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Naked barley – functional grain – functional food

by

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1 Naked barley

1.1 Naked barley – a plants history

Barley is an ancient plant crop that played an important role in human nutrition in Asia, Africa and Europe for many millennia. *Hordeum vulgare L. subsp. spontaneum* (=wild barley) is believed to be the ancestor of today's barley. However, the question where the roots of barley really are is controversially discussed. The most common theory is that the spread of barley started in present-day Israel, northern Syria, southern Turkey, eastern Iraq and western Iran.

The earliest archaeological discoveries of barley stem from Syria and Iran around 8000 B.C. With the movement of civilizations accompanied by the establishment of trade routes the use and cultivation of barley arrived Europe. Barley was a popular foodstuff in ancient Greek and Italy used as a component of porridge or unleavened bread. Greek scholars such as Hippocrates or Pliney the Elder considered barley a healthy and nourishing food which was also used for medical treatments. In ancient Rome gladiators (who were also called "hordearii" which means "barley men") believed that barley could increase strength and stamina and thus preferred it to other cereals. With the fall of the Roman Empire barley lost its reputation and became the major food of the poor and slaves. Barley reached Spain around 5000 B.C and spread over today's German and France. A remnant of those days is a bread called "bolon" or "boulon" which is still prepared in parts of France.

Around 3000 BC six-rowed, hulless barleys were grown on the British Isles. In England and Scotland barley was a major food especially for poor people form the 15th century onwards.

Around 2000 BC barley was introduced in Norway and Scandinavia. In many regions of Northern Europe barley was grown till the 20th century and thus was a common ingredient of several foods. At the same time it spread over Asia; in Korea, China, Tibet, Japan and India it still is part of the traditional kitchen (Newman and Newman, 2006).

1.2 Taxonomy

Botanically barley falls into the family of the grasses (*Poaceae=Gramineae*) tribe *Triticeae*, genus *Hordeum*. It is a diploid (2n=14) mainly self-pollinating crop. In contrast to domesticated variations wild barley has a brittle spike where the ripe spikelets separate from each other easily; this favours the spread of the seed but complicates harvest. Non-brittle variations can be harvested unproblematically but survive only under domestication.

A barley head consists of triplets of spikelets that are alternately arranged on the rachis. Due to the morphology of the spikelets one can distinguish between two and six-rowed barleys. Two-rowed barleys have two lateral, unfertile spikelets in each triplet and thus only two rows of fertile spikelets. In six-rowed barleys all six spikelets are fertile and awned (Zohary and Hopf, 2000). The differences emerge from a mutation of Vv on the chromosomes 2 and Ii on chromosome 4 which confer fertility to the lateral spikelets. Normal two-rowed barley is VVii; normal six-rowed barley is vvII (Gymer, 1978).

In naked barley varieties the expression of the naked caryopsis gene (nud) prevents the intergrowth of husks and caryopsis. Consequently, the kernels are not hulled by glumes and thus released while threshing. To this end, naked barley (Hordeum vulgare var. nudum) requires no further dehulling. The removal of the fibre-rich hull proportionally increases the

levels of protein, starch and micro-nutrients in naked barleys (McGuire and Hockett, 1981; Newman and Newman, 2005). Naked barleys can either be two- or six-rowed (*Hordeum vulgare subsp. distichum var. nudum* and *Hordeum vulgare subsp. vulgare var. nudum*), albeit two-rowed barleys are predominantly hulled. Beside the morphology of the heads, barley varieties can also be distinguished according to the shape and surface of their kernels. Kernels of two-rowed barleys are straight and symmetrical whereas kernels of six-rowed barleys are asymmetrical and slightly bent. Naked barley kernels have a dwarfed surface and a constantly narrow furrow (Zohary and Hopf, 2000).

Various hull pigments lead to different kernel colors. Anthocyanins, melanins (Lundqvist et al., 1996) and carotenoids are responsible for blue and purple, black and yellow kernel colours, respectively. The presence of these secondary plant metabolites goes along with additional health benefits depending on the properties of the particular molecules. Anthocyanins belong to the group of polyphenols and are as well as carotenoids known for their antioxidative power. The latter function as free radical scavengers and therefore may protect against oxidative damage. In association with lutein and zeaxanthin carotenoids act together with other phytochemicals against cancer, cardiovascular risk and other diseases (Mares-Perlman et al., 2002; Calvo, 2005).

1.3 Starch in Naked barley

Starch is the major component in barley grains; the dry matter of barley consists of 60-70% starch. Starch itself is composed of two types of glucose polymers; the highly branched amylopectin and the linear amylose. According to the ratio of amylose and amylopectin barley can further be classified. Normal naked barley contains 25-30% amylose, waxy varieties less than 15% and high-amylose cultivars more than 35%

amylose (Ajithkumar et al., 2005; Bhatty, 1986, 1999). Among the waxy cultivars there are also zero-amylose waxy barleys that contain no amylose (Izydorczyk et al., 2000). The different barley varietis have distinct physicochemical properties and also differ from each other in their ß-glucan content, which is highest in high-amylose and waxy barley (Izydorczyk, Storsley et al., 2000). The ratio of amylose and amylopectin strongly influences the quality of the obtained baking products. Amylopectin is responsible for crystallinity of starch; the crystalline order emerges from the organisation of the double helices formed by the amylopectin molecules. Consequently, crystallinity is higher in waxy cultivars that contain high levels of amylopectin. Flour of waxy hulless barley has excellent water absorbing properties and thus is an appropriate thickening agent (Bhatty, 1997). Moreover waxy cultivars lead to a sticky texture in the final product. The waxy endosperm character is controlled by a recessive wax gene on chromosome 7HS (Ajithkumar, Andersson et al., 2005; Bhatty, 1986b; Bhatty, 1999; Dendy and Dobraszczyk, 2001a; Dendy and Dobraszczyk, 2001b; Yanagisawa et al., 2006; Yasui et al., 2002). The swelling power and fragility of starch are inversely related to the amylopectin level; (Sandhya Rani and Bhattacharya, 1995) came to the conclusion that starch granules that contained high levels of amylose could resist swelling and disintegration during agglutination whereas starch granules that contained little amylose disintegrated at lower temperatures and could not resist swelling. Generally starches with lower amylose levels exhibit lower pasting temperature, a higher water binding capacity and peak viscosity and a more pronounced breakdown than starches that contain higher levels of amylose. Peak viscosity is absent in high-amylose starches (Gao et al., 2009; Zheng et al., 1998).

