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Modifying Wheat Bran for Food Applications - Effect of Wet Milling and Enzymatic Treatment

Metropolia University of Applied Sciences
Bachelor of Engineering
Bio and Food Technology
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Preface

This thesis was written and conducted at VTT Technical Research Centre of Finland during the spring of 2012. As the research process was diverse and challenging, I found it extremely rewarding for my future bioprocessing challenges.

Firstly, I would like to give my special thanks to my instructor Juhani Sibakov at VTT for his extremely valuable advices and admirable assistance during the work.

Secondly, I would like to thank my instructor Mikko Halsas at Metropolia University of Applied Sciences, for all his professional advices and ideas during the work.

Also, I would like to thank several other people for their contributions to the work. These people are Carola Fortelius and Pia-Tuulia Laine at Metropolia University of Applied Sciences, Panu Lahtinen, Seppo Kuosmanen, Craig Faulds, Jaakko Pere, Kati Katina, Arja Viljamaa, Katri Hartikainen, Eero Mattila, Heljä Heikkinen, Piritta Niemi, Leena Pulkki, Tarja Eriksson, Helena Hakuli, Erna Storgårds, Outi Santala, Anu Saloheimo, Anita Laamanen and Jaana Rasanen at VTT.

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Abstract

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The aim of this study was to develop sustainable methods to modify the structure of wheat bran used in food applications. The methods of interest were wet milling and enzymatic treatment. The effect of these methods on a product quality was tested in a pilot scale baking process.

Wheat bran is composed of many nutritionally valuable components, such as dietary fibre, antioxidants and proteins. Due to its relatively low price, wheat bran holds a great potential as an ingredient to be added into food products. However, most of the previous studies have shown that the addition of wheat bran usually decreases the quality of food products. The focus of this study was on modifying the properties of wheat bran, so that it could be used as a filler ingredient. Besides the particle size reduction and enzymatic treatment, the stability of wheat bran-water suspensions was studied by following the phase separation. The aim of the modifications was to release valuable components from the wheat bran matrix, to concentrate the dietary fibre and to alter the physico-chemical properties of wheat bran.

Wet milling and subsequent centrifugation increased the fibre content of the wheat bran fractions and increased the proportion of insoluble fibre. The enzymatic treatment increased the amount of reducing sugars in the soluble fraction after centrifugation. The highest solubilisation was obtained by using enzymes Depol 740L and Ecopulp TX200A. In addition, the phase stability of the wheat bran water mixtures increased in proportion to the level of milling. The effect of modified wheat bran on the quality of the breads was not as good as wanted due to the decreased volume and hardness of the test breads. Similar results were obtained both with dry and wet milled wheat bran fractions. The negative effect of wheat bran on the bread's structure was most clearly seen with 20 % addition level.

As a conclusion, wheat bran could be used as a bakery ingredient and a source of dietary fibre, when the addition level is lower than 20 % (of the dry mass in dough). However, the protocol for particle size reduction and the chosen enzymes are of utmost importance. As the desired properties of bread seemed to decrease in proportion to added wheat bran, the effect of particle size, enzymatic hydrolysis and addition level would need further studies.

Keywords	wheat bran, particle size, wet milling, enzymatic treatment, baking, bread

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Tiivistelmä

Tekijä Nimi Sivumäärä Aika	Olli-Pekka Lehtinen Vehnäleseen muokkaus elintarvikesovellutuksiin märkäjauhatuksella ja entsymaattisella hydrolyysillä 63 sivua + 9 liitettä 20.5.2012
Tutkinto	Insinööri (AMK)
Koulutusohjelma	Bio- ja elintarviketekniikka
Suuntautumisvaihtoehto	Tuotekehitys ja laadunvalvonta
Ohjaajat	Tutkija, Projektipäällikkö, Juhani Sibakov (VTT) Lehtori, Mikko Halsas (Metropolia Ammattikorkeakoulu)

Tämän insinöörityön tarkoituksena oli kehittää menetelmiä vehnäleseen rakenteen muokkaamiseksi elintarvikesovellutuksia varten. Tutkittavat muokkausmenetelmät olivat vehnäleseen märkäjauhatus sekä entsymaattinen hydrolyysi. Muokkauksen vaikutuksia arvioitiin pilot-mittakaavan koeleivonnassa.

Vehnälese sisältää monia ravitsemuksellisesti arvokkaita komponentteja kuten ravintokuitua, antioksidatiivisia komponentteja sekä proteiinia. Vehnälesettä on hyödynnetty suhteellisen vähän elintarvikkeissa, mutta oikeanlainen muokkaus voisi mahdollistaa sen käytön ravitsemuksellista arvoa lisäävänä raaka-aineena. Aiemmat tutkimukset ovat osoittaneet, että muokkaamattoman vehnäleseen lisäys heikentää elintarvikkeiden ominaisuuksia. Insinöörityössä tutkittiin partikkelikoon pienentämisen ja entsymaattisen hydrolyysin lisäksi muokatun vehnälese-vesi -suspension stabiiliutta faasierottumisen funktiona. Menetelmien tavoitteena oli vapauttaa vehnäleseen matriisista arvokkaita komponentteja, kuten ravintokuitua ja proteiinia sekä tarkastella muuttuvia fysikaaliskemiallisia ominaisuuksia.

Märkäjauhatus lisäsi sentrifugoinnin jälkeisen kiinteän lesefraktion kuitupitoisuutta sekä liukenemattoman kuidun osuutta. Entsymaattinen hydrolyysi lisäsi vehnälese-vesi -suspension pelkistävien sokereiden pitoisuutta supernatanttiliuoksessa sentrifugoinnin jälkeen. Parhaan tuloksen antoivat entsyymit: Depol 740L ja Ecopulp TX200A. Vehnälese-vesi -suspension stabiilius parani myös jauhatusasteen myötä. Muokkausten vaikutus leivontaominaisuuksiin ei kuitenkaan antanut toivottua lopputulosta. Muokatun vehnäleseen lisäys huononsi leipien tilavuutta ja lisäsi kovuutta. Kun vehnäleseen lisäystaso oli 20 %, leipien ominaisuudet olivat merkittävästi huonommat verrattuna vehnäjauholeipään.

Johtopäätöksenä voidaan todeta, että vehnälesettä voidaan käyttää leipomotuotteissa nostamaan kuitupitoisuutta, kunhan lisäystaso ei ylitä 20 % taikinan kuivapainosta. Vehnäleseen partikkelikoon pienentäminen ja entsyymien valinta vaikuttaa oleellisesti tuotteen ominaisuuksiin. Koska ominaisuudet huononevat oleellisesti suhteessa lisättyyn vehnäleseeseen, lisätutkimukset ovat tarpeen partikkelikoon pienentämisen, valittavan entsyymin ja tuotteeseen lisättävän vehnäleseen määrän optimoinnin osalta.

Avainsanat	vehnälese, partikkelikoko, märkäjauhatus, entsymaattinen hydroyysi, leivonta, leipä

Table of Contents

1 Introduction

Wheat is one of the most important grains in the world (Gooding, 2009: 19). Annually, the global agriculture produces large amounts of wheat. In 2011, global wheat crop was about 683 million tons (VYR, 2012). In the same year, the Finnish wheat crop was 9.8 million tons (Tike, 2011). The global development of wheat production is shown in Figure 1.

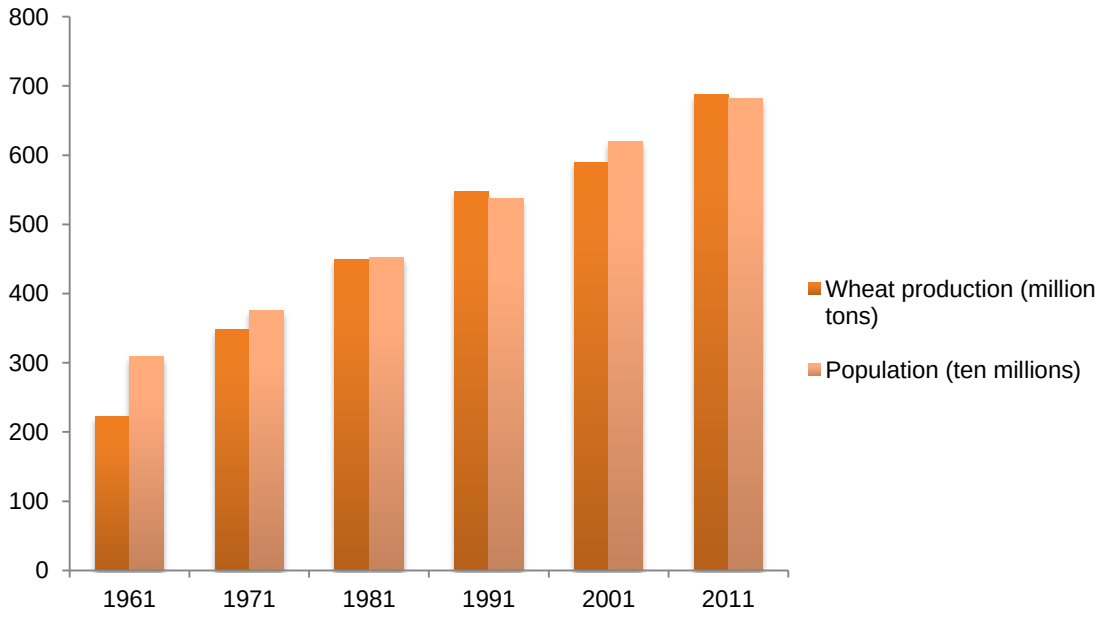

Figure 1. Total wheat crop has increased compared to the world's population in last decades. (FAO, 2012)

Wheat bran is a by-product of wheat milling industry. Milling of one million tons of wheat can yield 0.25 million tons of wheat bran (Javed et al., 2012). For example, in Finland, one of the biggest industrial mills: Fazer Mills and Mixes Ltd., produces around 20 000 tons of wheat bran every year. More than 95 % of it is used as animal feed. The price of the wheat bran is from 70 to 140 euros per kilo, depending on its quality (Arrajoki, 2012).

Wheat bran is mainly composed of cell wall material and contains many nutritionally valuable components, such as dietary fibre, proteins and phenolic compounds with antioxidative properties. It holds a great potential for food applications. However, only a little portion of the produced wheat bran is consumed as a food supplement (Javed et al., 2011). Thus, agro and food industry is looking for more valuable end-uses.

By reducing the particle size of wheat bran and modifying the solubility of the polymers, wheat bran could be used as a value added compound in food or paper applications. One possibility is to use the bran as a biofiller. The biofiller particles can be used to increase the bulk and lower the caloric density of food products or to improve the strength of paper. Particles rich in dietary fibre can also be used as ingredients for healthier food products. (Agrobio, 2010)

The aim of this study was to develop sustainable technologies to produce tailor made particles from wheat bran and to use them in food applications. The focus on this work was to solubilise different components, such as proteins, lipids and hemicelluloses. In addition, the structural properties of bran were modified by reducing its particle size. The particle size of wheat bran was reduced by ultrafine wet milling and different components were solubilised by enzyme hydrolyses. Modified wheat bran was tested in a pilot scale baking process. The quality of the products was tested with a sensory evaluation panel. Ultimate goal of this study was to produce dietary fibre rich products with good structure and sensorial quality.

2 Wheat

Wheat (*Triticum aesticum*) is among the oldest crops in the world. It has been cultivated since 10,000-8,000 B.C. The unique dough making potential of wheat was one of the main reasons for the transition from hunter-gatherer nomad to the settled agriculturalist. Nowadays, wheat is cultivated throughout the world, from Americas to most Eastern parts of Asia. (Wrigley, 2009: 1–6, Javed et al. 2011)

2.1 Wheat Quality

The quality of wheat affects the quality of flour and bran (hardness, flour yield, amount of starch damage and energy requirements in processing). The quality of wheat is a

structural and biochemical feature. It varies greatly among different wheat crops, strains, climate conditions, harvesting conditions and processing technology. (Bechtel et al., 2009: 51, Carson and Nancy, 2009: 97)

2.2 Composition

The wheat kernel consists of dietary fibre rich bran layers, starchy endosperm and fatty germ. Valuable nutritional compounds, such as micronutrients, phytochemicals and fibres are concentrated in germ and bran (Barron et al., 2006). The structure of the wheat grain is shown in Figure 2.

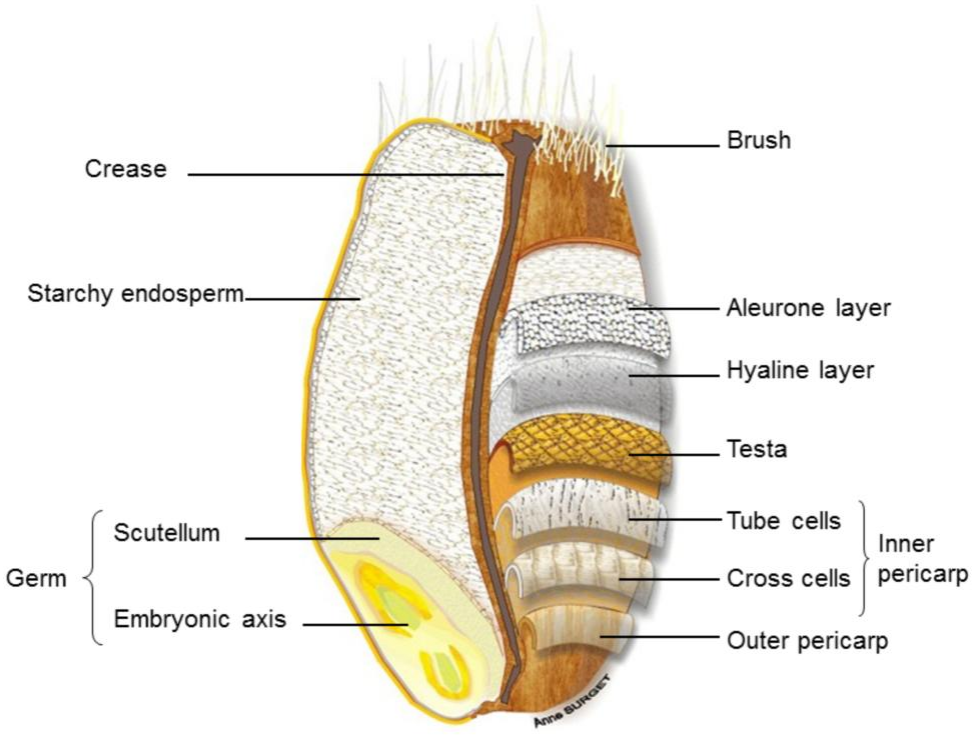

Figure 2. The structure of the wheat grain: different bran layers are illustrated from aluerone to outer pericarp (Surget and Barron, 2005). Courtesy of INRA, l'Institut National de la Recherche Agronomique, France.

The average length of the wheat kernel is 8 mm and the weight is about 35 mg. The size varies widely depending on cultivation properties. The crease is located nearly throughout the entire length of the kernel. Wheat kernels vary widely in texture and colour. The colour is related to pigment in the seed coat. (Hoseney, 1986: 1–2)

Wheat bran has several layers: outer and inner pericarp, testa/seed coat, hyaline layer and aleurone layer seen in Figure 2. The pericarp is composed of intermediate cells, cross cells and tube cells. The total pericarp comprises about 5 % of the grain. It consists of approximately 20 % cellulose, 6 % protein, 2 % ash, and 0.5 % fat. It is also rich in xylans and insoluble fibre (Hemery et al., 2007 and Hoseney, 1986: 4–5). Most of the pericarp's tissues have lignified walls. The uppermost layer of pericarp is called outer epidermis. It is 15-20 µm thick, and is composed of long, narrow cells that are arranged alternately (Brechtel et al., 2009: 56–60).

The testa layer (or seed coat) comprises about 1 % of the grain and is composed of mainly arabinoxylan and lignin. The proportion of cellulose is lower than in pericarp (Hemery et al. 2007). Testa contains almost all of the grain alkylresorcinols (Landberg et al., 2008), a class of phenolic lipids reported to exhibit antioxidant properties and anticancer activity (Kozubek and Tyman, 1999). Testa varies from 5 to 8 µm in thickness. The nucellar epidermis is about 7 µm thick and closely united to both the seed coat and the aleurone layer.

The aleurone layer is one cell thick, covering both the starchy endosperm and the germ seen in Figure 3. The aleurone contains most of the antioxidant potential of wheat grain (Mateo Anson et al., 2009), due to the high content of lignans and phenolic acids (Buri et al., 2004; Esposito et al., 2005; Zhou et al., 2004). Aleurone layer represents about 7 % of the wheat grain dry mass, but contains the major part of the B vitamins and about half of the mineral content (Antoine et al., 2003). Compared to the other peripheral layers, aleurone layer has high protein content, with a better balance of amino acids (particularly higher level of lysine) than the proteins of endosperm (Buri et al., 2004; Rhodes and Stone, 2002. The aleurone layer provides 80 % of the all niacin, 60 % of the all vitamin B_6 and 32 % of the all thiamine in whole wheat grain (Piironen et al., 2009: 183). Aleurone layer with all outer layers is removed during the milling of wheat flour (Hoseney, 1986: 5).

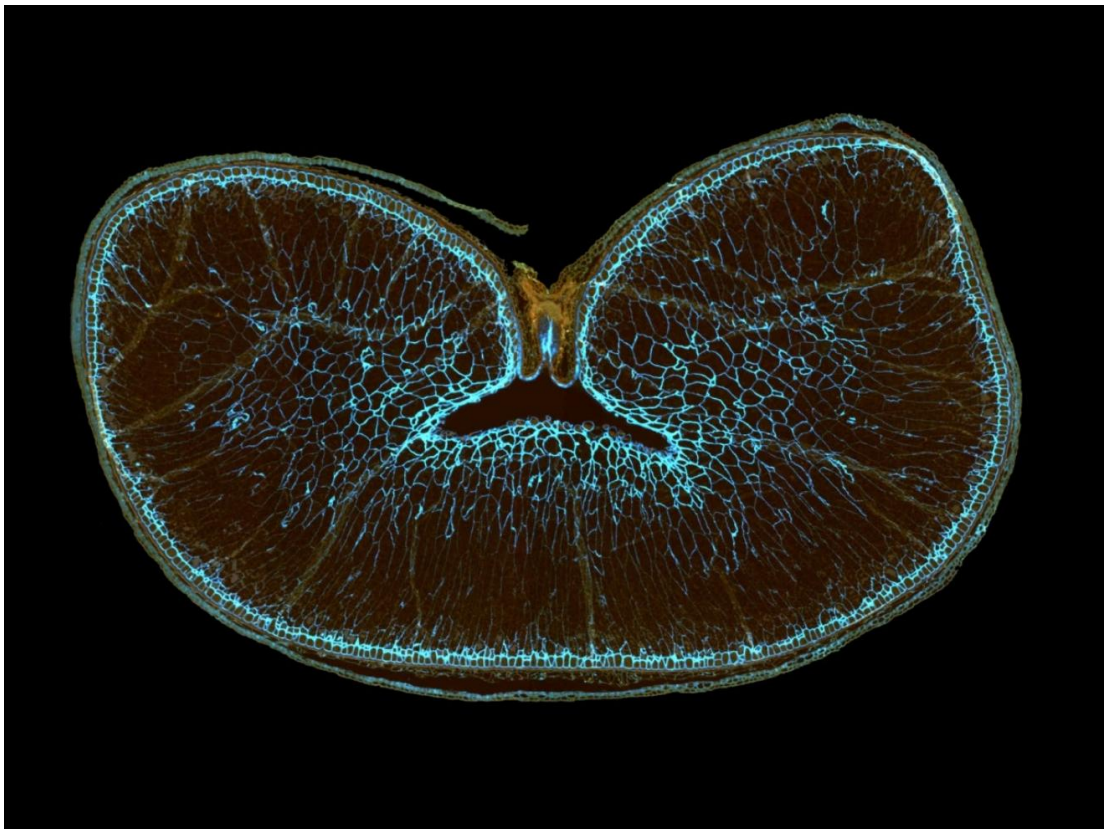

Figure 3. Cross-section picture of wheat kernel shows the aleurone layer covering both starchy endosperm and fatty germ (brown section in the middle). Courtesy of VTT Technical Research Centre of Finland (2011).

Next to the seed coat is the pigment strand. It consists of ragged cylinder of cells with lignified and oily contents. The structure of strand is similar among all white wheat varieties, and it is not distinguishable by colour. The pigments in the pigment strand have not been identified yet. (Bechtel et al., 2009: 60, 63)

The germ of wheat comprises 2.5 to 3.5 % of the kernel. The germ is composed of the embryonic axis and the scutellum. The scutellum functions as a storage organ for wheat. The germ contains 25 % protein, 18 % sugar and 16 % lipids**.** The sugars are mainly sucrose and raffinose. The germ does not contain starch but is rich in B vitamins. It also contains many enzymes. (Hoseney, 1986: 6–8)

The endosperm together with the aleurone layer contributes more than 80 % of the grain mass (Bechtel et al., 2009: 65). White flour is made of the endosperm, where cells are packed with starch granules embedded in a protein matrix. Most of the protein is gluten which is the storage protein of wheat. The starch granules are lens-shaped

and up to 40 µm in length. The starchy endosperm is composed of three types of cells: peripheral, prismatic and central. The cells vary in size, shape and location. The peripheral cells are the first row inside the aleurone layer. The prismatic cells are found inside of the peripheral cells, and the central cells are inside the prismatic cells. The endosperm cell walls are composed of pentosans, other hemicelluloses, and β-glucans (Hoseney, 1986: 8-9). In Figure 4, β-glucans are seen as long chinks coloured in Calcofluor blue.

