke et al. (2007) had similar results in that preference of whole wheat vs. refined wheat flour breads noted on a preliminary questionnaire related to increased scoring for the preferred bread during taste tests. Appearance preference for 100% whole wheat bread over refined (either white or darkened with caramel color) was shown. Panelists also showed increased liking for whole wheat bread when it was made sweeter with honey; possibly overcoming negative flavors in the whole wheat products.

In both of these studies, flavor and taste preferences were not due to dislike of darker whole wheat color. Texture was not a factor and flavor preference was strongly linked to pre-recorded liking of whole wheat vs. refined breads, so liking of whole wheat flavors may be linked to learned behavior. Therefore the anecdotal evidence of people liking whole wheat products less than refined wheat may only be true for population subgroups. Undeniably though, there are flavor differences between whole wheat and refined wheat products, and possibly also across different varieties of wheat such as red vs. white wheat. It is worthwhile to explore these differences.

## Measurement of Aroma and Taste Compounds in Grain and Wheat Ingredients

Both volatile and nonvolatile compounds found in wheat contribute to the aroma and taste of grain ingredients and products they make. Flavor particularly influences acceptance by consumers, and volatile aroma components generally are regarded as the most important parameters of food flavor quality (Heath and Reineccius, 1986). As far back as the 1970's, Maga summarized 24 volatiles associated with wheat including alcohols, aldehydes, and ketones (Maga, 1978). Negative aroma volatiles were identified by Seitz and Ram (Seitz and Ram, 2000) who measured methoxybenzene compounds, including 1,2-Dimethoxybenzene and 1,4-Dimethoxybenzene, causing musty, sour and insect odors in grain samples.

Other objectionable and bitter flavors in whole wheat ingredients and products, besides oxidation of lipids, may result from derivatives of nonvolatile phenolic

compounds. Phenolic compounds are the major secondary metabolites of plants and are formed from phenylalanine and tyrosine precursors. They occur in free as well as bound forms. Klepacka and Fornal summarized the classifications of diverse phenolic structures including anthocyanins and tannins contributing to sensory properties of foods (Klepacka and Fornal, 2006). Because flavor activity is strongest in soluble and free phenolic compounds, the bitter flavored tannins (water soluble polyphenols) and free phenolics would have a larger impact on sensory properties when compared to bound phenolic compounds.

Phenolic compounds found in wheat include ferulic, vanillic, gentisic, caffeic, salicylic, syringic, p-coumaric, and sinapic acids. Ferulic acid, the primary phenolic acid in the grain accounting for up to 90% of total phenolic acids, is found predominantly in the aleurone cell walls of the kernel (Naczk and Shahidi, 2006). While Siliani et al. showed that a positive correlation does exist between the amount of phenolic compounds and bitter intensity in olive oil (Siliani et al., 2006), individual phenolic compounds in wheat have not been shown to have the same bitter intensity. Heinio et al. profiled the flavor of rye milling fractions by sensory descriptive analysis. The inner endosperm was found to be mildest in flavor and the outer bran layers the most intense in flavor (Heinio et al., 2003). Free phenolic acids were found to be the most flavor active in rye grain. The lignan pinoresinol along with syringic acid produced bitterness whereas ferulic and sinapic acids caused germ-like flavors (Heinio et al., 2008). Syringic acid is not typically found above the taste threshold level in wheat bran (Maga and Lorenz, 1973).

Although hard white winter wheat is agronomically the same as the hard red winter wheat, anecdotal claims that red wheat is more bitter than white wheat have been stated for some time. For example, Perrin and Anthony (2001) claimed: "White varieties of hard wheat have less of the red, bitter-tasting, tannin in the seed coat. They offer slightly higher yields of flour that tastes sweeter than flour from red varieties." Wheat bran bitterness may be more variety dependent than color dependent. Chang and Chambers (1992) reported flavor differences in loaf breads made from HRW (Hard Red Winter) or HWW (Hard White Winter) wheat. Both HRW and HWW crust were similar in aroma and flavor characteristics including burnt, toasted, brown, and grain-like aromas and flavors with additional bitter flavor notes. Traditional fermentation of wheat bran has been shown to significantly decrease levels of tannin and phytic acid in coarse, medium, or fine particle size fractions (Hassan et al., 2008).

## Measurement of Aroma and Taste Compounds in Grain and Wheat

### *Products*

More than 540 compounds have been described in the complex aromatic fraction of bread (Chang et al., 1995). Volatile and nonvolatile compounds contribute to the flavor of bread including acids, alcohols, aldehydes, esters, ethers, ketones, furans, hydrocarbons, lactones, pyrazines, pyrroles, and sulfur compounds (Quilez et al., 2006). Early work by Lorenz and Maga attributed reduction in aldehydes and increase in ketones to reduced flavor and taste acceptability during bread staling (Lorenz and Maga, 1972).

They noted the ratio of aldehydes to ketones was reduced from 72.9:27.1 to 15.1:84.9 after 5 days of storage and concluded the decrease in aldehyde content contributed to the decreased flavor and taste acceptability of stale bread. More recently, Quilez et al. (2006) reported contradictory results. They found a positive sensory correlation between flavor scores in commercial baguettes and salt, pH (higher), alcohols (ethanol, 2-phenylethanol) and ketones (diacetyl, acetoin). A negative correlation was found with high titratable acidity (acetic, butyric), and aldehydes (hexanal, benzaldehyde, 2.4 decadienal) (Quilez et al., 2006).

When studying volatiles in bread, more compounds are found when organic solvent extraction or steam distillation methods are used for isolation than when headspace methods are used. Chang et al. (1995) measured volatile flavor components of breads made from different wheats, with and without bran. They found that 15 compounds were significantly higher in relative quantities in whole wheat bread than in white pan bread, including fruity odors (ethyl acetate, isoamyl acetate, 1-pentanol, and ethyl octanoate), green and grassy flavors (dimethyldisulfide, 2-octanone, and 1-hexanol), and increased levels of fermented and sour flavors (3-methylbutanal, 1-butanol, heptanal, and 1-octen-3-ol). Maeda et al. (2009) evaluated flavor compounds in straight dough and sourdough breads made from refined flour (70%) and polished bran fraction (30%) of outermost, middlemost, and innermost fractions of the same wheat. Both sourdough and straight dough crumb and crust made with the outermost bran fraction (C-1) had a very large increase in methoxybenzenes. As mentioned earlier, methoxybenzene compounds caused musty, sour and insect odors (Seitz et al., 2000).

Chang and Chambers (1992) reported flavor differences in loaf breads made from HRW or HWW wheat. They suggested that a phenolic-like flavor and aroma found in HWW crumb may have been due to phenolic acids present in the wheat bran; however they did not find a similar flavor in HRW crumb. Lang and Walker (1990) compared hamburger buns made with HRW and HWW wheat. In triangle tests, panelists were able to distinguish between red wheat and white wheat hamburger buns when different forms of wheat were used including cracked wheat, flaked wheat, wheat bran, and whole wheat, however panelists did not have a significant preference for either wheat color when used in hamburger buns. Stanyon and Costello (1990) noted biscuits with added wheat bran were stronger in wheat flavor than refined wheat biscuits resulting in increased bitter, metallic, or salty aftertastes. Not all bran fractions are alike in flavor. Heinio et al. (2003) demonstrated in rye bread that the outermost rye bran fractions significantly increased bitterness in crumb and caused an intense aftertaste and flavor in the crust. The inner shorts fraction resulted in a cereal-like flavor in bread, but without obvious bitterness or intense flavor.

While predisposition of the taster for whole wheat vs. refined wheat products influences liking for flavors associated with wheat bran, bitter compound tasting ability may also be a factor. Bakke and Vickers (2007) measured sensitivity to bitterness of sensory panelists by having them rate the intensity of 6-n-propylthiouracil (PROP). The study participants also rated liking of refined and whole wheat breads. PROP supertasters were more sensitive to bitter tastes and liked laboratory made whole wheat breads less than PROP medium and nontasters.

## Modifying Flavor and Taste Profiles through Processing

There are many processing methods that can modify phenolics in wheat that may have flavor impacts including chemical, thermal, enzymatic, microbial, and combinations of these processes. Monsalve-Gonzales and Prakash (2003) treated bran with an acid to reach pH 4-6 forming acidified bran, then treating the acidified bran with low levels of ozone to oxidize ferulic acid, preferably to vanillin to try to improve bran flavor. While the small amount of free ferulic acid in wheat bran would be available for oxidation reactions, the vast majority of bound ferulic acid would not, as ferulic acid is generally bound with ester linkages. Acid treatment breaks glycosidic bonds and solubilizes sugars while leaving ester bonds generally intact, while alkali treatment breaks the ester bond linking phenolic acids to the cell wall (Mathew and Abraham, 2004). Chemical treatments alone require high concentrations of acid or base and can lead to modifications of other components and bring about unwanted chemical changes in plant cell walls.

Combining pH and thermal treatments, Bredie et al. (2002) extruded wheat flour at different temperatures from 120 °C to 150°C and pHs (adjusted from 6.8 to 8.8 with sodium hydroxide) to investigate complex flavor generation in direct extrusion cooking of cereals. They measured volatiles by GC and assessed odors of the extruded products by trained assessors. Raising the pH increased the sulfur-containing volatiles formed in the Maillard reaction (Bredie et al., 2002). High pH produced strong burnt, sulfury odors, intermediate pH produced wheat-like and puffed wheat odors, and low pH extrudates (samples without sodium hydroxide addition) had cooked milk notes. Temperature also had an effect. Lower temperature extrudates had increased levels of

lipid-derived furans and aldehydes. Those compounds may have reacted at higher temperatures with other compounds formed during extrusion. Higher temperature extrudates showed increased levels of thiophenes, thiophenones, thiazolines, thiapyrans, polythiacycloalkanes, and sulfur-containing furans (Bredie et al., 2002).

A gentler and more specific method for modifying phenolic compounds than pH and thermal treatments is to use enzymes. Cinnamoyl or feruloyl esterases or cinnamic acid hydrolases hydrolyze the ester bond between hydroxyl cinnamic acids like ferulic acid and hemicellulose in plant cell walls (Mathew and Abraham, 2004). Feruloyl esterases isolated from different source organisms show substrate selectivity depending on xylan substitution and the presence of other hemicellulases. Catalytic efficiency of ferulic acid esterases is determined by the distance between the phenolic ring and the ester bond and the number and position of methoxy and hydroxyl substitutions on the benzene ring (Mathew and Abraham, 2004). Releasing ferulic acid from lignin in cereal bran has been accomplished using various enzymes singly and in combination including feruloyl esterases, cinnamoyl esterases, xylanases, arabinases, and arabinofuranosidases (Mathew and Abraham, 2006). Once freed, ferulic acid has many uses including use as a precursor for making vanillin, 4-vinyl guaiacol (an undesirable constituent in beer and soy sauce flavors), caffeic acid (Mathew and Abraham, 2006),or as a feed stock for making polymers, epoxides, alkylbenzenes, guaiacol and catechol (Mathew and Abraham, 2004).

Tannins are naturally occurring polyphenolic compounds that form strong complexes with protein and other macromolecules such as starch, cellulose, and minerals.

Not only do tannins contribute astringent or bitter flavors resulting in reduced feed intake, but they can bind macromolecules reducing digestibility and nutrient absorption (Aguilar et al., 2007). Modifying tannin properties with enzymes to improve flavor is performed commercially. Tannase enzymes hydrolyze the ester bonds of tannic acid and are used in the food industry to reduce haze and bitterness in juice. Industrially, hydrolysis of tannins by tannases produces gallic acid and propylgalate antioxidants for use in foods, cosmetics, hair products, adhesives, and the lubricant industry (Aguilar et al., 2007).

Just as there are multiple reasons consumers choose not to eat whole grain products, there are many different ways to modify whole grain products to make them more appealing in appearance, more palatable, and more healthful. Whole wheat in particular has the greatest potential for consumption increase since so many refined wheat products are currently available in whole wheat versions including bread, pasta, cereal, crackers, extruded snacks, cookies, and more. Wheat bran also has the potential of becoming a functional ingredient instead of an inert one if processed properly. Bound phytochemicals may also be made bioavailable through processing as well. The goal for this research program is to explore physical and chemical processing of wheat bran to improve its functionality and bioactivity.

## Program Objectives:

Develop processes for production of bioactive wheat bran ingredients with desirable functionality.

### *Specific Aims:*

In order to develop bioactive ingredients with improved functionality, wheat bran processing will achieve the following specific aims:

1) Improve functional characteristics and potential cholesterol lowering potential by maximizing bran physical properties of viscosity, water hydration capacity, and surface area. These parameters are good indicators of fiber structure expansion and accessibility, and will be used to guide process optimization.
2) Increase phenolic antioxidant bioaccessibility and bioavailability by maximizing the release of bound phenolics as free and soluble conjugate phenolics.
3) Achieve these physical and chemical characteristics simultaneously by first individually screening variables followed by combined treatment optimization. Variables include:
    a. Wheat bran particle size.
    b. Pre-processing: bran concentration, bran hydration time, hydration temperature, concentration of alkali treatment, and high shear dispersion.
    c. High Pressure Homogenization (HPH): Pressure, number of passes, temperature.

### *Expected Outcome and Anticipated Use of the Results*

We anticipate that activities under this objective will result in: (1) an understanding of the effects of chemical and mechanical treatments on the physical properties and bioaccessibility profiles of phytochemicals in processed wheat bran; (2) optimized processes for enhanced bioavailability of phytochemicals in processed wheat bran.

## *Project 1: Screening of Preprocess and High Pressure Processing Variables*

In order to modify functionality of wheat bran while improving bioavailability of phenolic antioxidants, the first step is to understand the effects of each individual process variable on the physical and chemical properties of the bran.

### Hypothesis

Individual variable prescreening will result in identification of variables important for modifying physical and chemical characteristics of wheat bran and their appropriate ranges for further combined optimization testing.

### Objectives

1) Test bran fractions including bran, red dog, bran + red dog combinations to determine the response of fractions and intact bran to processing conditions.
2) Test HPH variables including sample temperature, homogenization pressure, and number of passes to determine the effect on particle size reduction, viscosity, water hydration capacity, increase in free phenolics, and matrix water interactions (NMR).
3) Test preprocess conditions of dry grinding, prehydration, chemical pretreatment, and high shear mixing on bran properties to determine the effect on particle size

reduction, viscosity, water hydration capacity, increase in free phenolics, and matrix-water interactions (NMR).

4) Determine physical and chemical treatment conditions important for further optimization experimentation that will modify functionality of wheat bran while improving bioavailability of phenolic antioxidants.

## Materials and Methods

**Wheat Bran**: For preliminary screening of dry and wet particle size reduction and HPH testing, a 90% HRS, 10% HRW wheat blend from Archer Daniels Midland was milled in a commercial facility (ADM Milling Co., Minneapolis, MN). Bran, shorts, red dog, and flour fractions were all obtained and held at -40°C until use. For chemical treatment testing and process optimization, Soft White Wheat (SWW) bran was obtained from King Milling Company (Lowell, MI).

**Dry Particle Size Reduction** utilized rotor beater milling technology (Retsch GmbH, Model SR 300, Haan, Germany). Multiple passes through several screen sizes were utilized to prevent significant temperature rise in the bran during grinding. Bran was passed sequentially through screens of 0.75mm, 0.5mm, 0.25mm, and some of the samples through 0.12mm.

**Alkali Pretreatment:** 5% bran solutions were prepared (using 10g bran sample on dry basis, add solution to 200g) with appropriate alkali concentration and agitated in an Innova 4300 incubated shaker (New Brunswick Scientific, Edison, NJ) for the specified time and temperature. Solutions were then neutralized to pH 7 with 1N HCl.

**High Shear Mixing:** Samples were diluted to 2% bran in solution totaling 500ml and then mixed with high shear using a T25 Basic SI homogenizer with S25N-25F dispersing element (IKA Works, Wilmington NC). High shear mixing was performed prior to all high pressure homogenization (HPH) processing to reduce particle size and prevent clogging of HPH disruption chambers. The 0.5 to 1.0L samples were mixed for 5 minutes at 24,000 rpm. Viscosity and particle size distribution were typically measured at this time and samples frozen for further analysis. Remaining samples were high pressure homogenized. Additional wet grinding and milling was attempted on other high shear rotor stator type homogenizers (Carter model XMUC752BF; T25 Basic SI Homogenizer, IKA Works Inc., Wilmington, NC; or Greerco, Homogenizer model 1L, Colloid Mill W200V, North Andover, MA).

**High Pressure Homogenization** (HPH) was carried out on an M-110Y Laboratory Microfluidizer® Processor (Newton, MA). Homogenization pressure was controlled by adjusting compressed air pressure. Pressures from 10,000 psi to 23,000 psi were utilized Disruption chambers used to achieve homogenization included a 200 micron chamber followed by a 100 micron dispersion chamber in series. The 200 micron chamber reduced particle size enough to prevent plugging of the 100 micron chamber. Another configuration tested was a 75 micron emulsification chamber followed by a 200 micron chamber providing back pressure. Heat was generated during processing so chilled water cooled the processed solutions. Solution concentrations from 1% to 5% solids were utilized successfully, with 2% utilized most often. Single and multiple passes through the HPH were tested. Two passes through the HPH were standard. Particle size and

viscosity were measured immediately and the remaining sample was frozen for later analysis. **Figure 5** shows a flow chart of the entire process.

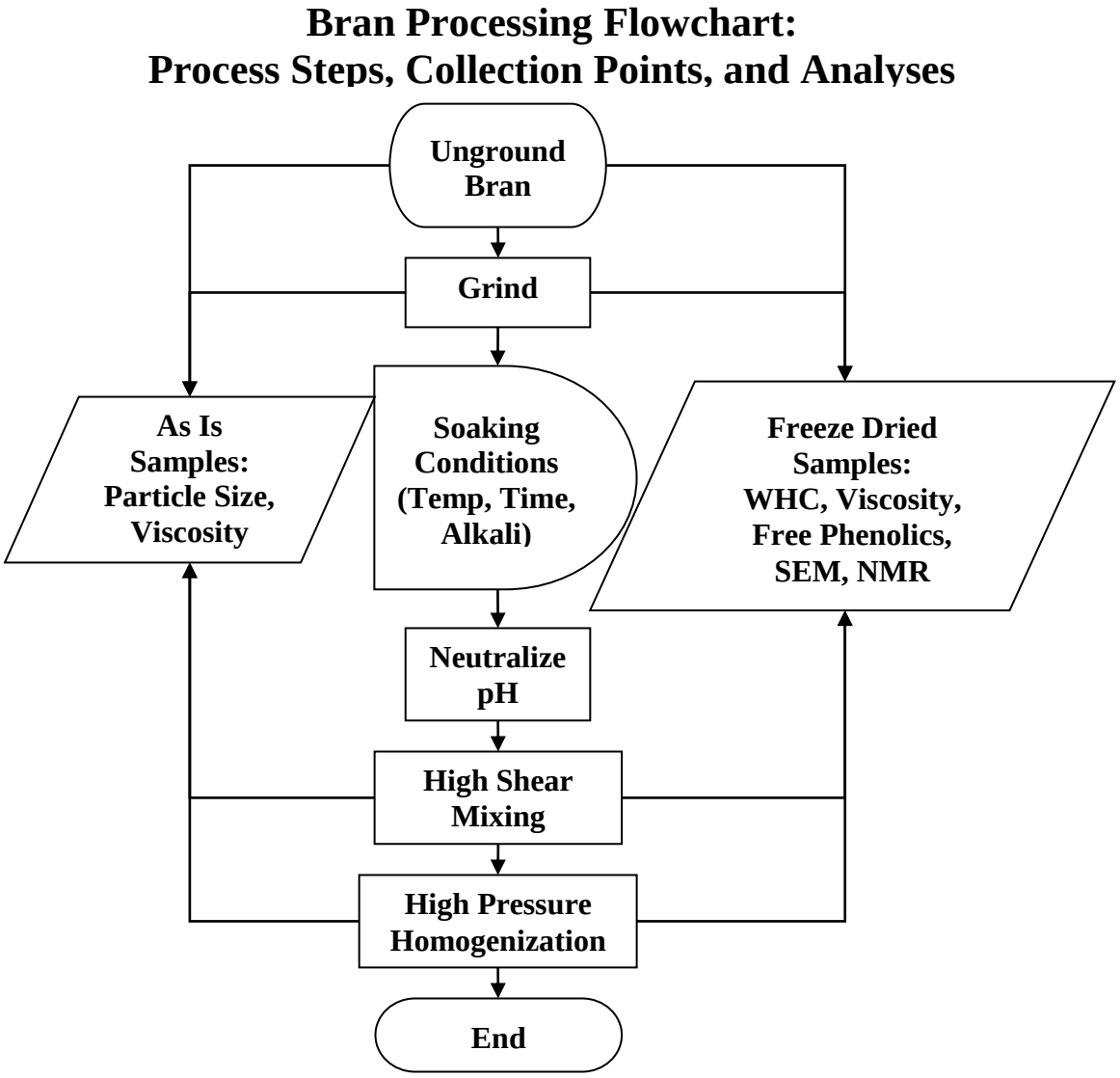


**Figure 5. Bran Processing Flowchart**

**Moisture Content** of duplicate samples was measured by AACC Moisture method 44-15A. Briefly, 2g of sample was weighed into an aluminum dish with cover. Sample was heated in a gravity oven at 130°F for 60 minutes. Dishes were removed from the oven, covered, and placed in a desiccator to cool. Moisture was calculated as loss in weight divided by original sample weight. Replicates must be within 0.2% moisture.

**Particle Size Distribution** was measured by two methods: AACC Method 66-20 using a Ro-tap sieve shaker (model RX 29), or laser diffraction particle size analyzer (LS-13-320, Beckman Coulter Inc., Brea, CA) using the Universal Liquid Module. For Ro-tap sieve analysis, 100g of bran or flour were sifted with tapping for 10 minutes.

**Viscosity** was measured on a Rapid Visco Analyzer (RVA, Newport Scientific). 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. 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.

**Freeze Drying** was performed on a Virtis Freezemobile freeze dryer. Models used include 12EL and 25EL (SP Industries, Gardiner, NY).

**Water Hydration Capacity** (WHC) was difficult to perform due to the limited sample size available and the extremely high WHC of processed samples making them difficult to hydrate without excess water. Therefore a modified micro-version of AACC method 56-30 was developed. Briefly, 0.1g of sample (A) of known moisture content (B) was combined with 1.0 ml distilled water (C) in weighed 2.0 ml microfuge tubes (D).

The sample was vortexed 3 times over 30 minutes to disperse and completely wet the sample. Tubes were centrifuged at 2000g for 10 minutes. The supernatant (E) (which included water extractable material) was decanted to a separate tube (F). The remaining hydrated pellet and tube was weighed (G). WHC was calculated as the water remaining in the sample divided by the sample dry matter weight (G-A-D) / (A*(1-B%)). Duplicate samples were run in each test and the result was the average. Less than 5% relative standard deviation is targeted, though most samples had less than 2% relative standard deviation among duplicate samples. Freeze dried samples were assumed to have 1% moisture content for calculation purposes.

**Scanning Electron Microscopy** (SEM) was utilized to provide a visual confirmation of bran modification. A Hitachi S3500N variable pressure scanning electron microscope (SEM) was utilized for selected process samples. Samples were critical point dried to remove all water. This was accomplished by dispersing in water in 1.5 ml microfuge tubes, mixing on a Roto Torque Tumbler 5 minutes at speed setting 5, then gradually replacing water with increasing concentrations of ethanol solution: 25%, 50%, 75%, 95%, and three times at 100%. After each tumbling, the samples were centrifuged and then re-dispersed in the next solution. Samples were placed on stubs and coated with gold in a Fullam Sputter-coater**.**

The Hitachi S3500N Variable Pressure Scanning Electron Microscope (SEM) had Quartz PCI digital imaging, photo CRT recording camera, stage with motor driven X & Y axes, standard Secondary Electron Detector (SED), Emitech K-1150 Cryogenic System with cryo-prep unit, and airlock interface to SEM. The electron beam was set at 5KV.

**Free Phenolic Extractions** from bran samples were performed according to established methods (Adom and Liu, 2002). Briefly, 1.0g defatted bran samples were extracted with 20 ml 80% ethanol for 1 hour with shaking each time. Samples were centrifuged and supernatant removed. Three extractions were performed and the pooled extracts were concentrated to about 5 ml by rotary evaporation under vacuum at 45°C. The sample was acidified with HCL (pH <2) and transferred to a centrifuge tube with water. Phenolic acids were extracted 3 times with 10 ml diethyl ether and vortexed. The ether fractions were removed, pooled, and evaporated. The extract was re-dissolved in methanol and samples were diluted for HPLC analysis.

**HPLC Measurements** of phenolic acids in sample extracts were performed using a RP-HPLC procedure on a Varian Pro Star HPLC with Polaris solvent delivery modules (Palo Alto, CA), employing a Phenomenex Luna phenyl-hexyl column (Torrance, CA), 250 mm x 4.6 mm, 5 μm. Elution was carried out using a binary gradient consisting of 1 mM TFA (A) and MeOH (B): Following a rinsing and equilibration step, initially concentration of 5% MeOH was ramped to 30% over 25 minutes, then from 30% to 50% MeOH from 25 to 35 minutes, then from 50% to 100% MeOH from 35 to 45 minutes, then held isocratically for 10 minutes at 100% MeOH, then ramped down to 5% over 5 minutes and held isocratically for 5 minutes. Pre flush volume was 30 μL. The injection volume was 10 μL, the column temperature 75°C, and the flow rate was maintained at 1 mL $min^{-1}$. Detection was carried out using a Varian ProStar 325 UV Vis detector (Palo Alto, CA) at 280nm.

**Matrix-Water Interactions**, which are closely related to the structure-function relationships in foods, were characterized using nuclear magnetic resonance (NMR) relaxometry. Water mobility is a function of the physical and chemical environments in which the water molecules are embedded. NMR technique lends itself best to the study of physiochemical properties of water inside samples. Untreated and processed bran samples were freeze dried and hydrated to specific moisture contents from 0.01% g H2O/g d.m. to 5.0 g H2O/g d.m. Samples were equilibrated for 24 hours and measured. Three sequences FID, CPMG and INVREC were applied to measure relaxation times T2*, T22, T21, and T1.

**Experimental Design and Analysis** utilized Minitab statistical software version 16 (Minitab Inc., State College, PA). The factorial design was analyzed by General Linear Model to determine main effects and interactions.

## Results and Discussion

### *Flour and Bran Fraction Particle Size*

**Table 2.** depicts typical fraction sizes for an experimental milling and sifting setup.

| Milling Fraction | From Break Rolls | From Reduction Rolls |
|---|---|---|
| **Bran** | **> 864 µ** | |
| **Shorts (plus germ)** | **< Bran and > 375 µ** | **> 240µ** |
| **Red Dog** | **< Shorts and > 136µ** | **< Shorts and > 136µ** |
| **Straight Grade Flour** | **< 136µ** | **< 136µ** |
| **Mill Feed: Combined Bran, Shorts (plus germ) and Red Dog** | | |

**Table 2. Typical Fraction Sizes for Experimental Milling and Sifting**

The measured quantity of each common milling fraction from the HRW blend is displayed in **Table 3**. The shorts fraction contained the germ which is about 3% of a wheat kernel. This means that the shorts fraction was really composed of about 25% germ. Shorts contain mostly medium sized bran pieces with some flour adhering to the bran. The particle size distribution results for the hard wheat blend are displayed in **Table 4**. It is interesting to note that large sized bran and shorts are detrimental to processing whole grain baked goods. Bran for whole grain products is often further ground to produce a fine grind bran or flour where the mill feed is ground to the same relative particle size as the flour fraction. The red dog fraction contains a large

proportion of wheat aleurone and more than 83% was passed through a 250μ sieve opening. Due to the high lipid content in the shorts, preliminary process screening experiments were conducted on the bran and red dog fractions only.

| | Mill Feed | | | Flour | Total % |
|---|---|---|---|---|---|
| | Bran | Shorts | Red Dog | | |
| **Mill Performance, Test Run 6/22/07** | **6.05%** | **11.58%** | **2.05%** | **80.32%** | **100.00%** |

**Table 3. Commercial Milling Fractionation of 90% HRS, 10% HRW Wheat Blend**

| US Sieve Size (overs) | Opening (microns) | Bran % | Shorts % | Red Dog % | Flour % | Mill Feed % |
|---|---|---|---|---|---|---|
| **30** | **>600** | 90.39% | 48.57% | 0.00% | 0.00% | 49.11% |
| **45** | **335-600** | 6.41% | 39.32% | 0.83% | 0.00% | 27.85% |
| **60** | **250-335** | 0.71% | 8.18% | 16.55% | 0.05% | 9.07% |
| **80** | **180-250** | 0.40% | 1.56% | 27.57% | 0.95% | 4.94% |
| **100** | **150-180** | 0.05% | 0.05% | 14.76% | 4.66% | 1.58% |
| **120** | **125-150** | 1.00% | 0.80% | 16.94% | 9.82% | 2.45% |
| **140** | **106-125** | 0.10% | 0.05% | 18.88% | 12.53% | 2.05% |
| **pan** | **<106** | 0.93% | 1.47% | 4.47% | 71.98% | 2.95% |
| **Total** | | 100.00% | 100.00% | 100.00% | 100.00% | 100.00% |

**Table 4. Particle Size Distribution of Hard Wheat Blend Fractions**

### *Dry Grinding of Bran and Red Dog*

The goals of dry grinding were to test the range of reduction possible as a preprocess variable and to optimize grinding size to achieve necessary particle size reduction for further processing while minimize processing cost and complexity.

Multiple dry and wet milling processes were tested including pin milling, hammer milling, rotor-beater type milling, and colloid milling. Reaction chamber limitations on the laboratory HPH require particle size reduction of bran to less than about 400 microns in order not to plug the 200 and 100 micron interaction chambers. The rotor-beater mill (Retsch, SR300) may utilize a range of screen sizes including 2.0, 1.0, 0.75, 0.5, 0.25, 0.20, and 0.12mm. Although a screen opening may be a circular diameter, particles are accelerated to high speeds in the rotor beater mill and produce a range of particle sizes. The 0.12mm screen produced an average bran particle size of 106μ, but it was nearly impossible to grind bran or red dog through the 0.12mm screen without frequent plugging. Grinding through the 0.2mm screen was very slow with significant loss to dust through the air collector. When grinding bran through the 0.25mm screen, a first pass through the 1.0mm (or 0.75mm) screen was necessary; whereas 0.5mm could be done in a single pass.

| Fraction (overs) | Opening (microns) | Bran, 0.75mm % | Bran, 0.5mm % | Red dog, 0.5mm % | Particle Size |
|---|---|---|---|---|---|
| **30** | **>600** | 5.89% | 0.07% | 0.04% | Large |
| **45** | **335-600** | 35.63% | 11.27% | 0.04% | |
| **60** | **250-335** | 18.65% | 26.24% | 4.82% | |
| **80** | **180-250** | 11.56% | 26.42% | 20.52% | Coarse |
| **100** | **150-180** | 3.72% | 10.32% | 29.05% | |
| **120** | **125-150** | 9.75% | 19.16% | 44.18% | |
| **140** | **106-125** | 3.77% | 2.21% | 0.52% | Fine |
| **pan** | **<106** | 11.03% | 4.31% | 0.83% | |
| **Total** | | 100.00% | 100.00% | 100.00% | |

**Table 5. Particle Size Distribution of Ground Bran and Red Dog Samples**

**Table 5** shows bran samples ground on a Retsch mill through 0.75mm screen, followed by sifting, then ground through a 0.5mm screen then sifted again. Red dog samples needed only one pass through the 0.5mm screen due to their smaller starting particle size.