1.4 β-Glucan in Naked barley

ß-Glucan is the major soluble fibre component of hulless barley (Bhatty, 1993). Barley ß-glucan possesses the advantages of dietary water soluble and dietary insoluble fibre. Both types of dietary fibre have different physiological properties; consequently, it is important to distinguish the two types, to estimate the benefits on human health realistically. The soluble fibre fraction is responsible for blood cholesterol and postprandial glucose modulation. It forms viscous solutions in water, which seem to be responsible for the physiological effects. Also the viscosity of barley flour is influenced by the ß-glucan content; higher levels of ß-glucan go along with increased viscosity.

Hulless barley contains high levels of ß-glucan and therefore is a potential ingredient for foods with health promoting properties; the concentrations reported in literature range from 3 to 8%. High ß-glucan contents go along with high levels of dietary fibre whereas ß-glucan and starch contents are inversely related. There seems to be no relationship between the kernel size and the ß-glucan content; consequently, it is not safe to say that smaller kernels with a proportionally higher hull volume naturally contain higher levels of ß-glucan. The variations in ß-glucan content might underlie other genetic variations.

Moreover, the ß-glucan content of barley strongly depends on the variety. Generally, high-amylose and waxy hulless barleys tend to contain more ß-glucan than zero-amylose and regular hulless barleys. Waxy barley cultivars posses higher levels of water and acid extractable ß-glucans than regular barleys (Ajithkumar et al., 2005; Izydorczyk et al., 2000)

Gao et al. (2009) analyzed the ß-glucan content of regular, waxy and high-amylose hulless barley starches and came to the conclusion that high-amylose and waxy hulless barley starches contained 8 or 7% of ß-Glucan, respectively; whereas regular hulless barley exhibited significantly less (4.6%) ß-Glucan. These results go along with the findings of other research works where 7.49%, 6.86%, 6.30% and 4.38% ß-glucan were detected in high-amylose, waxy, zero amylose waxy and normal barley, respectively. However, the ß-glucan content of regular barley can underlie a big variation; the ß-glucan content can range from 3 to 6%; to this end it is possible to identify regular hulless barley varieties with a relatively high ß-glucan content (Izydorczyk et al., 2000).

However, high ß-glucan contents must not necessarily go along with increased health enhancing properties. Research results suggest that ß-glucan extractability underlies a big variation in different hulless barley varieties. This strongly suggests that the ß-glucan content is no good parameter to predict the health promoting properties of hulless barley products if the extractability and/or digestibility are not considered (Xue et al., 1997).

In technological regards a high ß-glucan content in naked barley flours goes along with deteriorated baking qualities. The sparsely formed gluten network of naked barley is additionally weakened by ß-glucan that exhibits a high water binding capacity and thus decreases the water availability for the gluten network (Gill et al., 2002).

1.5 Milling

Barley is usually milled using pin, hammer or roller mills; each procedure has its advantages and disadvantages. The use of pin and hammer mill requires an abrasion step before milling; the abrasion process, where the outer layer of the endosperm is partially removed, goes along with lower levels of vitamins and essential amino acids in the final flour. A further disadvantage of pin mills is the high rate of starch damage caused by mechanical stresses and generation of heat. However, the combination of a pin mill and an air classification system facilitates the selection of starch, protein and ß-glucan rich flours (Klamczynski and Czuchajowska, 1999; Nowakowski et al., 1986). The use of roller-mills ends up in darker flour with a higher ash content caused by an increased brittleness of the hull and consequently a deteriorated separation of bran and shorts (Bhatty, 1993; Bhatty, 1997).

Bhatty (1997) studied the milling behaviour of hulless barleys with waxy and regular starch milled with a roller-mill under several conditions and evaluated the physicochemical properties of the obtained fractions.

lt is reported that waxy barleys produce more break flour and less reduction flour than non-waxy barleys. The higher percentage of break flour indicates that waxy barley has a softer endosperm than non-waxy varieties (Klamczynski and Czuchajowska, 1999).

1.5.1 Codex Alimentarius Austriacus (B 20)

Due to the reason that barley was used more and more for beer and feed production in Austria as well as in the area of today's European Union there is almost no legislation on milling products from barley.

Nevertheless the general maximum value for the moisture content for flour is specified with 15.5%.

Typing of the different mill streams is made by indicating the ash content in percent dry matter multiplied with 1.000 – even though examples are just given for wheat and rye. This matter of fact opens the way to have a look whether which kind of NB milling product is possible and necessary to produce and not which one is allowed.

The list of the different special milling products includes pearl barley and ground barley – two products emerging from the fact that the consumption of spelt barley milled without previous peeling would come along with digestive problems. (Codex-B20, 2001)

1.6 Bread and bakery products

The basic ingredients of bread are flour and water; nevertheless, it is common to ad further ingredients to improve the sensory and technological qualities: Yeast gives the crumb a soft and spongy structure, increases bread volume and the formation of flavour components; acidifiers augment the swelling power and solubility of pentosans and thus facilitate the development of the pentosan network. Malt flour supports the growth of yeast; moreover, its α- and ß-amylase activities yield maltose in the dough and thus deliver sugar for non-enzymatic browning reactions. Pregelatinized flours enhance the flours water binding capacity (Belitz et al., 2001; Cauvain, 1998).

Barley bread was available in ancient days as unleavened bread. Nowadays bread has to deliver more than satiety. Bread has to have a soft and well leavened crumb and a more or less crunchy crust as it is the case with many available types of wheat bread. Since barley is known to negatively influence bread volume and appearance it is most times used in composite flours. This was the case in a study where an addition of 5% naked barley flour resulted in a 14% lower loaf volume (Bhatty, 1986a). The author suggested adding to ad not more than 10% of naked barley flour to preserve bread quality, following the AACC straight-dough method.

Comparable results were described by (Cavallero et al., 2002). They used different hulless barley flour fractions as well as a water extracted fraction high in β-glucan to produce bread. The addition of these fractions to wheat flour dough resulted in increased mixing times and reduced bread volumes. A sensory evaluation showed that the bread with 20% water extracted fraction was scored as good as the wheat bread and

consequently significant better than the breads with the 50% addition of the flour fractions.