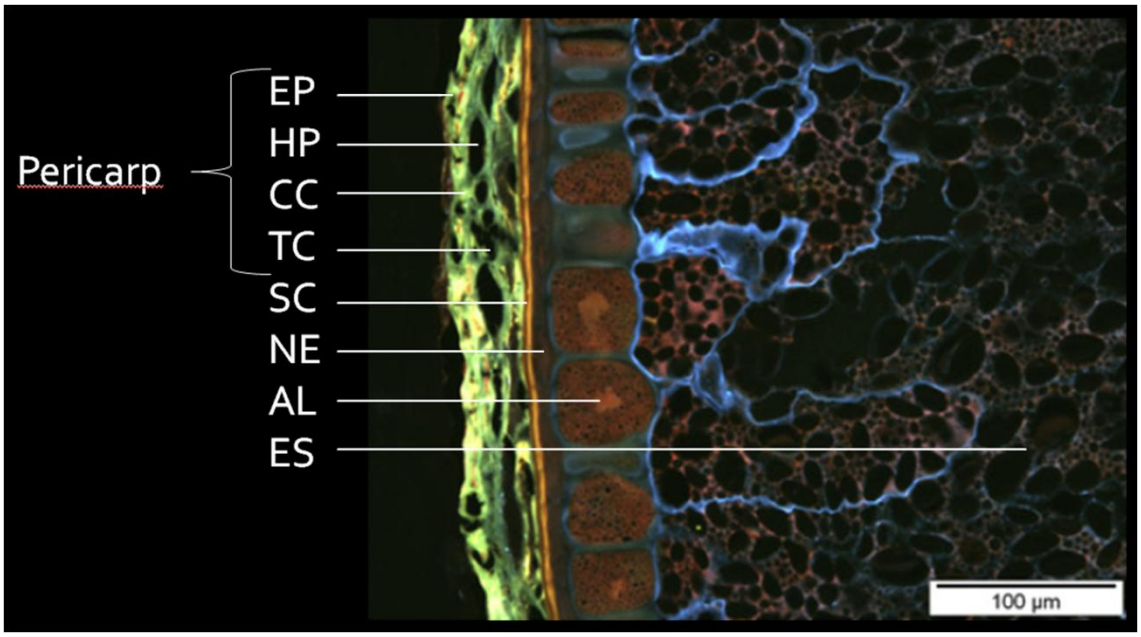

Figure 4. Tissues of a wheat kernel in a cross section picture, where β-glucan in the cell walls is stained with Calcofluor (blue) and protein with Acid Fuchsin (red) in a resin embedded sample. Starch granules are not stained and are seen as black spaces in the protein matrix. EP, epidermis; HP, hypodermis; CC, cross cell; TC, tube cell; SC, seed coat; NE, nucellar epidermis; AL, aleurone layer; ES, starchy endosperm. (Dornez et al., 2011, Hoseney, 1986)

2.3 Health Effects Related to Whole Wheat Grain

Whole wheat grain consists of bran, germ and endosperm. When refined, only carbohydrate rich endosperm is retained. This results in a big loss of many nutritionally valuable biochemical compounds such as dietary fibre, vitamins, minerals and antioxidative compounds which play an important role in reducing cardiovascular disease (CVD). Higher intake of unrefined carbohydrates, such as whole grain or bran,

is shown to be protective against insulin resistance and type two diabetes. (Mellen et al., 2008)

3 Wheat Bran

3.1 Production

Wheat bran is a by-product of wheat flour milling. The production of wheat flour starts with grain cleaning by use of sieves and aspirators. This removes both large and fine foreign particles. After the cleaning, wheat grain is conditioned and tempered. This kind of treatment toughens the outer casing and mellows the endosperm so that the grain will be in an optimal condition before milling. (Cornell and Hoveling, 1998: 43–49)

Production of wheat bran is carried out by milling and separation. Milling starts with the separation of husks from the kernels by an aspirator, which removes the lower density husks. After that, kernels are ground between two metal rolls. Roller milling is divided into two main categories, breaking and reduction. During the breaking, grain is opened up and the content, i.e. endosperm, is released so that separation can be done. The breaking is carried out by using rolls, which are spirally fluted and driven at different speeds. Heat in the rollers must be minimized with a cooler. (Cornell and Hoveling 1998: 43–49)

Afterwards, the reduction is carried out with on a set of rollers without flutings. The bran is flattened and endosperm material is reduced in size. Sieves of 0.25 and 0.13 mm are used to produce middlings (or dunst in UK) which consist of particles roughly intermediate in size between semolina and flour. Semolina is ground and coarse wheat middlings which are used for porridges and gruels (Turtia, 2001: 588). At this point, wheat bran (with germ) is separated from middlings (Cornell and Hoveling, 1998: 43–49). Furthermore, the separation of germ from bran can be achieved by rolls that cause flattening of the embryo and allow it to be separated by sieving.

3.2 Composition

Wheat bran usually accounts for 14-19 % of the grains weight (Maes and Delcour, 2002). It consists of outer layers: from pericarp to aleurone layer seen in Figure 2 and 4. Germ can also be included in bran depending on the milling process. Bran is rich in dietary fibre and lipids. Lipids are originated mainly from the germ. Wheat germ meal

consists mainly of the germ together with some bran middlings. Wheat bran has a complex structure due to its layer composition (Bechtel et al., 2009: 83). This makes it difficult to predict its behaviour during the treatments.

The composition of wheat bran depends greatly on wheat variety, cultivation conditions and separation methods. Separation methods determine how much starch is attached to the aleurone layer after the separation. Typical composition of wheat bran and wheat germ meal is shown in Table 1. Nevertheless, the composition may vary widely among different crops, and, according to another study, wheat bran contains approximately 45-48 % dietary fibre (Agrobio, 2011).

Table 1. The composition of wheat bran. Wheat germ meal consists of the germ together with some bran middlings. (Posner, 2009: 145)

Product	Minimum protein %	Minimum fat %	Maximum fiber %
Wheat bran	14.5	3.0	48.0-53.0*

* Source: Kamal-Eldin et al. (2009)

3.3 Carbohydrates

The major components of the bran are carbohydrates, especially arabinoxylan and other dietary fibre compounds. Arabinoxylan has important functional properties in bread making due to its partial water-extractability, viscosity, gelation, and water-binding capacity. (Hille and Schooneveld-Bergmans, 2004)

Kamal-Eldin et al. (2009) characterized two commercial wheat bran samples from Nordic countries. The dietary fibre content in those brans varied from 40 to 53 % of the dry matter, and starch content from 9 to 25 %. Around 55 % of the dietary fibre in wheat bran was arabinoxylan, while the rest was cellulose (9-12 %), lignin (3-5 %), fructan (3-4 %) and mixed linked β-glucan (2.2-2.6 %). The ash content of wheat bran samples was 5.5-6.5 %. Wheat bran also contains about 4-6 % di- and trisaccharides. These are mainly sucrose and raffinose (Hoseney, 1986: 95).

3.3.1 Celluloses

Celluloses are large polymers composed of D-glucose units combined by β-(1→4) linkages. The cellulose molecule can consist of 6 000 to 14 000 glucose units.

Cellulose makes up 30 % of wheat bran's cell walls (Stone and Morell, 2009: 319), and corresponds about 9.3-12.1 % of the dry weight of wheat bran (Kamal-Eldin et al., 2009). Cellulose is insoluble and resistant to many microorganisms and enzymes. Celluloses are often associated with lignin and other non-starchy polysaccharides (Hoseney, 1986: 89). Together with lignin and other fibres, cellulose forms a "lignocellulose"-matrix, which is highly resistant towards degradation (Zimmer, 2005: 249).

3.3.2 Hemicelluloses and Pentoses

Hemicelluloses are heteropolysaccharides consisting of several polymers. Their monosaccharide composition, glycosidic linkage, substitution pattern, and degree of polymerization may vary. Hemicelluloses are associated (e.g. by cross-linking) to other polysaccharides, proteins or lignin (Viikari et al., 1999: 1383) Thus, the definition of hemicellulose is not very clear. It usually refers to plant cell wall polysaccharides that can be extracted by alkaline solutions. According to Hoseney (1986: 90–91), hemicellulose and pentosans encompass the non-starchy and non-cellulotic polysaccharides of plants. However, the definition of hemicellulose may vary depending on the literature. For example, according to Hille and Schooneveld-Bergmans (2004) hemicellulose can be composed of arabinoxylans, arabinogalactans, cellulose, β-glucans, glucomannans and lignins. Nevertheless, hemicellulose constitutes one of the most complex polysaccharide matrixes which binds many grain components together (Shallom and Shoham, 2003).

Chemically, hemicelluloses are quite diverse, varying from short oligosaccharides to polymers that may contain pentoses, hexoses, proteins and phenolics. Hemicelluloses can be water-soluble or water-insoluble. For example, the water-insoluble hemicelluloses (or pentosans) make up about 2.4 % of the wheat endosperm (Hoseney, 1986: 90-91). Hemicellulases are classified according to the main sugar residue in the backbone, for example xylans (D-xylose) and glucans (D-glucose). The term pentosan is often used as a synonym for hemicelluloses, especially for xylans. (Hoseney, 1986: 90; Viikari et al., 1999: 1383). In cereals, hemicelluloses mostly consist of arabinoxylans. Among cereals, wheat bran and rye bran have the highest degree of arabinoxylans (Viikari et al., 1999: 1384).

3.3.3 Arabinoxylans

Arabinoxylans (AX) such as heteroxylans and pentosans, are polysaccharides composing of arabinose and xylose sub-units. AX have a basic backbone chain of β-D-xylopyranosyl residues linked through (1→4)-glycosidic linkages. They are the major non-cellulosic polysaccharides in cell walls. Around 64 % of wheat bran cell walls consist of AX (Stone and Morell, 2009: 320). AX corresponds about 22.4-29.8 % of the wheat bran dry weight (Kamal-Eldin et al., 2009). The chemical structure of AX and its subunits are shown in Figure 5.

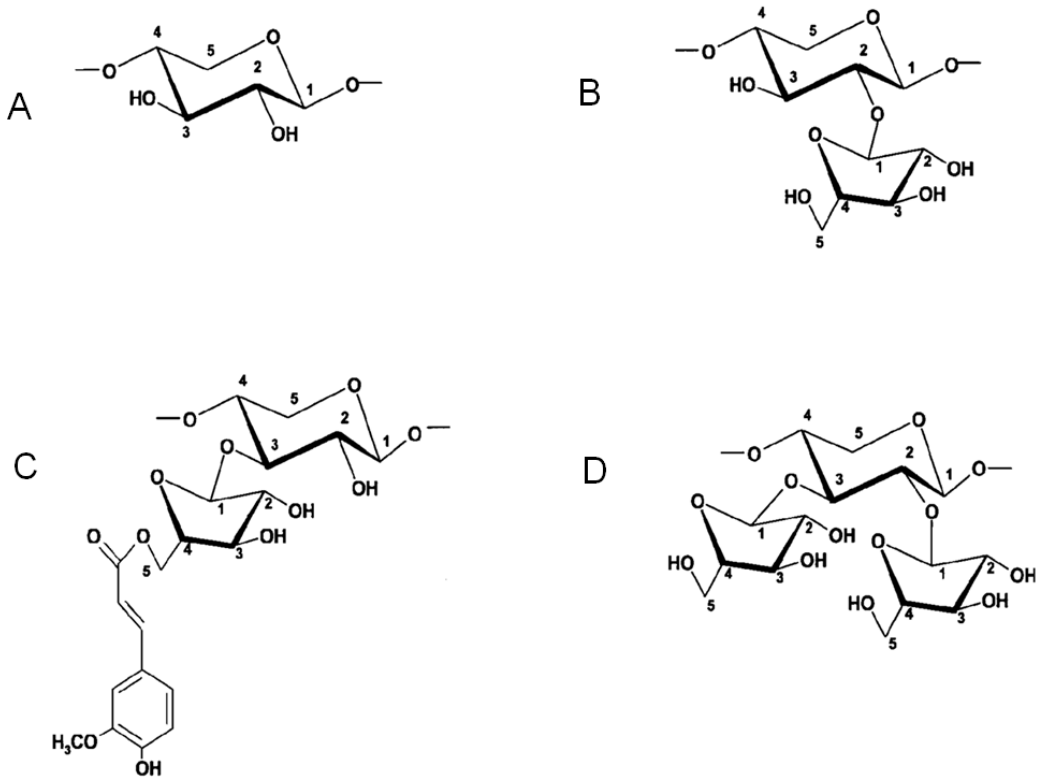

Figure 5. The structures of arabinoxylan with (A) unsubstituted, (B) monosubstituted, (C) monosubstituted with minor substituent and (D) disubstituted xylose residues. (Broekaert et al., 2010 (a) and (b))

AX has an important role in the preparation of dough and in bread baking performance when bran is used as an additive (Maes and Delcour, 2002). Functional properties of AX can be improved by modifying its structure by endoxylanases (Santala et al., 2011) or by an enzyme and microbe -combination in sourdough fermentation (Lappi et al., 2010). AX can be water-soluble or water-insoluble (Stone and Morell, 2009: 322). The

flexibility of AX molecules is dependent on the arabinose and xylose subunits ratio (Ara/Xyl). The AX in the endospermic tissues of wheat has intermediate Ara/Xyl-ratio about 0.5 to 0.7 (Broekaert et al., 2010(a)). Only small proportion of the AX is water-soluble (0.3-0.8 % of wheat grain dry weight). Thus, the majority of AX is water-insoluble, and solvents are needed to make it into soluble form (Stone and Morell, 2009: 328).

3.3.4 β-glucans

Cereal β-glucans (in wheat, oats, barley and rye) are polysaccharides which are composed of β-(1→3) and β-(1→4) linked D-glucose units. The chain has mostly cellulose-like β-(1→4) links and every third or fourth link is β-(1→3). This makes β-glucan soluble in water and highly viscous (Coultate, 2009). β-Glucans correspond about 2.2–2.6 % of the dry weight of wheat bran (Kamal-Eldin et al., 2009). At high concentrations, 4–10 % or more, β-glucans form thermo-reversible elastic gel networks (Stone and Morell, 2009: 332).

β-glucans are thought to be beneficial in relation to heart disease by lowering the cholesterol in blood. Their high viscous characters are believed to impede the reabsorption of cholesterol and bile acids from the small intestine (Coultate, 2009). The β-glucan of barley and oats has obtained a positive health claim related to its cholesterol lowering in the USA (FDA, 1997 and 2003) and Europe (EFSA, 2009).

3.4 Proteins

Wheat bran contains approximately 15.2–16.9 % protein. This is around 14 % of all the protein in wheat kernel. The amino acids in the wheat bran mostly consist of glutamic acid (18.6 %) and aspartic acid (7.2 %). (Shewry et al., 2009: 232)

3.5 Lipids

Wheat bran contains 5.5-5.6 % of lipids and wheat germ approximately 28.5 % of free lipids: 10.0-16.3 % in embryonic axis and 12.6-32.1 % in scutellum (Kamal-Eldin et al., 2009; Kim Chung et al, 2009: 371). These are mostly phospholipids, but there are also some glycolipids. In bran, 50 % of the lipids are unsaturated lipids with 18 carbons and two double bonds. The saturated lipids (16 carbons, without double bonds) account 19 % of the lipids (Hoseney, 1986: 99–100).

3.6 Phenolic Acids

Phenolic acids are a group of phenolic compounds that have an aromatic ring containing one or more hydroxyl groups. The total phenolic acid content in the wheat bran is around 4.5 $\mu g/g_{bran}$ on wet basis (Piironen et al., 2009: 202). Ferulic acid is an example of the hydroxycinnamic acids. These phenolic compounds are found richly in cereal bran (about 6.6 $mg.g^{-1}$, dry basis, in wheat bran). Usually about 80 % of ferulic acid (along with other hydroxycinnamic acids) is ester-linked to other constitutive elements of the cell wall, namely arabinoxylans (Giet et al., 2010).

Wheat phenolics have an effect on the pigmentation affecting the quality of flour and bread. In addition, the phenolic acids can influence flavour, texture, colour and the nutritional properties of foods. Several phenolic acids are known to bring greyish, brownish or greenish colours to food products (Piironen et al., 2009: 204). Recently, the antioxidant activity of the phenolic compounds has attained significant interest among researchers (e.g. Mateo Anson et al., 2009). According to epidemiological studies, consumption of wheat bran is associated with reduced risk of colorectal and gastric cancers due to its phenolic acids. Phenolic acids account for almost one third of the total intake of phenolic compounds in regular diet while flavonoids account the remaining two thirds (Piironen et al., 2009: 201–202).

3.7 Matrix

Wheat bran cell wall components such as cellulose, hemicellulose, proteins and phenolic compounds are bound to each other both covalently and non-covalently, constituting a complex network (Santala et al., 2011). Figure 6 shows the microscopic analysis of wheat grain matrix using different kinds of colour markers.

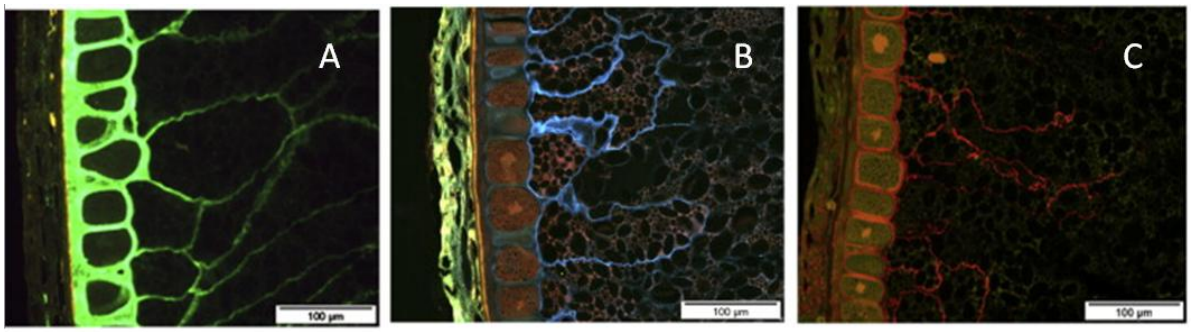

Figure 6. Microscopic analysis of wheat grain matrix. (A) Outer kernel layer stained with inactive fluorescently labeled xylanase probe. (B) Outer kernel layer stained with Acid Fuchsin - Calcofluor. (C) Outer kernel layer stained with mixed-linkage β-glucan antibody (Dornez et al., 2011).

3.8 Health Effects Related to Wheat Bran and Dietary Fiber

Wheat bran contains a high amount of dietary fibre, which is mainly located in the outer cell walls of the bran. According to the American Association of Cereal Chemists (AACC, 2001), dietary fibre is defined as "edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or glucose attenuation".

Studies show that the consumption of whole grain and dietary fibre is linked in the reduction of cardiovascular disease, diabetes and cancer (Montonen, 2005, Lindström, 2006, Fardet, 2010, Thomas and Pfeiffer, 2012). This is mainly caused by the specific structure of food and fibre itself. In addition, wheat bran consists of many antioxidant and anti-carcinogenic compounds, such as minerals, trace elements, vitamins, carotenoids, polyphenols and alkylresorcinols. Oligosaccharides of wheat bran may also act as prebiotics and improve the microbial flora in colon (Fardet, 2010, Karppinen et al., 1998).

Recently, EFSA (2010) has approved two health claims related to the consumption of wheat bran. The positive effect on human health can be due to the increase of faecal

bulk and the reduction of intestinal transit time. To substantiate these claims, the daily wheat bran fibre consumption should be more than 10 g.

4 Prosessing of Wheat Bran

Wheat bran must be modified in several steps, as the matrix is not easy to degrade. Previous studies have shown that there are feasible ways to reduce wheat bran particle size and modify its components, so that it could be used as value-added filler component. The most promising operations are the reduction of particle size, dry fractionation, surface modification, colour removal and different kinds of enzymatic treatments (Hemery et al., 2010 and 2011, Noort et al., 2010).

4.1 Reduction of Particle Size

Efficient reduction of the particle size releases valuable components from the wheat bran matrix but can also have negative effects. For example, in the study of Noort et al. (2010), the reduction of wheat bran particle size had an effect on dough-mixing properties, and bread-making quality. With particle size D_{50} = 48 µm (when more than 50 % of the particles were below 48 µm), the negative effects, such as smaller loaf volume and reduced mixing properties due to a gluten aggregation, were enhanced when milled bran was added to the dough. They concluded that it was caused by the fibre-gluten interactions. Two possible explanations for this were an increased interaction of the surface and the liberation of reactive components due to cell breakage. In their study, particle size reduction was carried out by using rotor mill (Retch ZM10), impact mill (Hosokawa Alpine 100 UPZ) and jet-mill (Hosokawa Alpine 100 AFG) (Noort et al., 2010).

In addition, several other techniques have been studied to reduce the particle size. For example, Hemery et al. (2011a) showed that different ultrafine grinding techniques efficiently decreased the particle size of wheat bran. Ultrafine particles were obtained in dry ambient and dry cryogenic grinding conditions using the Hosokawa Alpine 100 UPZ impact mill with a 0.3 mm sieve. One step of cryogenic grinding at −80 °C resulted in a particle size D_{50} = 50 µm. Similar results were obtained when performing the grinding three times at ambient temperature (25 °C).