During HPH processing, bran particles are forced through a 100μ opening and although only dilute solutions were tested under very high pressure, Large particle size bran > 400 microns sometimes clogged the chambers resulting in a shutdown of the equipment. Based on these observations, the Large bran particle size range was not acceptable and bran fractions were then minimally processed through a 0.5mm screen size. Because particle size was shown to be important for release of phenolics (Caprez and Fairweather-Tait 1982; Yu and Kies 1993) and physical properties (Chau et al. 2006), bran grinding through 0.25mm (fine) and 0.5mm (coarse) were chosen for further optimization.

### *Initial HPH Experiments with Bran and Red Dog*

Hard Wheat Bran and Red Dog samples were ground through a 120μ screen in the rotor beater mill to minimize risk of plugging during initial HPH testing. Bran and red dog were suspended in water at 3% concentration and prehydrated using a high shear mixer at 50% power for 5 minutes and constantly stirred during HPH to prevent particle settling. The HPH was operated at 13,000 psi and had a throughput of approximately 500 ml/min. Sample temperature increases during processing so chilled water was used to bring the processed sample back to 25°C within a few seconds and prior to the sample

exiting the machine. The 100µ dispersion chamber is for disruption and particle size reduction, while the 75µ chamber is primarily made for emulsification, however, due to the smaller opening of the 75µ chamber, it was also tested to attempt to minimize sample particle size.

**Figure 6** shows the bran fraction particle size reduction after cumulative HPH treatments. The small average starting particle size due to dry grinding through a 120µ screen was difficult to achieve and excludes approximately 15% sample loss during grinding so the sample was not completely representative of the original starting material. Samples did not appear to increase in viscosity as bran particle or red dog particle size was reduced. Due to pressure changes during cycling of the air piston intensifying pump in the homogenizer, Microfluidics recommends two passes through the laboratory homogenizer to ensure complete processing.

The 50-60µ average particle size for the 100µ 3X samples was unexpectedly high compared to claims of 2-3µ results from the manufacturer. The difference is due to the material. Bran and red dog are very difficult to grind to small size compared to typical emulsion droplets, cells, or non-fibrous solids tested by Microfluidics.

It was also surprising that after 20 minutes of continuous operation and roughly 20 passes during that time, the final samples differed less than expected. The main difference in bran was the reduction in particle size of the largest fractions, moving from 26.2% > 30.5 µm in the 75 µm 3X sample to only 8.5% > 30.5 µm in the 75 µm 20 min sample. There were no clogging issues with the 3% solids concentration in these samples, however there was also no visually distinguishable viscosity increase either.

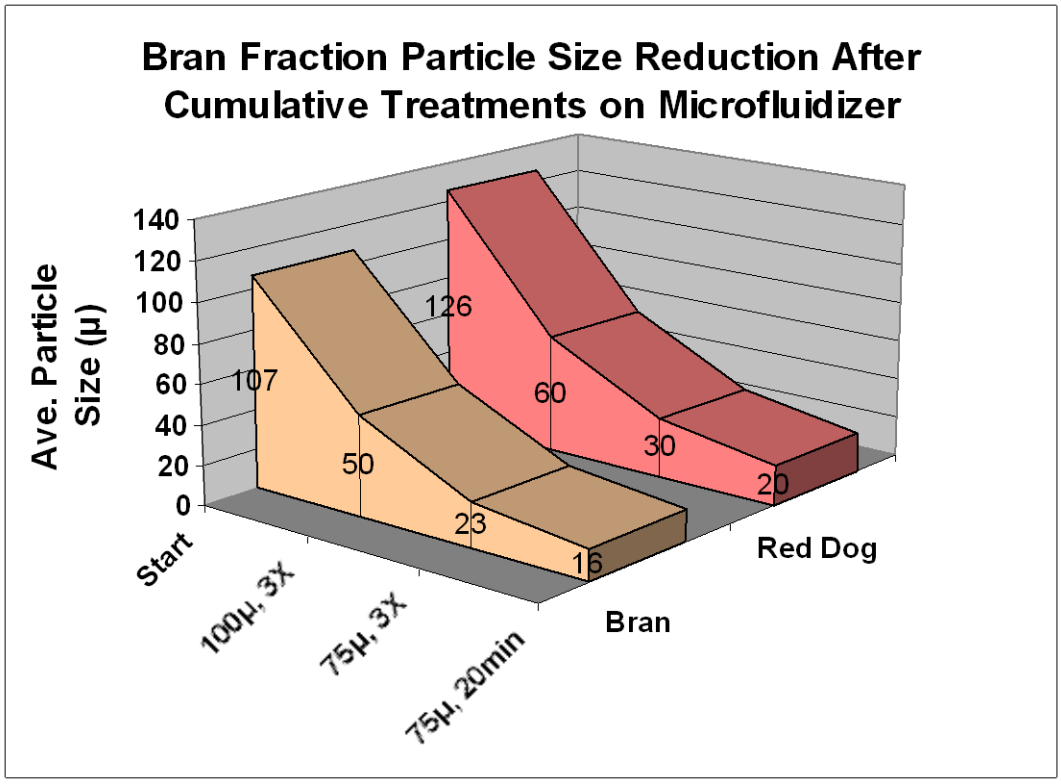


**Figure 6. HPH Particle Size Reduction of Bran and Red Dog Samples**

Visual examination of the bran and red dog samples by SEM indicated very different appearances. **Figure 7** shows SEM images of unprocessed and HPH bran. Native bran surface shown in A and B, both inside and outside, is mostly smooth, continuous, and solid in appearance. With such a large particle size and continuous surface it is difficult for enzymes to penetrate. Further processing to reduce particle size and increase surface available for reaction is necessary. In unprocessed bran, WHC averaged 1.95 ml/g. HPH bran from the 75µ 20 min sample in C and D shows torn and broken surfaces with holes and pores. Individual fibers are seen giving the surface a

fuzzy or furry appearance. This is due to the extremely high shear during HPH separating fibers and opening the bran structure. WHC doubled to 3.94 ml/g. Although the process was successful in showing some degradation of fiber structures, the lack of increase in viscosity compared to 10 ml/g WHC of other commercially available highly expanded fibers processed by HPH indicates the need for additional preprocessing to improve the effect of the final HPH treatment.

## SEM of Unprocessed and High Pressure Homogenized Bran

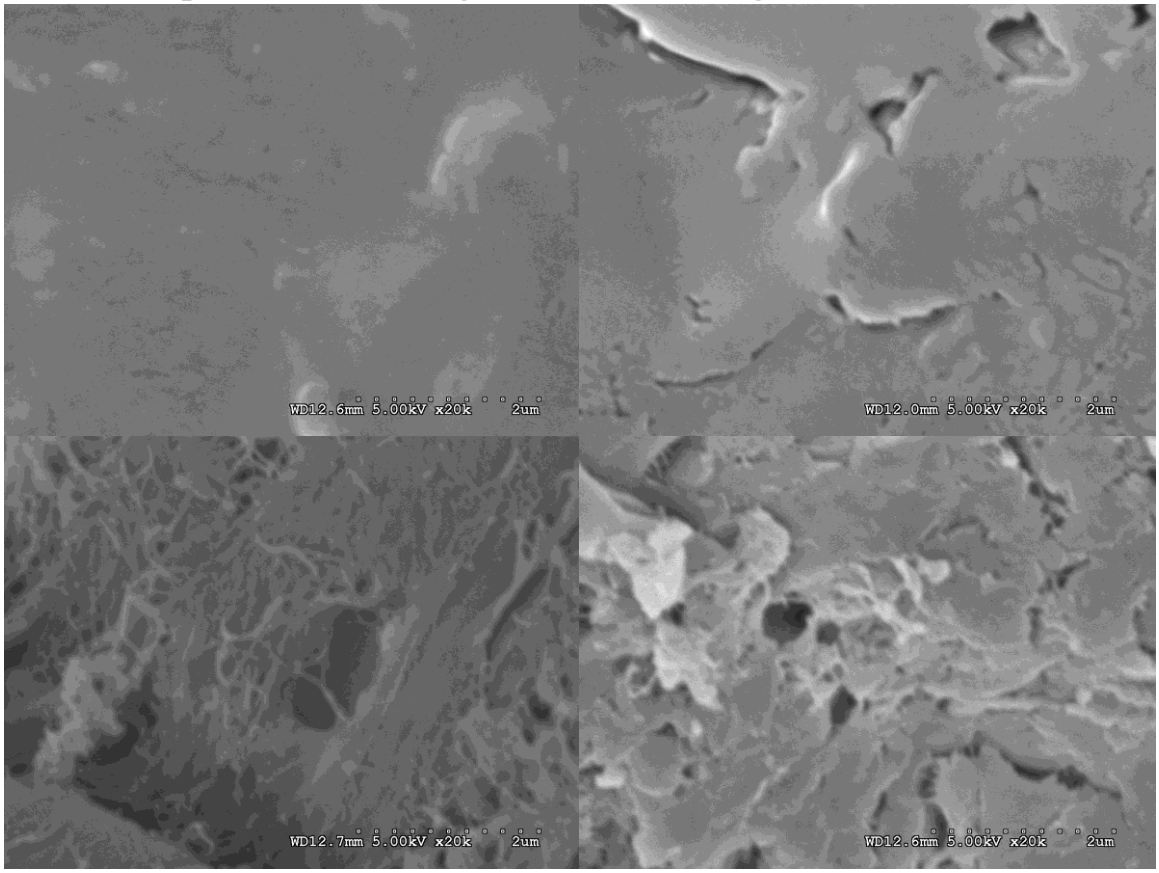


**Figure 7. SEM of Unprocessed and High Pressure Homogenized Bran**
A and B show unmodified bran outer and inner surfaces (20,000X).
C and D show pre-ground, high pressure homogenized bran (20,000X).

Testing at multiple temperatures (25°C, 50°C, and 75°C) did not significantly change the extent of particle size reduction or increase the sample viscosity. Additionally, the high temperature HPH operation caused problems with o-ring failure in the pump. Continued testing at 25°C process temperature decided. Processing continuously for 20 minutes is also impractical on a commercial basis. After consulting with Microfluidics, maximizing processing pressure was determined to be the most important factor to improve results in the minimum number of passes. In order to increase the operating pressure from 13,000 psi to the recommended maximum 23,000 psi, a new air compressor capable of supplying 120 psi at 57cfm continuously was purchased. Based on the preliminary test results of temperature, pressure, and number of passes, the HPH conditions moving forward were set at two passes at 25°C and 23,000 psi in the 100μ chamber.

### *Chemical Pretreatment of Bran: Experimental Screening Design*

**Table 6** shows the process variable conditions for the factorial screening design. Pretreatment of wheat bran with dilute Sodium Hydroxide (NaOH) to break ester bonds between phenolic acids and bran polysaccharides was examined along with hydration time and temperature conditions. Three bran particle sizes were also tested. Following treatment, the bran solutions were neutralized with HCl and mixed 5 minutes at 24,000

rpm with a high shear mixer. Particle size was measured before and after HPH processing.

| **Factors** | **Levels:** | **1** | **2** | **3** |
|---|---|---|---|---|
| Bran Grind | | Unground | thru 0.5mm | thru 0.2mm |
| Concentration of NaOH | | 0.0N | 0.1N | 0.2N |
| Time (hours) | | 6 | 12 | 24 |
| Temperature during soaking | | | 60C | |
| Homogenization Conditions: 2% bran solution, 2 passes, 23,000 psi, 25°C | | | | |

**Table 6. Chemical Pretreatment Screening Design**

Test results proved the inability to utilize the laboratory HPH on large particle samples. None of the unground wheat bran samples treated with 0.0N NaOH were able to pass through the interaction chamber without clogging. The high shear mixer was operated at 24,000rpm for 5 minutes and considerably reduced the average particle size of the samples treated with NaOH at higher levels. Particle size results are shown in **Table 7**. Interestingly, the highest concentration of NaOH did not produce the lowest particle size after HPH treatment. This may have been due to increased swelling of bran particles due to disruption of their fiber structure.

| Pretreatment Screening Design | | | | Ave Part Size (μ) | |
|---|---|---|---|---|---|
| **Bran Grind** | **NaOH Conc** | **Soak Time** | **Soak Temp** | **5 min Mix** | **2 Pass HPH** |
| none | 0.0N | 6 | 60C | 749.1 | X |
| none | 0.0N | 12 | 60C | 739.3 | X |
| none | 0.0N | 24 | 60C | 677.9 | X |
| none | 0.1N | 6 | 60C | 300.8 | X |
| none | 0.1N | 12 | 60C | 295.0 | X |
| none | 0.1N | 24 | 60C | 195.6 | X |
| none | 0.2N | 6 | 60C | 354.0 | X |
| none | 0.2N | 12 | 60C | 328.8 | X |
| none | 0.2N | 24 | 60C | 299.0 | X |
| 0.5mm | 0.0N | 6 | 60C | 559.2 | X |
| 0.5mm | 0.0N | 12 | 60C | 555.2 | X |
| 0.5mm | 0.0N | 24 | 60C | 567.6 | X |
| 0.5mm | 0.1N | 6 | 60C | 306.2 | X |
| 0.5mm | 0.1N | 12 | 60C | 230.7 | X |
| 0.5mm | 0.1N | 24 | 60C | 216.0 | 50.76 |
| 0.5mm | 0.2N | 6 | 60C | 265.8 | X |
| 0.5mm | 0.2N | 12 | 60C | 259.0 | X |
| 0.5mm | 0.2N | 24 | 60C | 248.1 | 51.88 |
| 0.2mm | 0.0N | 6 | 60C | 130.6 | 35.12 |
| 0.2mm | 0.0N | 12 | 60C | 188.0 | 33.75 |
| 0.2mm | 0.0N | 24 | 60C | 141.9 | 35.34 |
| 0.2mm | 0.1N | 6 | 60C | 110.1 | 41.47 |
| 0.2mm | 0.1N | 12 | 60C | 101.9 | 45.23 |
| 0.2mm | 0.1N | 24 | 60C | 91.7 | 49.08 |
| 0.2mm | 0.2N | 6 | 60C | 108.2 | 46.68 |
| 0.2mm | 0.2N | 12 | 60C | 102.5 | 48.88 |
| 0.2mm | 0.2N | 24 | 60C | 100.8 | 47.25 |

**Table 7. Particle Size Results of Pretreatment Screening Design**

### *Effects of Bran Grinding, Chemical Pretreatment, and HPH Processing on Bran Particle Size and Viscosity*

**Figures 8 and 9** show the effects of HPH processing on particle size distribution. Samples that received high shear mixing only (**Figure 8**) had differences in size distribution when the larger coarse bran particle size was used but not the smaller fine bran particle size. The high shear mixing had improved particle size reduction in the coarse bran after treatment with sodium hydroxide. The sodium hydroxide is breaking bonds within the bran structure and improving the effectiveness of the high shear mixer, though only to some extent as indicated by the finer particle size bran showing no difference in particle size due to sodium hydroxide treatment. This is most likely due to the high shear mixers rotor-stator gap limiting the further reduction in particle size.

High pressure homogenization, in contrast, reduced all samples to nearly equivalent particle size distributions regardless of pretreatment (**Figure 9**). This is important because on a commercial production basis, it is possible to use HPH to vastly reduce particle size without any chemical pretreatment. It is important to also note, however, that HPH alone did not achieve the increased viscosity (**Table 8**), water hydration capacity, or free phenolic targets compared to combined alkali + HPH treatments as shown in the next report sections.

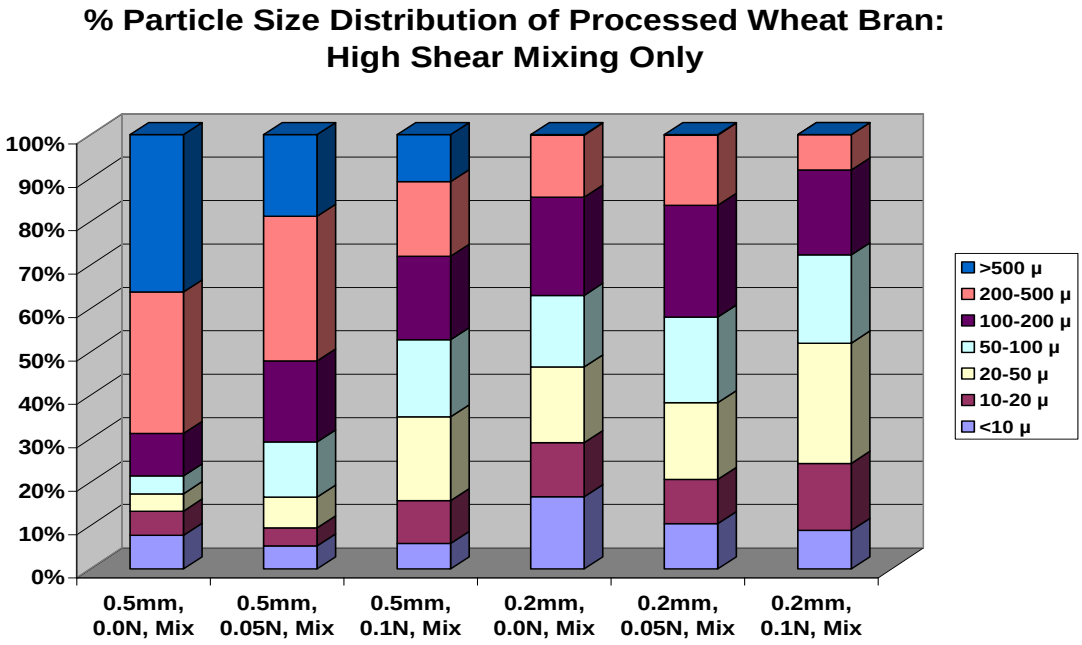


**Figure 8. Particle size distribution of processed bran without HPH is dependent on starting particle size and NaOH treatment**

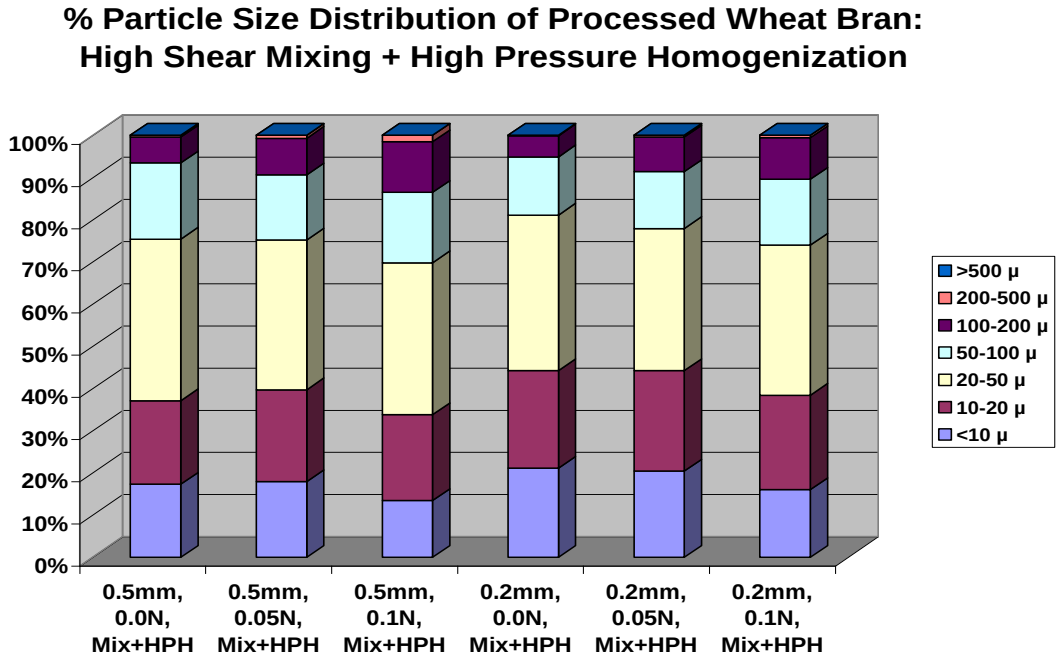


**Figure 9. Particle size distribution of processed bran with HPH is not dependent on starting particle size and NaOH treatment**

| Viscosity increase was principally due to NaOH treatment, but starting bran size was also significant | | | | | | |
|---|---|---|---|---|---|---|
| **Bran Grind** (through Retsch Mill screen) | **0.5mm** | **0.5mm** | **0.5mm** | **0.2mm** | **0.2mm** | **0.2mm** |
| **NaOH Concentration** (24 hour shaking at 60°C) | **0.0N** | **0.05N** | **0.1N** | **0.0N** | **0.05N** | **0.1N** |
| **High Shear Mixing** (24K rpm, 5 min) | **Mix** | **Mix** | **Mix** | **Mix** | **Mix** | **Mix** |
| **% Viscosity Increase** (vs. Control 2% soln.) | **Control** | **122%** | **212%** | **27%** | **71%** | **114%** |
| **High Pressure Homogenization** (2 passes, 23K psi) | **Mix+HPH** | **Mix+HPH** | **Mix+HPH** | **Mix+HPH** | **Mix+HPH** | **Mix+HPH** |
| **% Viscosity Increase** (vs. Control, 2% soln.) | **75%** | **169%** | **187%** | **-1%** | **59%** | **158%** |

**Table 8. Effect of Wheat Bran Processing Variables of Bran Grind, NaOH Concentration, and HPH on RVA Viscosity**

### *Effects of Bran Grinding, Chemical Pretreatment, and HPH Processing on the Release of Bound Phenolics*

Phenolic Analysis was measured by HPLC and is represented in **Figure 10** by the measured level of ferulic acid as the primary indicator of free phenolic levels**.** The chart clearly shows the dramatic increase in free ferulic acid due to the NaOH pretreatment. Pretreatment without NaOH showed minimal free ferulic acid. All samples shown underwent high pressure homogenization except the two unprocessed bran controls; CI and CII. Although not shown in **Figure 10**, samples prior collected to high pressure

homogenization were also analyzed and free ferulic acid levels were similar but slightly lower than their HPH processed counterparts**.**

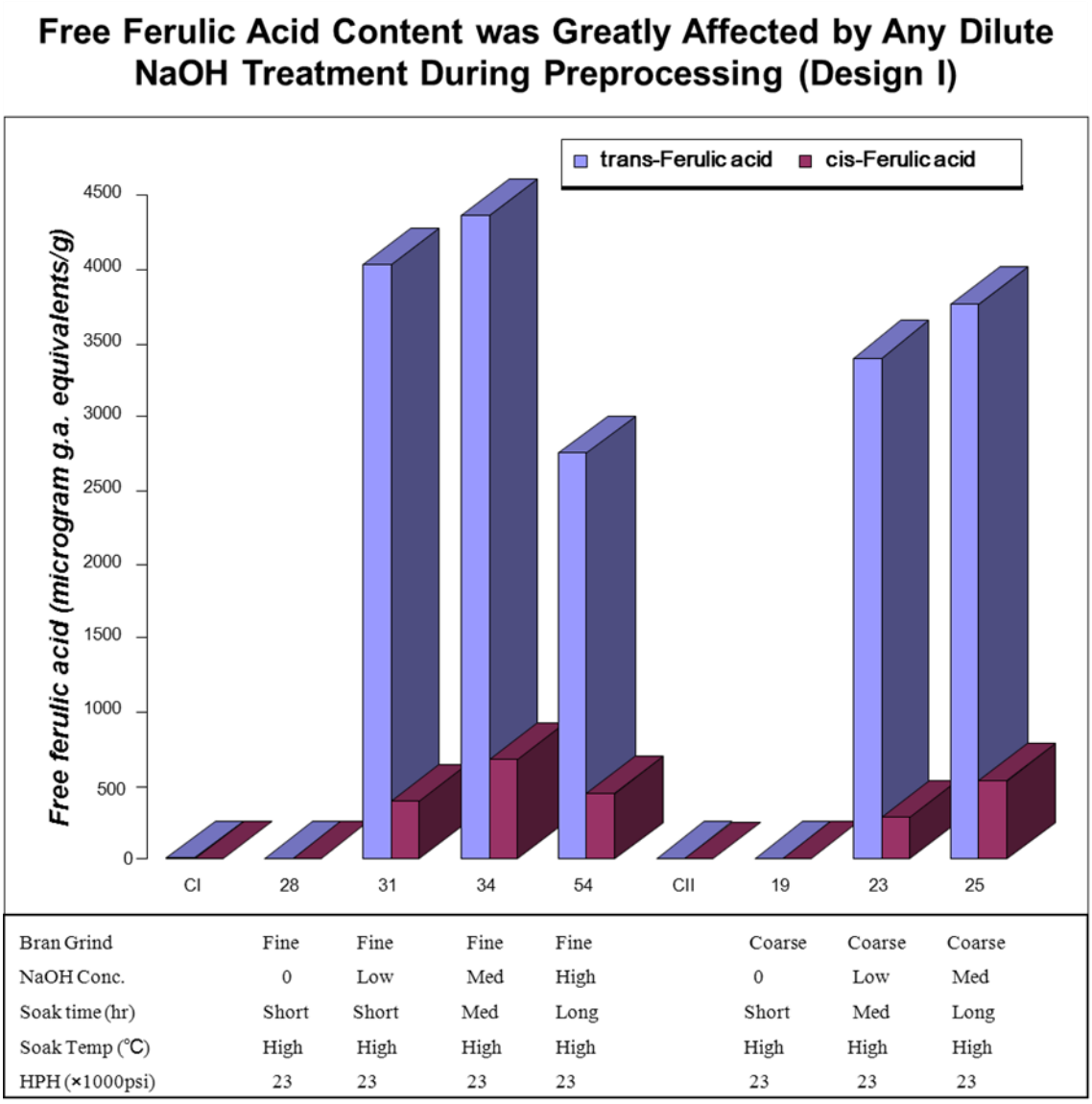


**Figure 10. Free Ferulic Acid Content of Processed Wheat Bran**

Free ferulic acid content of the most extremely processed sample (#54) was compared to a sample without NaOH treatment (#28) and a control intact bran sample (C1). HPLC analysis showed little to no free ferulic acid content in the C1 or #28 samples, whereas the #54 sample contained approximately 2700μg gallic acid equivalents/gram bran. HPH alone clearly was not releasing free phenolics whereas dilute alkali liberated acted by breaking ester bonds between phenolic acids and arabinose.

The untreated and HPH treated samples also did not develop high viscosity in solution, but the alkali treated HPH samples were much more viscous (**Table 8**). Preliminary WHC method development indicated a slight reduction in WHC in finely ground bran compared to coarse bran. Lundberg et al (2003) found a strong correlation between microsurface area and water retention capacity (WRC) of highly refined cellulose (HRC) samples. Their HRC process incorporated a similar dilute alkali soaking followed by HPH processing as the wheat bran samples of this research. The surface area maximum in their experiments corresponded to the WRC maximum for the same concentration of alkali and the curves were similar. Therefore WHC and viscosity will be good indicators of increased surface area of processed bran.

***Effects on the Matrix-Water interactions of HPH processed Wheat Bran***

The matrix-water interactions, which are closely related to the structure-function relationships in foods, were characterized using nuclear magnetic resonance (NMR) relaxometry. **Figure 11** shows T2* for the samples at lower moisture. Although samples treated with High NaOH level had longer relaxation times at 10% moisture (0.1g H2O/g dry matter), this is possibly due to an increase in soluble carbohydrates and sugars increasing proton mobility and shown as an increase in T2* relaxation time. The other treated samples all had lower water mobility than the two controls.

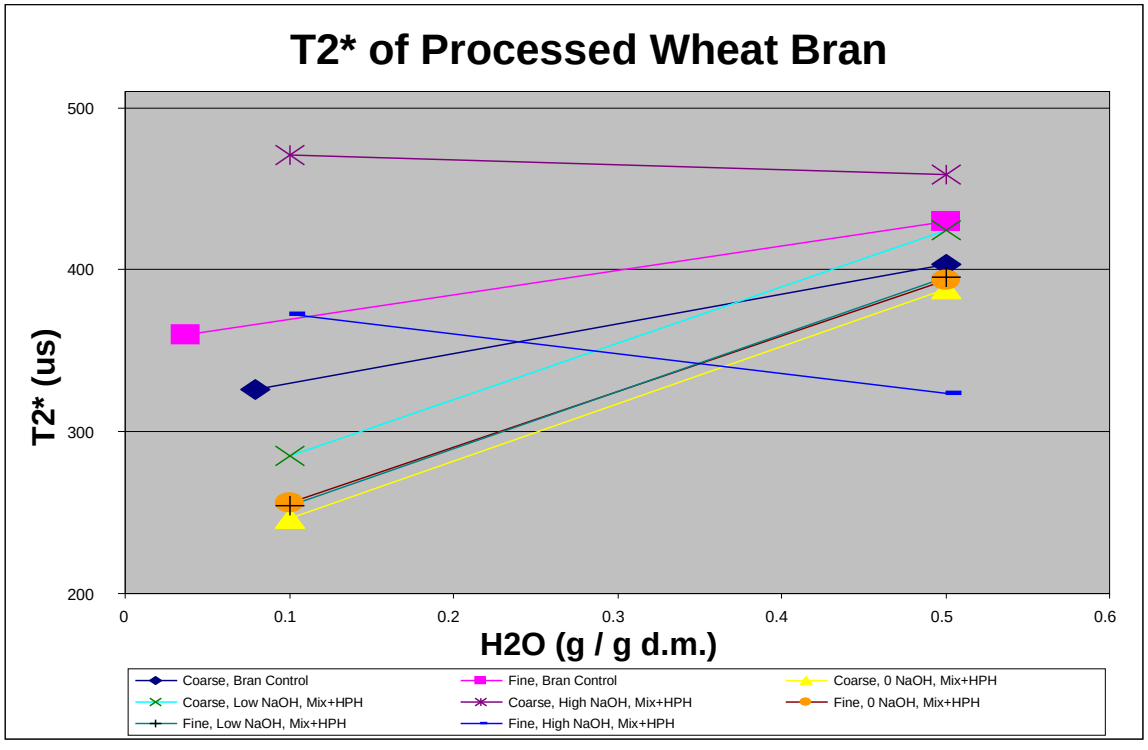


**Figure 11. T2* of Processed Wheat Bran**

The T1 relaxation (**Figure 12**) depends on the state of the sample. The low point in the "U" shaped curve is the transition between the glassy state (left of the low T1 point and rubbery state (right of the low T1 point) of the sample. The shortest relaxation times were for processed samples with no NaOH. Longest relaxation times were for the control samples. Increased bound or reduced free water in the processed samples was also seen physically as a harder more structured sample.