Quick bread was among other things made of 55g whole beaten egg, 40g fat and 1.5g baking powder per 100g flour in another survey (Klamczynski and Czuchajowska, 1999). Application of 100% barley flour did not reveal a reduction of bread volume whereas 100% waxy hulless barley flour lead to a reduced volume compared to wheat flour.

Gluten protein is important for bread quality. The concentration, water absorption capacity, elasticity and extensibility of gluten determine the baking quality of flour. Gluten stabilizes the gas-containing pores that are relevant for gas retention and loaf volume (Caballero et al., 2007). The sparsely formed gluten network of naked barley flour is responsible for its limited baking qualities leading to a less flexible dough and a reduced bread volume. The use of various enzyme classes can compensate poor baking qualities and improve the properties of dough and bread at different stages of the breadmaking process.

1.7 Literature

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2 Aim of the work

Naked barley is a crop with several interesting aspects along the food supply chain. Naked barley can be grown in a wide range of climatic conditions. Since naked barely threshes free of the hull, pearling is not necessary and therefore common milling systems can be used. Beta-glucan can be found in the caryopsis as well as in the endosperm. This condition makes naked barley flour a functional ingredient.

However, literature states that naked barley flour deteriorates bread quality by making the crumb more compact and thus decreases bread volume. This makes it difficult to bring the health benefit of β-glucan to the customer in sensory attractive bread.

Target of the work was to fill this gap in the food supply chain by developing sensory attractive bread with the health benefit of the naturally occurring β-glucan.

Following problems should be solved:

1. Adaptation of the bread baking process to the specific characteristics of naked barley flour concerning kneading and leavening.
2. Development of a sensorily optimized recipe for pure naked barley bread.
3. Characterization of naked barley varieties.
4. Generation of a model to predict baking quality of naked barley.

3 Naked Barley – Optimized recipe for pure barley bread with sufficient beta-glucan according to the EFSA health claims[1]

3.1 Abstract

Naked barley is an underutilized crop that is suitable for the production of functional food: it contains remarkable amounts of β-glucans, which are well known for their blood cholesterol and short-time blood sugar regulating properties and their impact on weight regulation. The aim of the present work was to develop naked barley bread with satisfying sensory characteristics and good baking qualities that could augment the intake of dietary fibre, especially β-glucans and therefore meet the requirements of the EFSA health claim for β-glucans.

The results of the multiple response optimization suggest that the elevated use of water, malt flour and margarine in pure naked barley bread augment the sensory attractiveness whereas the use of acidifier and pre-gelatinized flour has a negative effect on the sensory quality.

Keywords: Hordeum vulgare, hull-less barley, bread, multiple response optimization, beta-glucan

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Abbreviations: db, dry basis; MA, baking margarine; MF, malt flour; PGF, pre-gelatinized flour; AF, acidifier; DY, dry yeast DFT, dough fermentation time; PP, pan proofing; rH, relative Humidity

3.2 Introduction

Naked (Hordeum vulgare var. nudum) and hulled barley varieties share the same genetic background, except that the naked gene, nud, is expressed in the naked types. Naked barley may be two- or six-rowed, have short or long awns, vary in straw height, and occur in hooded (awnless)-type barley. Environmental growing conditions have major influences on kernel size and composition, but kernel size and shape are also genetically controlled characteristics (Newman and Newman, 2005).

The barley kernel consists of the caryopsis, the lemma, the palea, and the rachella. In contrast to hulled barleys, the caryopsis threshes free of the hull in the manner of common wheat. With the removal of the hulls, nutrients in the caryopsis increase in relative proportion because of reduction of fibre represented in the hulls (McGuire and Hockett, 1981). The caryopsis consists of the pericarp, testa, aleurone layer, endosperm and embryo. Incidences of increased protein and starch contents in naked barley genotypes are given by two studies conducted in the United States and Sweden (Xue et al., 1997; Oscarsson et al., 1997).

Naked barley is a good source of dietary fibre providing soluble and insoluble dietary fibre fractions (Bhatty, 1999; Izydorczyk et al., 2000). Mixed-linkage (1-3),(1-4)-β-d-glucans (hereafter termed as β-glucan) are a major part of the soluble dietary fibre (SDF) in barley. A previous study showed that the total β-glucan content is higher, whereas the insoluble dietary fibre content is significantly lower in naked barley (Xue et al., 1997) compared to hulled barley genotypes. Nevertheless, its content

generally underlies a natural fluctuation depending on the variety and conditions before and after harvesting (Ehrenbergerová et al., 2008). High-amylose and waxy hulless barley contains approximately 7 or 8% β-glucans, whereas regular hulless barley comprises significantly less (4.6%) (Gao et al., 2009; Tiwari and Cummins, 2008).

Together with arabinoxylans, a fraction of partly soluble nonstarch polysaccharides (NSP) occurring in the cell walls, β-glucan has a great impact on cereal processing and product properties.

The positive effects of cereal β-glucans have been recently reviewed by Wood (2010; 2007), with most of the data deriving from studies with oat β-glucans, followed by barley and rye. The mode of action of barley β-glucan in particular included the short time blood sugar regulating effects of bread (Cavallero et al., 2002; Vitaglione et al., 2009) and of chapatis (Thondre and Henry, 2009), beneficial effects on the weight management by increasing satiety (Nilsson et al., 2010; Granfeldt et al., 1994) and on serum cholesterol levels (Talati et al., 2009; Smith et al., 2008; Shimizu et al., 2008). In 2005 the US Food and Drug Administration (FDA) concluded that a cause and effect relationship between the consumption of β-glucan and coronary heart disease lowering properties exists (21CFR101.81). In Europe, the European Food Safety Authority (EFSA) has indicated that it will allow claiming "regular consumption of β-glucans contributes to maintenance of normal blood cholesterol concentrations" (EFSA, 2009) by assuming that barley β-glucans have the same effects than oat β-glucans. In order to bear the claim, EFSA demands a quantity in food of at least 3 g/day of β-glucans from oats, oat bran, barley, barley bran, or from mixtures of non-processed or minimally processed beta-glucans in one or more servings. The positive effects of β-glucans are mainly attributed to its high viscosity in aqueous solution and thus increasing the viscosity of the contents within the intestinal tract (Jalili et al., 2000). Viscosity in the small intestine is determined by the

concentration, molecular weight and solubility of β-glucan. At the moment, neither FDA nor EFSA substantiate their claims on the physicochemical properties but on a daily dosage of 3 g/day, although scientific evidence unequivocally links the effects of β-glucan with its viscosity (Wolever et al., 2010), which is indirectly decreased due to degradation of the molecular weight during food processing (Andersson et al., 2008; Tosh et al., 2008; Lan-Pidhainy et al., 2007; Åman et al., 2004; Andersson et al., 2004).