In the study of Zhu et al. (2010), ultrafine ball milling was used to decrease the particle size of wheat bran. As the particle size diminished, the hydration properties, such as water holding capacity, water retention capacity and swelling capacity of the wheat bran dietary fibre decreased. Ultrafine milling was carried out by using multidimensional swing high-energy nano-ball-milling with ZrO_2 balls (6-10 mm in diameter). After ultrafine milling, the average particle size was D_{50} = 344 nm. For wheat bran used as filler particle in food applications, this kind of ultrafine milling technology would not be cost-effective. Nevertheless, the study showed that the particle size can be reduced under the sub-micron level.

In a study of Zhu et al. (2010) ultrafine ball milling was used to decrease particle size of wheat bran dietary fibre. As the particle size diminished, hydration properties of dietary fibre such as water holding capacity, water retention capacity and swelling capacity of wheat bran dietary fibre decreased. Ultrafine milling was carried out by using multidimensional swing high-energy nano-ball-milling with ZrO_2 balls (6-10 mm in diameter). After ultrafine milling, the average the particle size was D_{50} = 344 nm. For wheat bran used as filler particle in food applications, this kind of ultrafine milling technology would not be cost-effective. Nevertheless, the study shows the conceivability of reduction of particle size.

4.2 Peeling and Polishing

Before milling, there are several methods that can be used to improve the quality of wheat bran. Debranning, degerming, peeling or pearling can be used to remove the outer pericarp layers and/or the embryo. Besides nutritionally valuable compounds, wholegrain cereals contain detrimental components, such as microorganisms, heavy metals, sand and pesticide residues. However, these are not uniformly distributed in the grain. For example, Fleurat-Lessard et al. (2007) showed that about 80 % of pesticide residues were present in the outer layers of wheat grain. The outer pericarp usually contains most of the contaminating bacteria and moulds. According to Laca et al. (2006), 87 % of the total microbial contamination was eliminated by removing only 4 % of the total grain weight using debranning.

In the separation steps, the bran particles can be fractionated according to size, shape, mass, density or dielectric properties. Several separation methods can be combined to obtain efficient separation of the tissues (Hemery et al., 2007). For example, by using efficient dry fractionation with grinding, air classification and electrostatic separation,

Mateo Anson et al. (2008) found out that wheat aleurone layer contained most of the antioxidant potential of wheat grain.

4.3 Fractionation

Hemery et al. (2011b) investigated an electrostatic fractionation of wheat bran. In their study, the ultrafine bran was charged by tribo-electrification and introduced in a chamber containing two high voltage electrodes, where bran particles were separated depending on their acquired charge, allowing positively and negatively charged fractions to be collected separately. The charge of the particles was influenced by their biochemical composition: particles rich in branched and cross-linked arabinoxylans (pericarp) were separated from particles rich in β-glucan, ferulic acid and para-coumaric acid (aleurone cell walls). The testa and the intracellular compounds from aleurone were not highly charged, neither positively nor negatively.

Bühler AG (Switzerland) has also developed an electric separation of wheat bran to separate mixtures of aleurone and other outer layer particles. The charged particles are separated from one from another due to their distinct dielectric properties and/or their different electrical polarization (Behrens and Bohm, 2004; Bohm and Kratzer, 2005). In addition, Bühler AG has a method, by which the aleurone fraction can be separated using biochemical and enzymatic actions (Bohm et al., 2003).

4.4 Increasing the Solubility of Polymers

After milling, the solubility of wheat bran polymers can be increased by enzymatic treatment or heating in a liquid solution. By increasing the solubility of polymers, the proportion of dietary fibre can be enriched. The AX of wheat bran can be cleaved to xylo-oligosaccharides (XO) by enzyme hydrolysis. The study of Damen et al. (2012) showed that XOs can possess prebiotic activity (Broekaert et al, 2011(b), Damen et al., 2012) and it can be used to improve the quality of bread in baking. XOs are water soluble oligosaccharides which can be added to food products without noticeable effect on their taste and texture (Broeakaert et al., 2011(b)). AX has been shown to be cleaved most efficiently by a xylanase originating from *Hypocrea jecorina. H. jecorina* is an anamorph of *Trichoderma reesei* (Chaverri and Samuels, 2003). The treatment resulted in an OX content of 2.1 % on dry basis and an average degree of polymerisation of 9 (Damen et al., 2012). This kind of treatment has a good potential in baking (see chapter 5.2).

There are different kinds of AX based on their solubilisation. Water-soluble arabinoxylan (WS-AX) refers to AX which can be solubilised in a sufficient amount of water at temperatures between 70 and 100°C. Solubility can be maintained after cooling by adding ethanol to a final concentration of 70 % at 70 °C. The WS-AX has an average degree of arabinose-substitution (A/X) between 0.15 and 0.70. Water-unextractable (WU-AX) refers to the AX which cannot be solubilised in water at temperatures between 70 °C and 100 °C. The WU-AX has an average degree of A/X between 0.35 and 1.0. (Broekaert et al., 2010(a))

Nevertheless, WU-AX can be partially be solubilised under alkaline conditions or by using endoxylanases. WS-AX has a remarkable viscous forming potential due to its large molecular mass (Broekaert et al., 2010 (b)). The degree of polymerisation (DP) for WS-AX exceeds from 50 to 15 000 and for WU-AX up to 200. The WS-AX content of bran and other milling fractions can be increased by enzymatic treatment. Enzyme treatment should also comprise at least of one endoxylanase which is selective for WU-AX (Broekaert et al., (2010b)).

In addition, a study of Broekaert et al. (2010(b)) showed a method in which cereal-based WS-AX was solubilised by endoxylanases to produce soluble arabinoxylo-oligo-saccharides. Endoxylanases were added to the insoluble fraction of the whole stillage obtained after distillation of ethanol-containing fermented mash. The soluble AX was subsequently recovered through the separation of the soluble AX from insoluble material. This process was not restricted to grain stillage, as any AX containing cereal material could be used as a raw material.

4.5 Colour Removal

The colour of wheat bran is usually seen as a negative property, especially if the bran is incorporated into bread or other food products. The content of phenolic acids in wheat flour correlates well with the colour. In addition, polyphenol oxidase is an endogenous enzyme, which is responsible for the unwanted browning of wheat products (Piironen et al., 2009: 204). Proper phenolic acid removal would ensure an efficient colour removal. Nevertheless, the removal of phenolic acids may also lead to a loss of antioxidant properties. On the other hand, the yellow pigment of wheat derives mainly from carotenoids (xanthophylls), their esters and aricin flavones. Carotenoids

can also contribute to the antioxidant properties. Thus, the removal of carotenoids may lead to a loss of beneficial properties of wheat bran (Piironen et al., 2009: 189).

In the study of Zheng et al. (2009), pentosan and dietary fibre fractions were obtained by treating wheat bran by pH regulation, extrusion and several other unit operations. The colour of the wheat bran was efficiently removed by an H_2O_2 solution. However, it is prohibited to use H_2O_2 in food processing and food products in Finland and the EU (MMM, 2011).

4.6 Enzymatic Treatment of Wheat Bran

Enzymes can be used to cleave the carbohydrates and proteins. In wheat bran, this usually results in increased solubility of polymers and release of xylo-oligosaccharides (Santala et al., 2011). The enzymatic modification of AX has been studied intensively, as it is the main component of wheat bran. However, there are several potential enzymes which can be used to modify the structure of wheat bran.

4.6.1 Hemicellulases

Hemicellulases are a diverse group of enzymes. They are the key components in the degradation of plant biomass. Their substrates are a heterogenous group of branched and linear polysaccharides called hemicelluloses. Hemicelluloses are bound together with hydrogen bonds to the cellulose microfibrils in the plant cell walls. Hence, hemicelluloses are hard to degrade into smaller units. The catalytic modules of hemicellulases are either glycoside hydrosylates or carbohydrate esterases (Shallom and Shoham, 2003). One subgroup of hemicellulases is xylanases (Viikari et al., 1999: 1385).

4.6.2 Xylanases

Xylanases are hydrolytic enzymes that cleave the backbone of arabinoxylan, decrease the degree of polymerisation, increase the solubility and release different kinds of xylo-oligosaccharides. Xylanases can be grouped into endo- and exoxylanases. Endo-xylanases catalyse the random hydrolysis of 1, 4-β-D-xylosidic linkages in xylans (Santala et al., 2011; Viikari et al., 1999: 1385). In industrial baking, xylanases are used for their ability to improve textural properties of bread (Santala et al., 2011).

Endoxylanase treatment can solubilise up to 50 % of the arabinoxylans in cell walls of wheat bran. In addition to released oligosaccharides, the treatment usually solubilises protein, e.g. 3 % of wheat bran dry matter. After the endoxylanase treatment, aleurone layer was completely disorganised. The treatment released 80 and 50 % of the carbohydrates in dissected aleurone layer and in the inner bran, respectively. The inner bran composed of the nucellus, seed coat, tubes cells and cross cells. In contrast, the outer bran, which was composed of hypodermis and epidermis, was much more resistant towards endoxylanase treatment. (Benamrouche et al. 2001).

4.6.3 Cellulases

Cellulases are enzymes that catalyse the degradation of celluloses to glucose. Cellulases are produced commercially by fungi or bacteria. Cellulose is degraded to glucose units through a synergistic action of several enzyme classes. For example, cellulase glycosidase catalyses the endohydrolysis of 1, 4-β-D-glucosidic linkages in celluloses (IFIS, 2009: 82). To obtain proper degradation of cellulose, both endo- and exocellulases are needed (Zimmer, 2005: 249–250). Endocellulases are a group of cellulases which cleave the internal glucosidic bonds of cellulose (Wen, 2007: 639). Exocellulases cleave from the end of the cellulose chains releasing oligosaccharides of varying lengths (Wen, 2007: 639; Gong, 1999: 1900).

4.6.4 Ferulic Acid Esterases

Enzymatic release of ferulic acid depends mainly on the breaking of its ester linkages by ferulic acid esterases (FAE). Esterases work in a synergy with arabinoxylan-degrading enzymes such as hemicellulases, including xylanases. Cellulases and proteases may also contribute to the opening of cross-linked structure in the cell wall matrix of bran. Purified FAE is still not commercially available. However, several enzyme complexes have FAE activity. Some examples of these enzymes are Depol 740 L (Biocatalysts), Pentopan 500 BG and Novo 188 (Novozymes), Pectinase PE (Catalysts) and Grindamyl S100 (Danisco). These complexes are used as filtration aids in brewing or for bread making. FAE activity is usually a side activity, the major being hemicellulase or cellulase activity, except for Depol 740 L, in which FAE activity is standardized to 36 Ug^{-1}. (Giet et al., 2010)

5 Application Potential in Food Industry

5.1 Increase of Bulk and Use as Emulsifier

Use of modified wheat bran can add several properties to the final food product. The target characteristics for the products in this study were reduced caloric density, stabilization of food colloids and a source of insoluble dietary fibre. Prevention of phase separation would be required especially in liquid and semi-liquid products, such as beverages, jams, jellies and yoghurts. Caloric density can be reduced when the particles are used to increase the bulk or when they are used to substitute fat in liquid or semi-solid products. The ultra-fine particles can be used as stabilisers to produce emulsions, foams or gels against phase separation. (Agrobio, 2010)

5.2 Baking

As an additive in dough, wheat bran is known to reduce the quality of bread resulting in a lower specific volume and denser crumb texture (Noort et al., 2010). Negative effects were notable when the amount of added wheat bran was 10-20 %. This gave more than 6 % total fibre in bread (Katina, 2003). Added wheat bran also caused changes in the flavour and colour, and reduces the shelf-life (Pomeranz et al. 1977). One of the most significant effects of added wheat bran is the disruption of gluten network. Fibre prevents the aggregation of gluten proteins, which results in a lower rise of dough (Salmenkallio-Marttila et al., 2001, Noort et al., 2010, Don et al., 2003). In addition, the study of Hartikainen (2011) showed that adding wheat bran into dough had a negative impact on the rheological properties of dough, the pasting of starch and the quality of bread. However, Katina et al. (2007) and Lappi et al. (2010) have shown that enzymatic treatment combined with sourdough fermentation can improve the baking quality of wheat bran.

On the other hand, milled wheat bran with reduced particle size can enhance firmness and mouth feel of the food product. For example, breads that contained finer wheat bran had lower specific loaf volume and darker crumb than breads that contain larger particles of wheat bran. The breads had also a smoother crust appearance and less gritty mouth feel. According to Zhang and Moore (1999), the best results were achieved when using milled wheat bran with the particle size 415 µm. When the particle size was smaller, the volume of dough and the mixing persistence decreased.

In addition, the studies of Damen et al., (2012) and Shewry et al. (2009: 228) have shown that bread quality can also be improved by adding wheat bran and xylanase into dough. Both the stickiness and volume of dough increased when raising the dosage of the xylanase enzyme. This was mainly caused by the release of xylo-oligosaccharides. The most significant improvement was observed when using an enzyme originating from *H. jecorina* with a dosage of 6 800 U/kg. The amount of wheat bran was 18 % of the total flour weight. Nevertheless, further addition of dietary fibre rich fractions decreased the volume of breads.

5.2.1 The Effect of Fibres on the Bread Quality

From the fibre components in wheat bran, arabionxylans can be used in food formulations as gelling agents, cryostabilizers, and as a source of prebiotics (Stone and Morell, 2009: 340). A study of Courtin and Delcour (2002) showed that arabinoxylans slowed down the hardening of the inner parts of bread. Water-soluble arabinoxylans increase the viscosity of the dough. During the bread making, there was a continuous change in the molecular mass of the arabinoxylans due to the action of endogenous xylanases in wheat flour. A part of the water-unextractable arabinoxylans became extractable, but a significant solubilisation could only occur when a greater amount of xylanase was added.

Arabinoxylans can also affect the loaf volume as well as the crumb and crust characteristics of bread (Stone and Morell, 2009: 338). The proportion of water insoluble arabinoxylans in wheat bran has a negative impact on the volume of bread (Biliaderis et al., 1995, Courtin and Delcour, 2002). In addition, insoluble arabinoxylans improved the shelf life of bread more than soluble arabinoxylans due to a slower hardening of the inner parts of bread. At the same time, the amount of insoluble arabinoxylans had a negative effect on the volume of bread (Courtin and Delcour, 2002). When treating wheat bran with xylanases at low water content, the highest degree of solubilisation of arabinoxylans from wheat bran can be reached at 40 % water content (Santala et al., 2011).

5.3 Beverages

Wheat bran can be used as an additive in beverages to increase the amount of dietary fibre. The study of Lyly et al. (2009) showed that a beverage enriched with wheat bran increased the satiety and feelings of fullness. The amount of wheat bran in the test

beverages was 5.5 %. In addition, two other studies have suggested that the satiating effect was caused by insoluble fibres of wheat bran (Delargy et al., 1995, Samra and Anderson, 2007).

5.4 Other Potential Application Areas

Modified wheat bran fractions, such as polymers and fibres can be used in paper and bioplastic applications as filler to improve the quality of the product. In paper industry, the particles can be used to decrease the weight of paper. As functional paper additives, the filler particles could be used to improve the retention, i.e. how well the filler materials are bound to the cellulose network, and thus increase the strength of paper. (Agrobio, 2010)

In paper applications, fillers could be used as edible packaging material or for eco-friendly products. Previous studies have shown that wheat bran can be modified in several operations to edible packaging material used as an inner or outer package. Edible packaging material could be used, for example, as packaging papers for burgers and sandwiches (Wang et al., 2011). In paper and bioplastic applications, the modified wheat bran particles can be used to improve recyclability by burning and decomposing.

6 Materials and Methods

6.1 Raw materials

The primary raw material of this study was wheat bran with germ produced by Fazer Mill & Mixes (Oy Karl Fazer Ab, Lahti, Finland). The wheat bran had a moisture content of 11.2 %. Other ingredients used in test baking, were Sunnuntai medium coarse wheat flour, Sunnuntai dried yeast and Sunnuntai lactose-free baking margarine, all supplied by Ravintoraisio Oy (Raisio, Finland). The appearance of untreated wheat bran is shown in Figure 7 and the composition is shown in Table 2.

Figure 7. Wheat bran ("as is").

Table 2. The chemical composition of wheat bran (Agrobio, 2010).

Product (%)	Protein	Dietary fiber	Extractives	Ash
Wheat bran	16.8	47.8	5.0	6.7

6.2 Reduction of Particle Size

The matrix of the wheat bran was broken down by wet milling and enzymatic treatment. These modifications opened the structure, reduced the particle and released valuable components from the cells. The particle size reduction of wheat bran was carried out by using the wet milling equipment Masuko Supermass Colloider MKZA10-15J (Masuko Sangyo Co., Ltd., Saitamaken, Japan) as shown in Figure 8.

The particle size reduction was carried out in seven sequential milling cycles. The milling cycles were labeled M1–M7, respectively. In addition, non-milled wheat bran ("as is") was used as a reference sample (named M0). The two first wet millings were performed at 15 % dry matter content. After the milling cycle M2, the dry matter content was diluted from 15 % to 10 % to achieve more efficient particle size reduction. The milling cycles from M5 to M7 were performed at 5 % dry matter content. The first four milling cycles (M1–M4) were performed with coarse grinding stones (MKE-10-46) and

following cycles (M5–M7) with finer stones (MKGA10-80). The rotation speed of the stones was 1800 rpm in all milling cycles.

In addition, ultrafine wheat bran was produced by using dry milling equipment: G-55 Turborotor mill (Görgens Mahltechnik GmbH, Dormagen, Germany). The dry milled wheat bran was used as a reference in the baking application trials.

Figure 8. The wet milling equipment, Masuko Supermass Colloider MKZA10-15J, was used to reduce the particle size of the wheat bran. The upper grinding stone was stationary and the lower stone was rotating.

6.3 Enzymatic Hydrolysis of Wheat Bran

Wet milled wheat bran was hydrolysed using different enzymes. The efficiency of the hydrolysis was determined by the solubilisation of reducing sugars. Hydrolyses were conducted in 200 ml scale, using 400 ml plastic bowls in a water bath supplied with a Thermostat E100 (Lauda GmbH, Königshofen Germany). During the hydrolysis, the bran-water mixtures were mixed with drill machine -type mixers (Heidolph GmbH,

Schwabach, Germany), as illustrated in Figure 9. In each hydrolysis, four different wheat bran samples were tested at the same time.

Figure 9. The set up for four parallel enzymatic hydrolysis bowls in a water bath supplied with drilling machine -type mixers.

The wet milled bran fractions for the enzymatic hydrolysis were chosen as shown below to ensure as big variation as possible between the samples:

1. Wheat bran without milling (M0, "as is")
2. Wheat bran milled three times (M3)
3. Wheat bran milled four times (M4)
4. Wheat bran milled seven times (M7)

The enzymatic hydrolyses of wet milled wheat bran were conducted with three different commercial enzymes at a 50 °C or 70 °C for 1 and 4 hours. The mixing speed was 50 rpm. The enzymes were Depol 740 L (Biocatalysts Ltd., Wales, UK), Econase CE (AB Enzymes Oy, Rajamäki, Finland) and Ecopulp TX 200A (AB Enzymes Oy). The

dosage of the enzymes used in hydrolyses and the experimental design for the enzymatic hydrolyses is given in Appendix 1. The enzyme activities and additional information are given in Appendix 2.

The first sample (4 ml) was taken after one hour of hydrolysis. The samples were cooled in an ice bath and centrifuged using the 5430R centrifuge (Eppendorf Ltd., Wesseling-Berzdorf, Germany) at 10 000 rpm for 5 min. After the centrifugation, the samples were boiled at 100 °C for 10 min to inactivate the enzymes.

The final samples were taken after four hours of hydrolysis. Volumes of the samples were approximately 196 ml after taking the 1 hour sample. The 4 hour samples were centrifuged by using a Sorvall RC-5C centrifuge (Sorvall Instruments Ltd., Delaware, USA) at 10 000 rpm for 10 min. The samples were weighted prior to centrifugation and the proportion of supernatants after centrifugation. After this, the samples were boiled at 100 °C for 10 min to inactivate the enzymes.

6.4 Chemical Composition of Wheat Bran

6.4.1 Moisture Content

The moisture content of wheat bran before and after the enzymatic hydrolysis was determined by dehydrating the samples at 130 °C for one hour. The weight of the wet sample was approximately 2 g. Although some samples contained up to 80 % water, one hour dehydration was proved to be sufficient, as only minor amount of water was removed during the second hour at 130 °C. The samples were weighted before and after the dehydration.

6.4.2 Reducing Sugars

The amount of reducing sugars was determined by using the DNS method according to the VTT method 3783-93 (VTT, 2001). In this method, 2-hydroxy-3,5-dinitrobenzoic acid reacts with reducing groups found in oligosaccharides. The amount of reducing sugars in the sample were calculated using glucose as a standard. Samples were diluted with de-ionized water. Each sample was measured as three replicates. The experimental design and concentrations of reducing sugars are given in Appendix 3.