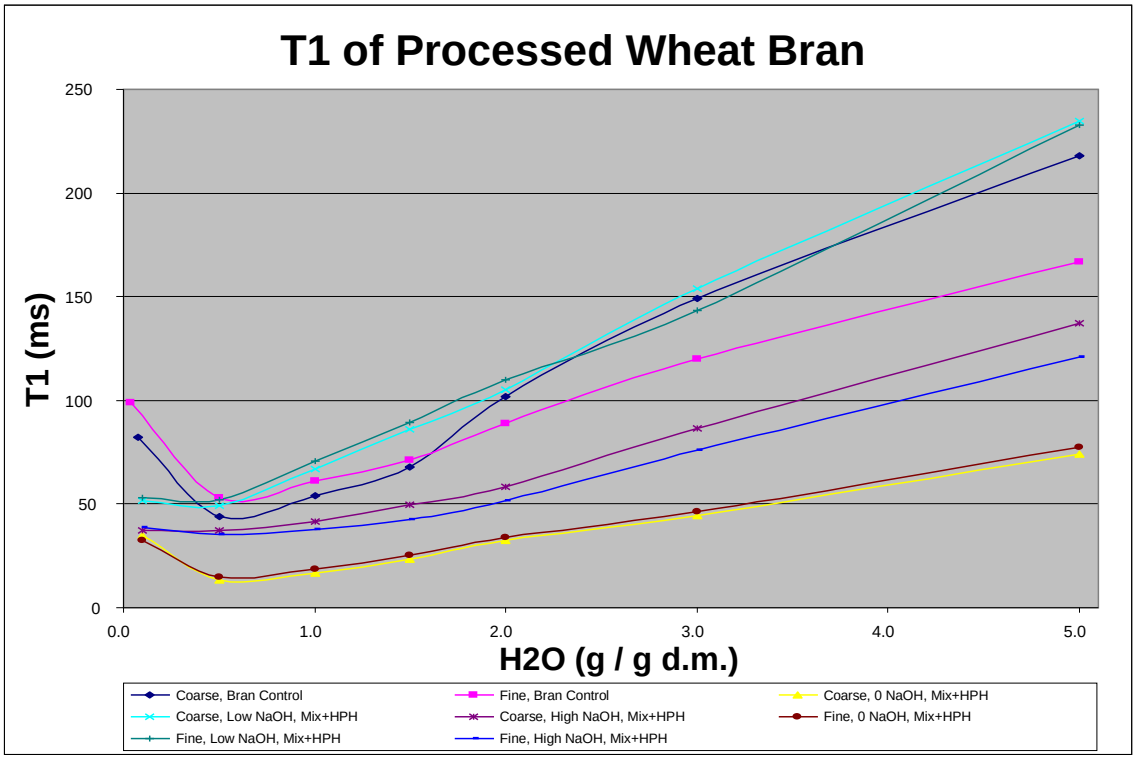


**Figure 12. T1 of Processed Wheat Bran**

T21 and T22 (**Figures 13 and 14**) have relevance at higher water content. Generally, lower relaxation times indicate more tightly bound water. The controls generally had longer relaxation times indicating more free water, but the results of processed samples varied depending on moisture content. One explanation is that the degree of processing affected the particle size (due primarily to HPH), soluble polysaccharide/sugar content, ability of the sample to gel, water hydration capacity and viscosity. Due to the complexity of the sample responses additional testing is needed to further elucidate effects of treatments.

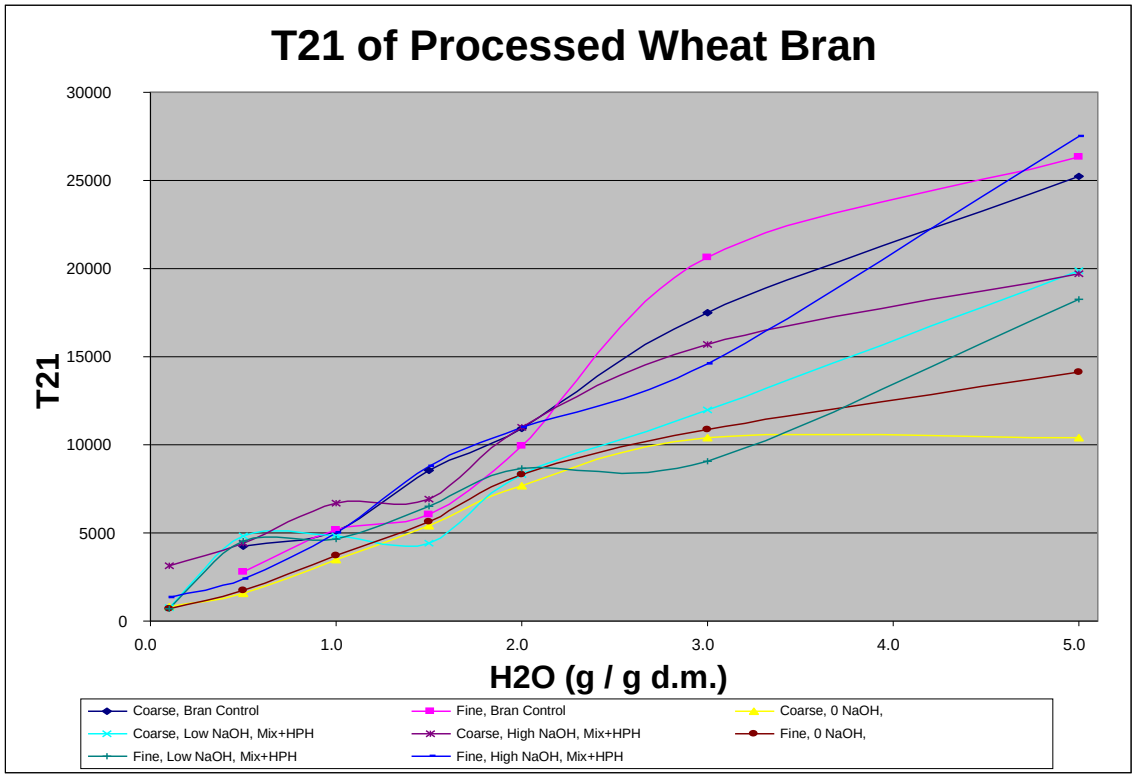


**Figure 13. T21 of Processed Wheat Bran**

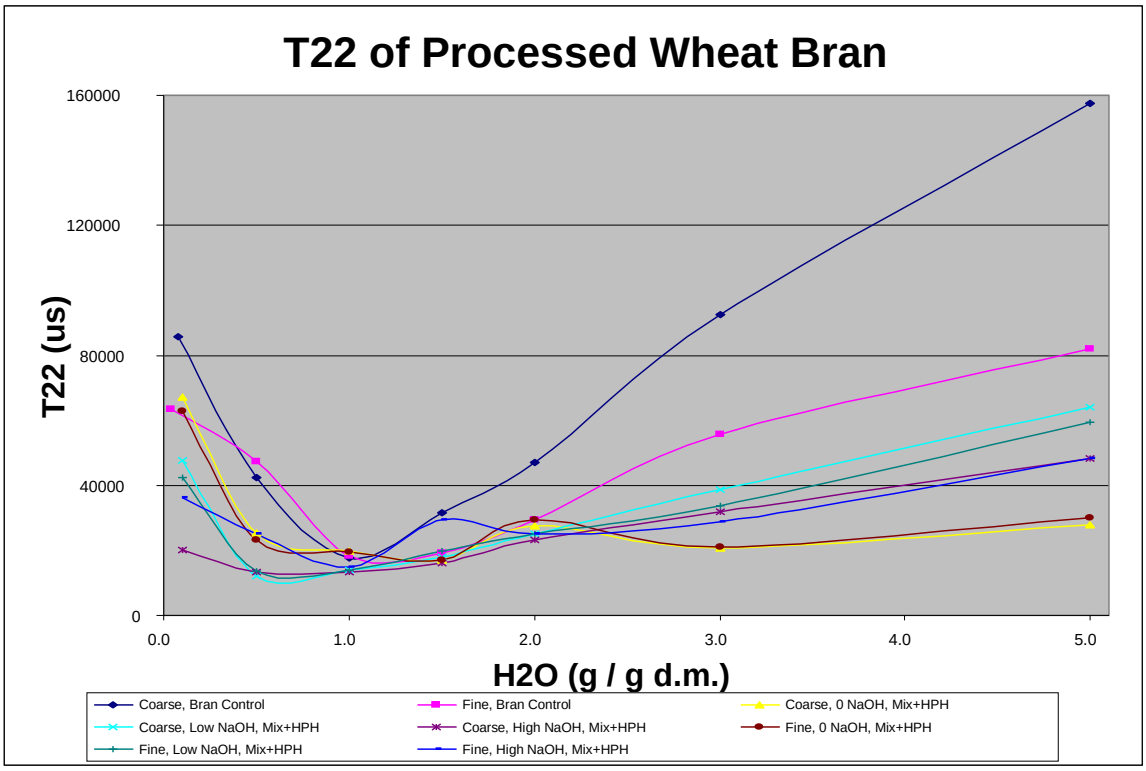


**Figure 14. T22 of Processed Wheat Bran**

## Conclusions

Bran Fraction Material and particle size: Unground bran could not be homogenized and pre-grinding was necessary. The large bran fraction from milling represents the most difficult to process and will be used for optimization. Bran grinding will be from 0.5mm to 0.2mm. Wheat bran without germ will be processed in future experiments.

High Pressure Homogenization: Higher Pressure of 23,000 psi and 2 passes will maximize bran modification while minimizing cost. Using a 2% bran solution will provide adequate sample size without clogging the homogenizer, but increased concentrations should be explored once optimized procedure was determined to increase process efficiency. Ambient temperature of 25°C will allow ease of handling and permit consistent process control.

Pretreatment conditions must all be optimized together for best results. NaOH concentration from 0.0N to 0.1N, reaction time from 6 to 24 hours, and reaction temperature from 30°C to 60°C will be optimized in a factorial design. Samples will be taken both before and after HPH processing to determine impact of pretreatment alone as well as the combined pretreatment plus HPH effect.

Viscosity and WHC results should have good correlation to surface area changes, so both Viscosity and WHC will be measured. Free ferulic acid will be measured as an indicator of free phenolics. Optimized samples will undergo additional characterization including SEM, soluble conjugate phenolics, and water extractable material (Wa-Ex).

Successful optimization will have the effects of maximizing viscosity, WHC, and release of bound phenolics in processed wheat bran.

## *Project 2: Combined Preprocess and High Pressure Homogenization Optimization*

### Hypothesis

Historical research cited and preliminary work on this program have shown the effectiveness in releasing free phenolics with a chemical (dilute alkali) pretreatment and the ability to increase viscosity and water hydration capacity (WHC) by combining alkali pretreatment with high pressure homogenization (HPH). We hypothesize that it is possible to optimize the combination of pretreatment and HPH conditions to achieve improved functionality wheat bran with increased bioavailability of phenolic antioxidants. Improved functionality in this case is defined as a large increase in viscosity and WHC in solution.

### Objectives

Utilizing variable ranges determined in Project 1, create statistical designs to evaluate preprocessing conditions (bran grind, NaOH concentration, temperature, time) combined with HPH to maximize viscosity, WHC, and free phenolics in processed bran samples. Additionally characterize selected samples for particle size, water extractable material, soluble bound phenolics, soluble fiber, and visual confirmation of modification by scanning electron microscopy (SEM).

## Materials and Methods

**Raw Materials:** Soft White Wheat (SWW) bran was obtained from King Milling Company (Lowell, MI), Sustigrain Barley and Ultrafiber stabilized wheat bran were obtained from ConAgra (Omaha, NE). All samples were stored at -20°C until use.

**Dry Particle Size Reduction** utilized rotor beater milling technology (Retsch GmbH, Model SR 300, Haan, Germany). Bran was ground in a single pass through the 0.5mm screen to obtain coarse bran samples. Fine bran samples were ground through the 0.5mm screen and then the 0.2mm screen.

**Alkali Pretreatment:** 5% bran solutions were prepared (using 10g bran sample on dry basis, add solution to 200g) with appropriate alkali concentration and agitated in an Innova 4300 incubated shaker (New Brunswick Scientific, Edison, NJ) for the specified time and temperature. Solutions were then neutralized to pH 7 with 1N HCl.

**High Shear Mixing:** Samples were diluted to 2% bran in solution totaling 500ml and then mixed with high shear using a T25 Basic SI homogenizer with S25N-25F dispersing element (IKA Works, Wilmington NC). High shear mixing was performed prior to all high pressure homogenization (HPH) processing to reduce particle size and prevent clogging of HPH disruption chambers. 0.5 to 1.0L samples were mixed for 5 minutes at 24,000 rpm. Viscosity and particle size distribution were typically measured at this time and samples frozen for further analysis. Remaining samples were high pressure homogenized.

**High Pressure Homogenization:** (HPH) was carried out on an M-110Y Laboratory Microfluidizer® Processor (Newton, MA). Homogenization pressure was

controlled by adjusting compressed air pressure. Air pressure and flow was targeted to achieve 23,000 psi during processing. Disruption chambers used to achieve homogenization included a 200 micron chamber followed by a 100 micron dispersion chamber in series. The 200 micron chamber reduced particle size enough to prevent plugging of the 100 micron chamber. Heat was generated during processing so chilled water cooled the processed solutions. Solution concentrations of 2% were targeted. Two passes through the HPH were standard. Particle size and viscosity were measured immediately and the remaining sample was frozen for later analysis. **Figure 15** shows a flow chart of the entire process.

**Moisture Content** of duplicate samples was measured by AACC Moisture method 44-15A. Briefly, 2g of sample was weighed into an aluminum dish with cover. Sample was heated in a gravity oven at 130°F for 60 minutes. Dishes were removed from the oven, covered, and placed in a desiccator to cool. Moisture was calculated as loss in weight divided by original sample weight. Replicates must be within 0.2% moisture.

**Particle Size Distribution** was measured by laser diffraction particle size analyzer (LS-13-320, Beckman Coulter Inc., Brea, CA) using the Universal Liquid Module.

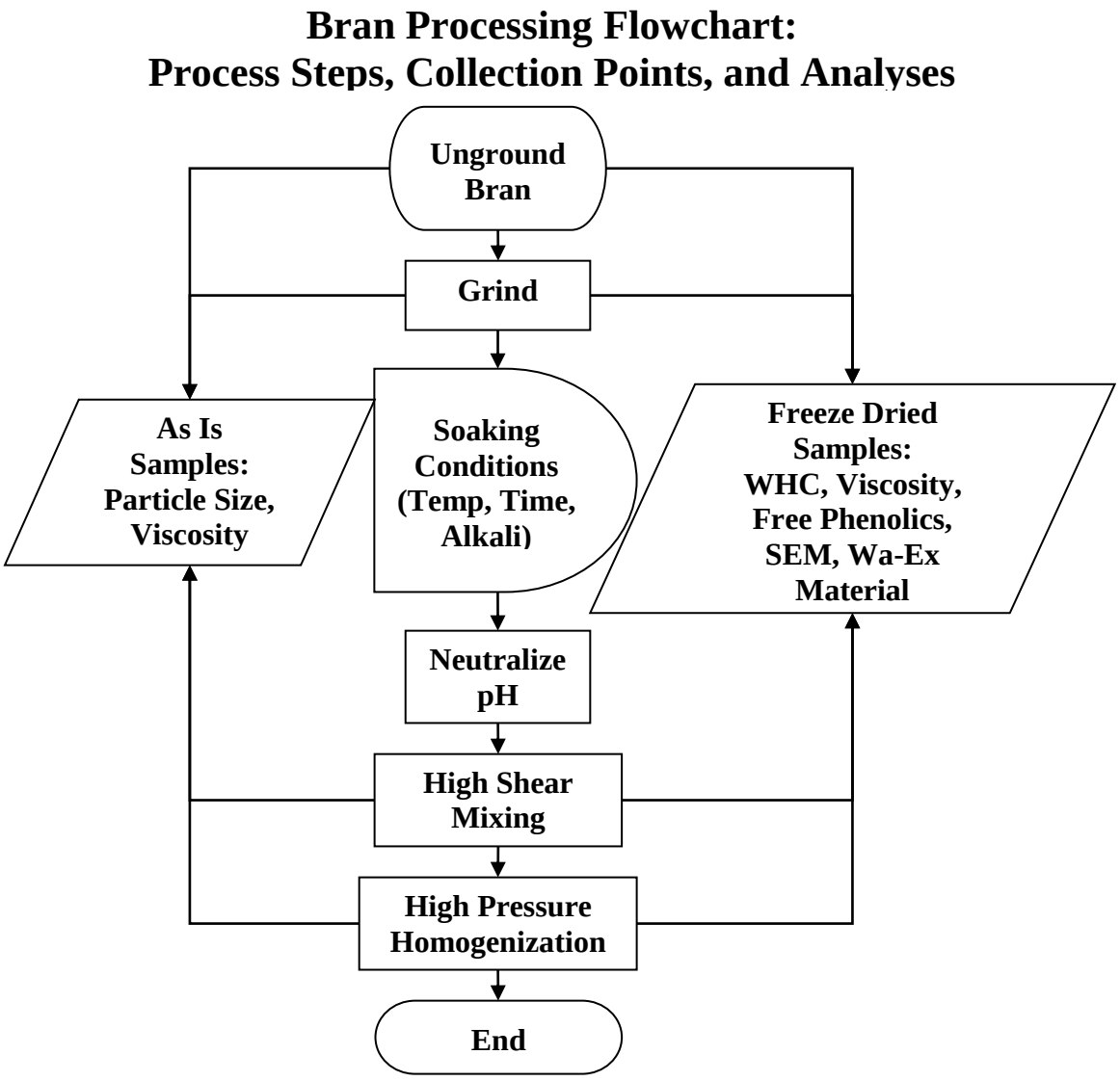


**Figure 15. Bran Processing Flowchart**

**Viscosity** was measured on a Rapid Visco Analyzer (RVA, Newport Scientific). 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. 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.

**Freeze Drying** was performed on a Virtis Freezemobile freeze dryer. Models used include 12EL and 25EL (SP Industries, Gardiner, NY).

**Water Hydration Capacity** (WHC) was difficult to perform due to the limited sample size available and the extremely high WHC of processed samples making them difficult to hydrate without excess water. Therefore a modified micro-version of AACC method 56-30 was developed. Briefly, 0.1g of sample (A) of known moisture content (B) was combined with 1.0 ml distilled water (C) in weighed 2.0 ml microfuge tubes (D). The sample was vortexed 3 times over 30 minutes to disperse and completely wet the sample. Tubes were centrifuged at 2000g for 10 minutes. The supernatant (E) (which included water extractable material) was decanted to a separate tube (F). The remaining hydrated pellet and tube was weighed (G). WHC was calculated as the water remaining in the sample divided by the sample dry matter weight (G-A-D) / (A*(1-B%)). Duplicate samples were run in each test and the result was the average. Less than 5% relative standard deviation is targeted, though most samples had less than 2% relative standard deviation among duplicate samples. Freeze dried samples were assumed to have 1% moisture content for calculation purposes.

**Water Extractable Content** (Wa-Ex) and **Water Unextractable Content** (Wa-Ux) of the samples were determined from the WHC test. Tubes with the hydrated samples were freeze dried and weighed (H). The percent Wa-Ux was calculated by subtracting the weight of the tube and dividing by the starting material weight (H-

D)/A*100. The percent Wa-Ex was simply 100-(Wa-Ux). While not completely accurate because a small amount of insoluble material was decanted with the supernatant, this quick method gave a reasonable result for comparing different samples.

**Phenolic Extractions of samples were conducted as follows:**

**Free Phenolics** were extracted from bran samples with 80% ethanol as follows: Approximately 0.100g of freeze dried bran samples were weighed on an analytical balance. Each sample was extracted three successive times with 1.5ml 80% ethanol under constant agitation for 30 minutes at 40°C. Samples were centrifuged at 10,000g for 10 minutes and supernatants of the three successive extractions were pooled. Extraction volume of the three sample extractions was adjusted to 5.00ml using 80% ethanol. 1.5ml of extract was centrifuged at 15,000g for 10 minutes to remove any insoluble suspended particles and 1.00ml was filtered, transferred to an HPLC vial, and frozen at -80°C until analysis. A further extract dilution of 2:5 achieved a concentration of 0.0080 g bran dry matter per ml extract, similar to soluble bound sample extractions.

**Soluble Bound Phenolics** were extracted in a two part procedure: First, 2.00ml of the Free Phenolic extract above was saponified with 1.00ml 6M NaOH (2M soln.) for 3 hours under constant agitation at 25°C. The solution was acidified with 1.00ml 6M HCL. 0.20ml of internal standard containing trans-2-Hydroxycinnamic acid and 3,4,5-trimethoxycinnamic acid was added. The 0.20ml delivered approximately 100ug of each standard in 80% ethanol. An additional 0.68ml ethanol was added to bring the volume to 4.88ml at 50% ethanol, and then adjusted to 5.00ml total volume with 50% ethanol. 1.5ml of extract was centrifuged at 15,000g for 10 minutes to remove any insoluble

suspended particles and 1.00ml was filtered, transferred to an HPLC vial, and frozen at -80°C until analysis. The procedure achieved a concentration of 0.0080 g bran dry matter per ml extract, similar to the free phenolic sample. This sample should theoretically contain measureable ferulic acid that was derived from both free and soluble bound sources, with the soluble bound phenolics liberated during the saponification process.

The second part of the procedure involved the re-extraction of the bran pellet with water to extract any remaining soluble bound phenolics that were not extracted in the first procedure with 80% ethanol. Each sample was extracted three successive times with 1.5ml HPLC grade water under constant agitation for 30 minutes at 40°C. Samples were centrifuged at 10,000g for 10 minutes and supernatants of the three successive extractions were pooled. Extraction volume was adjusted to 5.00ml using water. 2.00ml of the extract above was saponified with 1.00ml 6M NaOH (2M soln.) for 3 hours under constant agitation at 25°C. The solution was acidified with 1.00ml 6M HCL. 0.20ml of internal standard containing trans-2-Hydroxycinnamic acid (o-coumaric acid) and 3,4,5-trimethoxycinnamic acid (TMCA) was added. The 0.20ml delivered approximately 100ug of each standard in 80% ethanol. The total volume was then adjusted to 5.00ml with water. 1.5ml of extract was centrifuged at 15,000g for 10 minutes to remove any insoluble suspended particles and 1.00ml was filtered, transferred to an HPLC vial, and frozen at -80°C until analysis. The procedure achieved a concentration of 0.0080 g bran dry matter per ml extract, similar to the other samples.

Although bound phenolics were not tested at the time, the remaining pellet was preserved for future analysis of insoluble bound phenolics.

**HPLC Measurement** of phenolic acids in sample extracts was performed on a Shimadzu LC-6AD system equipped with an SIL-10AF autosampler and an SPD-M20A photodiode array detector according to the a procedure developed by Dobberstein and Bunzel (2010). This procedure utilized an RP-HPLC procedure employing a Luna phenyl hexyl column (250 mm × 4.6 mm i.d., 5 μm particle size, plus 3 mm × 4.6 mm i.d. guard column). Elution was carried out using a ternary gradient system made up of 1 mM aqueous TFA, ACN, and MeOH at a flow rate of 1.0 mL/min. The injection volume was 20 μL, and the separation was performed at 45 °C. Chromatograms were monitored at 280 nm. The following gradients were used eluent A, 1 mM aqueous TFA; eluent B, ACN/1 mM aqueous TFA [90/10 (v/v)]; and eluent C, MeOH/1 mM aqueous TFA [90/10 (v/v)]. Separation of phenolic monomers: initially 87% A, 13% B, and 0% C held for 10 min, linear over 10 min to 77% A, 20% B, and 3% C, linear over 5 min to 70% A, 25% B, and 5% C, linear over 5 min to 25% A, 50% B, and 25% C, following a 10 min equilibration step.

**Scanning Electron Microscopy** (SEM) was utilized to provide a visual confirmation of bran modification. A Hitachi S3500N variable pressure scanning electron microscope (SEM) was utilized for selected process samples. Freeze dried samples were used for analysis. Samples were placed on stubs and coated with gold in a Fullam Sputter-coater**.**

The Hitachi S3500N Variable Pressure Scanning Electron Microscope (SEM) had Quartz PCI digital imaging, photo CRT recording camera, stage with motor driven X &

Y axes, standard Secondary Electron Detector (SED), Emitech K-1150 Cryogenic System with cryo-prep unit, and airlock interface to SEM. The electron beam was set at 5KV.

**Factorial Experimental Designs** were generated with variable settings determined by screening during Project 1. The variables and settings for the first design are listed in **Table 9**. This design required 36 runs blocking by Soaking Temperature.

| Factors | Levels: | Low | Med | High |
|---|---|---|---|---|
| Bran Grind | | thru 0.5mm | | thru 0.2mm |
| Concentration of NaOH | | 0.0N | 0.05N | 0.1N |
| Soaking Time (hours) | | 6 | 15 | 24 |
| Soaking Temperature | | 30°C | | 60°C |
| Wet Particle Size Reduction | | Mix Only | | Mix + HPH |

**Table 9. Factorial Experimental Design to Optimize HPH Preprocess Conditions**

A second **Augmentation Factorial Design** was conducted to augment the first factorial design, add replication, and better optimize the results. In this design, bran grind was fixed at 0.5mm and the NaOH concentration range was tightened (0.05N, 0.075N, 0.1N). Soaking time was also fixed at 24 hours, but Soaking temperature was increased to three levels (30°C, 45°C, 60°C) Although this design had only 9 bran cells plus a control, Sustigrain barley flour and Ultrafiber stabilized wheat bran and germ products (ConAgra Mills) were also tested for a total of 30 cells.

**Experimental Design and Analysis** utilized Minitab statistical software version 16 (Minitab Inc., State College, PA). Factorial designs were analyzed by General Linear Model to determine main effects and interactions, or by Response Surface Regression to optimize factors for maximizing viscosity or WHC.

## Results and Discussion

### *Maximizing Viscosity of HPH Processed Wheat Bran through Experimental Design Testing*

Maximizing processed bran viscosity to achieve similar cholesterol lowering characteristics of oat and barley cereals is a goal of the project. Following the experimental design outlined in the Materials and Methods section, viscosity was measured immediately following processing, while WHC was measured on freeze dried samples. Viscosity and WHC results for the 36 samples are shown in **Table 10**.