It is anticipated, that due to legislation the interest of food producers and consumers in using naked barley for food purposes will increase, although despite the numerous health promoting properties of naked barley, barley flour is hardly used for human consumption at the moment. Reasons may be the poor baking properties of naked barley flours emerging from the sparsely formed gluten network. Additionally, the high concentration of β-glucan decreases the water availability for the gluten network and thus impairs the baking properties (Gill et al., 2002). The use of naked barley flour in bread is said to deteriorate the sensory properties, because it leads to a lower bread volume and a worse crumb structure. Thus, the maximum level of barley flour recommended by Bhatty (1986) was 10% based on flour for yeast leavened breads. As a compromise between sensory attractiveness and additional health promoting benefits, barley flours substituted other flours at maximum by equal parts in a study of Cavallero et al. (2002) albeit usual levels range from 15-20% (Škribić et al., 2009; Berglund et al., 1992).

The objective of this study was to develop an optimized formulation for bread based on 100% naked barley flour by using an experimental design and statistical multiple response optimization to attain a dough with technological feasible processing properties and bread with satisfying sensory attributes that contains sufficient β-glucan to increase the daily intake and to be in accordance with the scientific opinion of EFSA.

3.3 Experimental

3.3.1 Materials

Two-rowed winter naked barley cv. Hiberna (originally released in Germany in 1993 by BPZ Saatenring) was conventionally grown in 2008 in Raasdorf (16°35‘ E, 48°13‘ N), Austria, on trial fields of the Experimental Station Gross-Enzersdorf. The baking experiments were accomplished with the following ingredients: Baking margarine was obtained from Senna Nahrungsmittel (Austria), whereas malt flour, pre-gelatinized flour (Risofarin) and acidifier (Diarol) were from Stamag Stadlauer Malzfabrik GmbH (Austria). Salt was obtained from Gustosal Salinen (Austria) and dry yeast (Saf-Instant yeast) from Lesaffre Austria AG (Austria).

The naked barley cv. Hiberna was milled with a MLU 202 roller mill (Bühler, Switzerland) as described by Andersson et al. (2003). The barley was not conditioned before milling and the feed rate for milling was approximately 5 kg/h. Six flour fractions (B1-B3, C1-C3) from the starchy endosperm were collected and merged to give a straight-run white flour. Brans and shorts were collected separately, but not used in this study. The flour was stored at 4°C until use and allowed to equilibrate to room temperature before baking trials.

3.3.2 Analysis

3.3.2.1 Proximate analysis

Moisture and ash content of the barley flour were determined according to AOAC approved standard methods 940.56 and 920.153, respectively (AOAC, 1995). Crude protein was determined according to ICC standard method 105/2 (ICC, 1998) using the factor 5.83 x N for

conversion. Total starch (AOAC method 996.11), β-glucan (AOAC method 995.16) and dietary fibre content (AOAC method 991.43) were measured with enzymatic-gravimetric methods using commercially available test kits (Megazyme, Bray, Ireland). Water absorption was determined according to ICC-Standard method 115/1 (ICC, 1998). The β-glucan content was as well measured in bread, in order to proof if the claim on helping to maintain a normal cholesterol level is enabled. For this, the bread was immediately shock frosted after baking and analysed after thawing according to the manufacturer's recommendation (Megazyme, Bray, Ireland).

3.3.2.2 Analysis of dough characteristics

To find out whether the investigated factors affected dough handling, dough stickiness was measured with the TA-XT2iR Texture Analyzer (Stable Micro Systems™, Great Britain) using the SMS/Chen-Hoseney Dough Stickiness Rig after kneading and after DFT as described by Grausgruber et al. (2003) with 10 replications per dough.

3.3.2.3 Analysis of bread characteristics

After baking the breads were allowed to cool down and equilibrate at 20°C and 50% rH in a climate chamber (MMM, Medcenter Einrichtungen GmbH, Germany) for 24 h ± 2 h.

To characterize the impact of the investigated factors the final bread volume, color, circumference along and across the bread were measured, each in 3 replications. Bread volume was measured with the rapeseed method (Chopin, France) according to ICC-Standard method 131 (ICC, 1998) and expressed on the basis of 1 kg flour. Color of crumb and crust were measured in the CIELAB color space (Micro Color, Dr. Lange, Germany).

The texture of bread cubes (40×40×25 mm l×w×h) using only bread crumb was assessed with the TA-XT2iR Texture Analyzer (Stable Micro Systems™, Great Britain) as previously described (Grausgruber et al., 2008). Maximum force (Fmax), the force at 25% compression (F25) and relative crumb elasticity (elasticityrel), defined as proportion of Fres to Fmax, where Fres describes the force after holding at 40% compression for 60 s, were taken for statistical analyses (10 replications per bread).

Four trained persons assessed the sensory characteristics of the barley breads. Six parameters (appearance, browning, consistence of the crust, consistence of the crumb, mouthfeel and smell & taste) were scored from optimum (5) to poor (1).

3.3.3 Bread making

Considering preliminary trials the processing conditions were modified compared to ICC-standard method 131 (ICC, 1998) as follows: Each recipe was sized to give approximately 1600 g dough for three tin breads (135×95×70 mm, l×w×h). Firstly, DY was dissolved in 72 mL H2O (35°C) containing 52.63 g L-1 sugar. Secondly, the remaining dry ingredients were placed in a mixing bowl (KM020 Titanium Major, Kenwood, UK) and mixed with the kneading hook at speed level 1 for 1 min. The DY solution was added slowly followed by the melted MA and the sucrose-salt solution (75 g sucrose and 75 g salt L-1) at speed level 1 within 1 min. Dough formation was completed by kneading for a further 3 min at speed level 2.5. The dough was proofed at 30°C and 85% rH in a proofer (G66W, MANZ, Germany), scaled thereafter to 400 g portions and formed. Second proofing was performed in the baking tins at the same conditions as above. Finally, baking started at 230°C top and bottom heat for 35 min in a baking station (BS60/3W, MANZ, Germany). The oven was steamed

once after loading at the maximum level and the temperature was subsequently reduced to 180°C.