6.4.3 Protein Content

The protein content of the different wheat bran fractions was analyzed according to AACC Method 46-11A (AACC, 2000) and the VTT method 3726 (VTT, 2006). The analysis was carried out using Kjeldahl autoanalyzer (Foss Tecator Ab, Höganäs, Sweden). The weights of the samples were approximately 1.0 g. The protein concentration was calculated from the determined nitrogen content as Protein (%) = Nitrogen (%) x 6.25.

6.4.4 Insoluble and Soluble Fiber in Wheat Bran

The amount of soluble and insoluble dietary fibers was determined after the milling and enzymatic treatments. The analysis was carried out according to the VTT method for determining insoluble and soluble fiber in food products. This method was derived from the previous method: integrated total dietary fiber assay procedure, (K-INTDF: AOAC Method 2009.01) (VTT, 2009).

6.5 Particle Size Measurement

The particle size distribution, as well as D_{50} and D_{90} values indicating that 50 or 90 % of the particles have a diameter under a certain level, were analysed with a Beckman Coulter LS 230 (Beckman Coulter, Inc., CA, USA) using the wet powder module with distilled water.

6.6 Microscopic Analysis for Particle Distribution

After the wet milling, microscopic analysis was carried out by using a BX50 microscope equipped with 10x, 20x and 50x lenses and PCO CCD Sensicam® 12 bit cooled imaging (Olympus Ltd., Japan). The cell structures of wheat bran were stained with toluidine blue (Sigma Ltd., USA).

6.7 Analysis of Phase Separation

The stability of the each fraction was measured by phase separation. The stability of the different wheat bran fractions (M1, M2, M3 and M5) was analysed using Turbiscan Lab equipment (Formulaction SA, L'Union, France). The samples were placed into class vessels shown in Appendix 4. In each sample, the phase separation was measured after every 30 min for 5 hours. The volume of the samples was approximately 20 ml. The dry matter contents of the samples were 15 % (M1 and M2),

10 % (M3) and 7 % (M5). In addition, each sample was diluted to 5 % dry matter content for parallel analysis.

6.8 Food Applications

The suitability of wheat bran in bread as filler particles and source of dietary fibre was evaluated. The suitability of wheat bran particles was tested in a pilot scale baking process. The quality of the product was analysed by a sensory evaluation panel.

6.9 Particle Size Reduction for Test Baking

For test baking, 10.14 kg of wheat bran (with moisture content of 11.2 %) was diluted with tap water (all together 60 kg) to obtain a suspension with 15 % dry matter content. This suspension was milled using Masuko Supermass Colloider MKZA10-15J. The particle size of wheat bran was reduced by five sequential milling cycles (M1–M5). The grinding stones were coarse (MKE-10-46) in first three milling (M1–M3) cycles, and fine (MKGA10-80) in the latter milling cycles (M4–M5). The suspension was diluted to 10 % dry matter after M2 and to 7 % dry matter after M3.

Wheat bran fractions of approximately 14 kg each were collected after second (M2), third (M3) and fifth (M5) milling cycles. In addition, 11 kg of M3 was collected for enzymatic treatments. After wet milling, the fractions, other than 11 kg of M3, were centrifuged at 4000 rpm for 15 min by using Sorvall RC12BP centrifuge (Sorvall Ltd., USA) to separate supernatant from the insoluble residue. After this, the residues were collected. Approximately half of the dry matter was freeze dried and second half frozen at -20 °C. In addition, samples of the supernatants were collected for further analysis.

After centrifugation, the sample of 11 kg of M3 was hydrolyzed using Depol 740L enzyme. Depol 740L was chosen as a treatment enzyme due to its good efficiency and availability according to previous studies at VTT. The M3 mixture was first heated up to 50 °C using 10 liter mashing kettle (Tankki Oy, Finland). After the heating, hydrolysis was initiated by adding 13.5 ml of Depol 740L enzyme as given in Appendix 1. The first sample was collected after 1 hour for the DNS analysis. After 4 hours, the hydrolysis was finished. The 11 kg sample was centrifuged at 4000 rpm for 15 min by Sorvall RC12BP centrifuge. In addition, two samples with a volume of 20 ml were taken from

the supernatant. The supernatant samples were boiled at 100 °C for 10 min to inactivate the enzyme.

6.10 Test Baking

6.10.1 First Trial: Baking with Non-milled and Turbomilled Wheat Bran

In the first pilot scale baking, non-milled wheat bran (M0, "as is") and Turbomilled (Turbo) were added into normal wheat flour dough. In addition, wheat flour dough without added wheat bran was used as a reference. The recipe included wheat flour, salt, baking butter and dry yeast (Ravintoraisio Oy, Raisio, Finland). The addition levels of different wheat bran fractions were 0, 10 and 20 %. Thus, five different breads were baked.

First, the water binding capacity and the consistency of each wheat flour and bran mixtures were determined by using a Farinograph-E (Brabender GmbH, Germany) to determine the required water addition. In each analysis, the temperature was kept at 30 °C. The duration of the test was 20 min. The amount of each flour-bran mixture was approximately 50 g depending on its water content. The water contents of wheat flour and wheat brans (M0 and Turbo) were 11.15, 11.22 and 4.40 % respectively. Due to the minor difference between the water contents of wheat flour (11.22 %) and M0 (11.15 %), the water content was considered as 11.22 %. Due to the low water content of Turbo (4.4 %), it was considered as totally dry ingredient. The eligible consistency of dough was determined as 500 ± 65 farinographic units (FU). The amount of added water was determined using farinographic analysis. The experimental design and the determined water absorptions in the farinographic analyses are given in Appendix 5. The results from farinographic analysis are given in Appendix 6.

The doughs were prepared in order to have wheat bran at different levels: 0, 10 and 20 %. The optimal amount of added water for Turborotor milled wheat bran fractions were evaluated according to the results obtained from the farinographic analysis. The proportion of water was calculated from the total dry matter content without salt, baking margarine and dry yeast. The recipes for each doughs shown in Table 3.

Table 3. The recipes for each doughs used in the first baking with wheat bran "as is" (M0) and Turborotor milled wheat bran (Turbo). The portion of water is shown in the brackets.

	Dough 1	Dough 2	Dough 3	Dough 4	Dough 5
	Addition level 0 %	Addition level 10 %	Addition level 10 %	Addition level 20 %	Addition level 20 %
	No added bran	M0	Turbo	M0	Turbo
Wheat flour	2000	1800	1600	1800	1600
Water	1220 (61 %)	1280 (64 %)	1300 (65 %)	1420 (71 %)	1480 (74 %)
Salt	36	36	36	36	36
Baking margarin	60	60	60	60	60
Dried yeast	30	30	30	30	30
Specific wheat bran	0	200	400	200	400

The baking was started by warming up the dough in mixing machines (to yield so called "the dummy dough"). The dummy dough had the same recipe as the reference dough, except for salt, baking margarine and yeast. After warming up, the ingredients were mixed by using Diosna Spriral Mixer (Dieks & Söhne GmbH, Germany) for 8 min. After mixing, the temperatures of doughs were 25.0 °C (Reference), 24.0 °C (10 % M0), 24.0 °C (10 % Turbo), 23.9 °C (20 % M0) and 25.2 °C (20 % Turbo). The temperature in the bakery was 22.1 °C for Reference, 20.9 °C for 10 % M0, 21.4 °C for 10 % Turbo and 20 % M0 and 22.1 °C for 20 % Turbo. After mixing, the doughs were raised using Lillnord raising cabin (Lillnord, Denmark) at 35 °C for 10 minutes shown in Figure 10. The relative humidity inside the cabin was 75 %. After rising, the doughs were cut into 350 g pieces. After cutting, the pieces were twirled into balls using CR59 arching machine (Werner & Pfleiderer Ltd., Netherlands) and rolled up using BM51-1 long roller (Werner & Pfleiderer). The baking equipment is shown in Appendix 7.

After shaping, the doughs were placed into aluminium moulds and raised at 35 °C for another 60 min by using raising cabin as shown in Figure 11. The relative humidity was 75 % inside the cabin. After this, the doughs were baked using SvebaDahlen Classic DC33 oven (SvebaDahlen AB, Sweden) at 225 °C for 20 min. In the beginning of baking, the doughs were steamed for 15 seconds.

Figure 10. Breads were raised using Lillnord rising cabin at 35 °C and for 10 and 60 min respectively.

Figure 11. Breads were baked using SvebaDahlen oven at 220 °C for 20 min.

6.10.2 Second trail: Baking with Wet Milled Wheat Bran

In the second pilot scale baking, wheat bran fractions M2, M3, M5 and M3 treated with Depol 740L were tested as an additive to dough. In addition, wheat flour dough without added wheat bran was used as a reference. The addition level of wheat bran fractions was 20 % (dry mass basis) of dough. The water holding capacity and consistency of different wheat flour and bran mixtures were determined by the farinograph. The baking process was similar compared to the previous baking process. The experimental design for farinographic analysis is given in Appendix 5 and the recipes for doughs are shown in Table 4.

Table 4. The recipes for doughs used in the second baking with wheat bran M2, M3, M5 and M3 treated with Depol 740 L. The proportion of water is shown in brackets.

	Dough 1	Dough 2	Dough 3	Dough 4	Dough 5
	Addition level 0 %	Addition level 20 %	Addition level 0 %	Addition level 20 %	Addition level 20 %
	No added bran	M2	M3	M5	M3, Depol 740L
Wheat flour	2000	1221	1200	980	1180
Water	1220 (61 %)	620 (31 %)	620 (31 %)	240 (12 %)	460 (23 %)
Salt	36	36	36	36	36
Baking margarin	60	60	60	60	60
Dried yeast	30	30	30	30	30
Specific wheat bran	0	779	800	1020	820

As the experimental design for the test baking was complicated, a small error occurred. The amount of baking additives (salt, making margarine and dried yeast) were miscalculated as the wheat bran would have dry matter content 100 %. This was not correct, because the dry matter content of wheat bran fractions were 29.49 % (M2), 30.51 % (M3), 41.91 % (M5) and 31.82 % (M3, Depol). Hence, the amount of additives should have been proportional to the dry matter. This error was taken into consideration in the discussion of the results. After mixing the ingredients, the dough temperatures were 23.3 °C (Reference), 25.4 °C (M2), 23.4 °C (M3), 21.8 °C (M5) and 25.8 °C (M3, Depol). The temperature in the bakery was 21.6 °C for 20 % M2, 22.0°C for 20 % M3 and M5. Baked breads are shown in Appendix 8.

6.10.3 Firmness, Volume and Yield of the Breads

The firmness of breads was determined by TPA-Texture Analyser (TA-XT plus Texture Analyser, Stable Micro Systems Ltd., United Kingdom) with SMS P/36R probe. The breads were sliced into pieces and the crust was removed. Five samples of bread were analyzed to calculate the average firmness. The volume and the yield of breads was determined using Pregesbauer infrared device (Bread Vol Scan, Pregesbauer Ltd., Germany). The analysis was conducted using six different breads. Volumetric photos from the bread slices are shown in Appendix 8.

6.10.4 Sensory Evaluation

The effect of added wheat bran on the quality of the bread was analyzed by a sensory evaluation panel. The number of persons in the panel was 4. The location of the evaluation was the test bakery. The evaluation was conducted individually without conversation. In the evaluation form, the panel was asked to give their score for each sample. The scoring was from 1 to 5 with intervals of 0.5. Score 1 represented "very low" and score 5 "very high". The evaluation properties were:

1. Smoothness of pores in the slice
2. Resilience of the slice
3. Softness of the slice
4. Intensity of the flavor
5. Astringency of the flavor
6. Strength of after taste of the inside
7. Moisture in mouth

The panelists were advised to wash their mouth after every bread sample. The evaluation form is shown in the Appendix 9.

7 Results

7.1 First Wet Milling Trials

7.1.1 Particle Size Reduction

The most significant reduction in particle size was seen after the second milling cycle (M2) in both milling processes. The finest particle size (D_{50} = 12 µm and D_{90} = 28 µm) was obtained after the seventh milling cycle in the first milling process. Particle sizes (D_{50} and D_{90} values) after the first and second wet milling are shown in Figures 12 and 13, respectively**.** The corresponding particle size distributions are shown in Figures 14 and 15 at logarithmic scale.

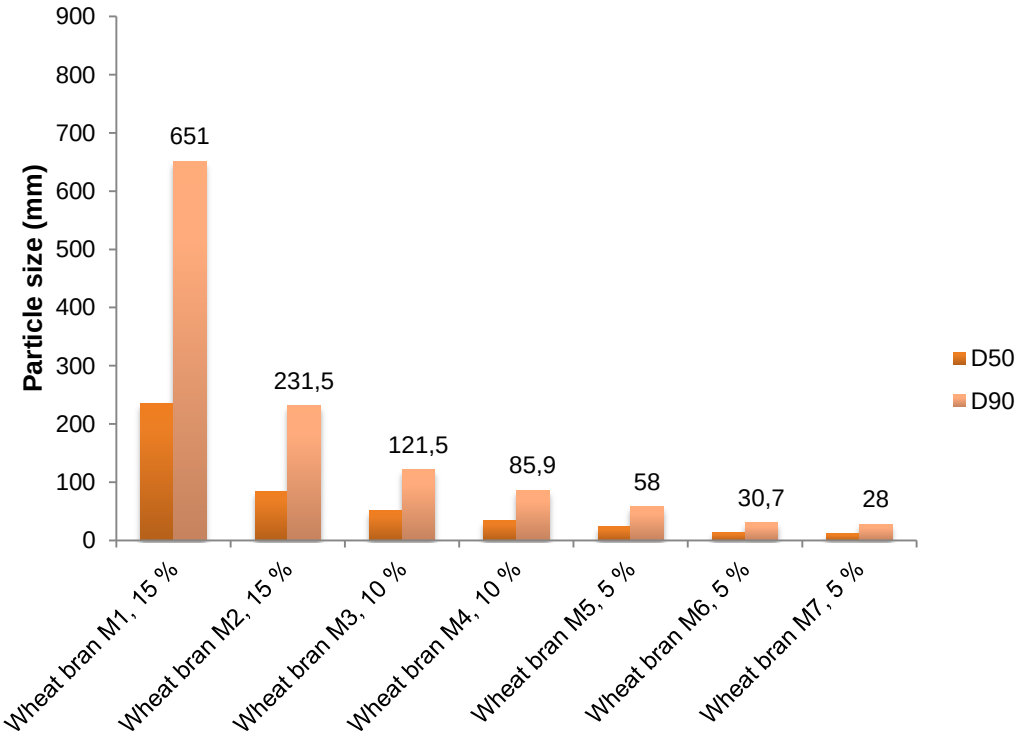

Figure 12. The particle sizes (D_{50} and D_{90} values) in the first wet milling trial after seven consecutive milling operations.

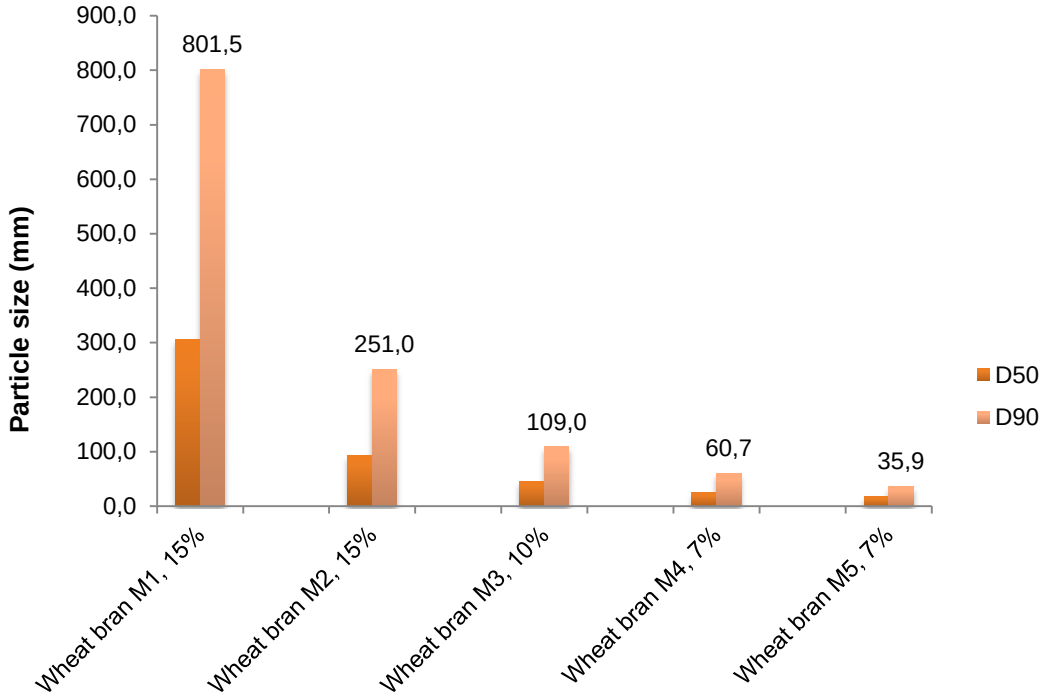

Figure 13. The particle sizes (D_{50} and D_{90} values) in the second wet milling trial after five consecutive milling operations.

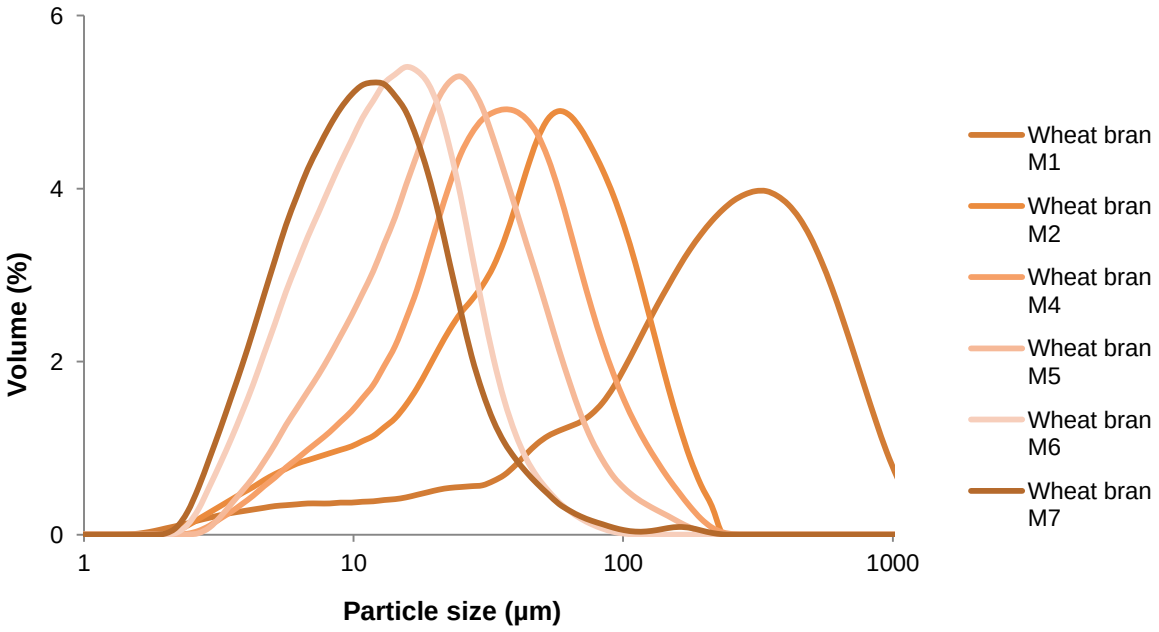

Figure 14. The particle sizes distribution in the first wet milling trial after seven consecutive milling operations, illustrated at logarithmic scale (1-1000 µm).

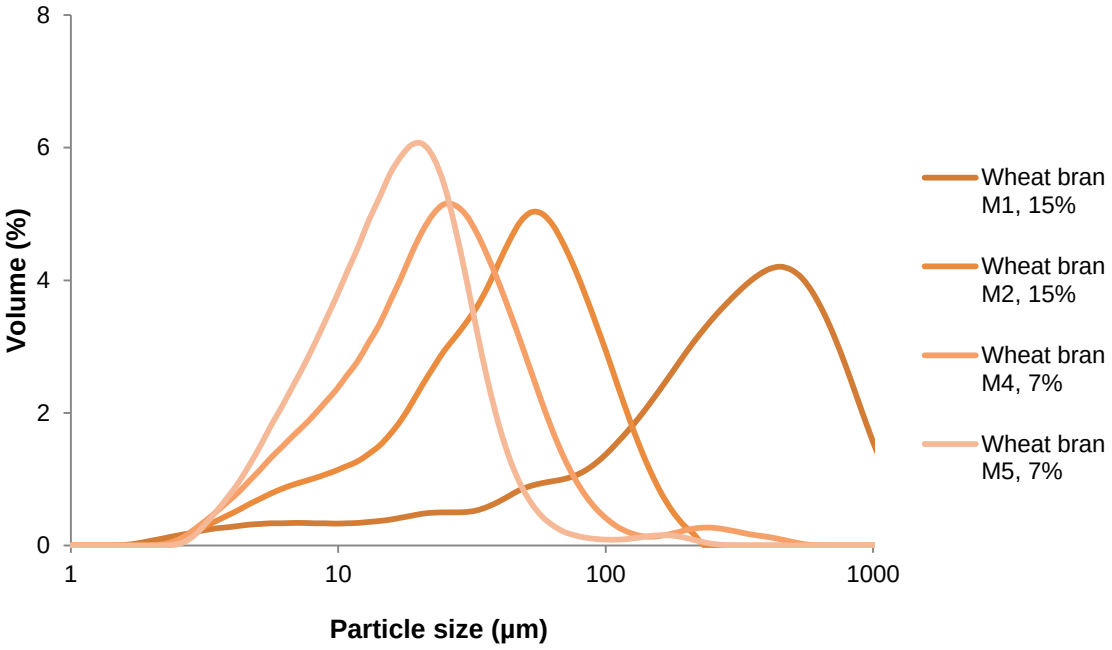

Figure 15. The particle sizes distribution in the second wet milling trial after five consecutive milling operations, illustrated at logarithmic scale (1-1000 µm).