Processed samples generated a wide range for both viscosity and WHC. At first look, increases in viscosity seemed correlated with increases in WHC. Main effects and interactions in the experimental design were analyzed to accurately define the relationships between the design variables and responses

**Table 11** shows the analysis of variance (ANOVA) of the factorial design indicating significant main effects of NaOH concentration ($p<.001$) and Soak Time ($p<.001$) on bran viscosity. Main effects are also illustrated in **Figure 16**. Soak temperature did not show up as significant probably due to the fact that the NaOH was effective at the lower temperature. The 60C did have a darker color and require less HCl to neutralize, indicating an increased level of reaction, but while viscosity will increase as more fiber becomes partially degraded, continued breakdown will eventually reduce viscosity. Additional testing at the midpoint of 45C was later added in the augmentation design to test this hypothesis. Bran grind was also not shown to be significant, but it is

preferable to begin with a larger particle size due to the increased cost of fine grinding bran.

| StdOrder | Soak Temp (C) | Grind (mm) | NaOH Conc (N) | Soak Time (hrs) | Sample No. | Visc HPH (mPa*s) | WHC (g/g dm) |
|---|---|---|---|---|---|---|---|
| 1 | 30 | 0.5 | 0 | 6 | 1 | 14.34 | 3.781 |
| 2 | 30 | 0.5 | 0 | 15 | 2 | 17.59 | 3.659 |
| 3 | 30 | 0.5 | 0 | 24 | 3 | 14.39 | 3.880 |
| 4 | 30 | 0.5 | 0.05 | 6 | 4 | 24.58 | 4.268 |
| 5 | 30 | 0.5 | 0.05 | 15 | 5 | 27.46 | 4.818 |
| 6 | 30 | 0.5 | 0.05 | 24 | 6 | 37.37 | 4.846 |
| 7 | 30 | 0.5 | 0.1 | 6 | 7 | 25.51 | 4.581 |
| 8 | 30 | 0.5 | 0.1 | 15 | 8 | 32.00 | 4.898 |
| 9 | 30 | 0.5 | 0.1 | 24 | 9 | 92.17 | 4.964 |
| 10 | 30 | 0.2 | 0 | 6 | 10 | 14.39 | 3.529 |
| 11 | 30 | 0.2 | 0 | 15 | 11 | 23.67 | 3.669 |
| 12 | 30 | 0.2 | 0 | 24 | 12 | 16.28 | 3.608 |
| 13 | 30 | 0.2 | 0.05 | 6 | 13 | 17.32 | 4.275 |
| 14 | 30 | 0.2 | 0.05 | 15 | 14 | 29.03 | 5.102 |
| 15 | 30 | 0.2 | 0.05 | 24 | 15 | 27.50 | 5.007 |
| 16 | 30 | 0.2 | 0.1 | 6 | 16 | 24.31 | 4.637 |
| 17 | 30 | 0.2 | 0.1 | 15 | 17 | 30.37 | 5.011 |
| 18 | 30 | 0.2 | 0.1 | 24 | 18 | 83.00 | 5.104 |
| 19 | 60 | 0.5 | 0 | 6 | 19 | 15.74 | 4.016 |
| 20 | 60 | 0.5 | 0 | 15 | 20 | 19.53 | 4.074 |
| 21 | 60 | 0.5 | 0 | 24 | 21 | 15.33 | 4.032 |
| 22 | 60 | 0.5 | 0.05 | 6 | 22 | 30.37 | 5.032 |
| 23 | 60 | 0.5 | 0.05 | 15 | 23 | 34.96 | 5.511 |
| 24 | 60 | 0.5 | 0.05 | 24 | 24 | 35.73 | 5.409 |
| 25 | 60 | 0.5 | 0.1 | 6 | 25 | 41.28 | 5.107 |
| 26 | 60 | 0.5 | 0.1 | 15 | 26 | 41.21 | 4.700 |
| 27 | 60 | 0.5 | 0.1 | 24 | 27 | 74.01 | 4.719 |
| 28 | 60 | 0.2 | 0 | 6 | 28 | 14.41 | 3.909 |
| 29 | 60 | 0.2 | 0 | 15 | 29 | 17.74 | 3.711 |
| 30 | 60 | 0.2 | 0 | 24 | 30 | 14.27 | 3.702 |
| 31 | 60 | 0.2 | 0.05 | 6 | 31 | 30.04 | 5.162 |
| 32 | 60 | 0.2 | 0.05 | 15 | 32 | 47.38 | 4.926 |
| 33 | 60 | 0.2 | 0.05 | 24 | 33 | 36.39 | 5.431 |
| 34 | 60 | 0.2 | 0.1 | 6 | 34 | 41.68 | 4.951 |
| 35 | 60 | 0.2 | 0.1 | 15 | 35 | 37.44 | 5.296 |
| 36 | 60 | 0.2 | 0.1 | 24 | 36 | 44.66 | 4.992 |

**Table 10. Experimental Design Results for Viscosity and WHC**

**General Linear Model: Ave. Viscosity vs. Soak Temp, Bran Grind, NaOH Conc., and Soak Time**

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Soak Temp | 1 | 4.64 | 4.64 | 4.64 | 0.14 | 0.718 |
| Bran Grind | 1 | 111.69 | 111.69 | 111.69 | 3.38 | 0.103 |
| **NaOH Conc.** | 2 | 6077.38 | 6077.38 | 3038.69 | 91.89 | **0.000** |
| **Soak Time** | 1 | 1363.84 | 1363.84 | 1363.84 | 41.24 | **0.000** |
| Soak Temp*NaOH Conc. | 2 | 158.99 | 158.99 | 79.50 | 2.40 | 0.152 |
| **Soak Temp*Soak Time** | 1 | 509.44 | 509.44 | 509.44 | 15.41 | **0.004** |
| Bran Grind*NaOH Conc. | 2 | 95.32 | 95.32 | 47.66 | 1.44 | 0.292 |
| Bran Grind*Soak Time | 1 | 71.58 | 71.58 | 71.58 | 2.16 | 0.179 |
| **NaOH Conc.*Soak Time** | 2 | 1732.11 | 1732.11 | 866.06 | 26.19 | **0.000** |
| **Soak Temp*NaOH Conc.*Soak Time** | 2 | 555.83 | 555.83 | 277.91 | 8.40 | **0.011** |
| Error | 8 | 264.54 | 264.54 | 33.07 | | |
| Total | 23 | 10945.35 | | | | |

**S = 5.75042 R-Sq = 97.58% R-Sq(adj) = 93.05%**

**Table 11. Analysis of Variance for Viscosity, Using Adjusted SS for Tests**

Several factor interactions were also significant including Soak Time and Soak Temperature (p=.004), NaOH concentration and Soak Time (p<.001), and the three way interaction of Soak Time, Temp, and NaOH (p=.011). The significant interactions indicate the multiple potential control points in the process for controlling the chemical treatment. Because time, temperature, and alkali concentration significantly interact, the settings can be adjusted to achieve an optimum amount of modification for a variety of different bran or fiber substrates. A surface plot of viscosity vs. NaOH concentration and soak time is shown in **Figure 17.** Maximum viscosity for the HPH processed wheat bran was obtained in this design at the highest concentration of NaOH and the longest soak time. This indicates that the factor set points may not be optimized to reach the highest

viscosity and increasing the NaOH concentration or soak time could result in an increased processed bran viscosity. Additional testing of NaOH concentration and temperature at the 24 hour soak time was done to augment the design. Results are presented in a later section.

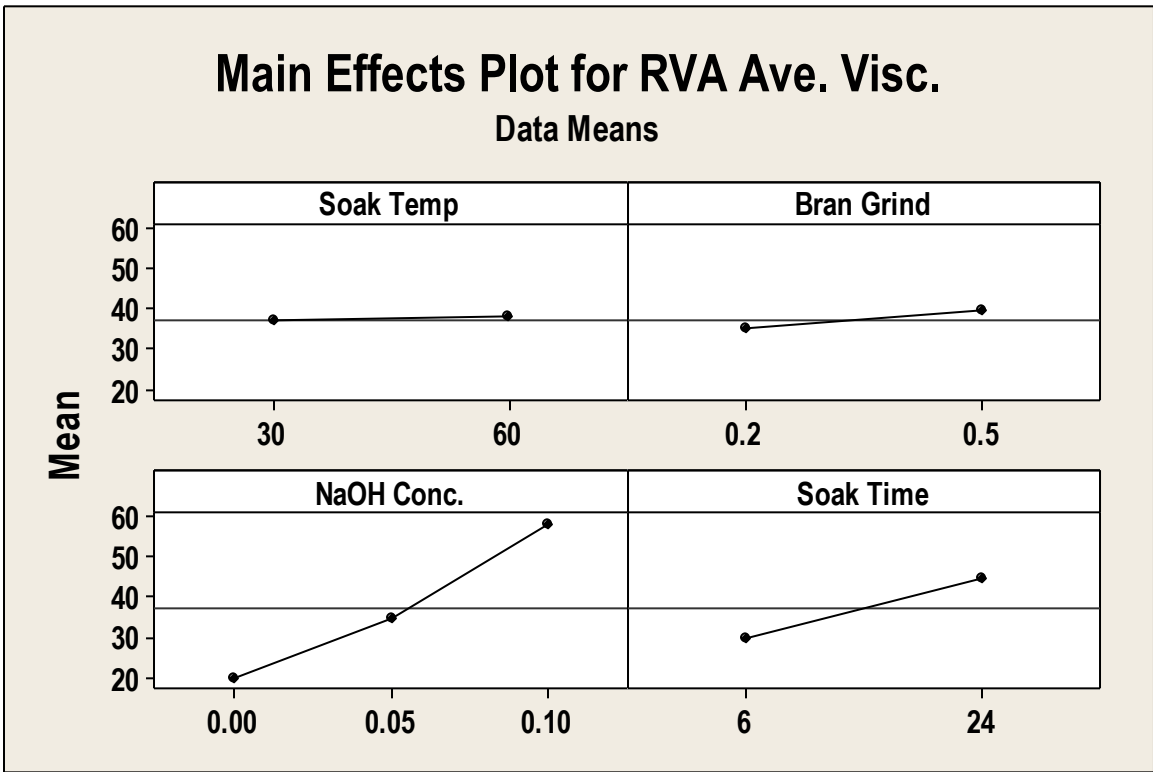


**Figure 16. Main Effects Plots for Average Viscosity in the Experimental Design**

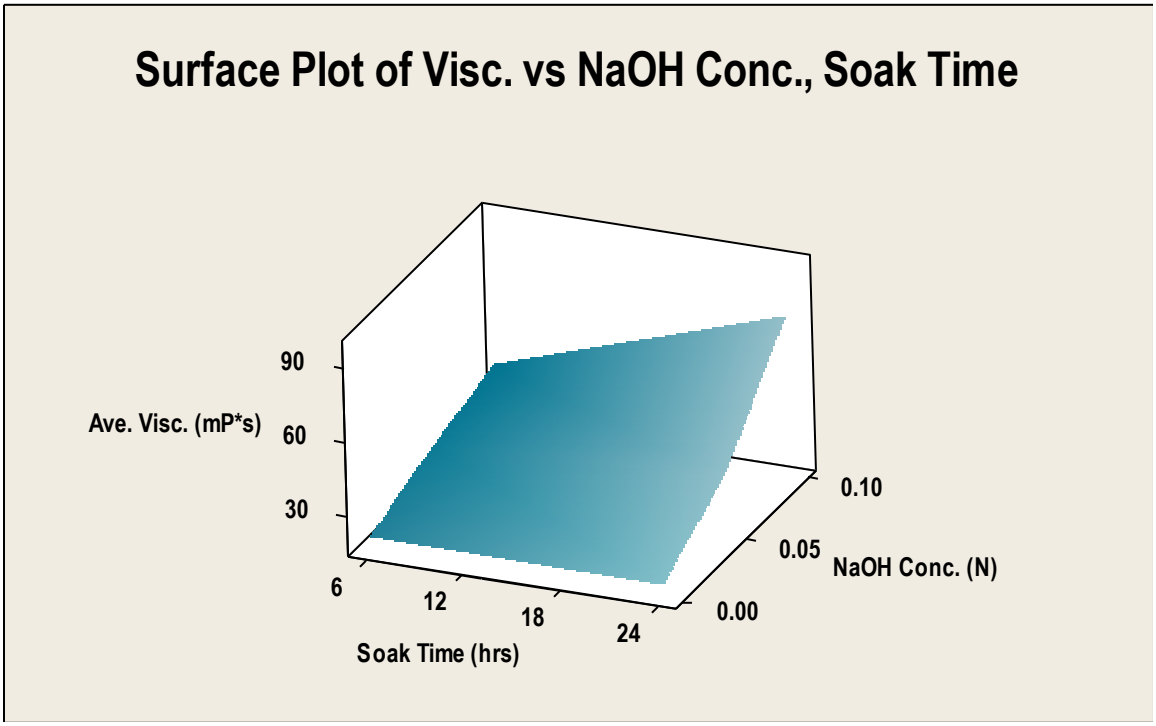


**Figure 17. Experimental Design Results Showing a Surface Plot of Viscosity vs. NaOH Concentration and Soak Time**

***Maximizing WHC of HPH Processed Wheat Bran through Experimental Design Testing***

Maximizing processed bran water hydration capacity may have similar function to viscosity for cholesterol lowering characteristics in that increased water hydration capacity (WHC) should contribute to increased viscosity. In addition, increased WHC is an important indication of structural modification during HPH treatment as well.

**General Linear Model: WHC vs. Soak Temp, Bran Grind, NaOH Conc., and Soak Time**

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| **Soak Temp** | 1 | 0.66050 | 0.66050 | 0.66050 | 79.21 | **0.000** |
| Bran Grind | 1 | 0.00449 | 0.00449 | 0.00449 | 0.54 | 0.484 |
| **NaOH Conc.** | 2 | 6.44075 | 6.44075 | 3.22037 | 386.23 | **0.000** |
| **Soak Time** | 1 | 0.24945 | 0.24945 | 0.24945 | 29.92 | **0.001** |
| **Soak Temp*NaOH Conc.** | 2 | 0.33131 | 0.33131 | 0.16565 | 19.87 | **0.001** |
| **Soak Temp*Soak Time** | 1 | 0.20673 | 0.20673 | 0.20673 | 24.79 | **0.001** |
| **Bran Grind*NaOH Conc.** | 2 | 0.13593 | 0.13593 | 0.06796 | 8.15 | **0.012** |
| Bran Grind*Soak Time | 1 | 0.00415 | 0.00415 | 0.00415 | 0.50 | 0.501 |
| **NaOH Conc.*Soak Time** | 2 | 0.26023 | 0.26023 | 0.13011 | 15.60 | **0.002** |
| Soak Temp*NaOH Conc.*Soak Time | 2 | 0.04407 | 0.04407 | 0.02203 | 2.64 | 0.132 |
| Error | 8 | 0.06670 | 0.06670 | 0.00834 | | |
| Total | 23 | 8.40429 | | | | |

**S = 0.0913129 R-Sq = 99.21% R-Sq(adj) = 97.72%**

**Table 12. Analysis of Variance for WHC, Using Adjusted SS for Tests**

**Table 12** shows the analysis of variance (ANOVA) of the factorial design (previously displayed in **Table 10**) indicating significant main effects of Soak Temperature ($p<.001$), NaOH Concentration ($p<.001$) and Soak Time ($p=.001$) on WHC. Main effects are also illustrated in **Figure 18**. Unlike viscosity, Soak Temperature was significant and increasing Soak Temperature increased WHC. Viscosity may be affected more by soluble bran material whereas larger insoluble bran fragments that don't contribute as much to viscosity may still have a large impact on WHC. Additional testing at the midpoint of 45C was later added in the augmentation design to expand understanding of the effect of Soak Temperature. Bran grind was again not shown to be significant and will be fixed at the larger size moving forward.

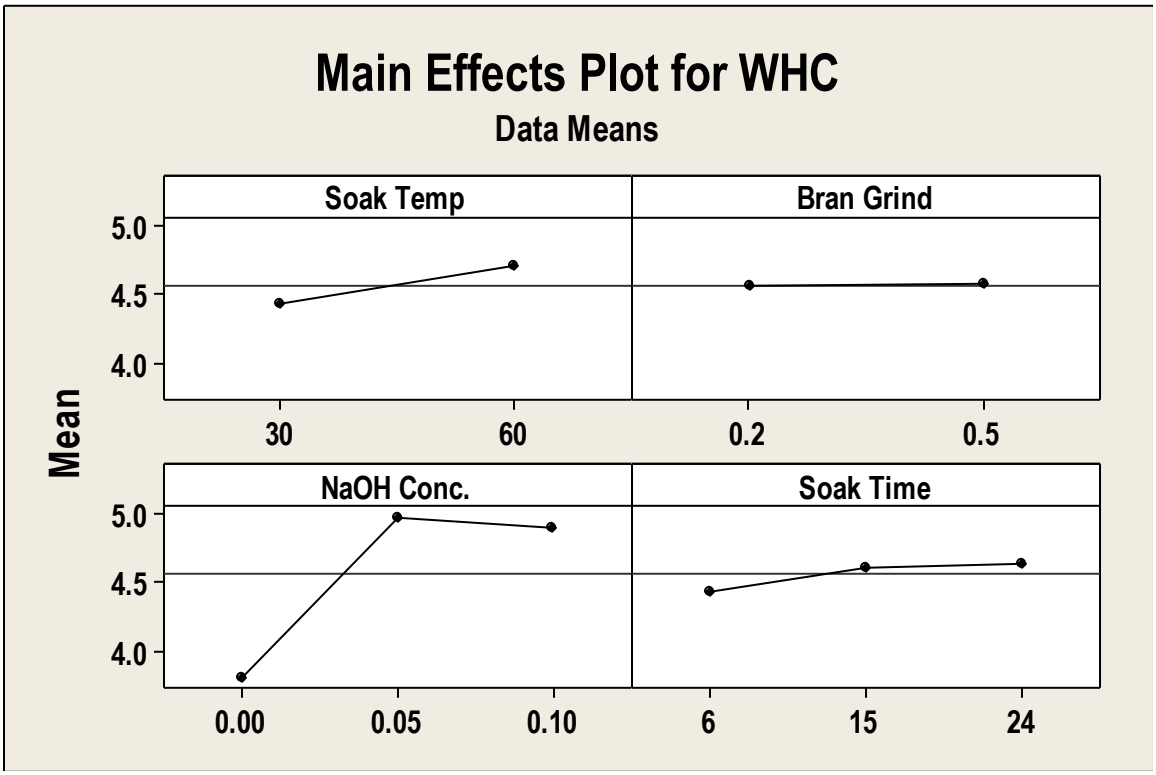


**Figure 18. Main Effects Plots for WHC in the Experimental Design**

Increasing alkali concentration and Soak Time also increased WHC, but the effect does not appear to be linear. This may be due to the fact that some soluble material is lost during supernatant removal after centrifugation and this material is more predominant when the chemical treatment is more severe, either through longer soak time or more concentrated alkali. This effect may be seen more easily by viewing the surface plot of WHC vs. NaOH concentration and Soak Time shown in **Figure 19.** As the NaOH concentration was increased, more bran material became solubilized and was seen in the supernatant during the WHC procedure. This removal contributed to the plateauing of the curve at high NaOH concentration.

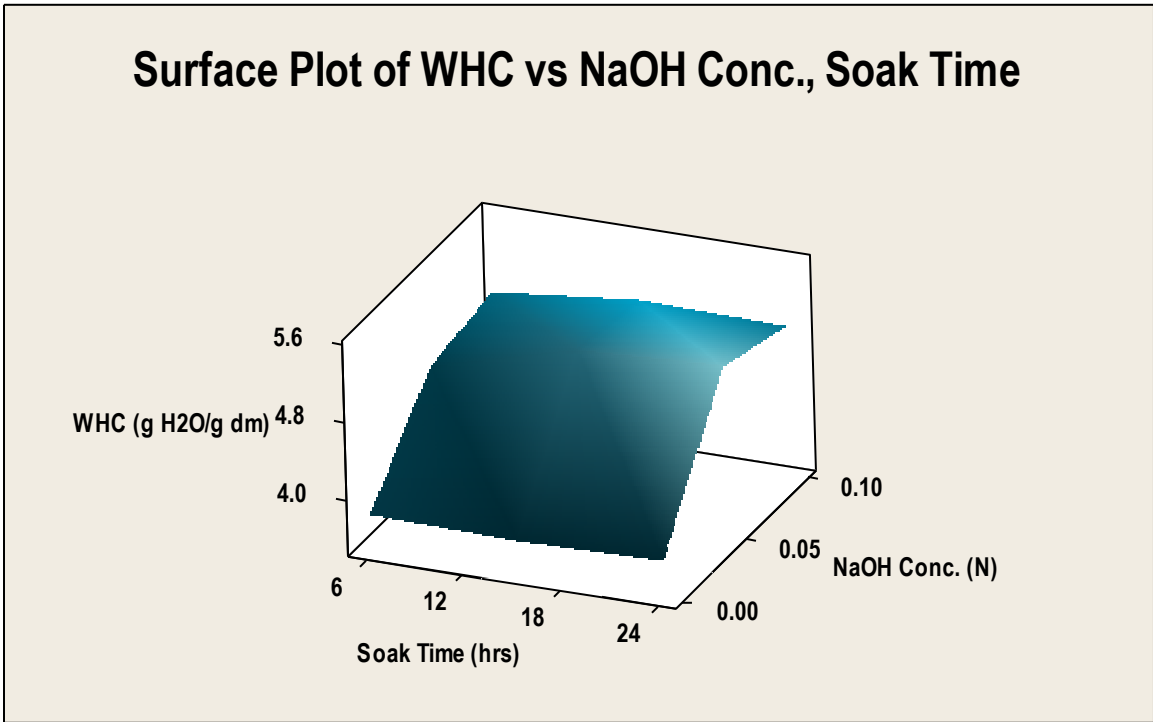


**Figure 19. Experimental Design Results Showing a Surface Plot of WHC vs. NaOH Concentration and Soak Time**

Several factor interactions were also significant including Soak Time and NaOH Concentration (p=.001), Soak Temperature and Soak time (p=.001), Bran Grind and NaOH concentration (p=.012), and NaOH Concentration and Soak Time (p=.002). As with viscosity, the significant interactions indicate the multiple potential control points in the process for controlling the extent of the pretreatment for optimizing WHC with the chosen bran material. A Maximum WHC for the HPH processed wheat bran appeared to be found within the factor ranges tested. Additional tests to augment the results and optimize process conditions were scheduled. Results are presented in the next section.

It is expected that a single optimized setting will not be able to maximize viscosity and WHC simultaneously. Therefore, viscosity and WHC will be analyzed separately to determine optimized processing parameters for each individually and, if reasonably similar, a single set of conditions will be chosen for further large sample production for additional physical, chemical, biological, and functional testing.

### *Maximizing Viscosity and WHC of HPH processed Wheat Bran with an Additional Augmentation Design*

A second experimental design was created to augment the first and further optimize the variable settings for maximizing viscosity and water hydration capacity of processed wheat bran. Experimental design conditions are shown in **Table 13**. For these experiments, bran size was ground through only the 0.5mm screen size based on the insignificance of bran size in the previous experimental design. Soak Time was also fixed at 24 hours to allow other variables to be screened. Soak Temperatures were expanded to three levels by adding a 45C midpoint. NaOH concentration was also tested at three levels, but only between the range of 0.05N and 0.1N. 0.0N controls were also tested. The other difference with this augmentation design is that two additional materials were tested including Barley Sustigrain; a whole grain barley variety with low endosperm content and high beta glucan level, and Ultrafiber; a heat treated stabilized wheat bran and germ. Barley Sustigrain and Ultrafiber results are presented separately from the wheat bran results.

| Augmentation Factorial Design to Optimize Pre-Processing Conditions (prior to HPH) | | | | |
|---|---|---|---|---|
| **Factors** | **Levels:** | **1** | **2** | **3** |
| Ingredient Type (S,U,B) | 3 | **Wheat Bran, .5mm (fixed)** | **Barley Sustigrain** | **Ultrafiber** |
| Concentration of NaOH | 3 | **0.05N** | **0.075N** | **0.1N** |
| Time (hours) | 1 | | **24 (fixed)** | |
| Soaking Temperature | 3 | **30C** | **45C** | **60C** |
| Homogenization variables: | | | | |
| Number of Passes | 1 | | **Maintained at 2** | Minimized to reduce cost |
| Concentration of bran | 1 | | **Held at 2%** | To minimize plugging |
| Pressure | 1 | | **Max 22,000 psi** | Maximum effectiveness |

**Table 13. Augmentation Factorial Design to Complete Optimization of Wheat Bran HPH Processing and Also Test Other Grain Materials**

**Table 14** shows particle size, viscosity, WHC, WaUx, and WaEx results from the Augmentation Factorial Design for wheat bran. **Tables 15 and 16** show particle size, viscosity, and WHC results for Barley Sustigrain and Ultrafiber respectively.

| Std Order | Run Order | Soak Temp (C) | NaOH Conc (N) | Part Size (Mix) | Part Size (HPH) | Visc Mix (mPa*s) | Visc HPH (mPa*s) | WHC (g/g dm) | %Un-Ex | %Ex |
|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 1 | 60 | 0.05 | 248.70 | 48.09 | 19.57 | 44.84 | 4.462 | 75.32 | 24.68 |
| 8 | 2 | 60 | 0.075 | 228.80 | 50.97 | 22.33 | 59.91 | 4.435 | 72.73 | 27.27 |
| 9 | 3 | 60 | 0.1 | 208.50 | 55.98 | 23.90 | 61.03 | 5.130 | 73.76 | 26.24 |
| 1 | 4 | 30 | 0.05 | 378.40 | 51.04 | 22.73 | 26.72 | 4.578 | 81.15 | 18.85 |
| 2 | 5 | 30 | 0.075 | 349.90 | 51.25 | 19.20 | 30.81 | 4.569 | 78.67 | 21.33 |
| 3 | 6 | 30 | 0.1 | 326.70 | 50.76 | 22.41 | 38.00 | 4.458 | 76.99 | 23.01 |
| 4 | 7 | 45 | 0.05 | 330.20 | 50.54 | 17.22 | 37.84 | 5.125 | 80.70 | 19.30 |
| 5 | 8 | 45 | 0.075 | 279.00 | 51.28 | 26.82 | 45.82 | 4.686 | 76.73 | 23.27 |
| 6 | 9 | 45 | 0.1 | 260.30 | 52.18 | 25.22 | 51.33 | 4.673 | 75.42 | 24.58 |

**Table 14. Factorial Augmentation Design for Wheat Bran.**

| Std Order | Run Order | Soak Temp (C) | NaOH Conc (N) | Part Size (Mix) | Part Size (HPH) | Visc Mix (mPa*s) | Visc HPH (mP*s) |
|---|---|---|---|---|---|---|---|
| 7 | 1 | 60 | 0.05 | 99.37 | 12.08 | 21.04 | 34.42 |
| 8 | 2 | 60 | 0.075 | 61.66 | 10.64 | 25.00 | 34.03 |
| 9 | 3 | 60 | 0.1 | 55.91 | 20.64 | 29.54 | 33.53 |
| 1 | 4 | 30 | 0.05 | 378.40 | 51.04 | 22.73 | 26.72 |
| 2 | 5 | 30 | 0.075 | 349.90 | 51.25 | 19.20 | 30.81 |
| 3 | 6 | 30 | 0.1 | 326.70 | 50.76 | 22.41 | 38.00 |
| 4 | 7 | 45 | 0.05 | 152.40 | 39.85 | 20.61 | 25.13 |
| 5 | 8 | 45 | 0.075 | 141.40 | 40.28 | 25.43 | 25.43 |
| 6 | 9 | 45 | 0.1 | 132.90 | 39.98 | 25.94 | 27.44 |

**Table 15. Factorial Augmentation Design for Barley Sustigrain.**

| Std Order | Run Order | Soak Temp (C) | NaOH Conc (N) | Part Size (Mix) | Part Size (HPH) | Visc Mix (mPa*s) | Visc HPH (mP*s) |
|---|---|---|---|---|---|---|---|
| 7 | 1 | 60 | 0.05 | 178.70 | 38.44 | 24.42 | 30.72 |
| 8 | 2 | 60 | 0.075 | 123.50 | 38.63 | 24.68 | 40.70 |
| 9 | 3 | 60 | 0.1 | 113.90 | 45.51 | 24.67 | 44.56 |
| 1 | 4 | 30 | 0.05 | 152.40 | 39.85 | 20.61 | 25.13 |
| 2 | 5 | 30 | 0.075 | 141.40 | 40.28 | 25.43 | 25.43 |
| 3 | 6 | 30 | 0.1 | 132.90 | 39.98 | 25.94 | 27.44 |
| 4 | 7 | 45 | 0.05 | 141.40 | 43.32 | 20.09 | 24.22 |
| 5 | 8 | 45 | 0.075 | 131.80 | 40.71 | 21.76 | 29.48 |
| 6 | 9 | 45 | 0.1 | 112.60 | 51.71 | 26.86 | 25.16 |

**Table 16. Factorial Augmentation Design for Ultrafiber.**

**Figures 20-25** depict the trends from the wheat bran processing in the Augmentation Design. **Figure 20** shows the effect of soaking temperature and alkali concentration vs. particle size. As seen in previous experiments, increasing either variable separately or both variables together resulted in a decrease in high shear mixed wheat bran particle size, but HPH treatment has a much larger particle size reduction effect as shown in **Figure 21**. The effects of soaking temperature and alkali concentration on solution viscosity prior to HPH treatment were somewhat inconsistent, but all viscosities were higher than the control that was soaked in water only (**Figure 22**). Once treated with HPH (**Figure 23**) the viscosities of all the samples increased and both soaking temperature and alkali concentration were effective singly and in combination at increasing viscosity of HPH processed wheat bran.

WHC results (**Figure 24**) may have been affected by error associated with transferring viscous supernatant from microfuge tubes that still contained suspended material. Compared to the Control, all alkali treated samples showed an increase in WHC. **Figure 25** shows the amount of Wa-Ex material that was removed in the supernatant, calculated from the amount of dried starting bran minus the dried pellet remaining after the WHC test. The optimized process sample contained approximately twice the water extractable material at 26% compared to the control processed sample with no alkali treatment at 13%. The Wa-Ex bran was composed of both soluble and insoluble suspended material in the supernatant. Increased supernatant viscosity suspended more insoluble bran particles and increased the Wa-Ex result slightly. In this way, both Wa-Ex and WHC results were both influenced by supernatant viscosity.