3.3.4 Experimental design

27 recipes for barley bread were assessed with a fractional factorial Plackett Burman experimental design. All ingredients were related to flour and are expressed in percent flour weight. The level of sucrose and salt was kept constant throughout the experiment and was 1.96 and 1.58%, respectively. The experimental factors studied were the two process parameters: DFT (min) and PP (min) and the six ingredient parameters: H2O, DY, AF, PGF, MF and MA. Each factor was tested at two levels namely a high level denoted by (+1) and a low level denoted by (-1) with three replications of the centre point (0) as listed in Table 3.1. The respective ranges of the factor levels have been evaluated in preliminary trials (data not published). The parameters used in the Plackett Burman design and their influence on dough, bread and sensory attributes are shown in Table 3.2. For statistical analyses, the average value of each response variable was used and experimental factors, which were significant at 5% level ($P < 0.05$) from the regression analysis were considered to have greater impact on the bread quality.

3.3.5 Optimization

Based on the results of the statistical design the multiple response optimization procedure was used to optimize the bread recipe. Table 3.3 shows the impact factors for each of the observed variables: mouthfeel, smell and taste had the greatest importance followed by circumference and volume, elasticityrel, appearance and consistence of the crust. Generally, the highest priority was ascribed to the sensory evaluation of

the naked barley bread whereas dough properties and colour of crust and crumb were graded less important.

3.3.6 Data analyses

All data were evaluated with STATGRAPHICS centurion XV® (Statpoint Technologies, Inc., Virginia) after eliminating outliers with Box-and-Whisker-Diagrams.

3.4 Results and Discussion

3.4.1 Proximate composition

The proximate composition of the barley flour is presented in Table 3.4. Total starch was the main constituent, followed by protein and soluble dietary fibre of which β-glucan content was measured as 3.16% db. Insoluble dietary fibre, lipids and ash were found in minority with contents of 2.4, 1.7 and 1.1% db, respectively. Water absorption was 57.2%.

3.4.2 Experimental Design

Preliminary tests outlined that pure barley bread of the cultivar Hiberna has a thin, cracked, light brown crust and a greyish, firm, compact and fine-pored crumb with a short bite and a dry mouthfeel during chewing.

The experimental design was used to identify ingredients that significantly affect the bread quality. As shown in Table 3.2 the wide variation of the response variables reflects the potential of optimization for a higher consumer preference. It is also reflected in Table 3.5 which

summarizes the results of the ANOVA that DFT, PP and DY had no significant influence on any of the response variables. DFT and PP were set relatively low because preliminary tests showed that a extensive fermentation negatively influenced bread quality. DFT beyond 40 min resulted in a very smooth dough leading to reduced bread volume and circumference. However, as a result of the optimization process DFT was close to the high level and PP to the low factor level. The main reason for this can be seen in their effects on bread volume. A longer DFT increased the volume of the bread whereas longer PP decreased it, although both effects were not statistically significant (P = 0.113 and 0.197, respectively). DY negatively influenced the appearance of the bread at higher concentrations (P = 0.0566) and moreover, at the highest level a reduced bread volume and circumference were observed. These negative effects might be caused by an excessive rising in combination with a dough unable to hold the incorporated air which finally ends up in a reduced volume and less appealing appearance of the bread.

H2O addition increased stickiness after kneading and stickiness after DFT which complicated dough processing. This finding is in agreement with others (Chen and Hoseney, 1995). A higher H2O addition softened the dough and consequently declined F25, Fmax and the elasticityrel , but had no impact on circumference and volume of the bread. With increasing amount of H2O the crumb became more bread like and darker and affected the appearance, consistence of the crust and the mouthfeel positively.

The use of MF exhibited several positive effects on naked barley bread: it softened the bread (lower F25 and Fmax) and improved elasticity (elasticityrel) which led to a more bread-like texture. Furthermore, it ameliorated the crust in terms of browning and consistence.

MA softened the bread in regards of physical and sensory attributes (lower Fmax, F25 and better consistence of the crumb) and led to a better

browning of the crust. On the other hand an increased use of MA made the dough stickier and thus more difficult to process and negatively influenced the crust consistence.

The elevated use of AF predominantly deteriorated the sensory and textural attributes. Higher levels of acid went along with a worse browning, consistence of crust and crumb, smell and taste. Of the textural parameters elasticityrel increased at higher AF levels.

PGF had no positive effects on any of the observed parameters. It hardened the dough and had a negative impact on the consistence of the crumb.

3.4.3 Optimization

Table 3.1 summarizes the levels of the investigated factors after running the multiple response optimization. To estimate the reliability of the multiple response optimization the predicted values for the naked barley bread were compared with the observed values. For instance, the predicted volume of the bread was 7% higher than the volume of the processed bread. The colour of the crust was 9% darker than predicted whereas the colour of the crumb was 2% lighter. Generally, the results of the sensory evaluation were better than statistically predicted which was positive because the emphasis of the experiment was set on sensory attractiveness.

Fig. 3.1 shows the naked barley bread processed according to the optimized formulation of the multiple response optimization. It illustrates the well developed bread volume, the light colour of the crumb and its evenly distributed crumb structure.

Fig. 3.2 shows that the β-glucan content of the naked barley bread is sufficiently high to meet the requirements of the recently passed EFSA health claim (3 g /day). One serving of bread (50 g) delivered 0.81 g of β-

glucan, as measured enzymatically. The nutritional recommendation for bread intake is set between 200 to 300 g of bread per day (Elmadfa et al., 2009). Consequently, an intake of naked barley bread according to the nutritional recommendation goes along with a β-glucan intake that is sufficiently high to have health promoting effects. Thus, an intake of four servings of naked barley bread a day can make a contribution to a reduced blood cholesterol level.

3.5 Conclusions

The basic function of food to satiate and to provide macro- and micronutrients has become in the Western industrialised countries less and less important. Concurrently, the discussion of increasing the healthiness of consumers by changing eating habits or incorporating ingredients with health benefits has increased quickly.

With increasing prosperity and/or educational knowledge additional attributes were ascribed to the consumption of food like sensorial sensation, social prestige or ethical reasons. Among these supplementary functions of food the health-awareness of consumers is used to a high degree by the food and marketing industry for the promotion of existing and newly developed food products.