7.2 Effect of Enzymatic Hydrolysis on the Solubilisation

Enzyme treatments increased the amount of supernatant after centrifugation compared to untreated wheat bran. The enzymatic hydrolyses were carried out at dry matter contents: 15 % (for M0 and M3), 10 % (for M4) and 5 % (for M7). In addition, the M0, M3, M4 and M7 fractions were diluted to 5 % dry matter content and treated with Depol 740 L. The treatment with Depol 740L and Ecopulp TX200A gave the highest yields of supernatant. With Depol 740L, the yield increased even more, when the treatment was carried out at low consistency (5 %). The yields of supernatant and insoluble residue after the enzymatic treatments are shown in Figure 16. Wheat bran samples without enzymatic treatment (M0) and wheat bran (M0) treated with Depol 740L at 15 % dry matter content, were not included due to their small yield of supernatant.

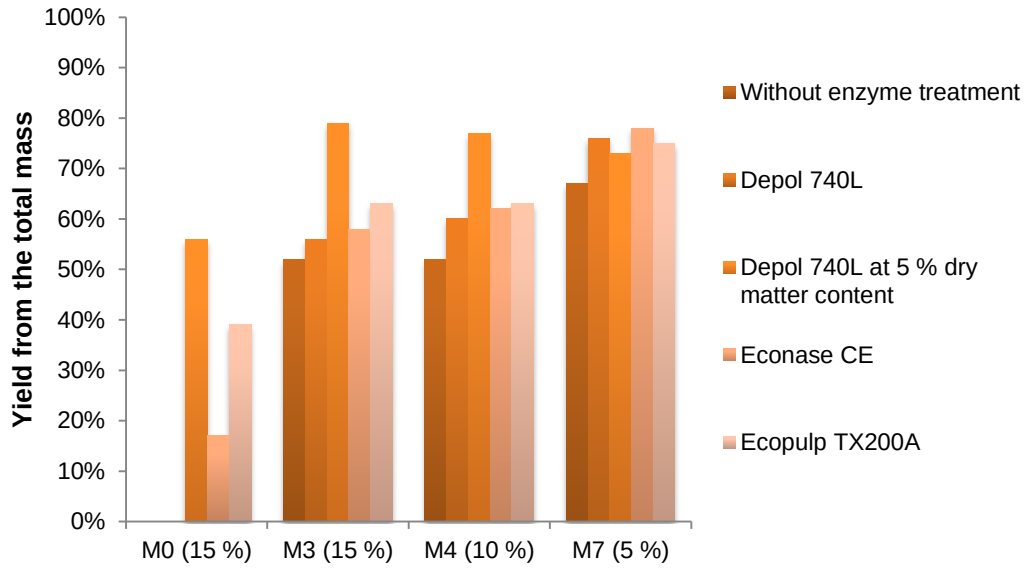

Figure 16. The yields of supernatants from differently milled wheat bran fractions after the centrifugation.

7.3 Chemical Composition of Wheat Bran after Enzymatic Treatment

Ecopulp TX200A released the highest amount of reducing sugars (24.8 g/l glucose equivalent) after 1 hour hydrolysis, whereas Depol 740 L seemed to be more efficient (34.5 g/l glucose equivalent) after 4 hours of hydrolysis. The amounts of reducing sugars after 1 and 4 hours are shown in Figures 17 and 18.

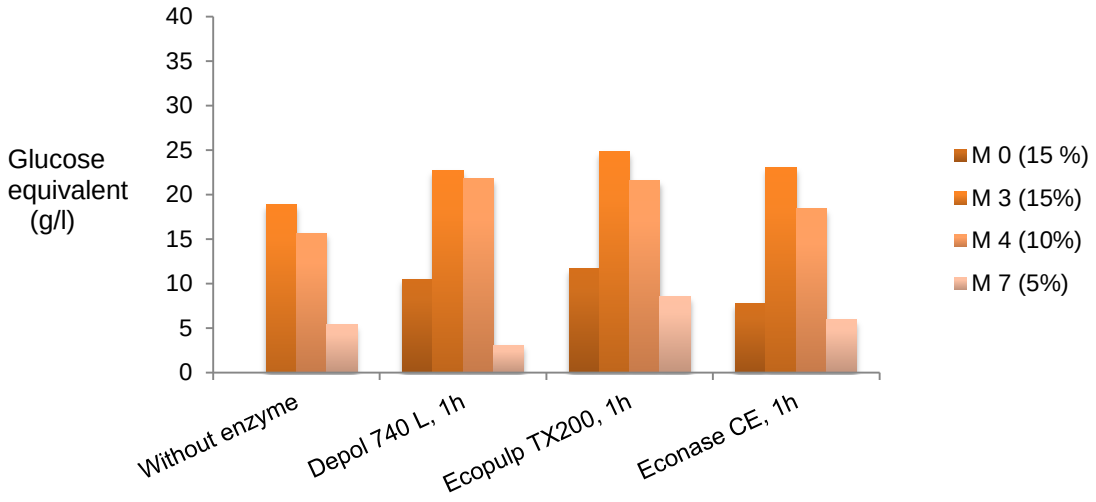

Figure 17. The amounts of reducing sugars in different wheat bran fractions after 1 hour of enzymatic hydrolysis. The dry matter contents used in wet milling are shown in brackets.

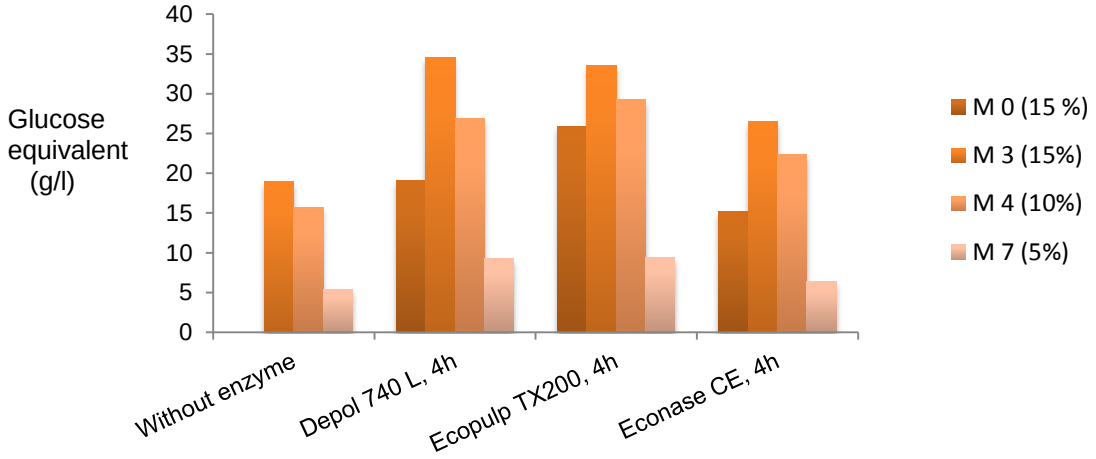

Figure 18. The amounts of reducing sugars in different wheat bran fractions after 4 hours of enzymatic hydrolysis. The water contents used in wet milling are shown in brackets.

Depol 740 L gave the highest protein content in different wheat bran fractions (at dry matter content 15 % for M3, 10 % for M4 and 5 % for M7). The protein content increased with both Depol 740 L and Econase CE after M3, indicating that some non-protein compounds were released due to the enzymatic treatments. The protein

contents of the insoluble residues after enzymatic treatments are shown in Figure 19. The protein content in wheat bran M0 treated with Depol at 15 % moisture content was not included due to the small yield of supernatant.

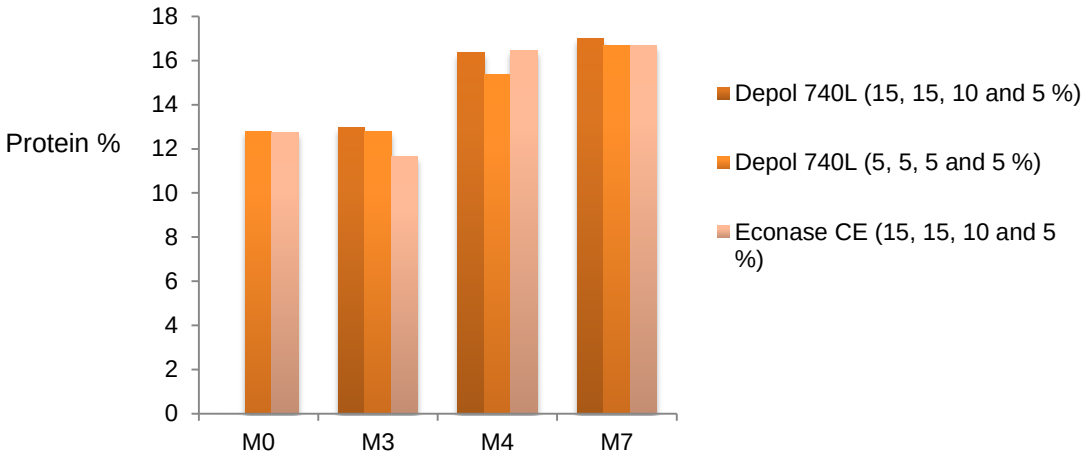

Figure 19. The protein content in different wheat bran fractions. Different moisture contents used in wet milling are shown in brackets respectively.

The amount of soluble fibre decreased after the wet milling and separation of supernatant from wheat bran (from 4.0 to 2.8 %). At the same time, the amount of insoluble fibre increased (from 50.0 to 55.3 %). Depol 740 L had the biggest impact on decreasing of the total dietary fibre. However, the milling and centrifugation seemed to have more significant impact on the dietary fibre content. The concentrations of insoluble and soluble fibres in the insoluble fractions of differently treated wheat brans are shown in Figure 20.

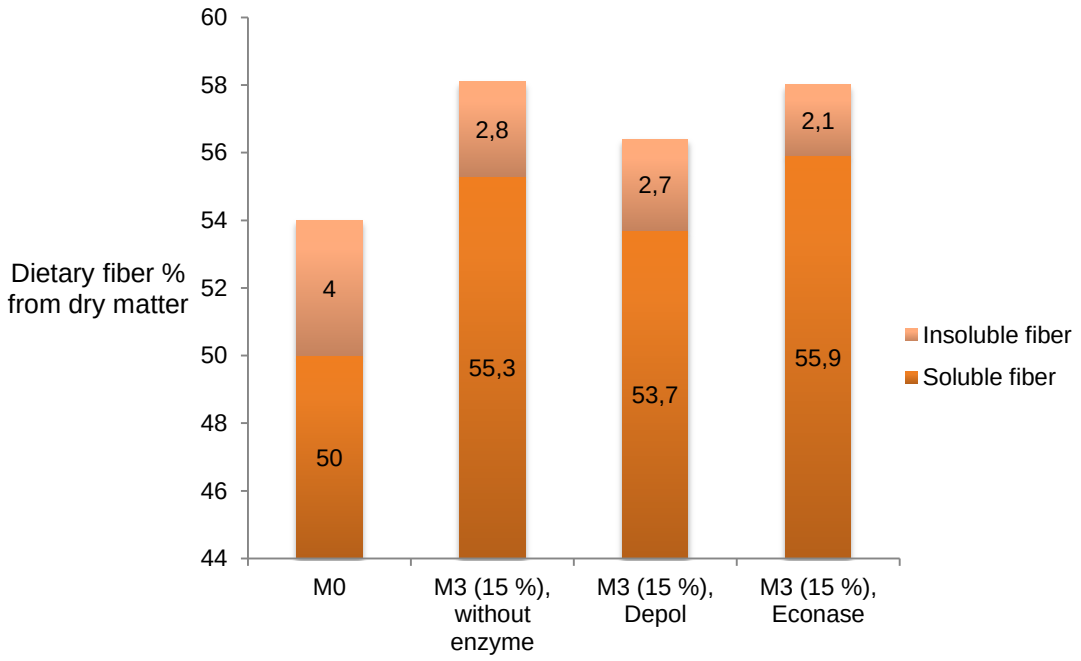

Figure 20. The amount of soluble and insoluble dietary fibre after enzymatic treatments. M0 is reference bran without enzymatic treatment.

7.4 Second Wet Milling

7.4.1 Moisture Content

The fractions M2 and M3 had almost the same moisture content (64.7 - 68.0 %), whereas M5 had much higher moisture content (78.8 %). Depol 740L did not have significant effect on the moisture content. The moisture contents of different wheat bran fractions after the second wet milling and centrifugation are shown in Figure 21.

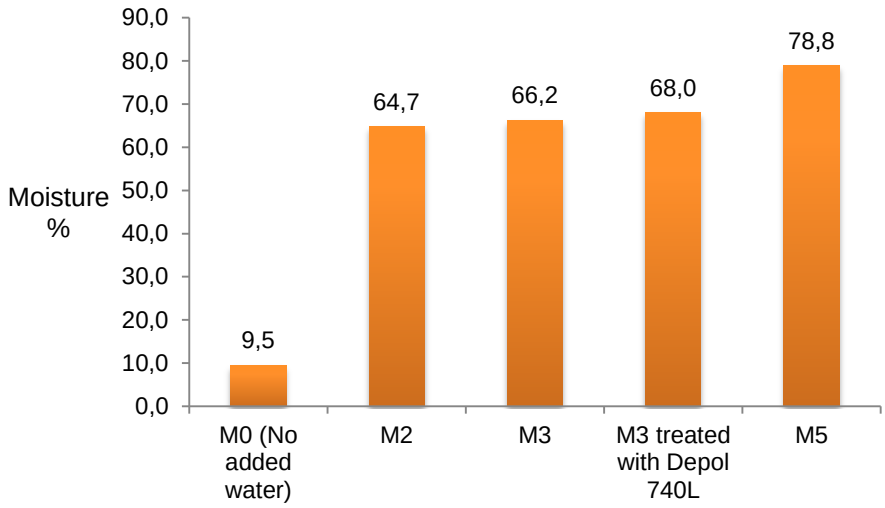

Figure 21. The moisture contents of the insoluble fractions of wet milled wheat brans after centrifugation.

7.4.2 Microscopic Analysis

The effect of wet milling on wheat bran cell wall degradation is shown in Figure 22. It can be seen that the cell wall matrix was partly opened in M2 and further destroyed in M3 and M5. In M5, the cell wall structures were not recognisable anymore.

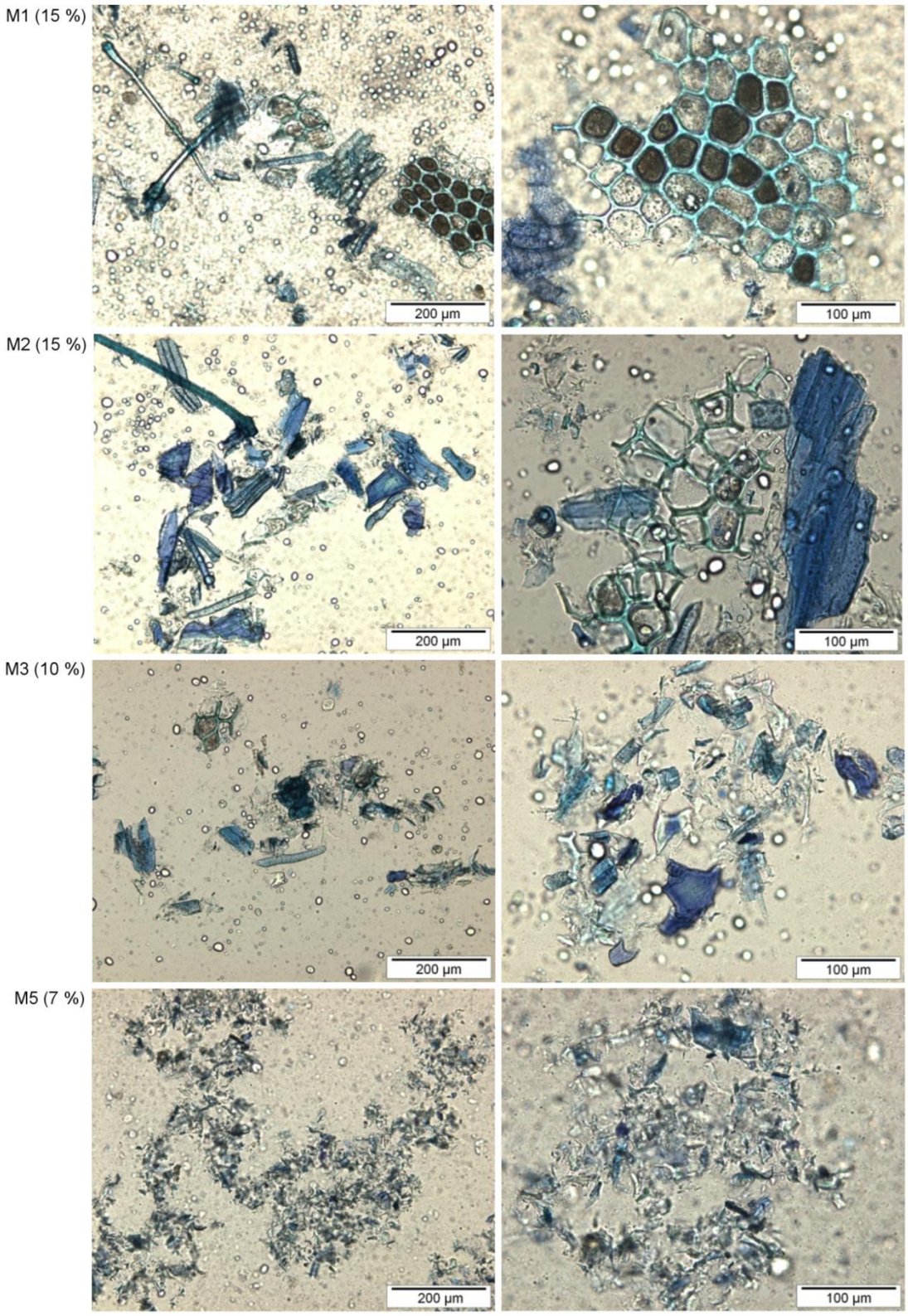

Figure 22. The microscopic pictures of the cell wall structures of wheat bran after milling cycles M1, M2, M3 and M5. The moisture content during the milling is shown brackets.

7.4.3 Analysis of Phase Separation Using Turbiscan

During the phase separation, the distance of sedimentation front from the bottom of the vessel descended. For wheat bran fraction M5, the distance was calculated as the width of clear area due to the phenomenon that water phase cleared under the dispersion. The results from phase the separation analysis of wheat bran mixtures with 15, 10 and 7 % dry matter contents are shown in Figure 23. The results from phase separation analysis with 5 % dry matter content are shown in Figure 24. The mixtures of wheat bran and the measurement vessels before and after the phase separation are shown in Appendix 4.

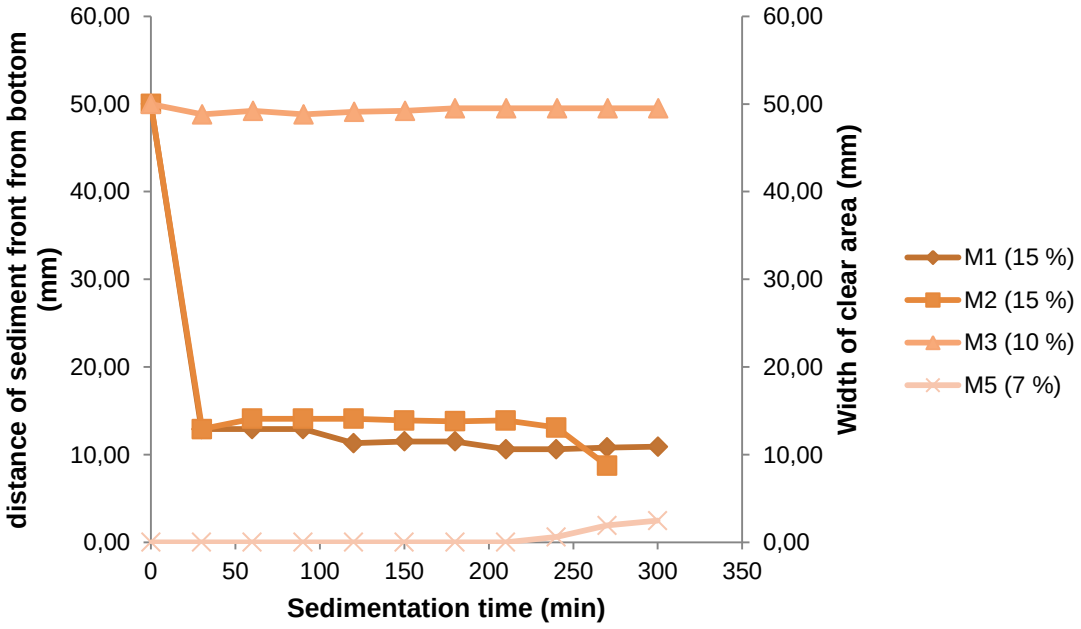

Figure 23. Phase separation of wet milled wheat bran fractions (M1, M2, M3 and M5) when the dry matter contents were 15, 10 and 7 %, respectively.