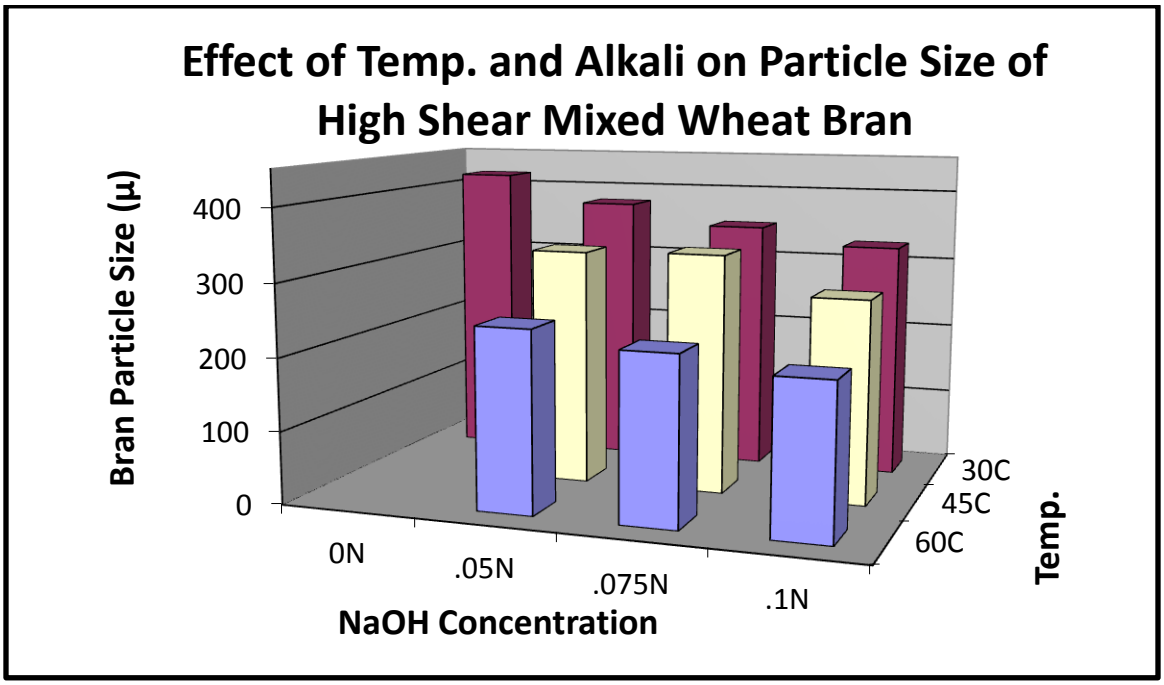


**Figure 20. Effect of Temperature and Alkali on Particle Size of High Shear Mixed Wheat Bran Prior to HPH Processing.**

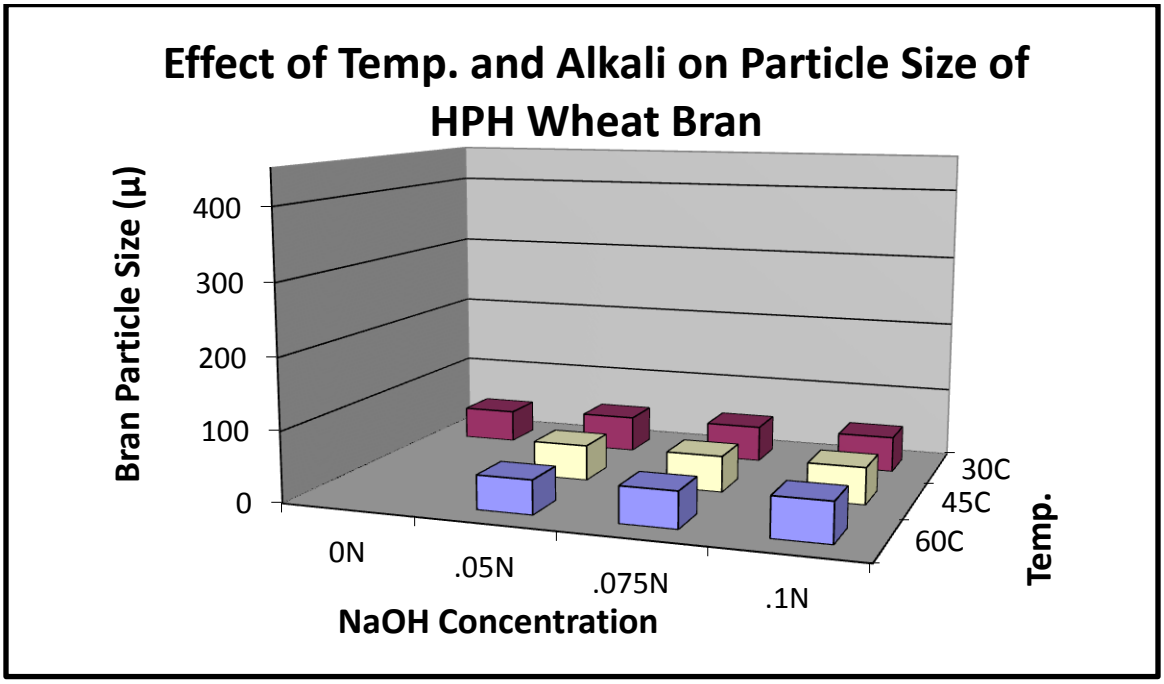


**Figure 21. Effect of Temperature and Alkali on Particle Size of HPH Wheat Bran.**

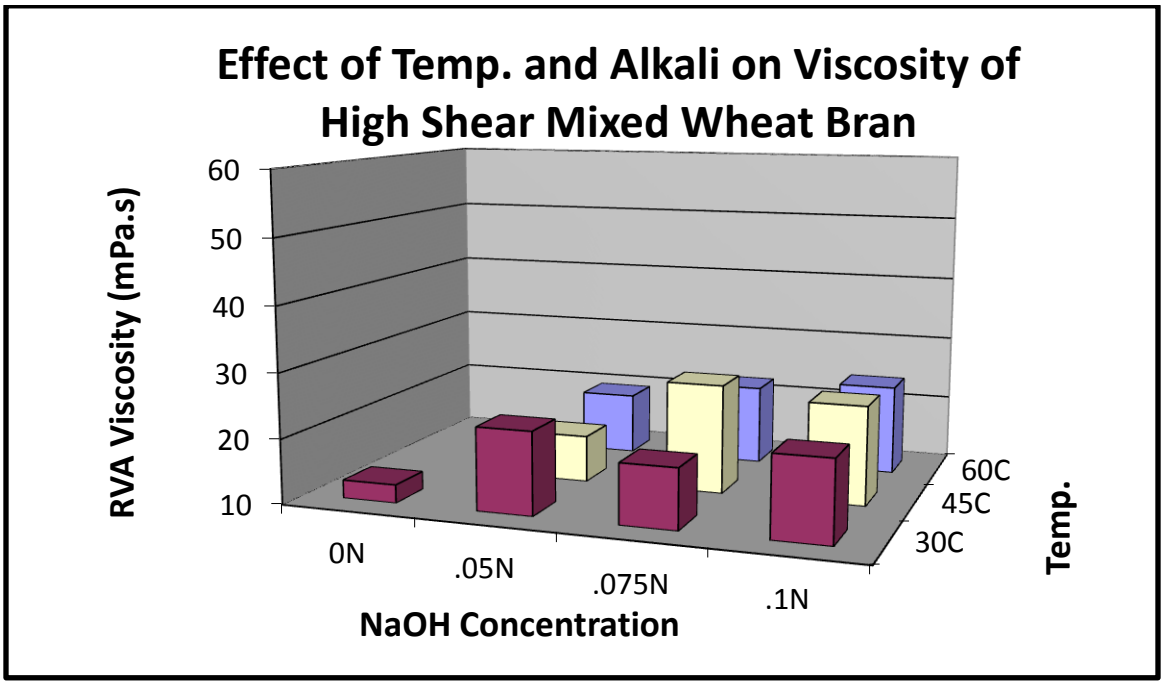


**Figure 22. Effect of Temperature and Alkali on Viscosity of High Shear Mixed Wheat Bran Prior to HPH Processing.**

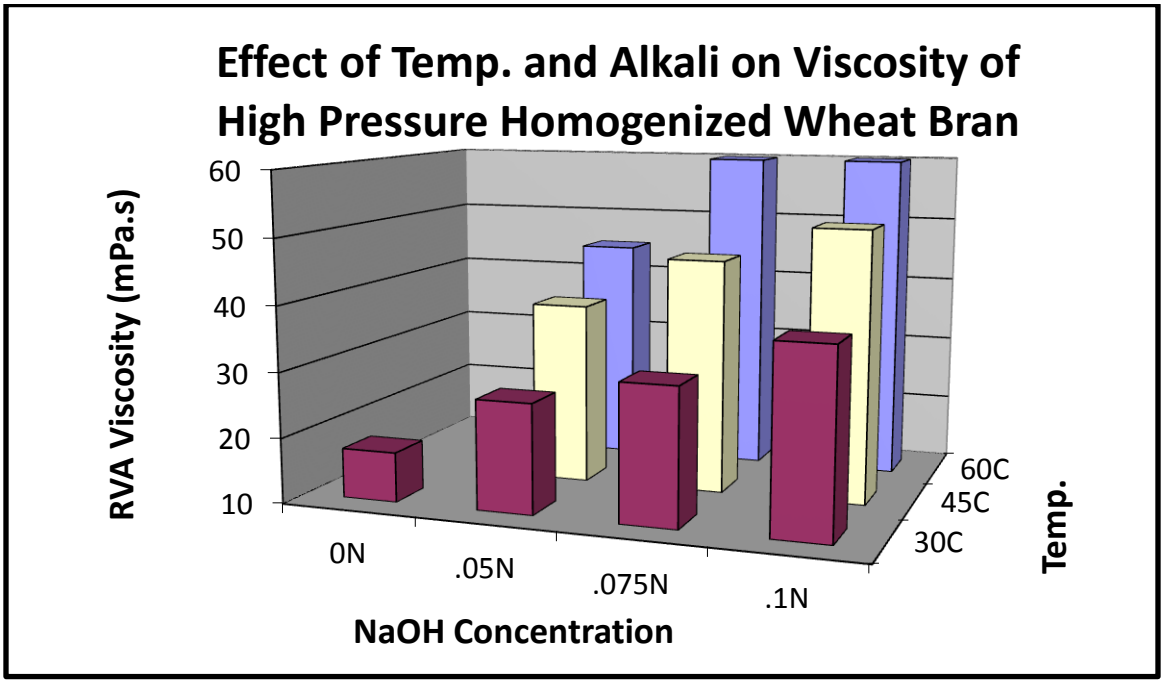


**Figure 23. Effect of Temperature and Alkali on Viscosity of HPH Wheat Bran.**

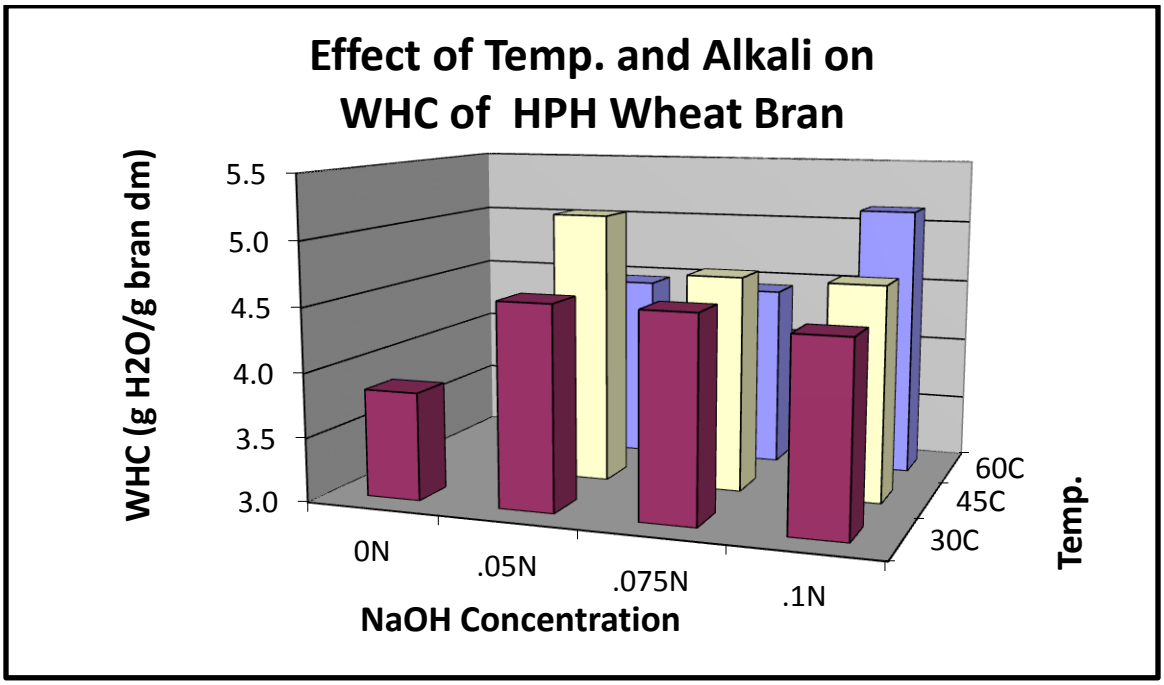


**Figure 24. Effect of Temperature and Alkali on WHC of HPH Wheat Bran.**

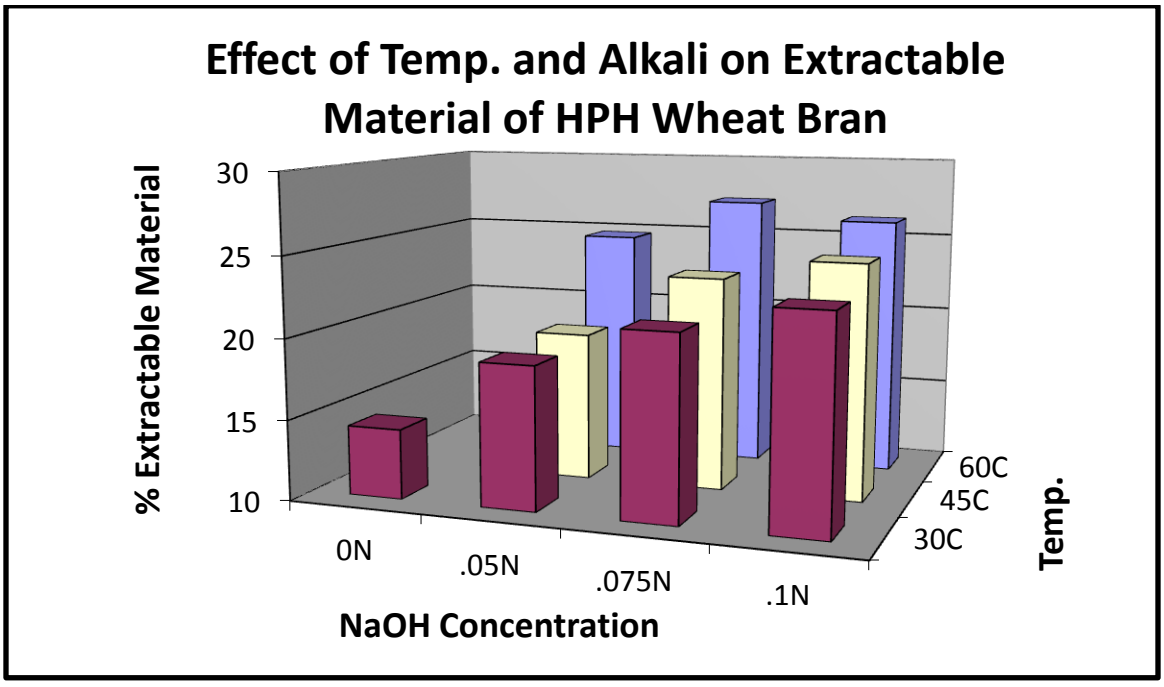


**Figure 25. Effect of Temperature and Alkali on Wa-Ex Material of HPH Wheat Bran.**

***Predicting Maximum Viscosity and WHC of HPH processed Wheat Bran through combined analysis of both Factorial and Augmentation Designs***

Combining the data from the first and second designs gave a more complete picture of the viscosity and WHC response to the processing conditions. Different optimum processing variable conditions exist to maximize viscosity or WHC. **Table 17** shows the process conditions, viscosity, and WHC results for wheat bran in combined Factorial experimental design and Augmentation design.

**Table 18** shows estimated regression coefficients for average viscosity in the combined optimization design. Soak temperature, NaOH concentration, and soak time were all significant main effects. Bran grind was not significant. Main effect plots for these variables are shown in **Figure 26**. Significant interactions of soak temperature vs. soak time and NaOH concentration vs. soak time were also seen. Analysis of variance for viscosity shown in **Table 19** shows the p values of the significant linear and interaction effects.

| Std Order | Blocks | Soak Temp (C) | Grind (mm) | NaOH Conc (N) | Soak Time (hrs) | Sample No. | Visc HPH (mPa*s) | WHC (g/g dm) |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 30 | 0.5 | 0 | 6 | 1 | 14.34 | 3.781 |
| 2 | 1 | 30 | 0.5 | 0 | 15 | 2 | 17.59 | 3.659 |
| 3 | 1 | 30 | 0.5 | 0 | 24 | 3 | 14.39 | 3.880 |
| 4 | 1 | 30 | 0.5 | 0.05 | 6 | 4 | 24.58 | 4.268 |
| 5 | 1 | 30 | 0.5 | 0.05 | 15 | 5 | 27.46 | 4.818 |
| 6 | 1 | 30 | 0.5 | 0.05 | 24 | 6 | 37.37 | 4.846 |
| 7 | 1 | 30 | 0.5 | 0.1 | 6 | 7 | 25.51 | 4.581 |
| 8 | 1 | 30 | 0.5 | 0.1 | 15 | 8 | 32.00 | 4.898 |
| 9 | 1 | 30 | 0.5 | 0.1 | 24 | 9 | 92.17 | 4.964 |
| 10 | 1 | 30 | 0.2 | 0 | 6 | 10 | 14.39 | 3.529 |
| 11 | 1 | 30 | 0.2 | 0 | 15 | 11 | 23.67 | 3.669 |
| 12 | 1 | 30 | 0.2 | 0 | 24 | 12 | 16.28 | 3.608 |
| 13 | 1 | 30 | 0.2 | 0.05 | 6 | 13 | 17.32 | 4.275 |
| 14 | 1 | 30 | 0.2 | 0.05 | 15 | 14 | 29.03 | 5.102 |
| 15 | 1 | 30 | 0.2 | 0.05 | 24 | 15 | 27.50 | 5.007 |
| 16 | 1 | 30 | 0.2 | 0.1 | 6 | 16 | 24.31 | 4.637 |
| 17 | 1 | 30 | 0.2 | 0.1 | 15 | 17 | 30.37 | 5.011 |
| 18 | 1 | 30 | 0.2 | 0.1 | 24 | 18 | 83.00 | 5.104 |
| 19 | 1 | 60 | 0.5 | 0 | 6 | 19 | 15.74 | 4.016 |
| 20 | 1 | 60 | 0.5 | 0 | 15 | 20 | 19.53 | 4.074 |
| 21 | 1 | 60 | 0.5 | 0 | 24 | 21 | 15.33 | 4.032 |
| 22 | 1 | 60 | 0.5 | 0.05 | 6 | 22 | 30.37 | 5.032 |
| 23 | 1 | 60 | 0.5 | 0.05 | 15 | 23 | 34.96 | 5.511 |
| 24 | 1 | 60 | 0.5 | 0.05 | 24 | 24 | 35.73 | 5.409 |
| 25 | 1 | 60 | 0.5 | 0.1 | 6 | 25 | 41.28 | 5.107 |
| 26 | 1 | 60 | 0.5 | 0.1 | 15 | 26 | 41.21 | 4.700 |
| 27 | 1 | 60 | 0.5 | 0.1 | 24 | 27 | 74.01 | 4.719 |
| 28 | 1 | 60 | 0.2 | 0 | 6 | 28 | 14.41 | 3.909 |
| 29 | 1 | 60 | 0.2 | 0 | 15 | 29 | 17.74 | 3.711 |
| 30 | 1 | 60 | 0.2 | 0 | 24 | 30 | 14.27 | 3.702 |
| 31 | 1 | 60 | 0.2 | 0.05 | 6 | 31 | 30.04 | 5.162 |
| 32 | 1 | 60 | 0.2 | 0.05 | 15 | 32 | 47.38 | 4.926 |
| 33 | 1 | 60 | 0.2 | 0.05 | 24 | 33 | 36.39 | 5.431 |
| 34 | 1 | 60 | 0.2 | 0.1 | 6 | 34 | 41.68 | 4.951 |
| 35 | 1 | 60 | 0.2 | 0.1 | 15 | 35 | 37.44 | 5.296 |
| 36 | 1 | 60 | 0.2 | 0.1 | 24 | 36 | 44.66 | 4.992 |
| 37 | 2 | 60 | 0.5 | 0.05 | 24 | 37 | 44.84 | 4.462 |
| 38 | 2 | 60 | 0.5 | 0.075 | 24 | 38 | 59.91 | 4.435 |
| 39 | 2 | 60 | 0.5 | 0.1 | 24 | 39 | 61.03 | 5.130 |
| 40 | 2 | 30 | 0.5 | 0.05 | 24 | 40 | 26.72 | 4.578 |
| 41 | 2 | 30 | 0.5 | 0.075 | 24 | 41 | 30.81 | 4.569 |
| 42 | 2 | 30 | 0.5 | 0.1 | 24 | 42 | 38.00 | 4.458 |
| 43 | 2 | 45 | 0.5 | 0.05 | 24 | 43 | 37.84 | 5.125 |
| 44 | 2 | 45 | 0.5 | 0.075 | 24 | 44 | 45.82 | 4.686 |
| 45 | 2 | 45 | 0.5 | 0.1 | 24 | 45 | 51.33 | 4.673 |
| 46 | 2 | 30 | 0.5 | 0 | 24 | 46 | 17.59 | 3.841 |

**Table 17. Combined Factorial Design and Augmentation Design for Wheat Bran.**

**Estimated Regression Coefficients for Ave. Viscosity**

| Term | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 26.1687 | 7.390 | 3.541 | **0.001** |
| Block 1 | 15.0947 | 5.213 | 2.895 | **0.007** |
| Block 2 | -8.9858 | 5.615 | -1.600 | 0.120 |
| **Soak Temp** | 13.1761 | 4.789 | 2.751 | **0.010** |
| Bran Grind | 1.2136 | 1.685 | 0.720 | 0.477 |
| **NaOH Conc.** | 14.0141 | 1.972 | 7.105 | **0.000** |
| **Soak Time** | 8.2137 | 2.064 | 3.979 | **0.000** |
| Soak Temp*Soak Temp | 0.8689 | 7.038 | 0.123 | 0.903 |
| NaOH Conc.*NaOH Conc. | -0.3406 | 3.233 | -0.105 | 0.917 |
| Soak Time*Soak Time | 2.8462 | 3.575 | 0.796 | 0.432 |
| Soak Temp*Bran Grind | 0.1281 | 1.685 | 0.076 | 0.940 |
| Soak Temp*NaOH Conc. | 0.7943 | 1.970 | 0.403 | 0.690 |
| **Soak Temp*Soak Time** | -4.3079 | 2.064 | -2.087 | **0.045** |
| Bran Grind*NaOH Conc. | 0.6224 | 1.972 | 0.316 | 0.755 |
| Bran Grind*Soak Time | 1.5512 | 2.064 | 0.751 | 0.458 |
| **NaOH Conc.*Soak Time** | 7.8780 | 2.357 | 3.342 | **0.002** |

**S = 10.1125 PRESS = 7734.36**
**R-Sq = 79.07% R-Sq(pred) = 47.24% R-Sq(adj) = 68.61%**

**Table 18. Response Surface Regression: Ave. Viscosity vs. Soak Temp, Bran Grind, NaOH Conc., and Soak Time.**

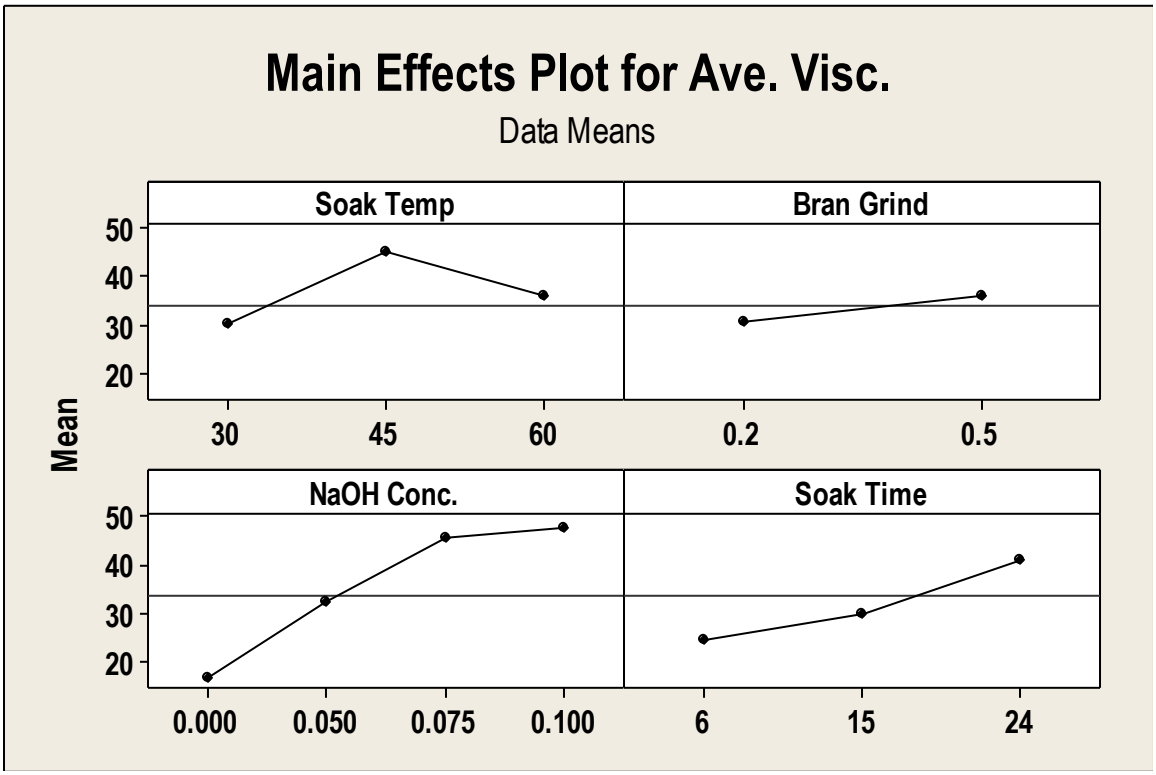


**Figure 26. Main Effects Plots of Average Viscosity in the Combined Design.**

**Analysis of Variance for Ave. Viscosity**

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Blocks | 2 | 772.0 | 942.51 | 471.26 | 4.61 | **0.018** |
| Regression | 13 | 10820.4 | 10820.39 | 832.34 | 8.14 | **0.000** |
| **Linear** | 4 | 9052.8 | 7906.16 | 1976.54 | 19.33 | **0.000** |
| Square | 3 | 66.5 | 67.40 | 22.47 | 0.22 | 0.882 |
| **Interaction** | 6 | 1701.2 | 1701.18 | 283.53 | 2.77 | **0.029** |
| Residual Error | 30 | 3067.9 | 3067.90 | 102.26 | | |
| Total 45 | 14660.3 | | | | | |

**Table 19. Analysis of Variance for Ave. Viscosity**

**Figure 27** depicts viscosity contour plots for all two way variable interactions from the experimental design. Relationships are fairly linear such that increasing the variable levels of sodium hydroxide concentration or soak time, would continue to increase viscosity. In order optimize process settings to maximize viscosity and water holding capacity, optimization plots were also created.

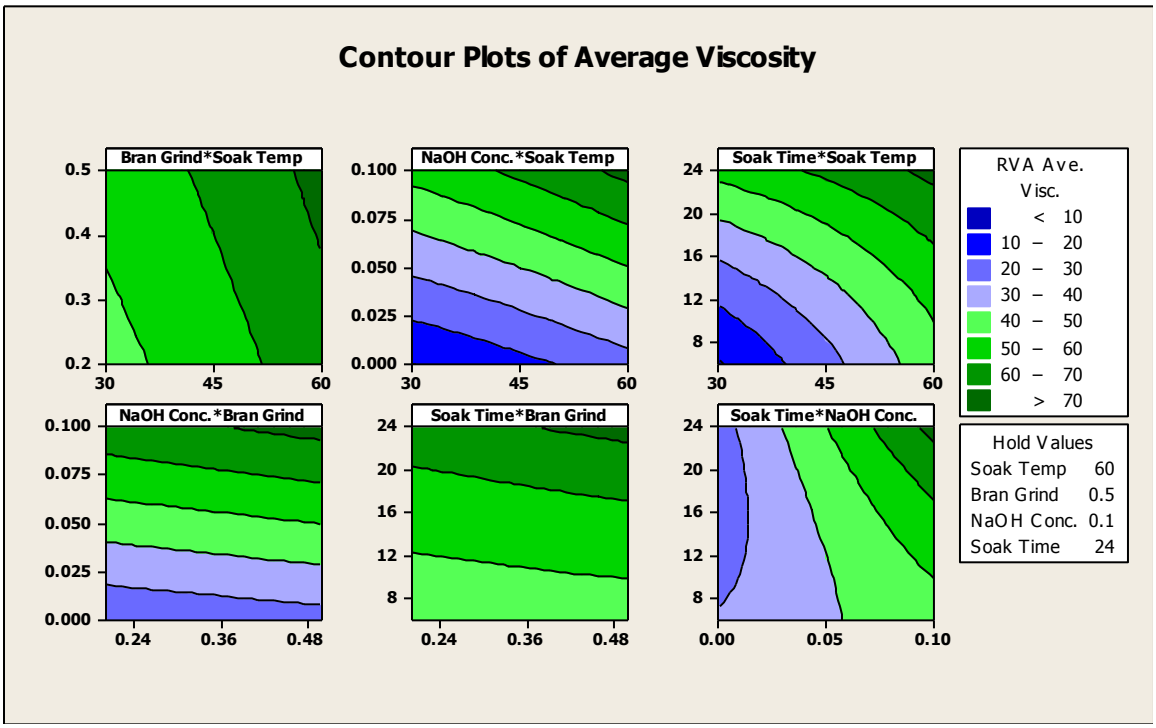


**Figure 27. Contour Plots of Two Way Interactions for Average Viscosity in the Combined Design.**

**Figure 28** shows an optimization plot of the experimental design response surface regression results. The red vertical lines correspond with the bracketed red numbers for each variable setting and can be moved left or right to determine the results for different

variable scenarios within the design. For maximizing viscosity, it is clear from the figure the maximum viscosity was achieved at the highest level setting of each variable within the design. By increasing the NaOH concentration and Soak Time, it is expected that viscosity would continue to increase. As insoluble fiber structures are broken down by the chemical treatment, the physical homogenization treatment will be more effective at converting insoluble fiber to soluble and further increasing solution viscosity. **Table 20** lists the specific variable set points for maximizing viscosity within the design.

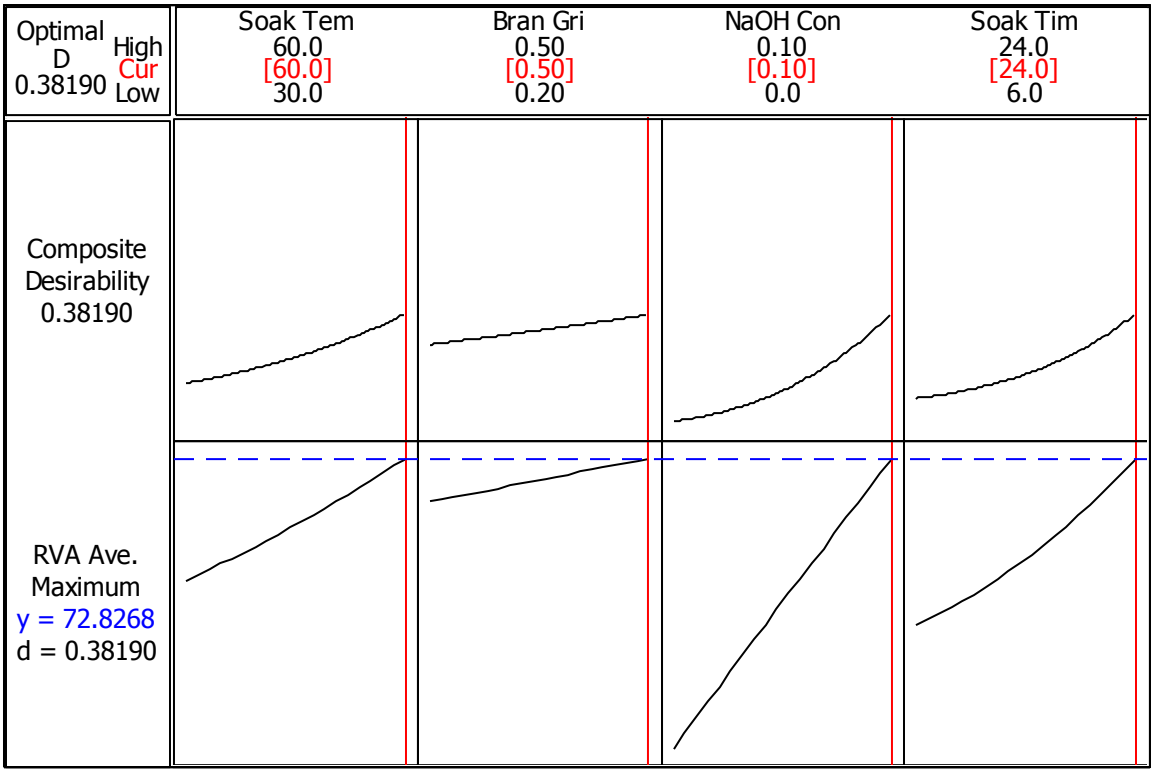


**Figure 28. Optimum Settings for Maximizing Viscosity within the Combined Factorial and Augmentation Designs.** Red bracketed numbers indicate current settings for each variable shown by the red vertical line. The blue dashed line is the viscosity resulting from the combination of variable settings.

| Soak Temperature | Bran Grind | NaOH Concentration | Soak Time |
|---|---|---|---|
| **60.0°C** | **0.50 mm** | **0.10 N** | **24.0 hours** |

**Table 20. Optimum Settings for Maximizing Viscosity within the Combined Factorial and Augmentation Designs.**

**Estimated Regression Coefficients for WHC**

| Term | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 5.05087 | 0.18381 | 27.478 | **0.000** |
| Block 1 | 0.08049 | 0.12967 | 0.621 | 0.539 |
| Block 2 | 0.16150 | 0.13967 | 1.156 | 0.257 |
| Soak Temp | 0.09962 | 0.11912 | 0.836 | 0.410 |
| Bran Grind | 0.00758 | 0.04192 | 0.181 | 0.858 |
| **NaOH Conc.** | 0.55039 | 0.04906 | 11.218 | **0.000** |
| Soak Time | 0.10195 | 0.05135 | 1.986 | 0.056 |
| Soak Temp*Soak Temp | -0.18082 | 0.17505 | -1.033 | 0.310 |
| **NaOH Conc.*NaOH Conc.** | -0.56450 | 0.08041 | -7.020 | **0.000** |
| Soak Time*Soak Time | -0.07544 | 0.08893 | -0.848 | 0.403 |
| Soak Temp*Bran Grind | 0.02124 | 0.04192 | 0.507 | 0.616 |
| Soak Temp*NaOH Conc. | 0.00093 | 0.04901 | 0.019 | 0.985 |
| Soak Temp*Soak Time | -0.09281 | 0.05135 | -1.808 | 0.081 |
| **Bran Grind*NaOH Conc.** | -0.10484 | 0.04906 | -2.137 | **0.041** |
| Bran Grind*Soak Time | -0.01314 | 0.05135 | -0.256 | 0.800 |
| NaOH Conc.*Soak Time | 0.02107 | 0.05864 | 0.359 | 0.722 |

**S = 0.251541 PRESS = 4.44230**
**R-Sq = 86.89% R-Sq(pred) = 69.31% R-Sq(adj) = 80.33%**

**Table 21. Response Surface Regression: WHC vs. Soak Temp, Bran Grind, NaOH Conc., and Soak Time.**

In determining the optimum settings to maximize WHC, the data was analyzed in a similar manner as the Viscosity results. **Table 21** shows the response surface regression results for WHC from the combined Factorial and Augmentation designs. NaOH concentration was a significant main effect and interaction effect. Analysis of variance for WHC in **Table 22** shows the p values of the significant linear and interaction effects. Main effect plots for these variables are shown in **Figure 29**.