The perception that "healthy" foods are boring foods, the lack of information on packages as well as family pressures were seen as major barriers for dietary changes as observed by Baghurst (1992) in Australia. A few years later, these barriers have not changed much (Lopez-Azpiazu et al., 1999).

A further barrier for increasing the consumption of whole grain in particular is the still increasing knowledge about the biochemical mechanisms behind the health benefits. In the late 80ies, Bhatty (1986)

concluded that the removal of β-glucan by plant breeding could enhance the use of naked barley in food and feed applications, whereas nowadays it is assumed that exactly the contrary is the case.

Since then, in most studies attention was mainly focused on the polysaccharide moiety, while the potential role of whole grain antioxidants was considered less up to now. The newly introduced term "dietary fibre-antioxidants" assumes that the beneficial effects attributed to the cereal dietary fibre are due not only to the polysaccharide moiety, but also to the associated polyphenolic compounds (Vitaglione et al., 2008).

The present experiments showed that the baking quality of naked barley flour is sufficient to bake pure naked barley bread. Thus, naked barley is an interesting alternative for commonly used grains and could contribute to a higher diversity in human nutrition.

Facts which strongly support the use of naked barley are the non-uniform distribution of dietary fibre within the kernel and especially its high content of β-glucans. As a consequence, white barley flour fractions contain a sufficiently high amount of soluble dietary fiber. This being the case, there is no need for using wholemeal flour to obtain high dietary fiber cereal based products when naked barley is utilized for food production. Thus, the current preference of consumers for white bread may be met more easily until eating habits have changed due to an increased health consciousness and scientific evidence.

3.6 Figures

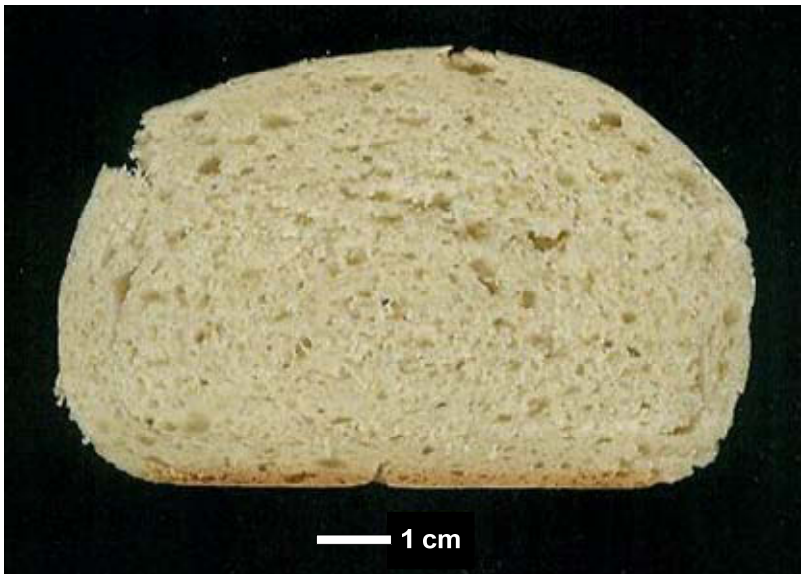

Fig. 3.1 Pure naked barley bread produced according to the optimized recipe.

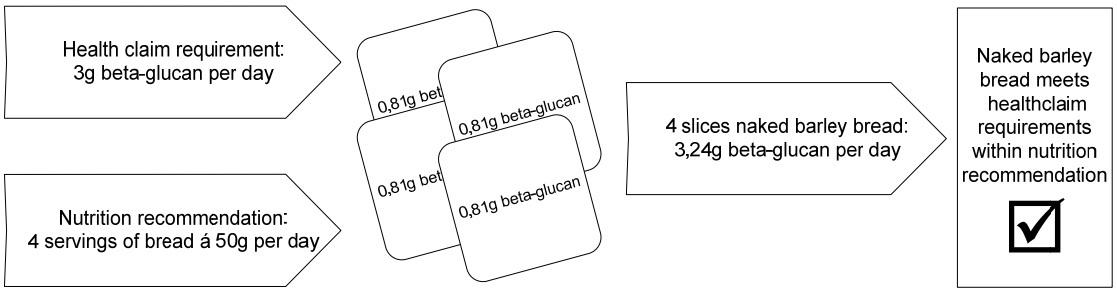

Fig. 3.2 Pure naked barley bread meets the health claim requirements for beta-glucan within the nutrition recommendation.

3.7 Tables

Abbreviations: db, dry basis; MA, baking margarine; MF, malt flour; PGF, pre-gelatinized flour; AF, acidifier; DY, dry yeast DFT, dough fermentation time; PP, pan proofing; rH, relative Humidity

Table 3.1 Levels of the factors used in the experimental design for the production of barley bread and for the optimized recipe. Values of ingredients are expressed as percent flour weight.

factor level	factor							
	DFT [min]	PP [min]	H_2O [%]	DY [%]	AF [%] [%]	PGF	MF [%]	MA [%]
-1	10	10	60.32	1.27	0.00	0.00	0.00	0.00
0	15	15	63.35	1.58	0.40	1.50	1.00	2.50
+1	20	20	66.38	1.89	0.80	3.00	2.00	5.00
optimized	17.5	10	66.27	1.30	0.01	0.08	2.00	1.05

Table 3.2 Plackett Burman design matrix (2^8*3/32) with observed values for physical and sensory attributes