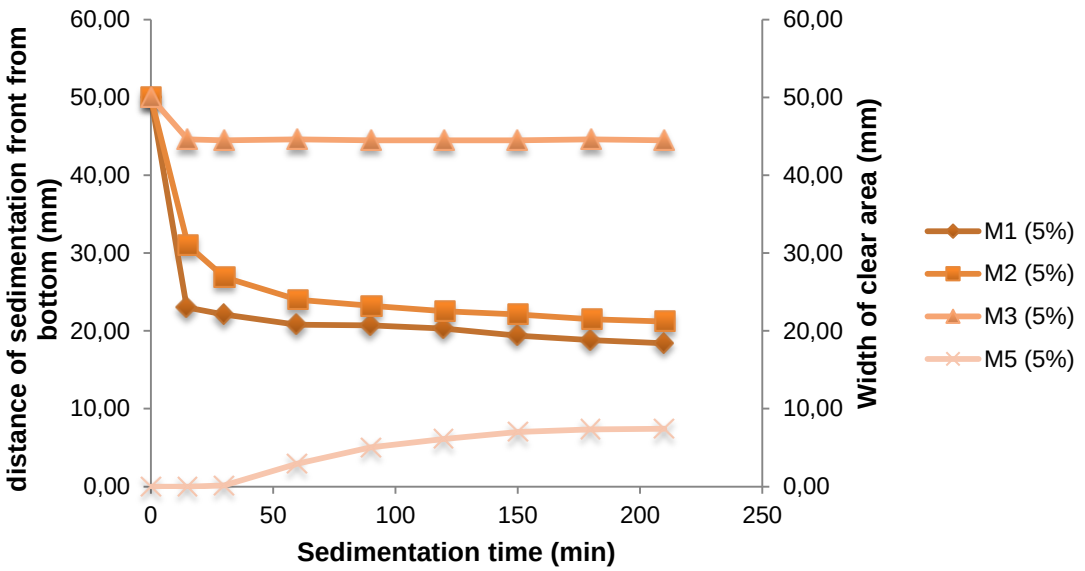

Figure 24. Phase separation of wet milled wheat bran fractions (M1, M2, M3 and M5) when the dry matter content was kept constant (5 %) in each sample.

7.5 Effect of Milled Wheat Bran on Bread Quality

The added wheat bran had a negative effect on bread colour. The baked breads with different wheat bran fractions are shown in Appendix 8.

7.5.1 Volumes and Densities of Breads

In both baking trials, the volume of the breads decreased in proportion to added wheat bran. In addition, Depol 740 L seemed to lower the volume even more. The reference bread had 16 % lower bread volume in second baking compared to the first baking trial. The volumes of the breads are shown in Figures 25 and 26. The densities of the breads are shown in Figures 27 and 28.

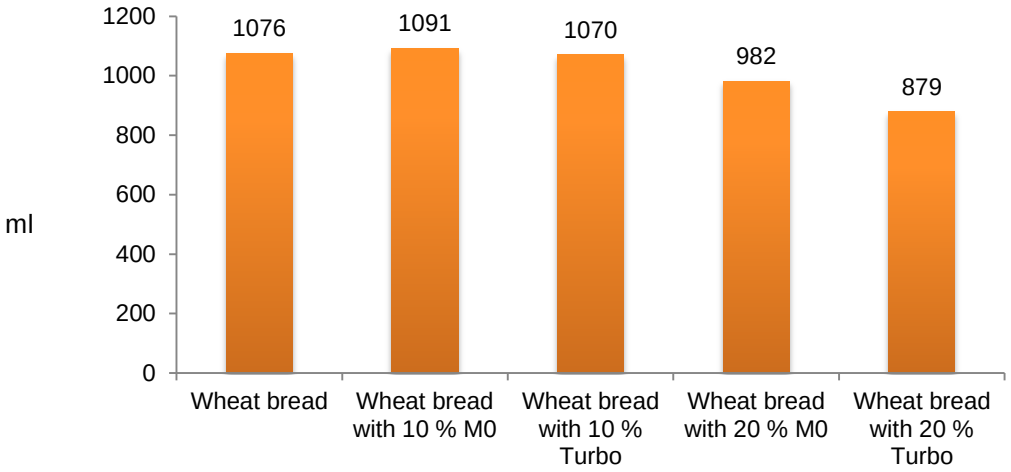

Figure 25. The volumes of the breads in the first baking trial.

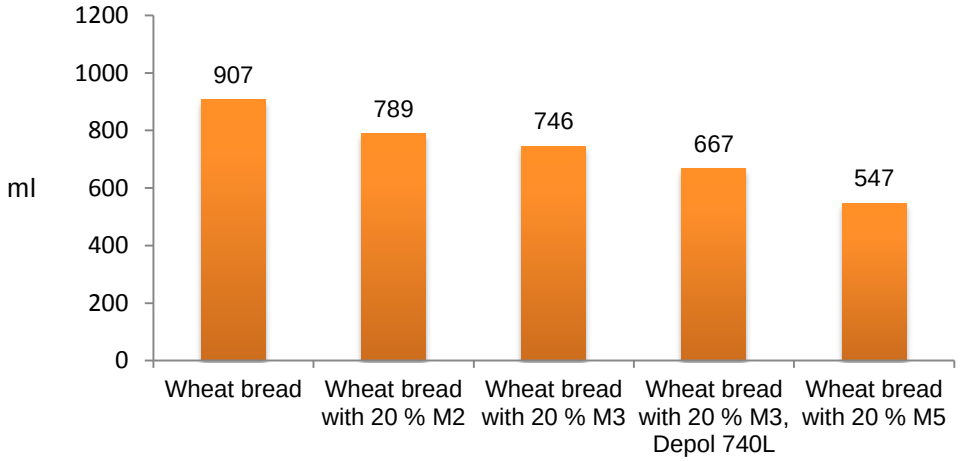

Figure 26. The volumes of the breads in the second baking trial.

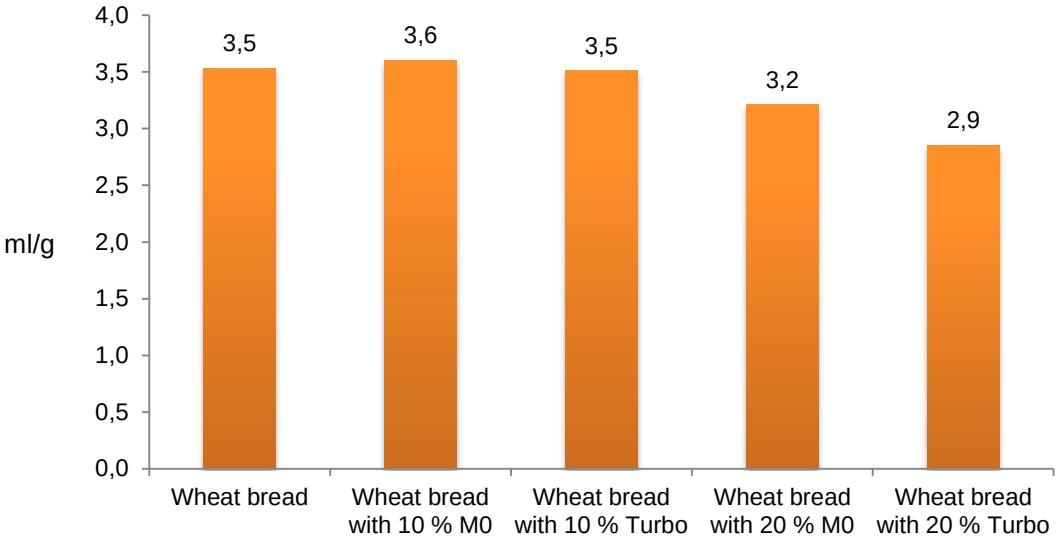

Figure 27. The densities of the breads after the first test baking.

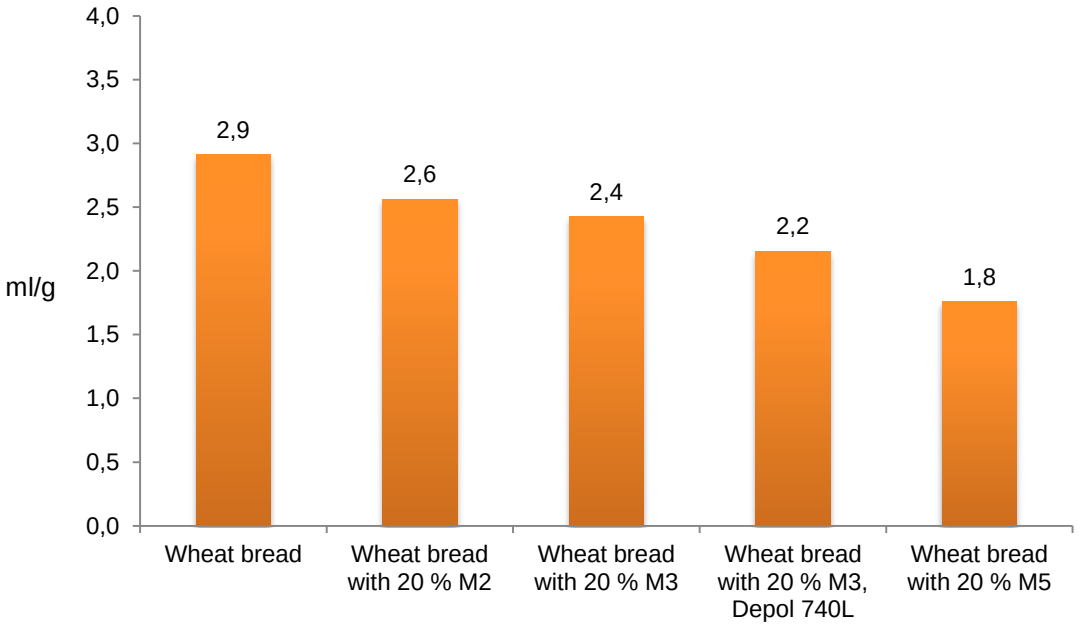

Figure 28. The densities of the breads after the second test baking.

7.5.2 Hardness of Breads

The wheat bread without added wheat bran was the softest. The hardness of the breads increased in proportion to added wheat bran fractions. Turbomilled wheat bran gave significantly softer breads compared to wet milled fractions. Enzymatic treatment seemed to make the breads harder. The hardness of the breads is shown in Figures 29 and 30.

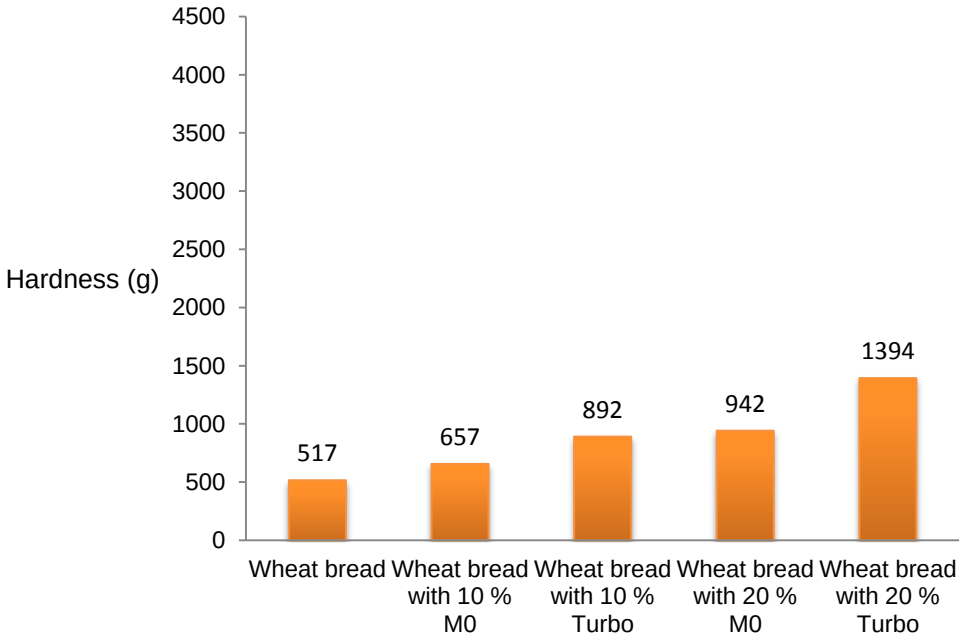

Figure 29. The Hardness of the breads incorporated with dry wheat bran fractions (M0 and Turbomilled).

Figure 30. The Hardness of the breads incorporated with wet milled wheat bran fractions (M2, M3, M3 + Depol 740 L and M5).

7.5.3 Quality of Breads in Sensory Evaluation

The softness of bread slice decreased with the addition of wheat bran. Wheat bread with 20 % Turbomilled wheat bran had the lowest softness of the slice in the first test baking. The wheat bread with 20 % M5 wet milled bran fraction had the lowest softness in the second baking. The astringency of the flavour seemed to increase in proportion to added wheat bran. The qualities of the different breads are shown in Figures 31 and 32.

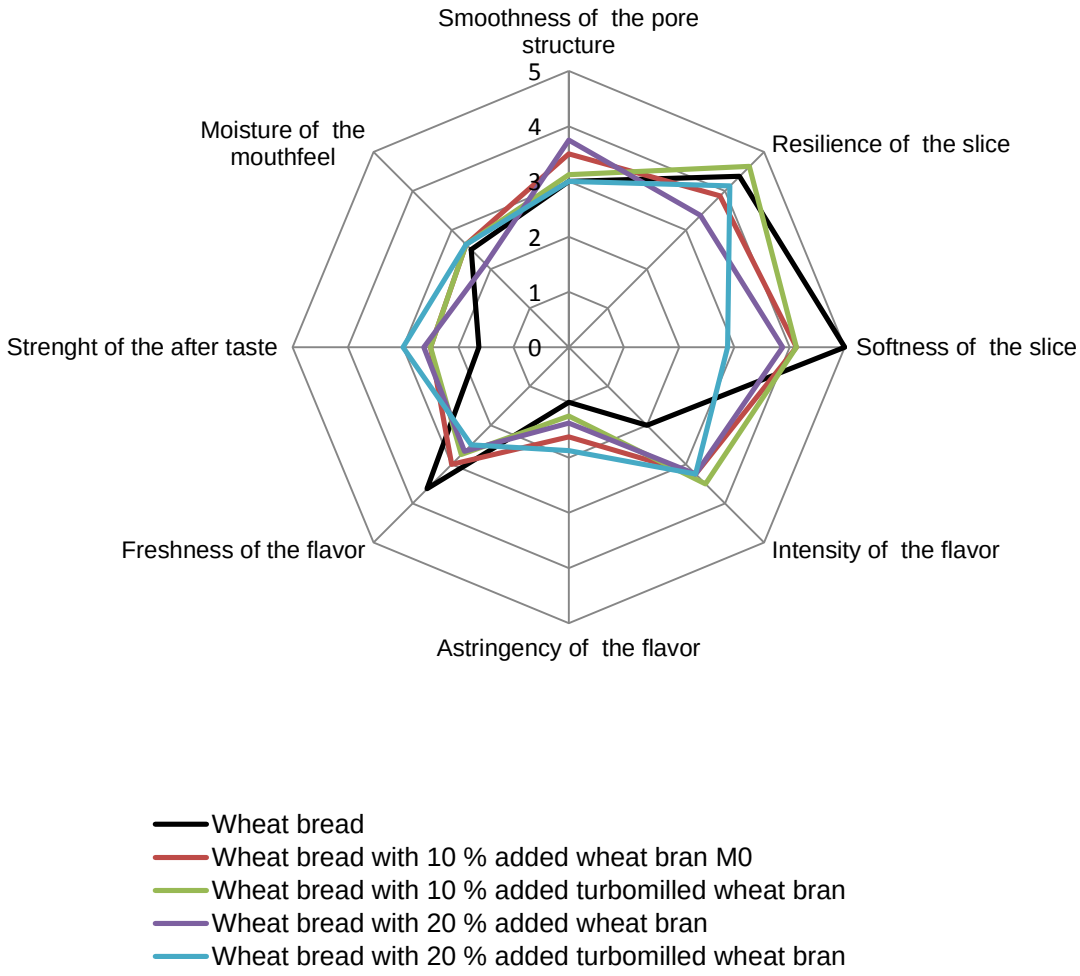

Figure 31. The result from sensory evaluation of the first test baking.

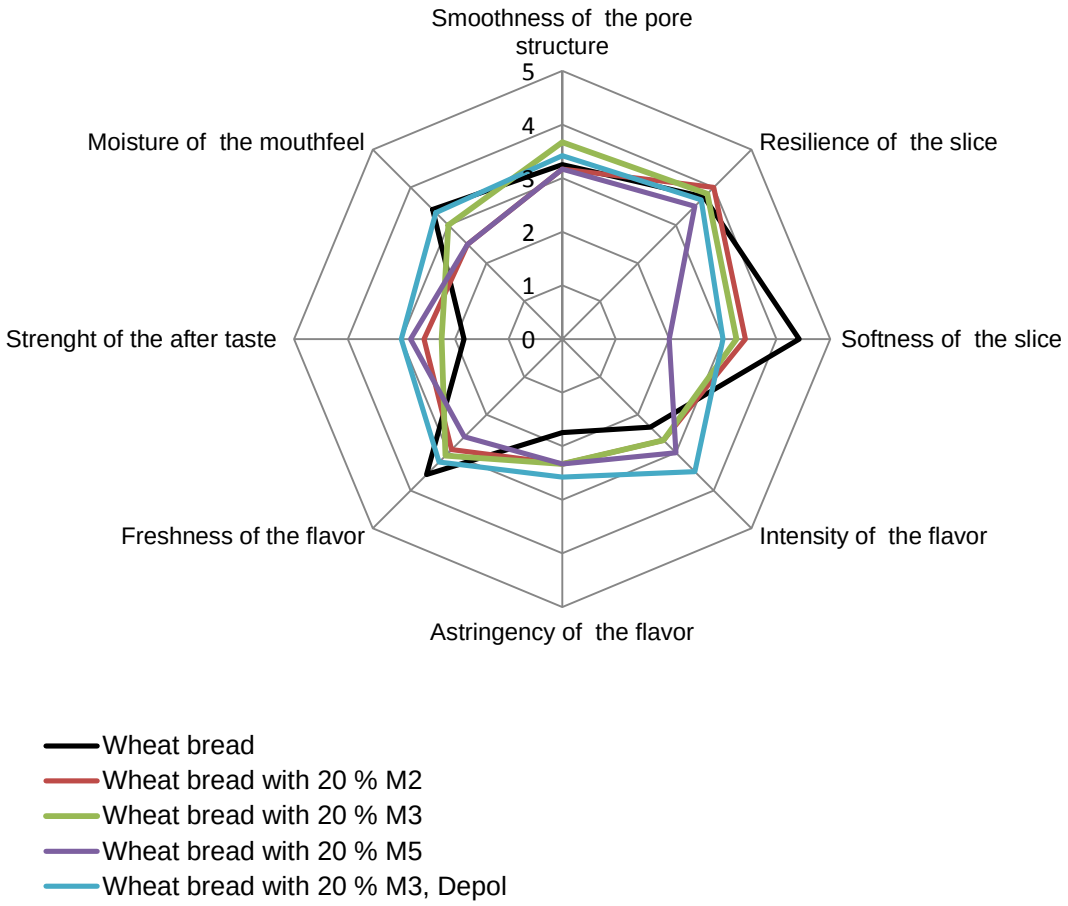

Figure 32. The results of sensory evaluation of the second test baking.

8 Discussion

8.1 Wet Milling and Particle Size Reduction

Wet milling reduced the particle size of wheat bran significantly (from initial 2-3 mm down to 10 µm). Although, the particle size reduction seemed to be efficient with only few milling cycles, the clearest change in phase separation was observed between M3 and M5 (See chapter 7.4). This shows that the critical concentration for smooth solutions was somewhere between 50 and 10 µm.

8.2 Microscopic Analysis

The microscopic analysis showed that the cell structure and matrix of wheat bran became more scattered after each milling cycle. After the two first milling cycles (M1–

M2), the cell wall structures were still present. However, after the fifth milling cycle (M5), solid cells were not present anymore.

8.3 Enzymatic Hydrolysis

Wet milling enabled a better efficiency for the enzymatic hydrolysis, due to the better availability of binding sites for the enzymes. Based on the results from the analysis of insoluble and soluble fibres, greater amount of oligosaccharides was released during the enzymatic hydrolysis. This resulted in an increased amount of reducing sugars after 1 and 4 hour hydrolysis (see M3 and M4 fractions in Figures 15 and 16). In addition, greater solubilisation was observed when the yield of the supernatants was higher. The lower amount of reducing sugars in wheat bran fraction M7 was caused by a high level of dilution (only 5 % dry matter).

The highest amount of reducing sugars was released when using Depol 740L or Ecopulp TX200A. As predicted, the amount of reducing sugars increased when the enzyme treatment was continued from one to four hours. Based on these results, Depol 740L was chosen to be used in the test baking and also partially due to its good availability and relatively low price. A study of Giet et al. (2010) showed that ferulic acid could be released by using Depol 740L for colour removal. This effect was not observed in this study, but could be studied further as a decolouring method or as a method for increasing the antioxidant capacity of wheat bran.