**Analysis of Variance for WHC**

| **Source** | **DF** | **Seq SS** | **Adj SS** | **Adj MS** | **F** | **P** |
|---|---|---|---|---|---|---|
| Blocks | 2 | 0.7145 | 0.41827 | 0.20913 | 3.31 | **0.050** |
| Regression | 13 | 11.8612 | 11.86122 | 0.91240 | 14.42 | **0.000** |
| **Linear** | 4 | 7.9950 | 8.37532 | 2.09383 | 33.09 | **0.000** |
| **Square** | 3 | 3.3486 | 3.27145 | 1.09048 | 17.23 | **0.000** |
| Interaction | 6 | 0.5176 | 0.51760 | 0.08627 | 1.36 | 0.261 |
| Residual Error | 30 | 1.8982 | 1.89819 | 0.06327 | | |
| Total | 45 | 14.4739 | | | | |

**Table 22. Analysis of Variance for WHC.**

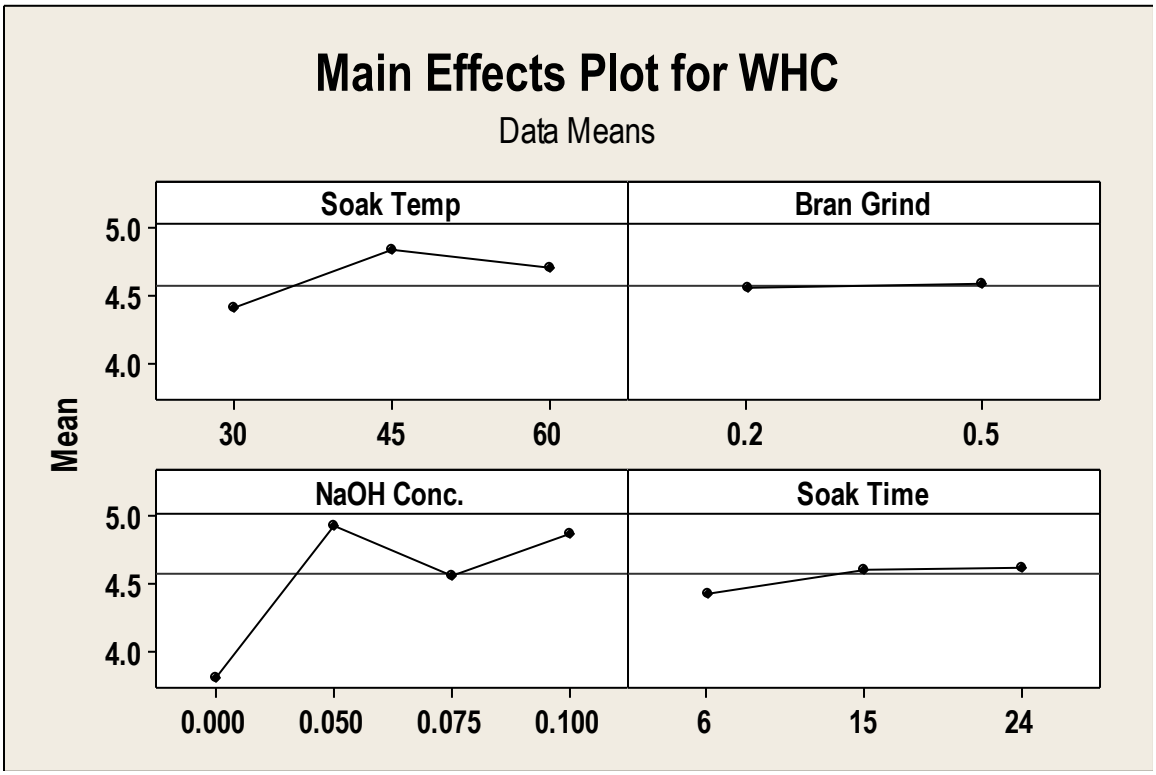


**Figure 29. Main Effects Plots of Water Hydration Capacity in the Combined Design.**

It was shown earlier that particle size regardless of treatment is reduced and normalized by high pressure homogenization as shown in **Figure 10**, but the chemical treatment was responsible for creating a porous structure seen visibly in **Figure 7**. As chemical treatment creates more porous bran structures, WHC increases. More water is trapped within porous particles with a large internal surface area. HPH processing has been shown previously to increase surface area and average pore diameter of fiber to create a large water retention capacity, similar to WHC (Lundberg et al., 2006; Lundberg et al., 2003). If the chemical treatment is too severe, degrading the ability of wheat bran to entrap water, WHC will decrease. This effect was seen within the Factorial design and

the Augmentation design was added to help show more detail in the critical region for maximizing WHC.

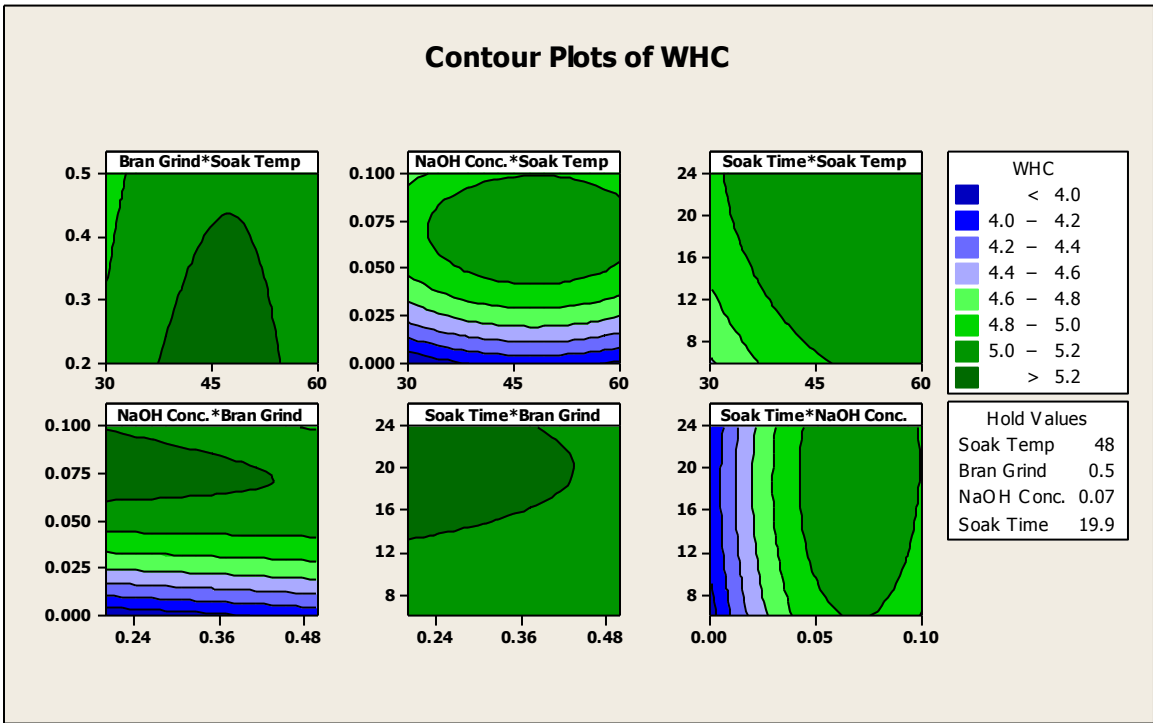


**Figure 30. Contour Plots of Two Way Interactions for Water Hydration Capacity in the Combined Design.**

The contour plots of all two way variable interactions for WHC are depicted in **Figure 30**. Curved contour lines show the nonlinearity of the interaction results with dark green spaces indicating the highest WHC. The conditions to maximize WHC are contained within the design variable ranges. Within the experimental design, the combination of chemical and physical treatments resulted in a nonlinear response where

WHC increased to a maximum and then began to decrease as the intensity of chemical treatment increased.

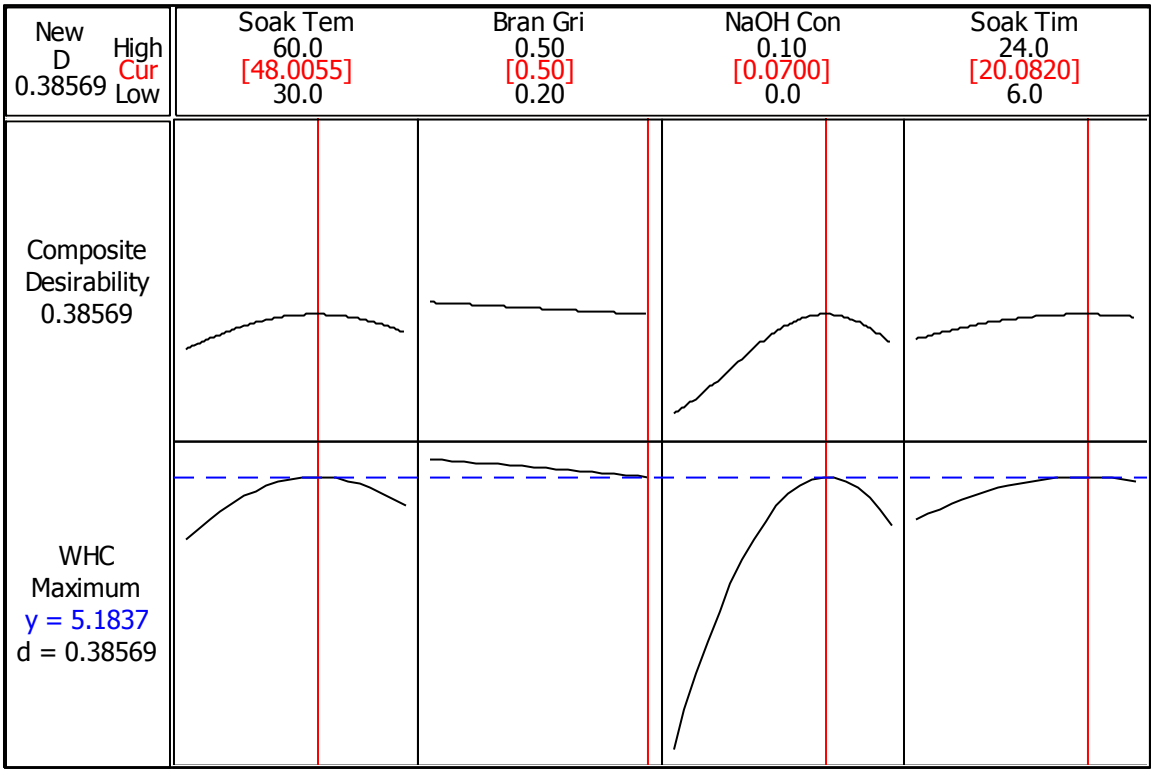


**Figure 31. Optimum Settings for Maximizing Water Hydration Capacity within the Combined Factorial and Augmentation Designs.** Red bracketed numbers indicate current settings for each variable shown by the red vertical line. The blue dashed line is the viscosity resulting from the combination of variable settings.

**Figure 31** shows an optimization plot of the experimental design response surface regression results. The red vertical lines correspond with the bracketed red numbers for each variable setting and can be moved left or right to determine the results for different variable scenarios within the design. When maximizing WHC, because of the nonlinearity of the results, the optimum settings fall within the variable ranges. While

bran particle size was not significant and was also later fixed at the 0.5 mm grind size for reducing processing costs, **Table 23** shows optimized settings for maximizing WHC at both bran particle sizes.

| Soak Temperature | Bran Grind | NaOH Concentration | Soak Time |
|---|---|---|---|
| 45.0°C | 0.20 mm | 0.08 N | 22.6 hours |
| **48.0°C** | **0.50 mm** | **0.07 N** | **20.0 hours** |

**Table 23. Optimum Settings for Maximizing Water Hydration Capacity within the Combined Factorial and Augmentation Designs.**

Two different sets of conditions have now been determined that maximize viscosity and WHC separately, but neither will result in an optimum set of conditions to simultaneously maximize both. The process conditions for further bran modification can be chosen from either set or a combination that attempts to achieve the highest values for both relative to the importance of each. For example, **Figure 32** uses an equal weighting for the importance of viscosity and WHC to generate optimum settings for the combined result. The optimum settings for maximizing viscosity and WHC combined are shown in **Table 24**. The assumption that WHC and viscosity are equally important for the processed wheat bran is changeable within the analysis.

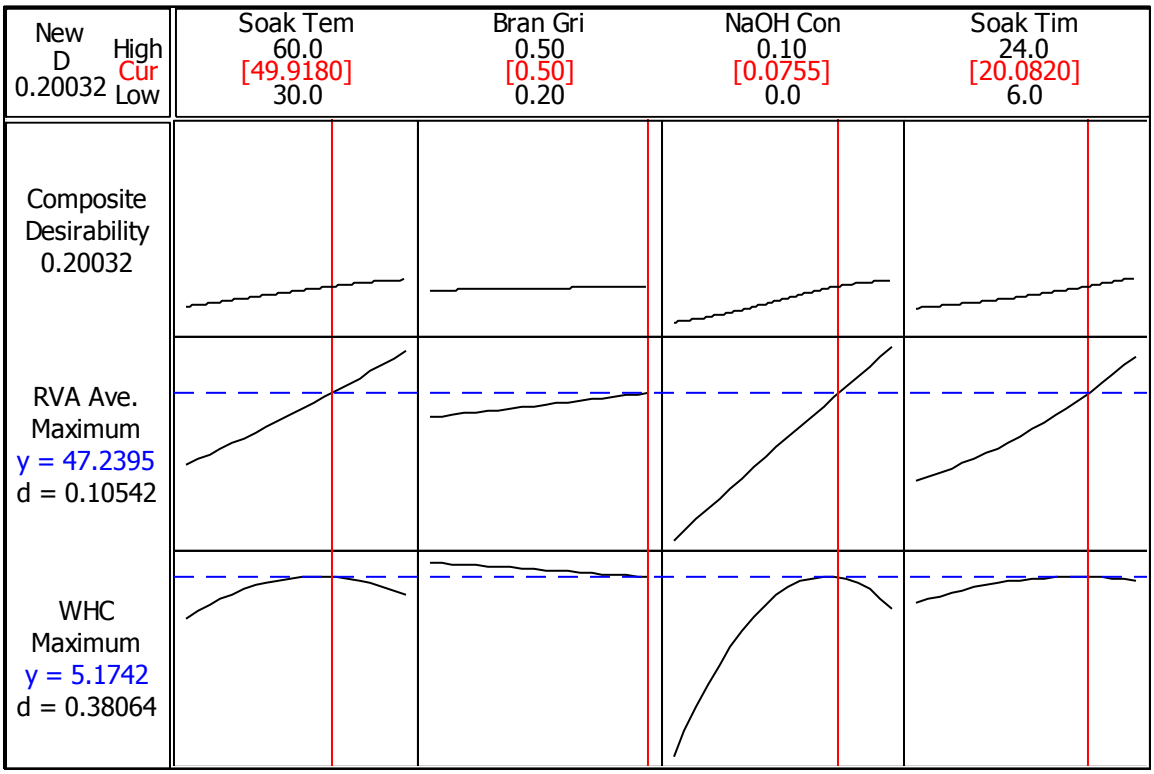


**Figure 32. Optimum Settings for Maximizing Viscosity and Water Hydration Capacity Simultaneously.** Red bracketed numbers indicate current settings for each variable shown by the red vertical line. The blue dashed lines show the resulting viscosity and WHC for the optimized settings chosen.

**Figure 32** shows that as the chemical pretreatment increased toward a maximum within the design, WHC increased to a peak and then lowered while viscosity continued to increase. Higher combined chemical pretreatment variable settings increased the breaking of covalent bonds including ester linkages between phenolic acids and arabinoxylan chains (Kim et al., 2006) allowing the high pressure processing to break apart the bran structures with solubilization of fiber that was once engaged in holding water. While the increase in soluble fiber continued to contribute to increasing solution viscosity, WHC was reduced.

| Soak Temperature | Bran Grind | NaOH Concentration | Soak Time |
|---|---|---|---|
| **49.92°C** | **0.50 mm** | **0.0755 N** | **20.08 hours** |

**Table 24. Optimum Settings for Maximizing Viscosity and Water Hydration Capacity Combined.**

***Bound Phenolic Release from Wheat Bran using Optimized Process Setponts for Maximizing Viscosity***

Based on the preliminary screenings and experimental design results, wheat bran preprocessing and processing conditions have been determined that maximize the physical properties of viscosity and water hydration capacity while enabling the release of bound phenolics from the bran structures. Wheat bran processed in both the Optimization design and Augmentation design was analyzed for free phenolics and soluble bound phenolics. Process variable settings included the optimized settings to maximize bran solution viscosity shown in **Table 20** as well as controls to show the effect of chemical treatment or HPH treatment alone on the release of bound phenolics.

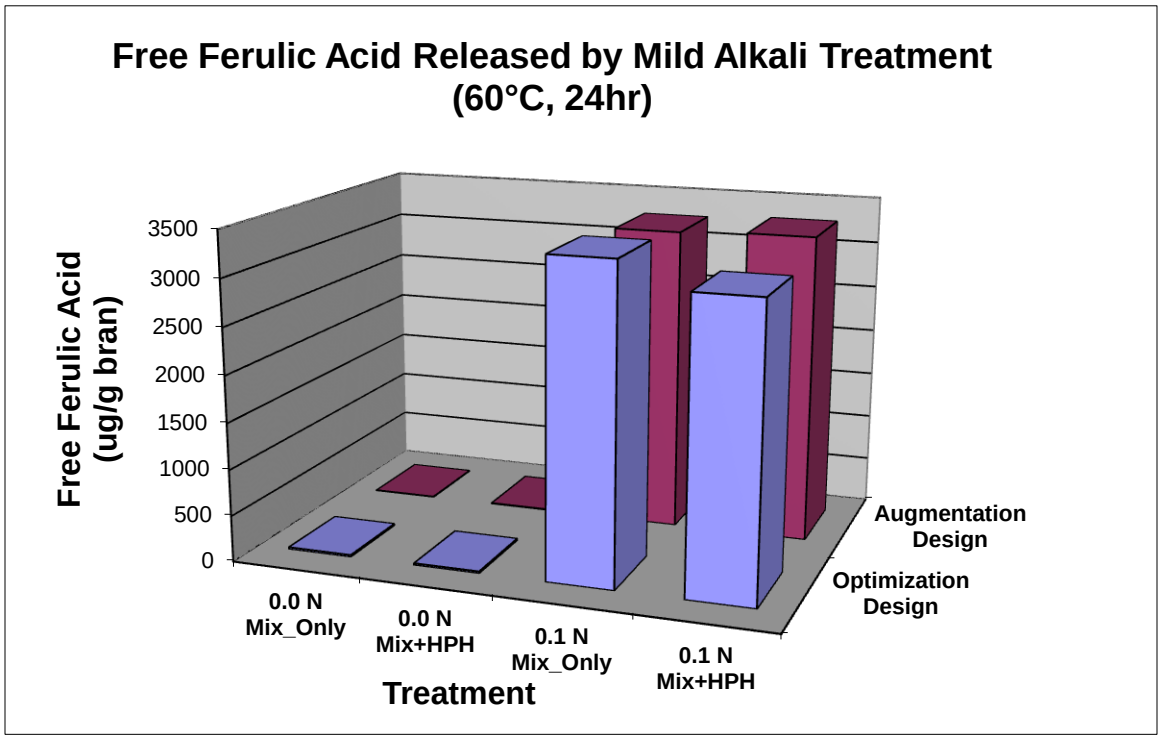


**Figure 33. Free ferulic acid content of processed wheat bran.**

Measurement of the free ferulic content of the Optimization design and Augmentation design bran samples (**Figure 33**) showed consistent results from both designs. The NaOH treatment was the key factor in releasing bound phenolics in these similarly treated samples from the two designs. Levels were very similar even though months separated the processing of the experimental designs.

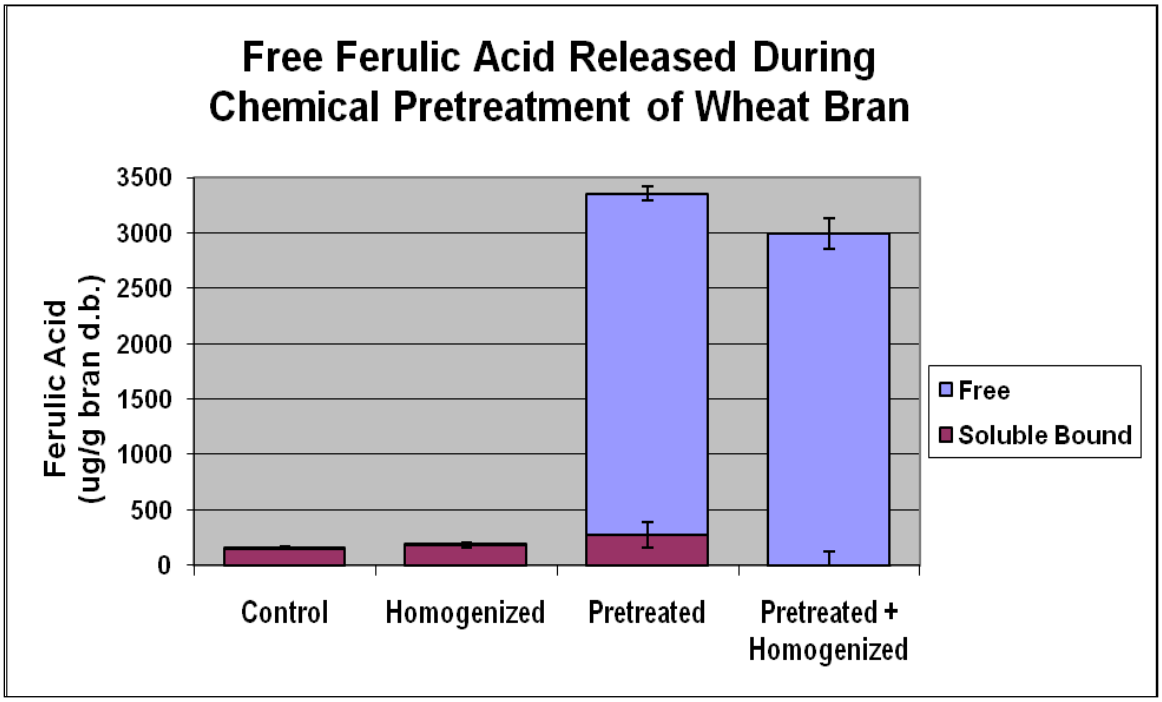


**Figure 34. Averaged free and soluble bound ferulic acid content of processed wheat bran from Optimization and Augmentation designs.**

**Figure 34** shows the averaged free and soluble bound ferulic acid from different samples with similar treatments in both the Optimization and Augmentation designs. There was difficulty in accurately determining the soluble bound ferulic acid in one of the samples. The amount was determined by subtracting free ferulic acid extracted with 80% ethanol from a saponified extract sample. The saponified sample contained both free and soluble bound phenolics and the total should have been higher than the free ferulic acid alone. However, in the case of one of the Pretreated + Homogenized samples, the total amount was less than the free amount leaving a negative value. This is likely due to sample handling error or loss of measurable ferulic acid due to precipitation during. The

extracted pellet was re-extracted with water and saponified to yield another soluble bound portion and added to the first to achieve a total soluble bound fraction. The soluble bound amount was only a minor portion of the total ferulic acid released by alkali treatment during processing, or around 10% of the total, but represented the majority of the soluble phenolics in the samples not reacted with alkali.

### *Soluble Fiber Content of Alkali Treated HPH Processed Wheat Bran*

Increased soluble fiber content in processed wheat bran was expected based on the results of increased viscosity and WHC. **Figure 35** shows the changes in soluble and insoluble fiber content of unprocessed bran, alkali treated high shear mixed bran, and alkali treated bran from the optimized processing profile that received HPH treatment (Iris Zhang, personal communication, August, 2009). Alkali treatment helped increase the soluble fiber while the insoluble fiber decreased. Additional HPH treatment further increased the soluble fiber. The total fiber content (soluble + insoluble fiber) was different across the measured samples, but the increase of viscosity producing soluble fiber in the alkali treated HPH samples is important and could lead to increased health benefits when utilized in food products similar to viscosity producing soluble barley beta glucan or soluble oat fiber.

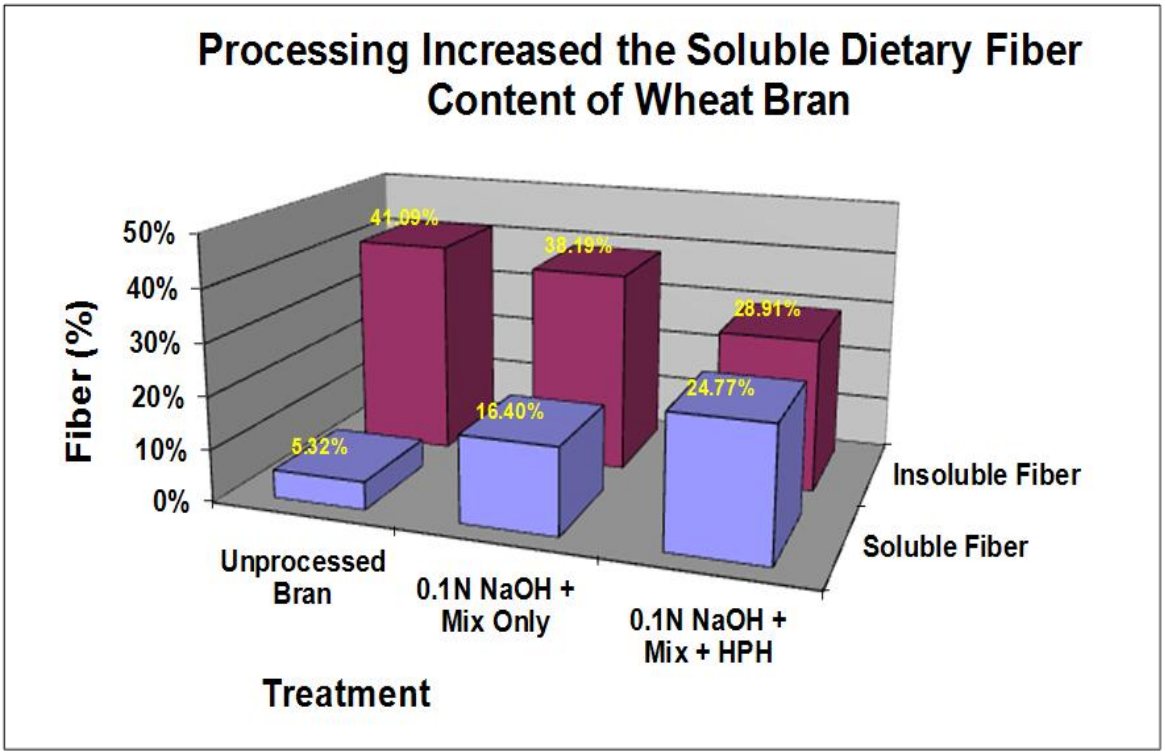


**Figure 35. Averaged free and soluble bound ferulic acid content of processed wheat bran from Optimization and Augmentation designs.**

***Visualization of Bran modifications using Scanning Electron Microscopy***

The flowchart shown in **Figure 36** indicates sample collection points 3a-3d during bran processing for use in Scanning Electron Microscopy. Sample 3a was a control bran sample that received hydration with no alkali treatment (0N) or high pressure homogenization (Mix only). Sample 3b shows the effects of high pressure homogenization without chemical pretreatment (0N, Mix+HPH). Sample 3c shows the effect of chemical pretreatment without high pressure homogenization (.1N, Mix only). Sample 3d shows the combined effect of chemical pretreatment followed by high pressure homogenization (.1N, Mix+HPH).