Run	Variables								Dough characteristics			Bread texture							Sensory attributes					
	DFT	PP	H_2O	DY	AF	PGF	MF	MA	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	0	0	0	0	0	0	0	0	4.4	15.8	14.7	216.0	2289.7	35.3	36.9	0.34	78.9	74.8	2	2	2	2	3	1
2	1	-1	1	-1	-1	-1	1	1	5.0	23.8	24.1	223.2	5273.3	22.1	22.5	0.35	70.2	72.4	4	4	3	4	3	4
3	1	1	-1	1	-1	-1	-1	1	5.0	29.7	29.9	198.3	1908.9	42.8	45.5	0.34	73.0	73.5	2	3	2	3	2	3
4	-1	1	1	-1	1	-1	-1	-1	4.0	16.2	12.3	219.1	2223.9	33.2	37.8	0.32	85.3	73.8	4	1	3	2	3	1
5	1	-1	1	1	-1	1	-1	-1	5.0	14.8	11.8	221.2	2320.2	42.4	48.4	0.36	73.5	72.5	3	2	3	1	3	4
6	1	1	-1	1	1	-1	1	-1	4.1	13.8	13.4	210.8	2175.9	46.5	51.8	0.34	83.9	75.0	2	1	3	2	2	2
7	1	1	1	-1	1	1	-1	1	4.1	21.0	18.9	202.5	2275.2	43.6	44.3	0.28	76.4	73.1	2	2	2	2	3	2
8	-1	1	1	1	-1	1	1	-1	5.0	22.5	14.9	203.6	1969.4	31.2	35.2	0.38	76.2	71.1	3	2	4	2	3	4
9	-1	-1	1	1	1	-1	1	1	4.1	41.1	31.6	200.7	2190.1	31.5	34.0	0.32	73.8	72.7	3	3	2	4	3	1
10	-1	-1	-1	1	1	1	-1	1	4.1	10.8	10.5	185.0	2472.2	58.1	60.4	0.34	79.3	76.7	1	2	1	1	1	1
11	1	-1	-1	-1	1	1	1	-1	4.0	12.1	12.9	209.3	2313.8	60.3	67.5	0.32	82.4	74.2	1	1	2	2	1	1
12	-1	1	-1	-1	-1	1	1	1	5.1	19.1	17.1	195.6	1940.5	37.8	40.6	0.38	69.0	70.8	3	4	1	3	2	3
13	-1	-1	-1	-1	-1	-1	-1	-1	5.0	11.0	8.6	209.3	2331.3	51.7	56.0	0.37	80.2	76.8	2	2	4	1	2	3
14	0	0	0	0	0	0	0	0	4.4	16.0	17.4	211.3	2362.3	38.6	41.5	0.35	79.1	73.1	2	2	2	2	1	2
15	-1	1	-1	1	1	1	-1	-1	4.0	10.8	8.8	197.0	1804.9	72.4	78.2	0.33	82.8	74.8	1	1	1	1	1	2
16	-1	-1	1	-1	1	1	1	-1	4.1	11.5	10.8	200.6	1953.1	37.3	41.8	0.33	83.4	72.8	4	1	4	1	4	2
17	1	-1	-1	1	-1	1	1	1	5.1	16.6	12.3	214.5	2242.0	37.0	40.4	0.37	70.6	69.9	2	3	1	2	1	3
18	-1	1	-1	-1	1	-1	1	1	4.1	16.2	15.8	217.0	2229.7	43.6	45.8	0.33	79.0	73.4	2	2	1	1	1	1
19	-1	-1	1	-1	-1	1	-1	1	5.0	28.0	29.2	203.1	2218.0	35.0	36.6	0.35	71.2	71.2	3	3	2	3	2	3
20	-1	-1	-1	1	-1	-1	1	-1	5.0	19.5	16.8	140.9	1642.1	40.5	44.1	0.37	78.4	73.4	2	3	4	3	3	3
21	1	-1	-1	-1	1	-1	-1	1	4.0	16.2	13.7	204.9	2127.2	44.1	45.3	0.32	76.9	73.7	2	2	1	1	1	1
22	1	1	-1	-1	-1	1	-1	-1	5.0	14.8	11.0	221.7	2577.5	58.6	65.2	0.36	77.8	73.4	2	1	4	1	2	3
23	1	1	1	-1	-1	-1	1	-1	5.0	34.3	30.5	210.2	2051.3	27.1	30.5	0.37	77.8	70.2	2	2	3	3	1	3
24	-1	1	1	1	-1	-1	-1	1	5.0	21.9	18.9	214.2	2317.5	31.1	33.1	0.32	72.0	72.3	2	3	3	3	2	3
25	1	-1	1	1	1	-1	-1	-1	4.1	15.6	11.9	180.6	2642.3	40.4	45.2	0.31	81.5	73.6	2	1	4	1	2	2
26	1	1	1	1	1	1	1	1	4.1	25.1	22.3	214.8	2298.0	37.0	38.8	0.32	75.3	71.7	1	2	1	2	2	1
27	0	0	0	0	0	0	0	0	4.5	22.1	21.7	214.1	2341.2	34.8	37.5	0.34	81.3	73.0	2	2	2	2	3	2

[Low (-1); High (+1); Centre point (0)] *A*: ph-value; *B*: stickiness after kneading; *C*: stickiness after dft; *D*: circumference/kg flour; *E*: volume/kg flour; *F*: F 25; *G*: F_{max}; *H*: elasticity $_{rel}$; *I*: L*crust; *J*: L* crumb; *K*: appearance; *L*: browning; *M*: consistence crust; *N*: consistence crumb; *O*: mouthfeeling; *P*: smell & taste

Table 3.3 Direction of optimization and impact factor for each parameter of the multiple response optimization.

observed variable	direction of optimization		impact factor
	minimize	maximize	
dough			
ph-value		X	1
stickiness after kneading	X		2
stickiness after dft	X		2
bread			
circumference / kg flour		X	4
volume / kg flour		X	4
F 25	X		3
F_{max}	X		3
$elasticity_{rel}$		X	4
L* crust	X		2
L* crumb		X	2
sensory			
appearance		X	4
browning		X	2
consistence crust		X	4
consistence crumb		X	3
mouthfeeling		X	5
smell & taste		X	5

Table 3.4 Proximate composition of naked barley flour (cv. Hiberna).