8.4 Chemical Composition

The determined chemical composition corresponds to the composition referred from the literature. The increase of moisture content due to the milling stage is caused by the superior water binding properties of smaller particles (D_{50} = 84.4 for M2, D_{50} = 51.0 for M3 and D_{50} = 23.7 for M5).

The protein content in different wheat bran fractions increased in proportion to milling stage in the insoluble residue, after the removal of supernatant. This was caused by the degradation of the matrix and release of other compounds than proteins. The amount of soluble fibre in the supernatant increased after the enzymatic hydrolyses. Due to the degradation of matrix after the wet milling, the total dietary fibre content increased in the insoluble residue, after the removal of supernatant. In addition, wet

milling increased the content of soluble fibres due to the hydration and break-down of insoluble fibres.

8.5 Analysis of Phase Separation

During the phase separation the distance of sedimentation front from the bottom of the vessel descended. The stability of the water-bran mixtures increased in proportion to the milling stage. At 5 % dry matter content, the phase separation was faster than at higher consistencies. For the wheat bran fraction M5, different kind of phenomenon was observed: the water phase was below the sedimented particles. This was most probably caused by the air bubbles and/or fat bound to the dispersed particles. Most likely, this was caused by the high level of homogenisation due total cell degradation (see chapter 7.3). This altered the physical properties of water-bran mixture into a distinct level as the water holding capacity decreased. Decrease in the water holding capacity was also observed by the study of Zhu et al. (2010) when they compared large particles to sub-micron level particles (D_{50} = 344 nm).

8.6 Effect of Wheat Bran on Baking

The volume of the breads decreased in proportion to the addition level of wheat bran. In addition, the volume seemed to decrease in proportion to milling stage but the result could have been affected by the amount of added baking additives (salt, baking margarine and dried yeast) and the low baking temperature in the second baking. The main source of error was the incorrect amount of added dried yeast due to the production of CO_2 which affects the volume. The low temperature inhibited the growth of the yeast and its CO_2 production. Nevertheless, the volume lowering trend was seen in the results.

The hardness of the breads increased in proportion to added wheat bran fraction and the level of addition. This was mainly caused by the lower volume as the density increased. Contradictory to expectations, the hardness of the bread with 20 % M3 wet milled bran treated with Depol 740L had greater hardness compared to the bread with 20 % M3 bran without enzyme treatment. This phenomenon could have been explained by an incorrect addition of yeast and needs to be studied further.

As the study of Noort et al. (2010) predicted, particle size reduction had a negative effect on bread baking quality such as lower specific volume and denser crumb texture.

However, the study of Noort et al. (2010) showed that negative effects were enhanced with an ultra-fine particle size (D_{50} = 48 µm). Now the negative effect was observed also with larger particles (D_{50} = 93 µm). Zhang and Moore (1999) presented their best results at 415 µm particle size, which is larger than the fractions studied in this study.

In addition, the results of this study were similar to the results discovered by Katina et al. (2003) where negative effects were notable when wheat bran was added at 10 or 20 % level. For future research, the effect of particle size reduction and enzymatic treatment in combination with sourdough fermentation on the quality of breads could be studied, as Katina et al. (2007) and Lappi et al. (2010) have shown in their studies.

8.7 Quality of Breads in Sensory Evaluation

The sensory evaluation gave some indications how to develop the taste of wheat breads supplemented with bran. The quality of the reference bread was set as a target due to its relatively large volume and softness. As predicted in the study of Noort et al. (2010) and Katina et al. (2003), the quality of bread was negatively affected with the addition of wheat bran. In the present study, the most significant changes were observed in softness, freshness of the flavour and strength of the after taste. The softness correlated well with the results from the texture analysis. This indicated that addition of wheat bran fractions made harder bread with stronger flavour.

9 Conclusions and Future Prospects

As discovered in this study, the modification of wheat bran holds a good potential for food applications. However, the particle size reduction protocol and choose of enzyme is of utmost importance. Milled wheat bran can provide an efficient way to increase of the dietary fibre content in food products, but it also affects the mouth feel, colour and bitterness of the product. In addition, the change in the proportion and effect of insoluble and soluble fibre content after enzymatic treatment has to be considered. As the desired properties of the bread seem to decrease in proportion to added wheat bran, the adding level which still fulfils the desired qualifications (such as high content of dietary fibre) would need further research. In other words, the optimal consistency and particle size of wheat bran should be studied further. Due to the small mistakes during the second baking trial, an additional test baking and sensory evaluation would be needed. In addition, the wet milled bran fractions could be tested in extruded snacks and beverage applications.

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Amount of Enzymes and Experimental Design for Enzymatic Hydrolysis

Desired efficiency: 100 nkat

Desired amount of enzyme for 1 g of wheat bran:

Depol 740L:

100 nkat ÷ (11 837 nkat/ml) = 0.00844 ml → 8.44 µl/g_{bran}

Econase CE:

100 nkat ÷ (49 000 nkat/ml) = 0.00204 ml → 2.04 µl/g_{bran}

Ecopulp TX200A:

100 nkat ÷ (64 292 nkat/ml) = 0.0015 ml → 1.55 µl/g_{bran}

Amount of added enzyme in different wheat bran stages for volume 200 ml (200 g):

Depol 740 L

M0 (15 %)
Amount of dry matter: 200 g x 0.15 = 30 g
Enzyme needed: 30 g x 8.44 µl/g_{bran} = 253 µl

M3 (15 %)
Amount of dry matter: 200 g x 0.15 = 30 g
Enzyme needed: 30 g x 8.44 µl/g_{bran} = 253 µl

M4 (10%)
Amount of dry matter: 200 g x 0.10 = 20 g
Enzyme needed: 20 g x 8.44 µL/g_{bran} = 169 µL

M7 (5%)
Amount of dry matter: 200 g x 0.05 = 10 g
Enzyme needed: 10 g x 8.44 µl/g_{bran} = 84 µl

Depol 740 L
M0 (5%)
Amount of dry matter: 200 g x 0.05 = 10 g
Enzyme needed: 10 g x 8.44 µl/g_{bran} = 84 µl

M3 (5%)
Amount of dry matter: 200 g x 0.05 = 10 g
Enzyme needed: 10 g x 8.44 µl/g_{bran} = 84 µl

M4 (5%)

Amount of dry matter: 200 g x 0.05 = 10 g
Enzyme needed: 10 g x 8.44 µl/g_{bran} = 84 µl

M7 (5%)
Amount of dry matter: 200 g x 0.05 = 10 g
Enzyme needed: 10 g x 8.44 µl/g_{bran} = 84 µl

Econase CE

M0 (15 %)
Amount of dry matter: 200 g x 0.15 = 30 g
Enzyme needed: 30 g x 2.04 µl/g_{bran} = 61 µl

M3 (15 %)
Amount of dry matter: 200 g x 0.15 = 30 g
Enzyme needed: 30 g x 2.04 µl/g_{bran} = 84 µl

M4 (10 %)
Amount of dry matter: 200 g x 0.10 = 20 g
Enzyme needed: 20 g x 2.04 µl/g_{bran} = 41 µl

M7 (5 %)
Amount of dry matter: 200 g x 0.05 = 10 g
Enzyme needed: 10 g x 2.04 µl/g_{bran} = 20 µl

Ecopulp TX200A

M0 (15 %)
Amount of dry matter: 200 g x 0.15 = 30 g
Enzyme needed: 30 g x 1.55 µl/g_{bran} = 47 µl

M3 (15 %)
Amount of dry matter: 200 g x 0.15 = 30 g
Enzyme needed: 30 g x 1.55 µl/g_{bran} = 47 µl

M4 (10 %)
Amount of dry matter: 200 g x 0.10 = 20 g
Enzyme needed: 20 g x 1.55 µl/g_{bran} = 31 µl

M7 (5 %)
Amount of dry matter: 200 g x 0.05 = 10 g
Enzyme needed: 10 g x 1.55 µL/g_{bran} = 16 µL

Amount of added enzyme in wheat bran stage M3 with volume 11 l (11 kg)

M3 (15 %)
Amount of dry matter: 11 000 g x 0.15 = 1650 g
Enzyme added: 1650 g x 8.44 µl/g_{bran} = 13.926 µl = 13.9 ml

Experimental Design for Enzyme Hydrolysis

Set up number	Enzyme					Duration	Temperature
1.	Depol 740 L	M0 (15%)	M3 (15 %)	M4 (10 %)	M7 (5%)	4 h	50 °C
2.	Depol 740 L	M0 (5 %)	M3 (5 %)	M4 (5 %)	M7 (5%)	4 h	50 °C
3.	Econase CE	M0 (15 %)	M0 (15 %)	M4 (10 %)	M7 (5%)	4 h	50 °C
4.	Ecopulp TX 200A	M0 (15 %)	M3 (15 %)	M4 (10 %)	M7 (5%)	4 h	70 °C

Enzyme Information

Depol 740 L (batch 100621505)
Xylanase activity (2010): 11 800 nkat/ml

Econase CE (batch 200103001)
Xylanase activity: 49 000 nkat/ml

Ecopulp TX 200A (batch 1032044231)
Xylanase activity (2011): 64 300 nkat/ml

Experimental Design for DNS

Without Enzyme	Dilution
M3 (15%)	1:50
M4 (10%)	1:50
M7 (5%)	1:10
Depol	
M0 (15%), 1 h	1:40
M3 (15%), 1 h	1:40
M4 (10%), 1 h	1:40
M7 (5%), 1 h	1:50
M0 (15%), 4 h	1:40
M3 (15%), 4 h	1:100
M4 (10%), 4 h	1:40
M7 (5%), 4 h	1:40
Econase	
M0 (15%) 1h	1:50
M3 (15%) 1h	1:50
M4 (10%) 1h	1:50
M7 (5%) 1h	1:10
M0 (15%) 4h	1:100
M3 (15%) 4h	1:100
M4 (10%) 4h	1:100
M7 (5%) 4h	1:10
Ecopulp	
M0(15%) 1h	1:50
M3 (15%) 1h	1:50
M4 (10%) 1h	1:50
M7 (5%) 1h	1:10
M0(15%) 4h	1:100
M3 (15%) 4h	1:100
M4 (10%) 4h	1:100
M7 (5%) 4h	1:10
Glucose standard	
Glucose (10 %)	1:10
Glucose (20 %)	2:10
Glucose (30 %)	3:10
Glucose (40 %)	4:10
Glucose (50 %)	5:10

Vessels of Phase Separation

Before phase separation

After 5 hours of phase separation

Experimental Design for Farinographic Analysis

Baking with wheat flour (as reference), wheat bran M0 and wheat bran turbomilled with 10* and 20 % addtion.

	Moisture (%)	Sample weight (g)	Consistency (FU) **	Water absorption (%)
Wheat flour	11.22	48.42	486	61.0
Wheat flour with 10 % M0	11.22	48.42	528	64.0
Wheat flour with 20 % M0	11.22	48.42	545	71.0
Wheat flour with 20 % Turbo	10.50	48.02	562	73.0

* Water absorption for wheat flour with 10 % Turbo was estimated from previous results.
**The eligible consistency was determined as 500 ± 65 FU.

Baking with wheat bran M2, M3, M5 and M3 treated with Depol 740L with 20 % addtion.

	Moisture (%)	Sample weight (g)	Consistency (FU) *	Water absorption (%)
Wheat flour with 20 % M2	29.49	60.99	542	30.0
Wheat flour with 20 % M3	30.51	61.87	478	30.0
Wheat flour with 20 % M5	41.91	74.01	520	11.0
Wheat flour with 20 % M3, Depol	31.82	63.04	533	22.0

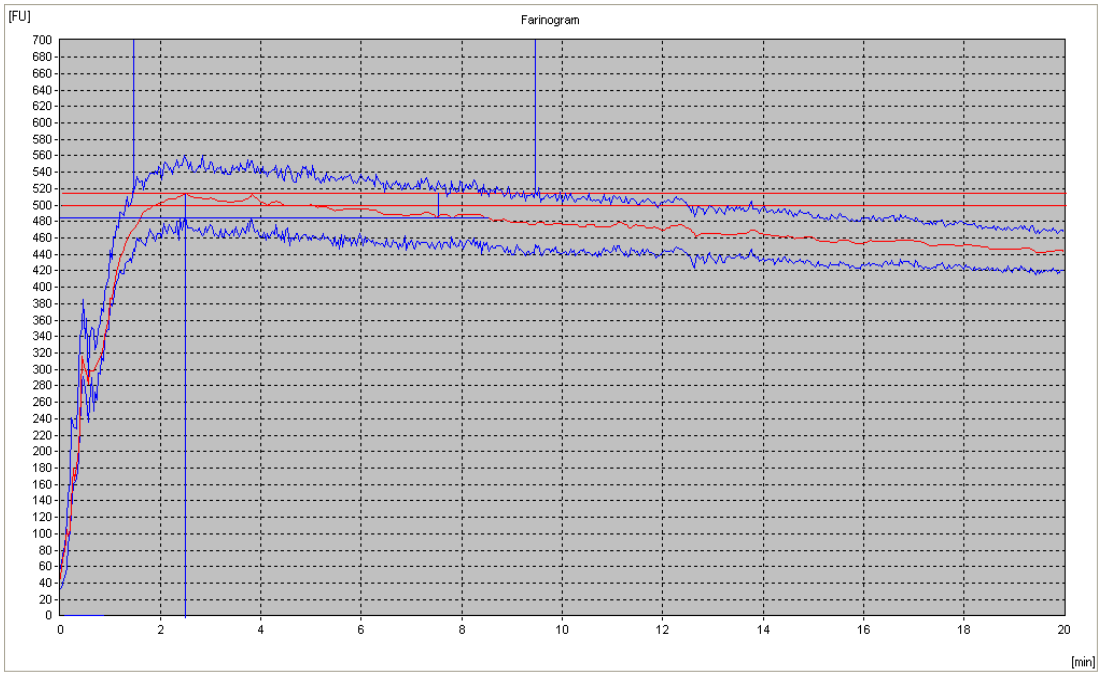
*The eligible consistency was determined as 500 ± 65 FU.