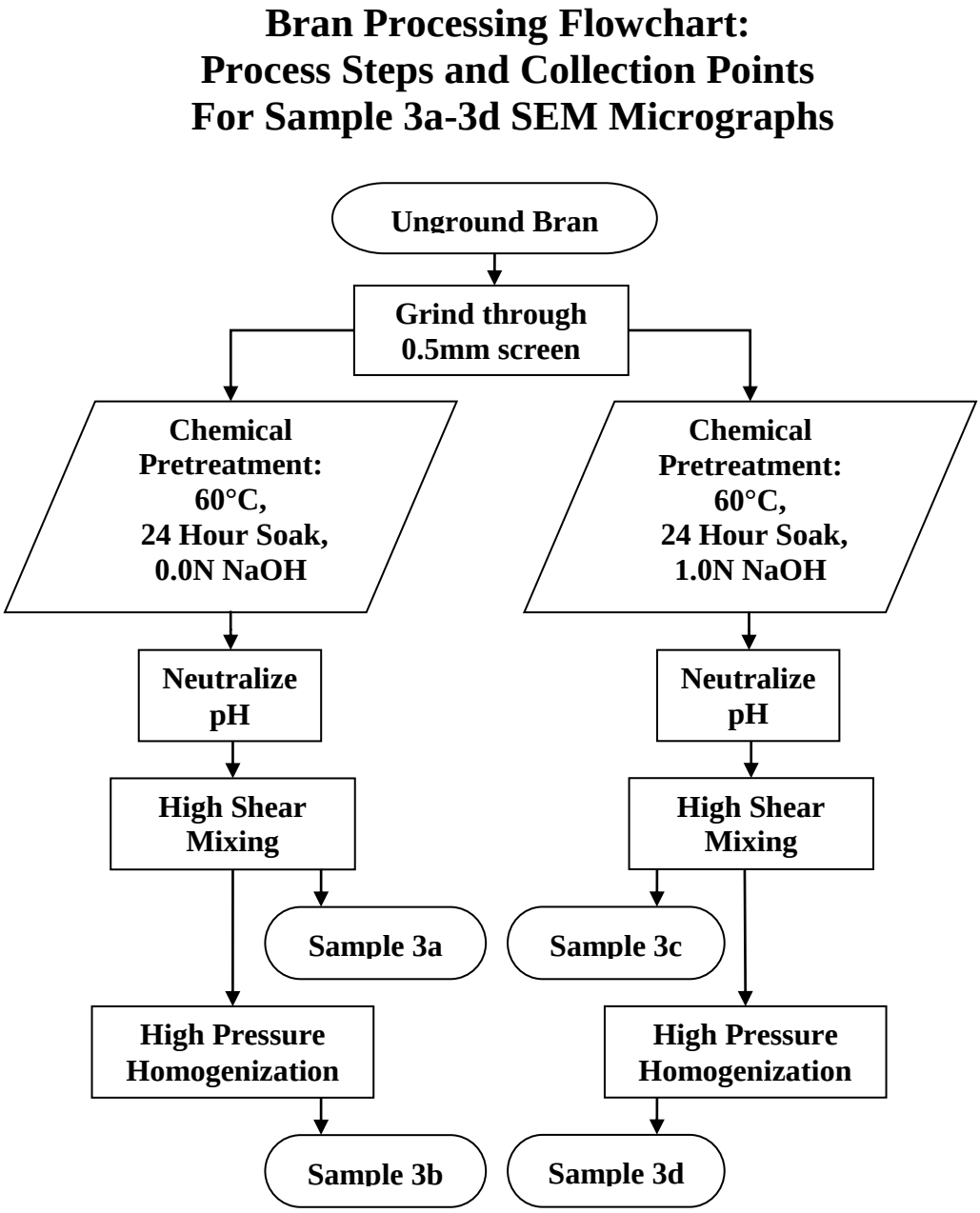


**Figure 36. Bran Processing Flowchart for Sample 3a-3d SEM Micrographs.**

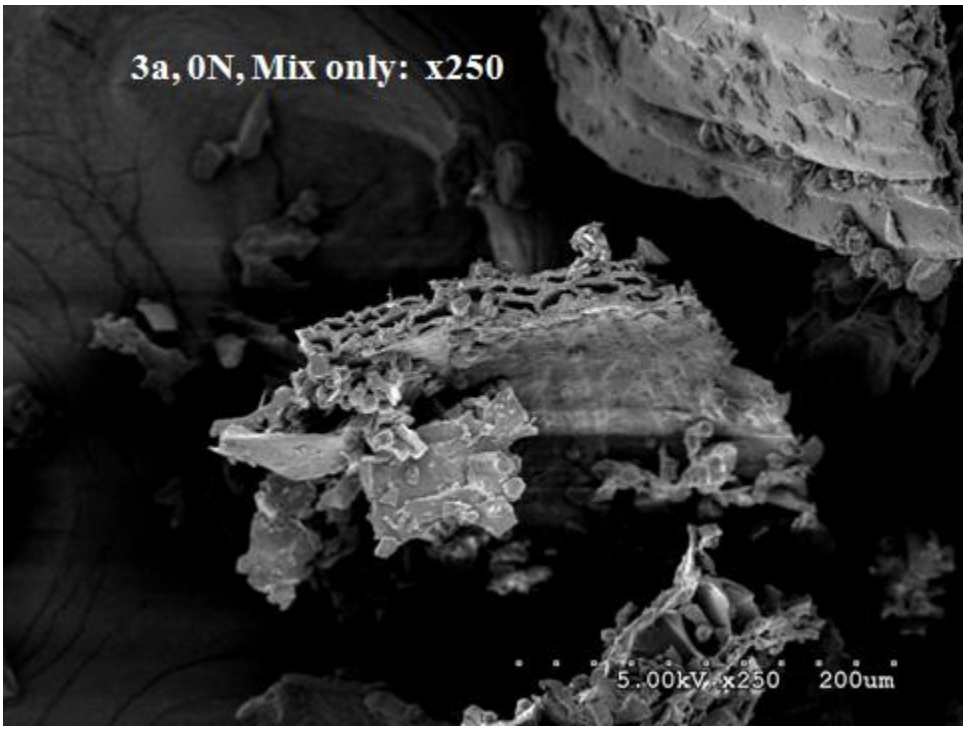


**Figure 37. SEM of Processed Wheat Bran: Sample 3a, 0N, Mix Only, x250.**

Starting with the lowest magnification of bran fragments seen in **Figure 37**, large pieces of bran with multiple layers including cross cells are seen in the center of the micrograph. The whole and partial structures in Sample 3a indicate the limited nature of the pretreatment to degrade the bran. Physical high shear mixing without chemical treatment or HPH reduced the particle size but did not extensively degrade the structures. The average particle size was greater than 200 microns. Near the bottom of the micrograph, aleurone cells are visible and magnified in **Figure 38**.

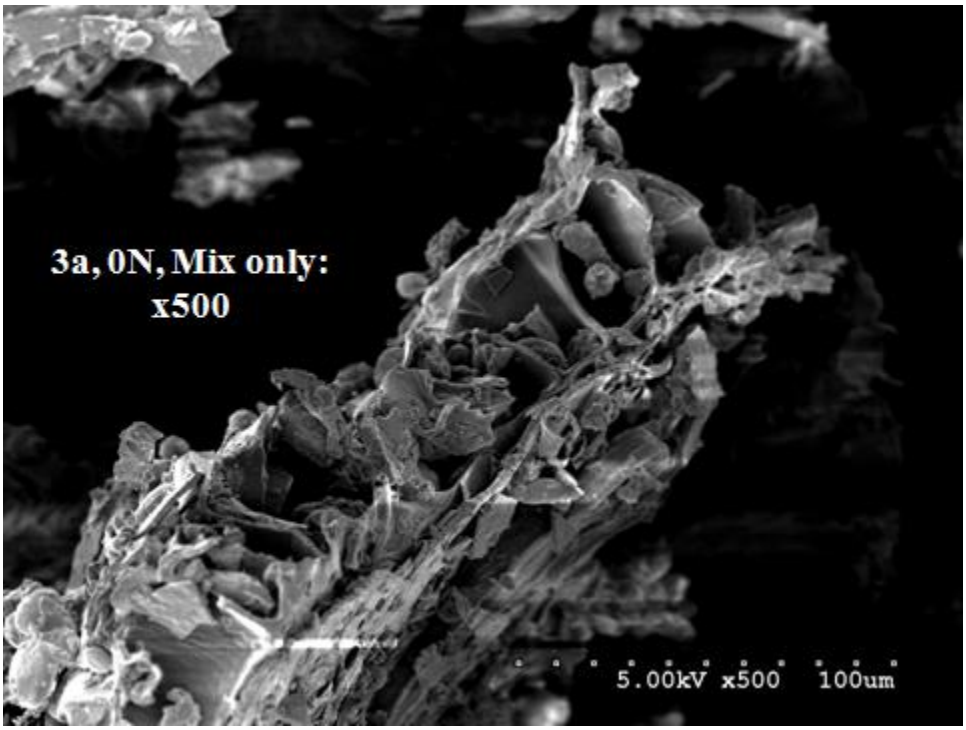


**Figure 38. SEM of Processed Wheat Bran: Sample 3a, 0N, Mix Only, x500.**

At 500 times magnification, **Figure 38** shows the broken aleurone cells of sample 3a magnified from the previous figure. The bran fragment appears ripped apart during the high shear mixing. Some individual starch granules are visible. In the image, the lower surface of the aleurone cells shows the cross cell layer and outer pericarp (beeswing bran) similar to the illustration in **Figure 3**. It is clear that the hydration and high shear mixing treatment, without chemical treatment or HPH does not degrade bran structures enough to create a large increase in viscosity or release of bound phenolic antioxidants previously shown in **Figures 17 and 11** respectively.

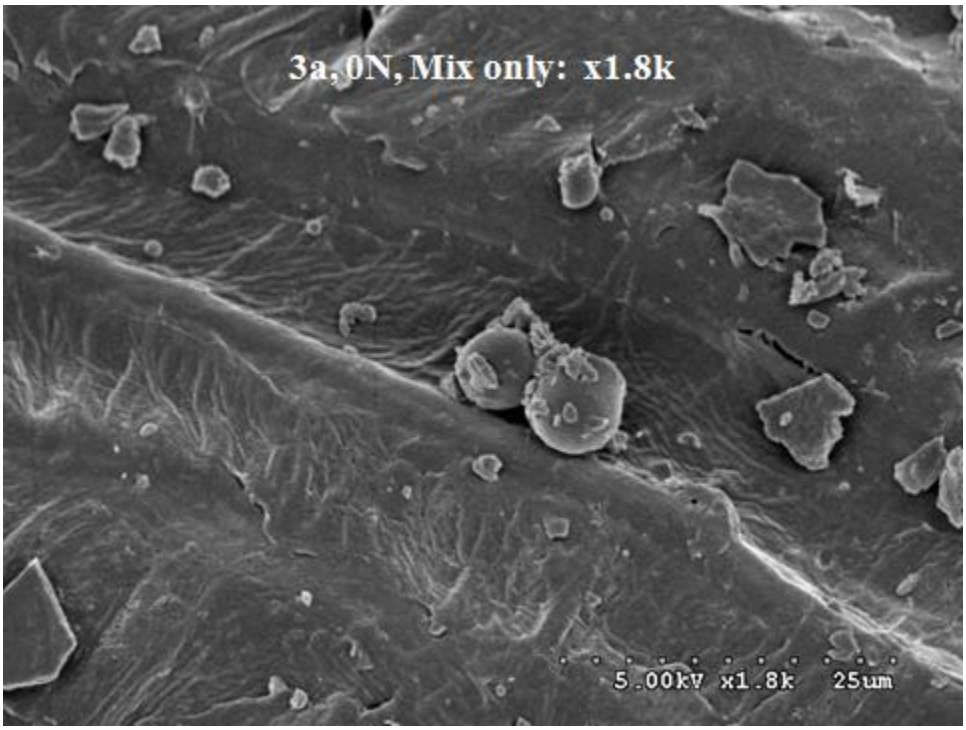


**Figure 39. SEM of Processed Wheat Bran: Sample 3a, 0N, Mix Only, x1.8k.**

**Figure 39** shows an inner bran surface from Sample 3a containing multiple small bran fragments and two small spherical starch granules. The surface is rough and contains ridges. It appears that this is the surface of the aleurone layer that faces inward toward the endosperm. The surface is rough, but mainly intact with few only a few breaks in the continuous surface.

**Figure 40** shows an outer bran surface from sample 3a that is magnified to 10,000 times and to 20,000 times in **Figure 41**. The surface is smooth with many small bran pieces visible on the surface that were separated during the high shear mixing step. The surface has no holes or broken sections to indicate breakdown of fiber structures during processing.

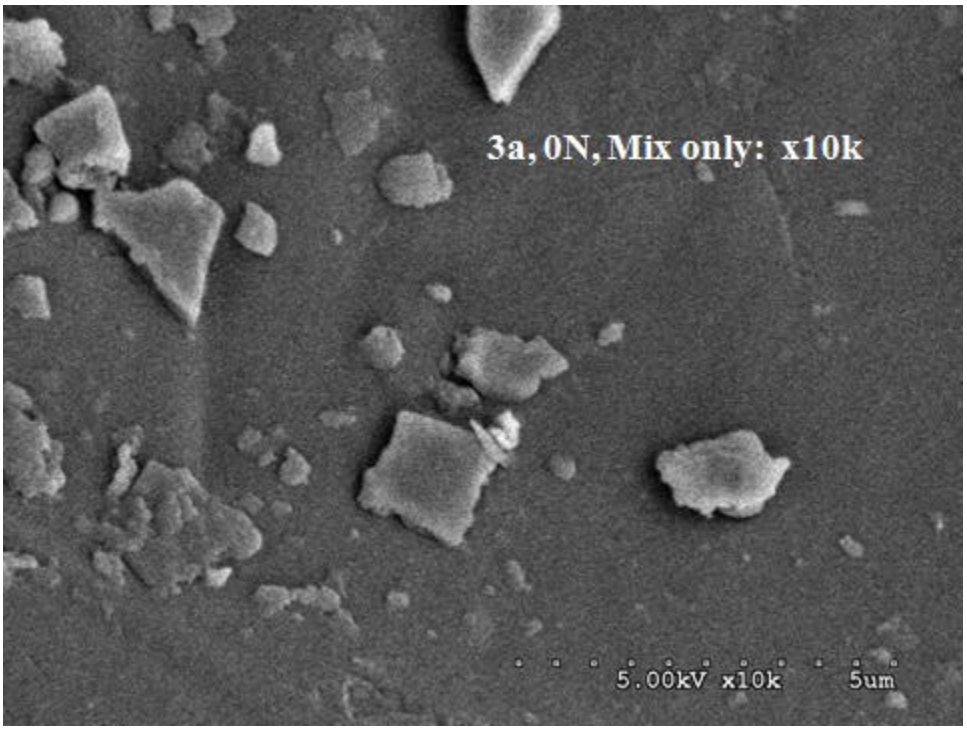


**Figure 40. SEM of Processed Wheat Bran: Sample 3a, 0N, Mix Only, x10k.**

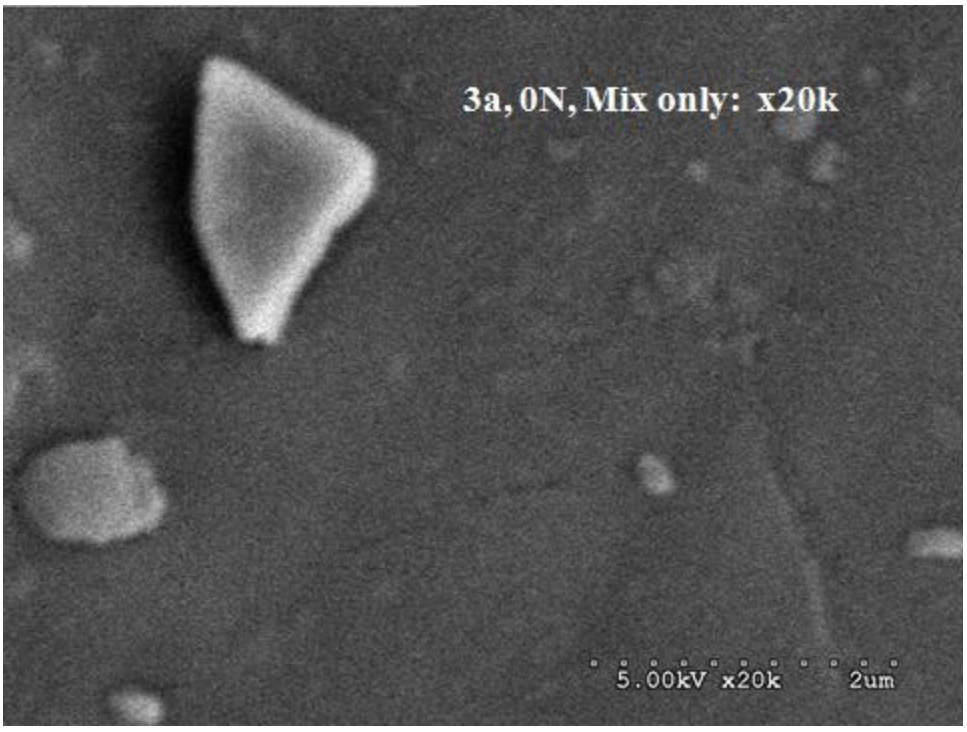


**Figure 41. SEM of Processed Wheat Bran: Sample 3a, 0N, Mix Only, x20k.**

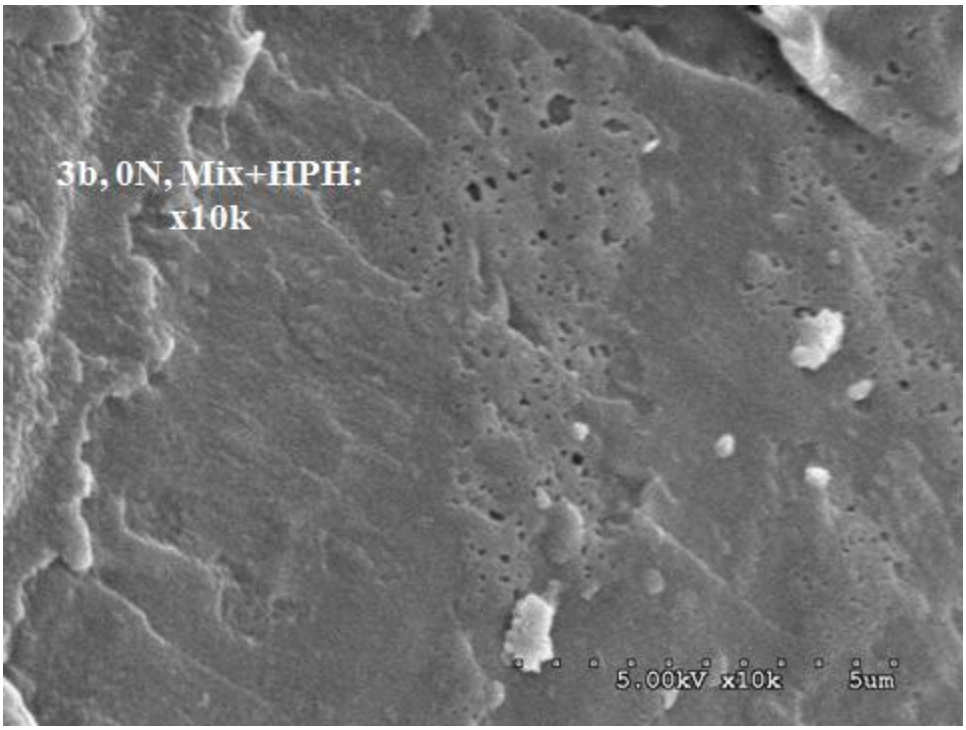


**Figure 42. SEM of Processed Wheat Bran: Sample 3b, 0N, Mix+HPH, x10k.**

**Figures 42, 43, and 44** show the effect of HPH treatment without chemical pretreatment on wheat bran Sample 3b. The difference between 3a and 3b is easily seen as holes and pores appear on the surface of the bran. Intense pressure and shear during HPH processing increase disruption of bran structures. Particle size was greatly reduced and the previously smooth and continuous bran surface is pitted and cratered. Though particle size was greatly reduced by HPH, solution viscosity and bran WHC was only moderately increased. Physical disruption alone through HPH did not release bound phenolics as previously shown.

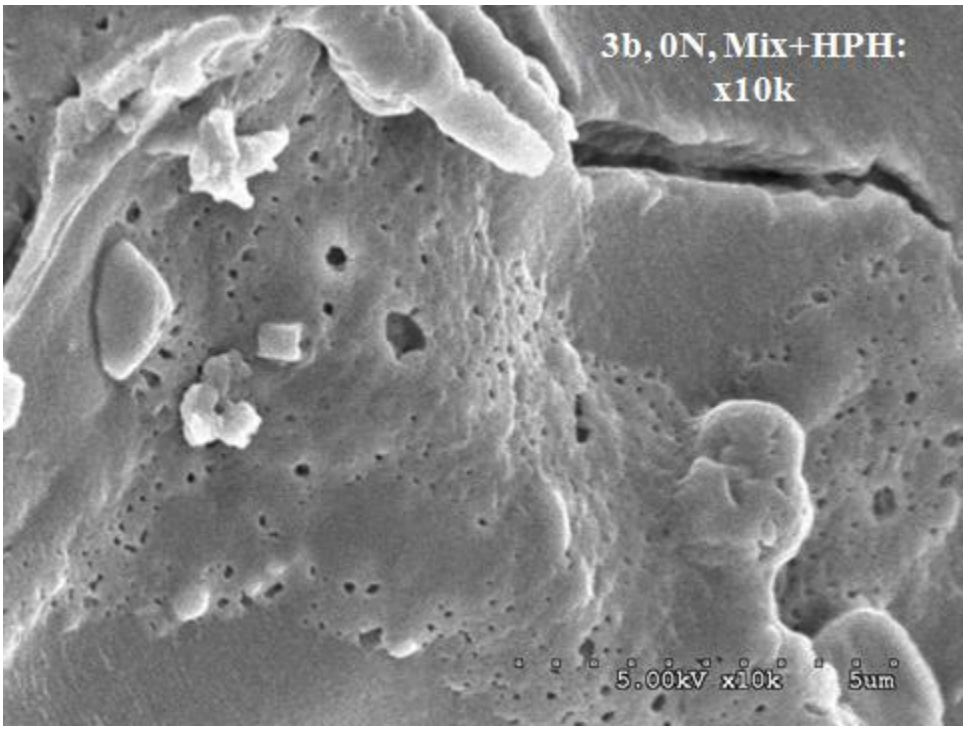


**Figure 43. SEM of Processed Wheat Bran: Sample 3b, 0N, Mix+HPH, x10k.**

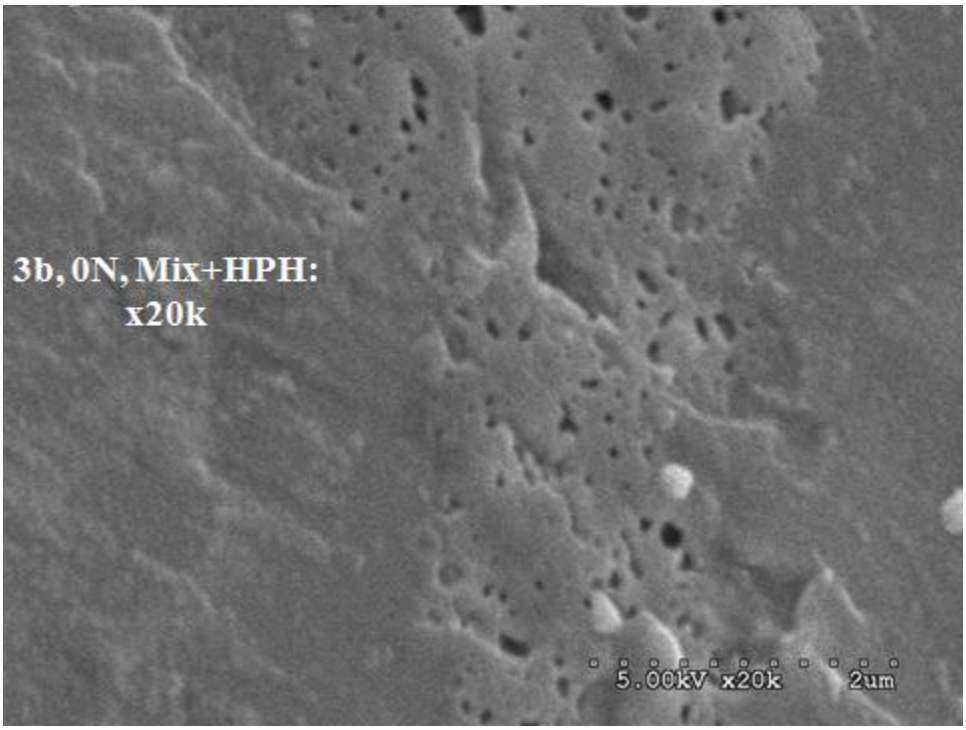


**Figure 44. SEM of Processed Wheat Bran: Sample 3b, 0N, Mix+HPH, x20k.**

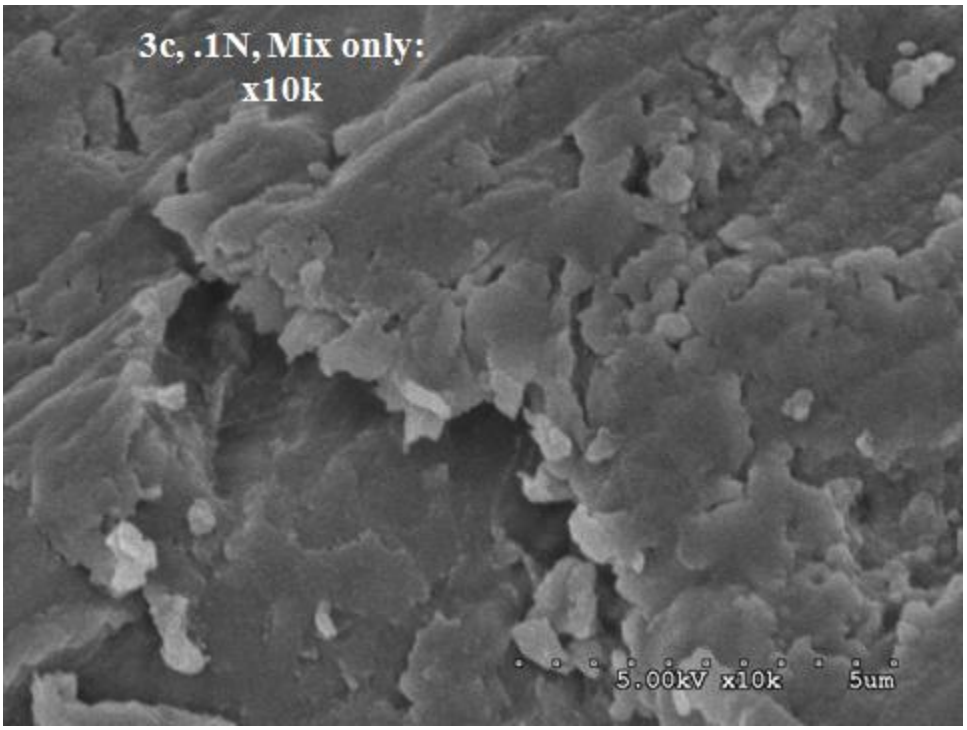


**Figure 45. SEM of Processed Wheat Bran: Sample 3c, 0.1N, Mix Only, x10k.**

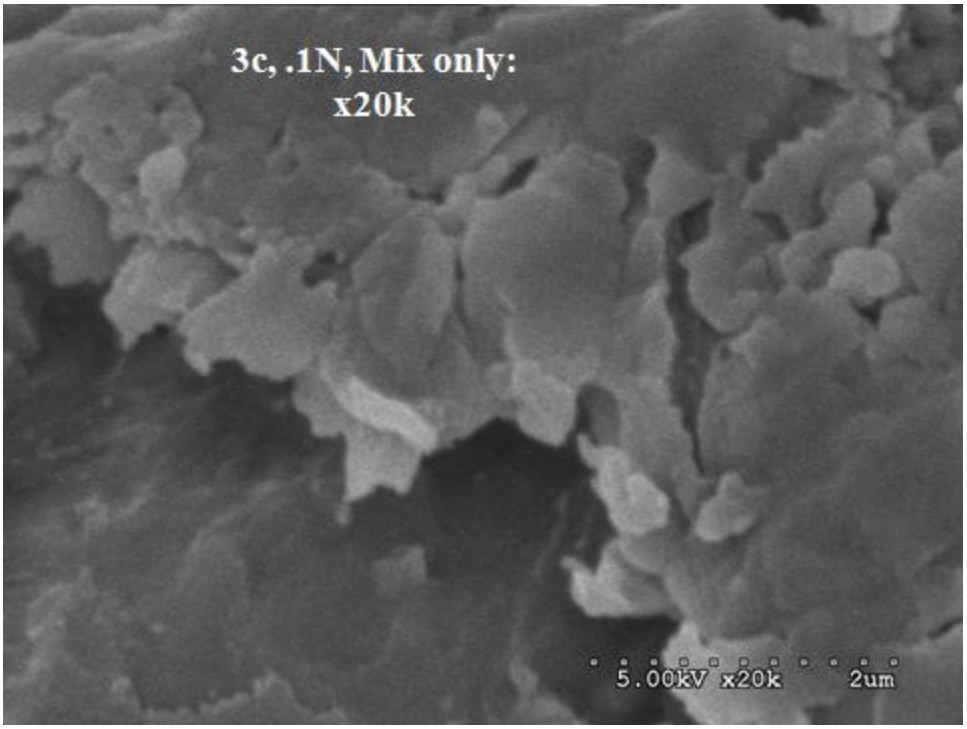


**Figure 46. SEM of Processed Wheat Bran: Sample 3c, 0.1N, Mix Only, x20k.**

Unlike the physical HPH treatment where disruption in bran surfaces created small holes and pores, the chemical pretreatment of Sample 3c shown in **Figures 45 and 46** creates a weakened structure that allows high shear mixing to break apart into smaller pieces. As previously described, the alkali treatment hydrolyzes ester linkages between arabinoxylan and phenolic acid molecules weakening the structure and freeing the bound phenolics. Even without HPH treatment, free phenolics increased dramatically (**Figure 11**) and a moderate increase in viscosity and WHC was found. Weakening the bran structure using the chemical pretreatment made the HPH treatment much more effective.