Data Collected from Farinographic Analysis

Consistency for wheat flour

486 FU with water absorption 61.0 %

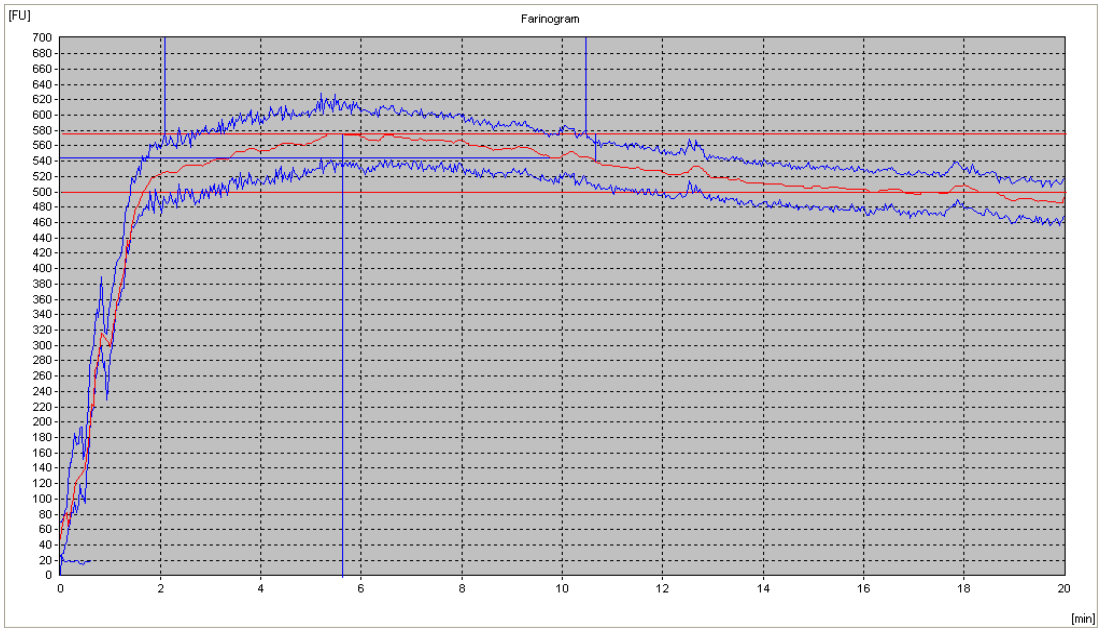
Moisture content 11.2 %



Consistency for wheat flour with 10 % M0

528 FU with water absorption 64.0 %

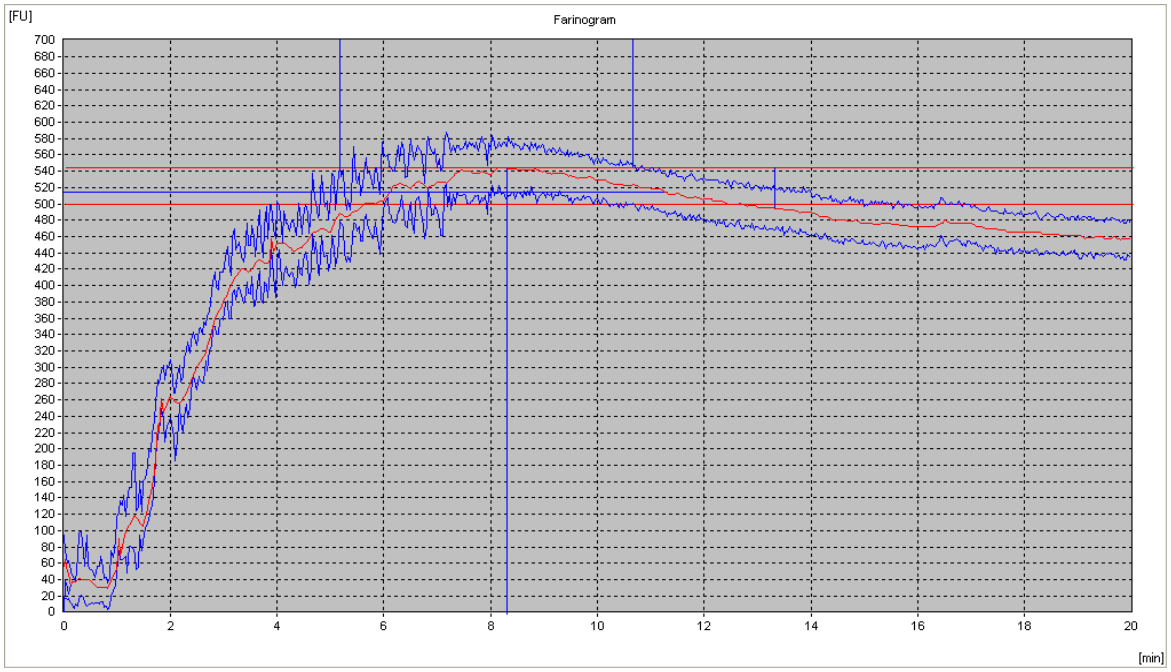
Moisture content 11.2 %



Consistency for wheat flour with 20 % M0

545 FU with water absorption 71.0 %

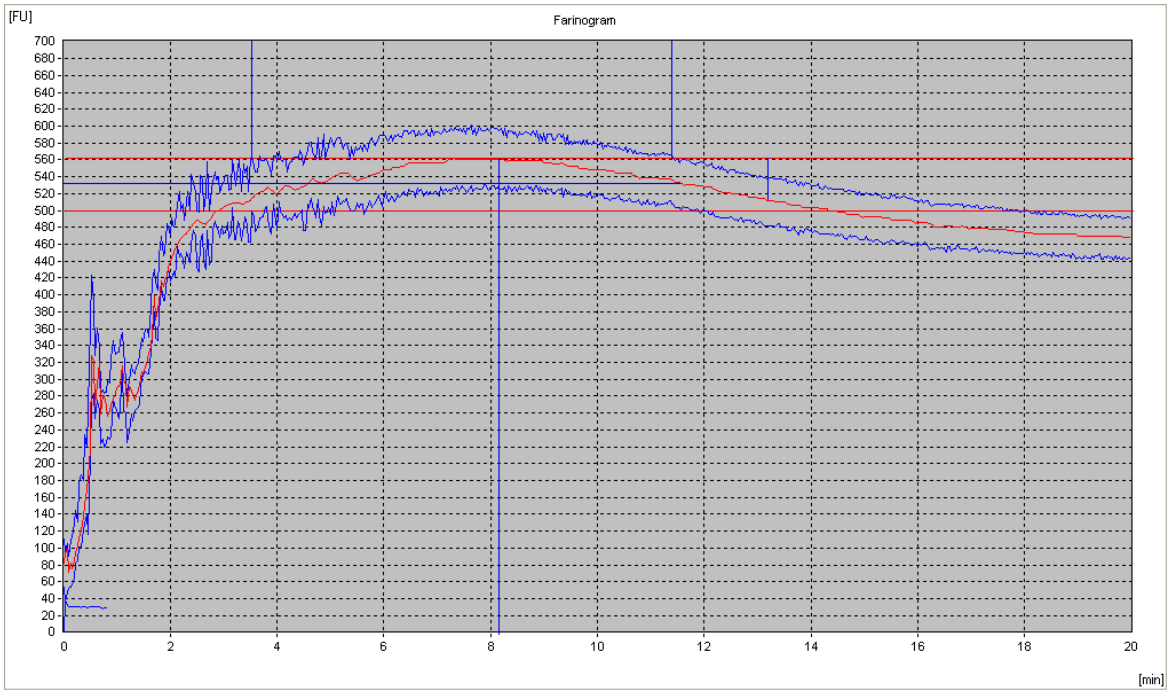
Moisture content 11.2 %



Consistency for wheat flour with 20 % Turbo

562 FU with water absorption 73.5 %

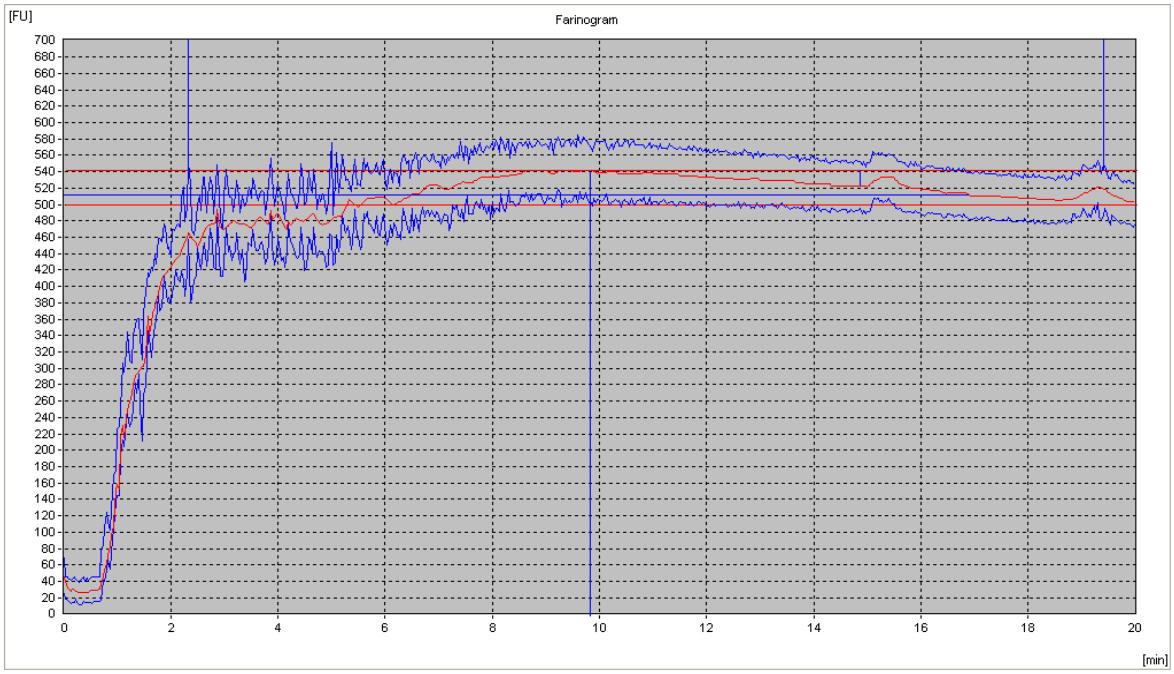
Moisture content 10.5%



Consistency for Wheat flour with 20 % M2

542 FU with water absorption 30.0 %

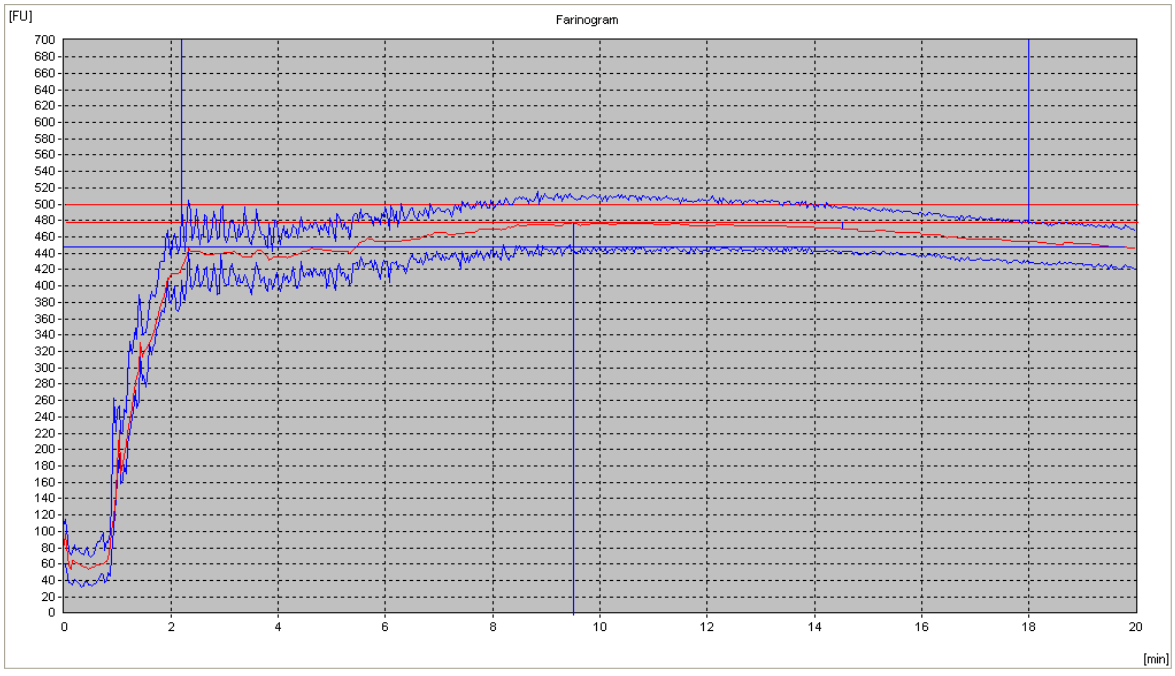
Moisture content 29.49 %



Consistency for wheat flour with 20 % M3

478 FU with water absorption 30.0 %

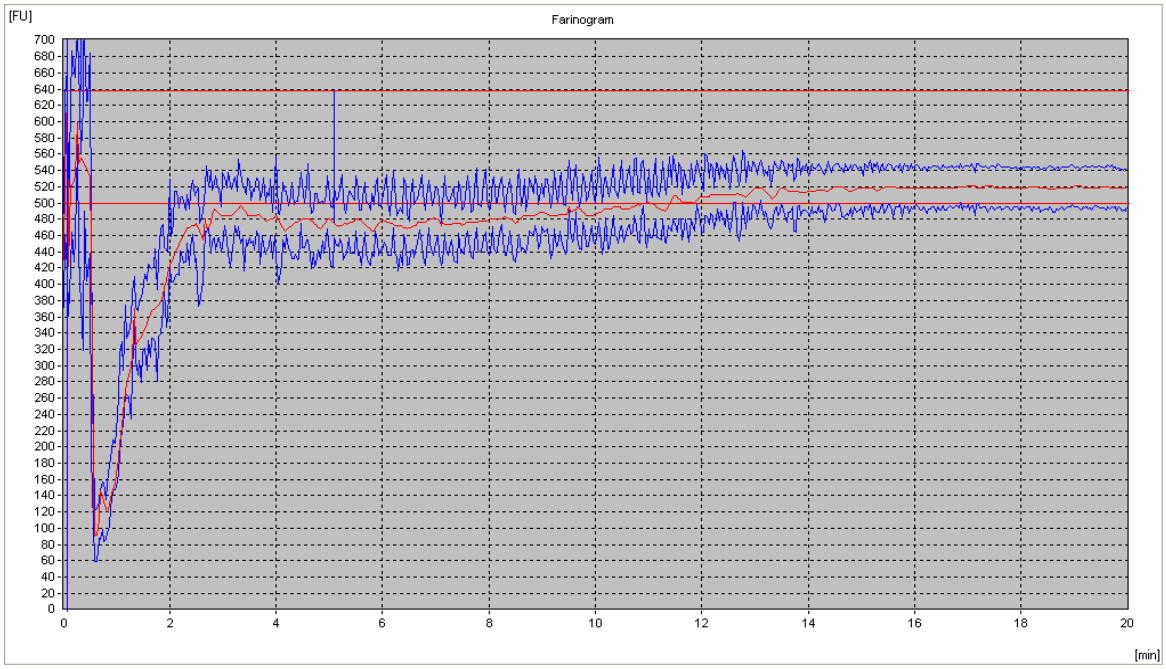
Moisture content 30.51 %



Consistency for wheat flour with 20 % M5

520 (corrected) FU with water absorption 11.0 %

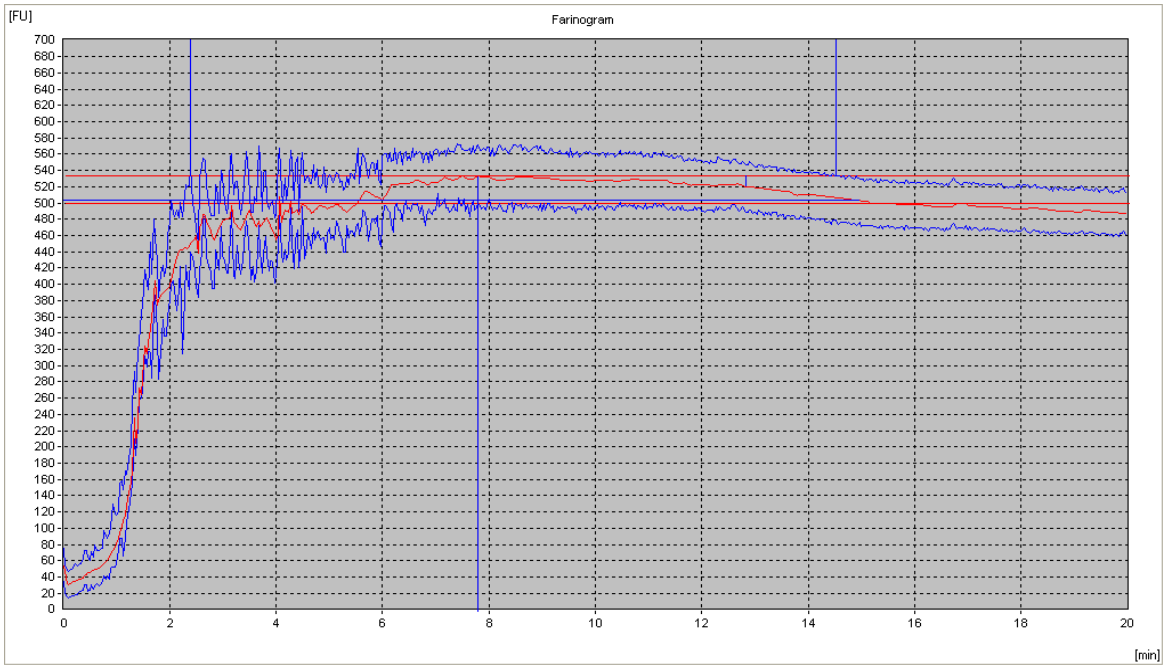
Moisture content 41.91 %



Consistency for wheat flour with 20 % M3, Depol

533 FU with water absorption 22.0 %

Moisture content 31.82 %



Baking Equipment

CR59 Arching machine.

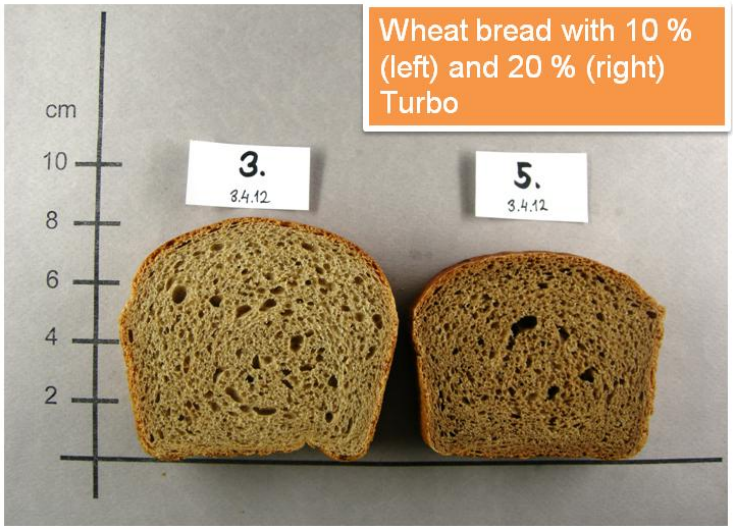
BM51-1 Long roller.

Baked Breads

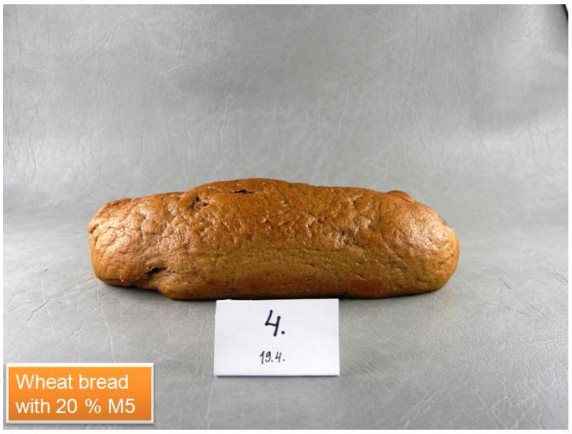
First test baking


Wheat bread, reference
cm
10
8
6
4
2
1.
3.4.12


Wheat bread with 10 % (left) and 20 % (right) M0
cm
10
8
6
4
2
2.
3.4.12
4.
3.4.12


Wheat bread with 10 % (left) and 20 % (right) Turbo
cm
10
8
6
4
2
3.
3.4.12
5.
3.4.12

Second test baking



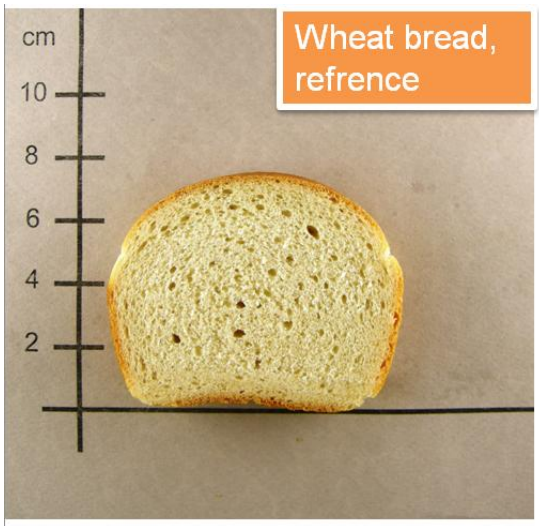

Wheat bread, reference


Wheat bread with 20 % M2


Wheat bread with 20 % M3


Wheat bread with 20 % M5


Wheat bread with 20 % M3, Depol


cm
10
8
6
4
2
Wheat bread, refrence


cm
10
8
6
4
2
Wheat bread with 20 % M2

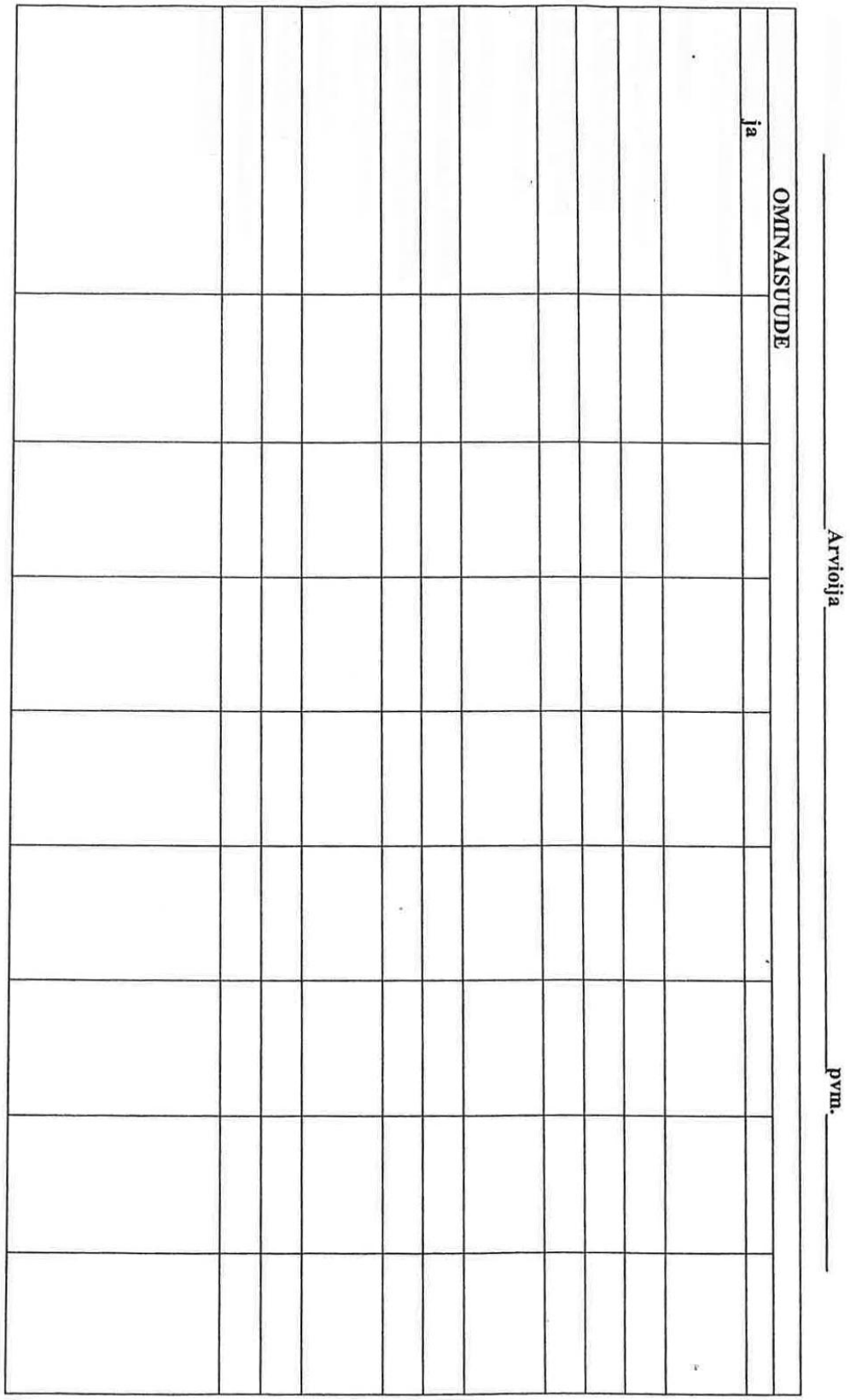

cm
10
8
6
4
2
Wheat bread with 20 % M3


cm
10
8
6
4
2
Wheat bread with 20 % M5


cm
10
8
6
4
2
Wheat bread with 20 % M3, Depol


cm
10
8
6
4
2
Wheat bread with 20 % M3 (left) and 20 % M3, Depol (right)

Sensory Evaluation Form



Leipien arvioinnit

- Arvioinneissa voi käyttää myös puolikkaita numeroita.
- Arvioi yksi leipä kerrallaan.
- Tee arviointi itsenäisesti, älä keskustele.
- arvioin leivät annetussa järjestyksessä
- Arvioi murenevuus leikkaamalla leivästä pala (miten paljon muruja pöydällä, hajoaako rakenne leikattaessa).
- **Huuhtele suusi vedellä näytteiden välillä ja pidä pieni tauko.**

ULKONÄKÖ

1. Viipaleen huokosrakenteen tasaisuus
5 erittäin tasainen huokosrakenne
4 melko tasainen huokosrakenne
3 hieman epätasainen huokosrakenne
2 melko epätasainen huokosrakenne, isoja yksittäisiä huokosia
1 erittäin epätasainen huokosrakenne, isoja onkaloita leivässä

RAKENNE

2. Viipaleen kimmoisuus (sormin), sormin painelemella.
5 erittäin kimmoisa
4 kimmoisa
3 melko kimmoisa
2 hiukan kimmoisa
1 ei lainkaan kimmoisa (viipale murtuu jo vähäisestäkin taivuttelusta)

3. Viipaleen pehmeys (sormin painamalla)
5 erittäin pehmeä
4 pehmeä
3 melko pehmeä
2 hiukan kova
1. kova

FLAVORI

4. Sisuksen flavorin voimakkuus
5 erittäin voimakas haju ja maku
4 melko voimakas haju ja maku
3 ei voimakas eikä heikko haju ja maku
2 melko heikko haju ja maku
1 heikko haju ja maku

5. Sisuksen maun pistävyys
5 erittäin pistävä
4 pistävä
3 melko pistävä
2 hieman pistävä
1 ei lainkaan pistävä

6. Sisuksen maun raikkaus
5 erittäin raikas
4 melko raikas
3 ei raikas eikä tunkkainen
2 melko tunkkainen
1 tunkkainen

7. Sisuksen jälkimaun voimakkuus
5 erittäin voimakas ~~haju ja maku~~ jälkimaku
4 melko voimakas haju ja maku
3 ei voimakas eikä heikko haju ja maku
2 melko heikko haju ja maku
1 heikko haju ja maku

8. Suutuntuman kosteus (sormin ja suussa)
5 erittäin kostea
4 kostea
3 melko kostea
2 hiukan kostea
1 ei lainkaan koskea