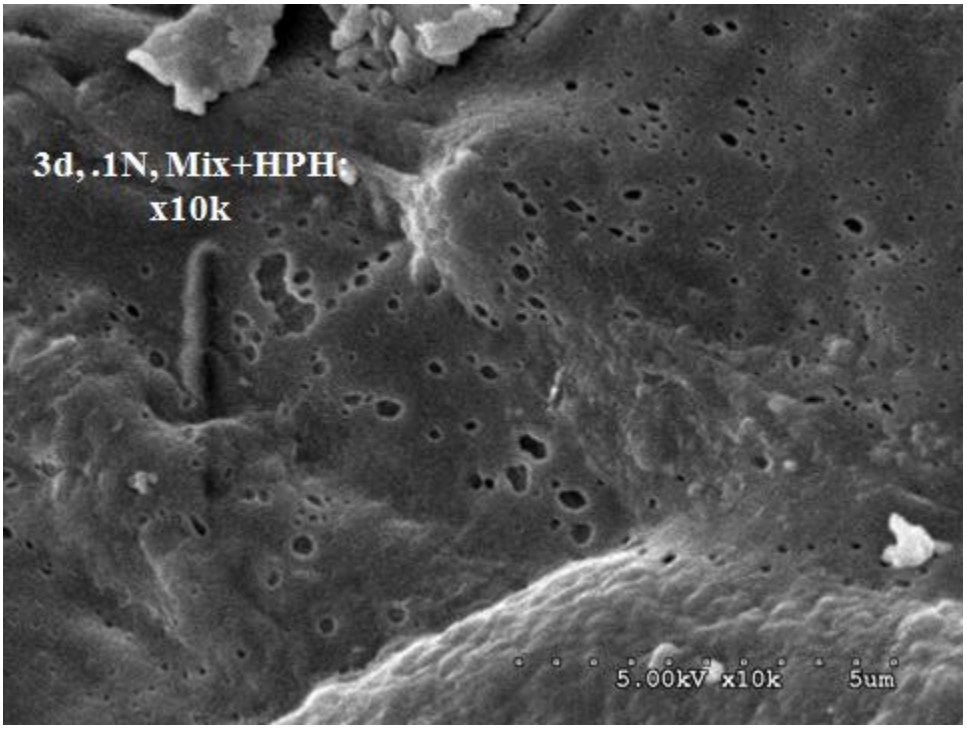


**Figure 47. SEM of Processed Wheat Bran: Sample 3d, 0.1N, Mix+HPH, x10k.**

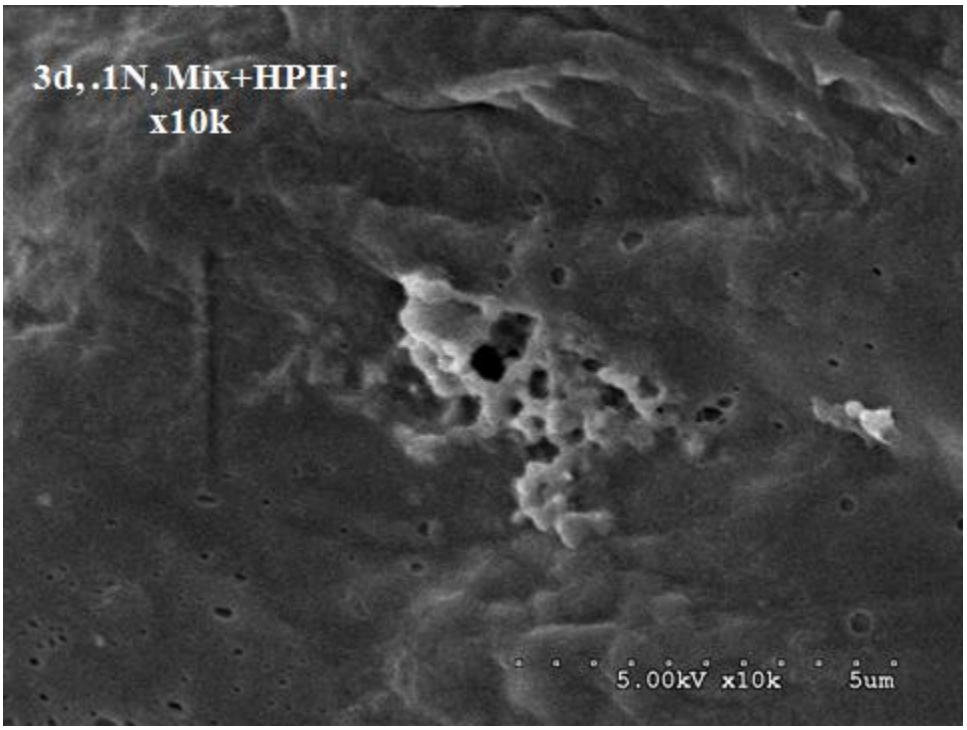


**Figure 48. SEM of Processed Wheat Bran: Sample 3d, 0.1N, Mix+HPH, x10k.**

**Figures 47 and 48** show the combined chemical pretreatment followed by HPH in Sample 3d at a medium magnification of 10,000 times. Surfaces that were once smooth and solid are now disrupted with larger dimples, holes, and craters. Bran layers have undergone additional particle size reduction and also layer separation. Additional detail of these two figures is shown by increasing the magnification to 20,000 times in **Figures 49 and 50**, and again to 50,000 times in **Figures 51 and 52**. 100 to 500 nanometer holes were common throughout the sample surface. The material that was removed by the processing treatments went into solution either as soluble or suspended material greatly increasing the viscosity and WHC.

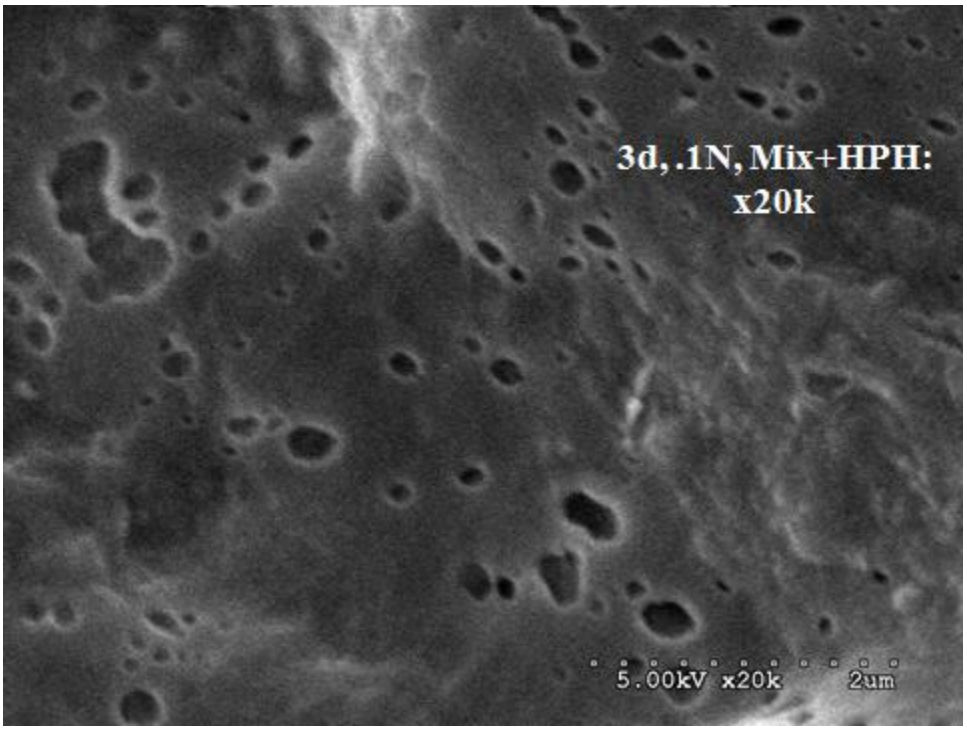


**Figure 49. SEM of Processed Wheat Bran: Sample 3d, 0.1N, Mix+HPH, x20k.**

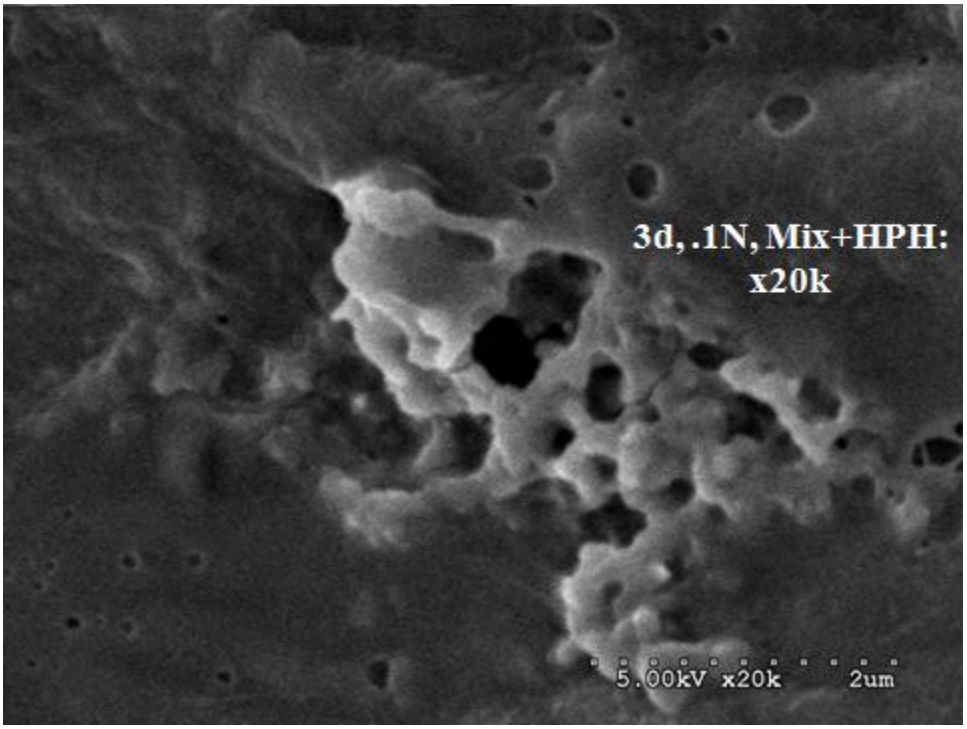


**Figure 50. SEM of Processed Wheat Bran: Sample 3d, 0.1N, Mix+HPH, x20k.**

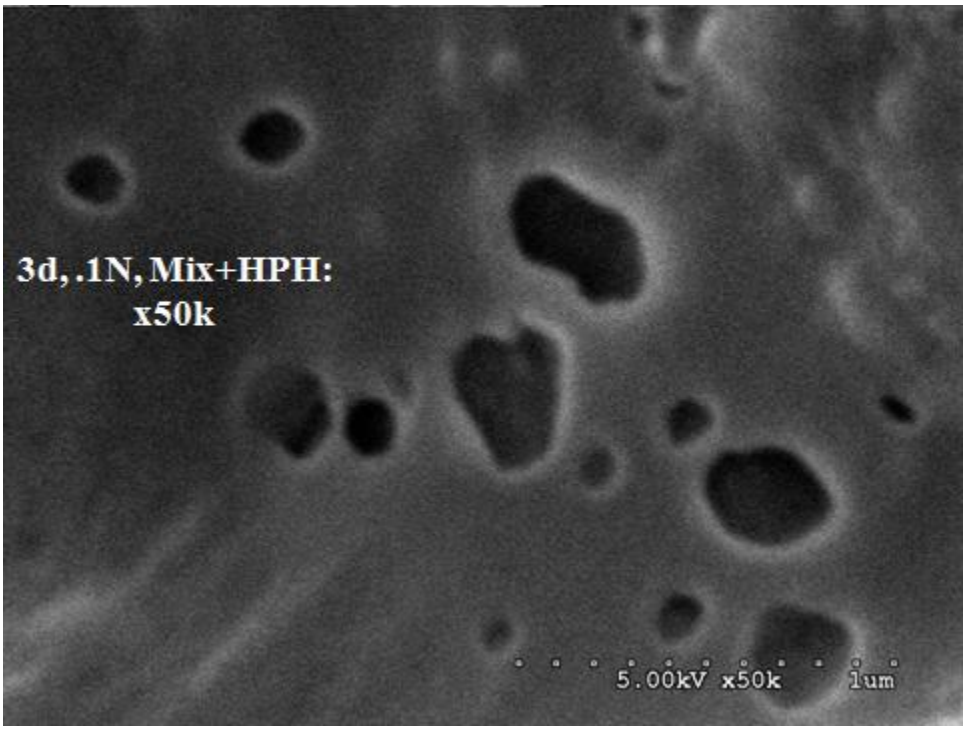


**Figure 51. SEM of Processed Wheat Bran: Sample 3d, 0.1N, Mix+HPH, x50k.**

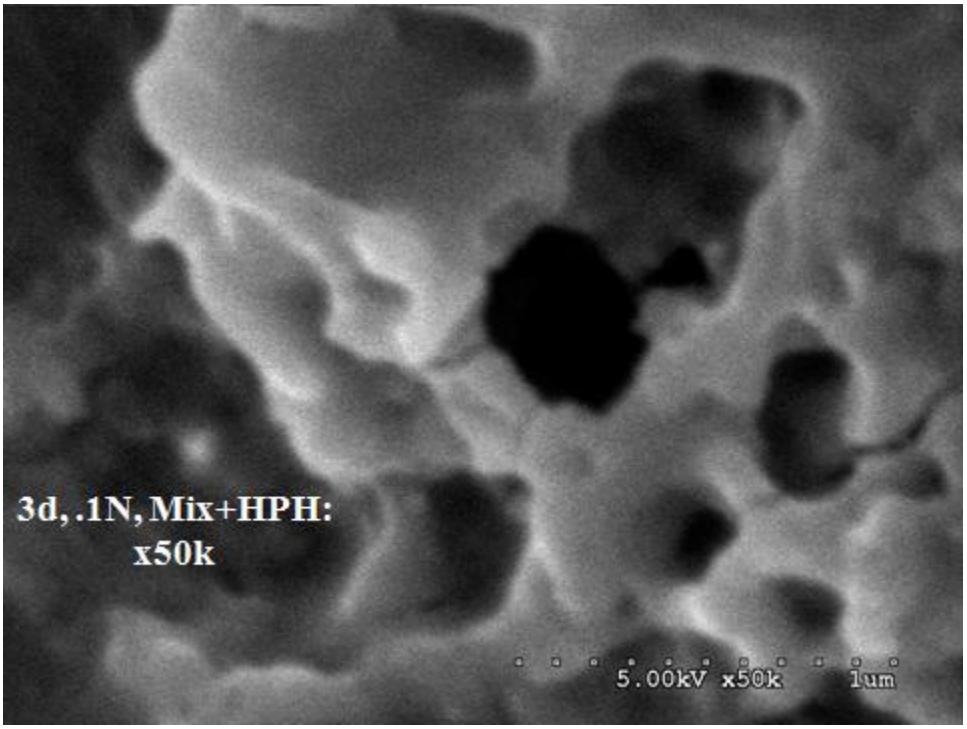


**Figure 52. SEM of Processed Wheat Bran: Sample 3d, 0.1N, Mix+HPH, x50k.**

## Conclusions

Earlier experimentation proved that chemical treatments were responsible for maximizing release of bound phenolics and the combination of chemical and physical treatments were important for maximizing viscosity and WHC. In this work, statistical experimental designs were evaluated to optimize preprocessing and processing conditions to maximize bran solution viscosity, WHC, and free phenolics. Optimum conditions for maximizing viscosity were not identical to the conditions for maximizing WHC, so an augmentation design was added to increase results resolution. Optimization pretreatment conditions leading to maximum viscosity and WHC were determined and treated bran had the following modifications: 500% increase in viscosity, 200% increase in WHC, 200% increase in Wa-Ex material, a 300 fold increase in free and soluble-bound phenolics, and a 500% increase in soluble fiber. Visual examination by SEM confirmed structural modification measurements.

With this phase completed, additional samples were needed for testing and developmental work by collaborators on this program. The next phase of research begins with producing large quantities of bran samples for the next phase. Optimization process conditions for maximizing solution viscosity were chosen for samples moving forward. They are: 0.1N NaOH treatment at 60°C for 24 hours prior to neutralization and HPH. Three kilograms of treated bran was requested.

## *Project 3: Large Scale Sample Preparation of Optimized Process Bran*

### Large Samples Needed for Collaborative Research

Previous work to develop processes to improve the functionality and bioactivity of wheat bran were successful based on physical and chemical measurements. The optimized process created wheat bran samples with increased potential for bioavailability of bioactive components and physiological benefits due to the changed physical properties of the bran. Increased sample size was needed for further study and to provide to collaborating researchers.

### Objectives

1) Utilizing the wheat bran pretreatment and high pressure homogenization (HPH) process settings to maximize viscosity and water hydration capacity (WHC) determined in Project 2, process 3 kg of wheat bran for additional analysis, animal feeding studies, and preliminary baking studies.
2) Explore process options for separating and concentrating the processed bran suspension and produce additional quantities of bran fractions for further research.

## Materials and Methods

**Raw Materials:** Soft White Wheat (SWW) bran was obtained from King Milling Company (Lowell, MI), Sustigrain Barley and Ultrafiber stabilized wheat bran were obtained from ConAgra (Omaha, NE). All samples were stored at -20°C until use.

**Dry Particle Size Reduction** utilized rotor beater milling technology (Retsch GmbH, Model SR 300, Haan, Germany). Bran was ground in a single pass through the 0.5mm screen to obtain coarse bran samples.

**Wet Grinding/Milling** was accomplished using a high shear rotor stator type homogenizer. The T25 Basic SI homogenizer with S25N-25F dispersing element (IKA Works, Wilmington NC) was used prior to all high pressure homogenization (HPH) processing to reduce particle size and prevent clogging of HPH disruption chambers. Large 5L samples were mixed for 10 minutes at 24,000 rpm.

**Alkali Pretreatment:** 5% bran solutions were prepared (using 500g bran sample on dry basis, creating 10 Liters solution) with appropriate alkali concentration and agitated in an Innova 4300 incubated shaker (New Brunswick Scientific, Edison, NJ) for the specified time and temperature of 24 hours at 60°C. Solutions were then neutralized to pH 7 with 1N HCl and diluted to 12.5 Liters to achieve 4% bran solutions for HPH processing. A total of 3 kg of bran dry matter was processed.

**High Pressure Homogenization** (HPH) was carried out on an M-110Y Laboratory Microfluidizer® Processor (Newton, MA). Homogenization pressure was controlled by adjusting compressed air pressure. Air pressure and flow was targeted to achieve 23,000 psi during processing. Disruption chambers used to achieve homogenization included a

200 micron chamber followed by a 100 micron dispersion chamber in series. The 200 micron chamber reduced bran particle size to prevent plugging of the 100 micron chamber. Heat was generated during processing so chilled water cooled the processed solutions. This study utilized 4% bran solution concentration and three passes through the homogenizer to ensure complete homogenization of samples.

**Moisture Content** of duplicate samples was measured by AACC Moisture method 44-15A. Briefly, 2g of sample was weighed into an aluminum dish with cover. Sample was heated in a gravity oven at 130°F for 60 minutes. Dishes were removed from the oven, covered, and placed in a desiccator to cool. Moisture was calculated as loss in weight divided by original sample weight. Replicates must be within 0.2% moisture.

**Particle Size Distribution** was measured by laser diffraction particle size analyzer (LS-13-320, Beckman Coulter Inc., Brea, CA) using the Universal Liquid Module.

**Paper Filtration of Processed Bran Solution** was tested using Whatman Grade 1 and Grade 4 qualitative paper discs in a Buchner funnel mounted to a suction flask.

**Evaporative Concentration of Processed Bran Solution** was tested using a small "homemade" dairy evaporator (Dairy Pilot Plant, University of Minnesota Dept. of Food Science and Nutrition). The sample was indirectly heated under vacuum and as vapor was removed and condensed, the sample was concentrated, cooled, and freeze dried. The bran sample was condensed from about 2.9% solids to about 5.5% solids.

**Laboratory Scale Separation of Processed Bran into More Soluble and Less Soluble Fractions** was tested on a Beckman J2-MC High Speed Floor Model Centrifuge

(Beckman Coulter Inc., Brea, CA). Samples were centrifuged at 10,000g for 30 minutes. Supernatant and pellet were separated, pooled, and freeze dried.

**Large Scale Separation of Processed Bran into More Soluble and Less Soluble Fractions** was accomplished by passing processed bran solution through a desludging centrifuge (Westfalia Clarifier/Separator, Model SB7, Oelde, Germany) creating a more "soluble" fraction and a more "insoluble" fraction. Bran solution was processed approximately 2 liters per minute, with flushing of the solids after 4 minutes. Centrifuge speed was 9200 rpm. Intake was adjusted to achieve roughly equal amounts of soluble and insoluble fractions. Clean incoming water was used to flush the insoluble fraction during continuous operation and this diluted the samples. The insoluble fraction was passed through the separator a second time and the soluble fraction was added to the first pass soluble fraction. All materials were freeze dried.

**Freeze Drying** was carried out using multiple VirTis EL series Freezemobile freeze dryers (SP Scientific, Warminster, PA). Processed bran samples were either shell frozen in jars and dried using manifold trees, frozen in trays and dried in a tray dryer, or frozen into round cake pans, placed into 7.2 Liter stainless steel vessels with acrylic lids (Millrock Technology, Kingston, NY) and attached to a freeze dryer manifold port. Dried samples were quickly ground in a food processer and combined into one lot for further study and analysis.

## Results and Discussion

### *Increased Scale Production of Optimized Processed Bran*

In order to provide processed bran samples for chemical analysis, animal feeding studies, bioactivity and bioavailability analysis, and test baking studies, a large quantity of viscosity optimized processed bran was needed. Previous sample size contained only 10 grams of bran dry matter, so a 300 fold increase was a large challenge. Although the HPH equipment remained the same and could process approximately 60 ml per minute per pass, this was not the bottleneck in the process as it could run continuously until processing was complete. The alkali pretreatment conditions were modified the most.

**Figure 53** shows a flowchart of the entire bran process. First, the bran quantity needed to be increased and it was decided to utilize 500 grams of bran dry matter in each of six batches that were reacted in 5 gallon pails with tightly closing lids. 5% bran solutions were made with 10 liters total. This was the same bran concentration as previously tested samples, but now much larger quantity. After alkali treatment, the samples were neutralized and diluted to 12.5 liters creating 4% bran dry matter concentration in the solution, twice the concentration of previous experimental designs.

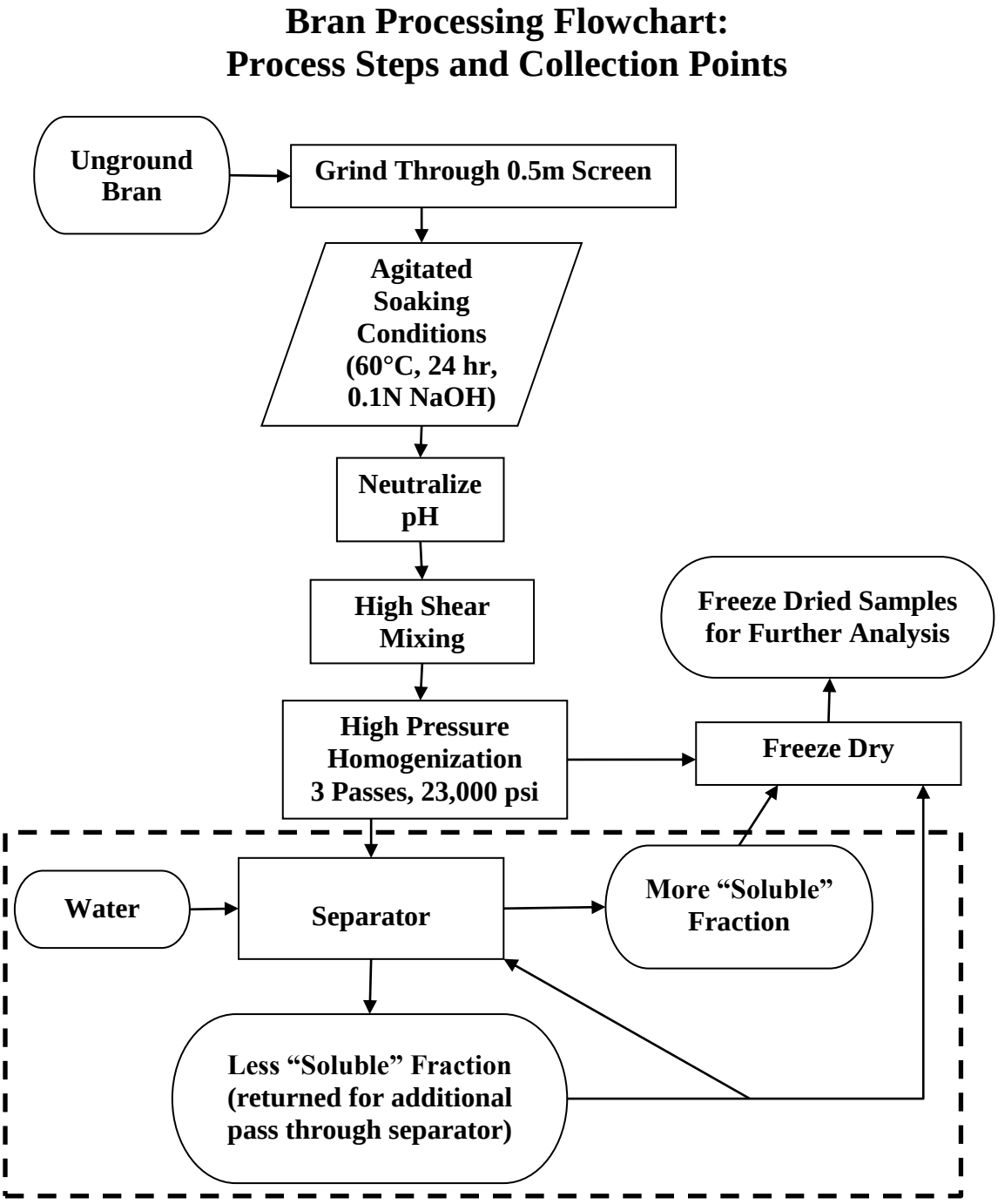


**Figure 53. Processing Flowchart for Large Scale Sample Preparation of Optimized Processed Bran and Fractionated Bran.**

The concerns with increasing the concentration of the solution for HPH treatment were clogging of the chambers with too much bran material, and processed bran viscosity too high to pump. Preliminary testing showed that neither was an issue. Though the solution viscosity after HPH processing was high, the powerful HPH pump had no issues with either pumping or clogging when processing the more viscous bran solution. Additionally, three passes through the HPH were utilized instead of two. This ensured the complete homogenization processing of the more concentrated solution.

Three kilograms of optimized process bran were processed and freeze dried and stored at -40°C for further testing. An additional three kilograms of control bran was freeze dried and also held at -40°C for further testing.

***Separating or Concentrating the Soluble Fraction of Optimized Process Wheat Bran***

After learning that more than half of the optimized processed bran fiber content was insoluble as shown in **Figure 35**, it was decided to experiment with processing techniques that could separate or concentrate the soluble fraction. The soluble fraction will contain the free and soluble bound phenolics so the ability to concentrate that fraction would be beneficial. Gallaher et al. (1993) suggested that materials moderately increasing the viscosity of intestinal contents could be effective in reducing plasma cholesterol, primarily by disrupting cholesterol absorption/reabsorption in the small intestine. Native wheat bran does not create viscosity in solution or in in-vitro intestinal simulations (Dikeman et al., 2006), but it is hypothesized that the modified wheat bran in this research may have a measurable cholesterol lowering effect as that of oat or barley

beta glucan soluble fiber (Dongowski et al., 2002) or other high viscosity soluble fibers like hydroxypropyl methylcellulose (Carr, et al., 1996).

Several methods to separate or concentrate the soluble bran fraction were evaluated including filtration, evaporation, and centrifugation. On a larger scale, membrane filtration, evaporation, and centrifugation are commonly used to separate and concentrate food ingredients. Laboratory testing of qualitative paper filters indicated that membrane filtration would not work. Laboratory samples were filtered using Whatman qualitative Grade 4 circles in a Buchner funnel mounted to a suction flask. When first tested, Grade 1 filter paper (11 micron particle retention) clogged almost immediately and even Grade 4 (25 micron particle retention) was very slow to filter. Although particle size analysis indicated roughly 50% of the processed bran was larger than the filter paper retention size, the bran clogged the paper almost immediately and 872 ml of 2.5% bran solution took many six hours to filter. Of the 24.28g of bran recovered from filtration after freeze drying, the "soluble" fraction passing through the filter was 52.6% of the total and the "insoluble" retentate was 47.4%. The viscous fibrous nature of the bran solution would also clog membranes of a commercial membrane filtration system, so separation or concentration by membrane filtration was not possible.

Evaporation to concentrate the bran solution to remove water was also tested. Approximately 8 liters of viscosity optimized bran solution was processed. The evaporative concentration of the bran solution nearly doubled the solids content, from about 2.9% to about 5.5% bran solids, but the increased temperature caused browning reactions to occur. As evaporation progressed, the bran solution became more and more

difficult to concentrate. On a larger scale, indirect heating, increased vacuum and better temperature control might limit sample heating, browning, oxidation, and degradation, but concentration to high solids content would still be limited by the highly viscous, high WHC nature of the bran in solution. Heating the free phenolic compounds in the solution, even under vacuum, would cause reactions detrimental to bioactivity. Other forms of concentration, such as microwave assisted vacuum drying or microwave assisted freeze drying may improve the likelihood of commercial success of a dried ingredient that would otherwise be too costly to produce and dry by means of freeze drying alone.

Laboratory centrifugation of the bran solution at 10,000g for 30 minutes proved to be a simple way of separating a more soluble fraction from a more insoluble fraction. A total of 2.2 kilograms of solution was centrifuged. Supernatants and pellets were pooled and freeze dried. The starting concentration of bran dry matter in solution was 3%. The solids content of the "soluble" supernatant fraction was 1.87%, while the solids content of the "insoluble" pellet fraction was 15.2%. For this process, the soluble fraction comprised 57% of the total bran material and 43% remained in the insoluble fraction.

Based on the success of the laboratory centrifuge test, an additional four kilograms of optimized processed bran was made for the purpose of centrifuge separation using a desludging dairy separator. The process is shown at the bottom of **Figure 53**. The processed bran solution was fed into the separator centrifuge which was rotating at 9200 rpm. More "soluble" material travelled out as supernatant, while more "insoluble" bran collected in the separator. Every 4 minutes, the solution in-feed stopped and water flushed the insoluble fraction from the separator. The insoluble fraction was passed

through the separator a second time both fractions were freeze dried. This separation yielded 68.8% soluble fraction and 31.3% insoluble fraction and was somewhat arbitrary based on the settings of the separator and the second pass of the insoluble fraction. Because one the goal of the separation was to obtain as much of the soluble phenolic material and viscosity producing soluble fiber as possible in one fraction, the extra pass of the insoluble fraction was desired.

Due to minor losses during separation processing, 2645 grams of freeze dried soluble fraction and 1250 grams of freeze dried insoluble fraction were produced and held at -40°C for further testing.

## Conclusions

A large quantity of three kilograms of viscosity optimized processed bran was needed for additional experimentation by collaborators in this research program. The process was scaled up to produce the bran without changing its functionality. Additional testing to separate or concentrate the more functional soluble fiber and phenolic content was conducted. Filtration, evaporation, and centrifugation methods were tested. Only centrifugation yielded positive and scalable results. An additional four kilograms of processed bran was separated into more soluble and more insoluble fractions for further research study.

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