Constituent	% db
Dry Matter	88.00 ± 0.12
Ash	1.12 ± 0.02
Protein N x 5.83	9.62 ± 0.10
Fat	1.66 ± 0.02
Total starch	69.61 ± 1.54
Total *β*-glucan	3.16 ± 0.18
Dietary fibre, unsolube	2.36 ± 0.24
Dietary fibre, soluble	4.27 ± 0.03

Table 3.5 Summary of Regression analysis of Plackett-Burman design for prediction of significant variables. Significant positive effects are marked with pos. and negative ones with neg., both are shown with the P-Value at the 95% confidence level.

observed parameter		factor							
		DFT	PP	H_2O	DY	AF	PGF	MF	MA
	R^2	[min]	[min]	[%]	[%]	[%]	[%]	[%]	[%]
dough									
ph-value	98.78					neg. 0.0000			
stickiness after kneading	59.05			pos. 0.0070					pos. 0.0182
stickiness after DFT	57.84			pos. 0.0209					pos. 0.0065
bread									
circumference / kg flour	34.24								
volume / kg flour	37.29								
F 25	87.43			neg. 0.0000		pos. 0.0010	pos. 0.0006	neg. 0.0004	neg. 0.0034
F_{max}	87.66			neg. 0.0000		pos. 0.0017	pos. 0.0005	neg. 0.0007	neg. 0.0003
elasticity $_{rel}$	84.87			neg. 0.0107	neg.	0.0000		pos. 0.0049	neg. 0.0227
L* crust	89.87					pos. 0.0000			neg. 0.0000
L* crumb	69.83			neg. 0.0039		pos. 0.0043		neg. 0.0041	
sensory									
appearance	53.86			pos. 0.0049					
browning	90.32					neg. 0.0000		pos. 0.0067	pos. 0.0000
consistence crust	74.83			pos. 0.0139		neg. 0.0139	neg. 0.0483		neg. 0.0000
consistence crumb	62.42					neg. 0.0162		pos. 0.0162	pos. 0.0162
mouthfeeling	37.90			pos. 0.0095					
smell & taste	80.93					neg. 0.0000			

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4 Improvement of pure naked barley bread by using commercial bread enzymes[2]

4.1 Abstract

Naked barley is an ancient crop with several benefits. It can be grown in many different climate conditions and the milling technology is comparable to that of established crops. Moreover, it contains considerable amounts of β-glucan in the endosperm, which makes it possible to use naked barley as a source for functional food. Nevertheless, the lack of gluten requires a modified handling of the baking process.

The objective was to further improve an existing recipe for naked barley bread and thus ameliorate its sensory properties by using commercially available enzymes. Thereby, the effect on physical and sensory attributes on pure naked barley bread was studied.

It was proved that the application of maltogenic amylase, cellulase, gluco-amylase and xylanase improved naked barley bread.

Keywords: Hordeum vulgare, hull-less barley, bread, bread improvers, enzymes, enzyme combination, factorial 24-experimental design, multiple response optimization, beta-glucan

Abbrevations: A-AMYL, Alpha-Amylase; CEL, Cellulase; G-AMYL, Gluco-Amylase; GO, Glucoseoxidase; LACC, Laccase; LIP, Lipase; M-

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AMYL, Maltogenic-Amylase; rH, relative Humidity; XYL-M, (Endo-1,4-) Xylanase; XYL-5, (Endo-1,4-)Xylanase;

4.2 Introduction

In contrast to hulled barley naked barley is free threshing (Bhatty, 1986); consequently, pearling before milling is unnecessary. Furthermore, the removal of the fibre-rich hull proportionally increases the levels of protein, starch and nutrients in naked barley. Moreover, naked barley is rich in soluble and insoluble dietary fibre fractions. It contains 1.6-2.1 % arabinoxylan and 1.2-1.5 % beta-glucan (Andersson et al., 2003).

The baking qualities of naked barley flour are sufficient for bread production (Kinner et al. 2010). Problems that go along with the use of naked barley flour are the less flexible dough and the reduced volume of naked barley bread. The presence of fibre rich fractions weakens the gluten network and thus deteriorates gas retention which has a negative impact on the texture and appearance of the bread (Skrbic et al., 2009; Trogh et al., 2004a and 2004b; Gill et al., 2002a and 2002b; Bhatty, 1986).

Different kinds of enzymes are used to compensate poor baking qualities of flour. Amylases raise the sugar content in the dough by degrading starch during the dough phase. Yeast converts the sugars into alcohol and carbon dioxide. Carbon dioxide expands in the dough during the oven spring and contributes to a higher volume of dough and bread (Caballero et al., 2007). Amylases extend shelf-life by reducing the retrogradation of starcggh (Primo-Martin et al., 2008; Caballero et al., 2007; Si and Drost-Lustenberger, 2002; Rosell et al. 2001; Champenois et al. 1999).

Xylanases raise loaf volume, prevent staling and soften the crumb by converting water-insoluble hemicelluloses (e.g. arabinoxylans) and thus increase water binding capacity (Caballero et al., 2007; Basinskiene et al.,

2007; Meyer, 2006; Jiang et al., 2005; Guy and Sahi, 2003; Si and Drost-Lustenberger, 2002).

Glucoseoxidases increase loaf volume and the elasticity and adhesiveness of the crumb. The conversion of glucose leads to hydrogen peroxide which supports the formation of disulfide-crosslinks in the gluten network. The activity of glucoseoxidases is oxygen dependent and thus highest during kneading when most oxygen is introduced into the dough (Caballero et al., 2007; Dagdelen and Gocmen, 2007).

Application of Laccase in wheat bread increases the specific loaf volume, the firmness of the crumb and crispness of the crust by catalyzing the oxidative gelation of arabinoxylans (Flander et al., 2008; Caballero et al. 2007).

The combination of two or more enzymes may bring synergistic or antagonistic effects. As observed by Gambaro et al. (2006) and Si and Drost-Lustenberger (2002), the combination of amylase and xylanase together with maltogenic amylase reduced staling and increased dough volume without increasing dough stickiness.

The combination of laccase with xylanase enhances the availability of water for the gluten network which makes the dough softer and more expandable (Flander et al. 2008). In contrast, the coexistence with glucoseoxidase lowers the positive effects of xylanase because its degradation products are oxidized by glucoseoxidase (Collar et al. 2000).

Finally, the promising use of enzymes depends on suitable concentrations and combinations; consequently, it is necessary to study the dose depended effects of each enzyme previous to the investigation of putative synergistic effects.

4.3 Experimental

4.3.1 Materials

The naked barley Hora (BVAL350010) is a yellow summer variety and was conventionally grown in 2008 in Raasdorf (16°35‘ E, 48°13‘ N), Austria, on trial fields of the Experimental Station Gross-Enzersdorf. Milling was accomplished in a pilot plant by fractionated rollermilling (break roller mill Josef Oser, Austria; roller mill E. Happle & Sohn, Germany; plan sifter, Hohm, Austria). The milling system is constructed to gain 14 flour fractions of which are five break flours (>1000 µm), three semolina (500-1000 µm) and six small middlings (<500 µm). These fractions are automatically unified into three flours. Two bran fractions and one middling fraction are collected separately.

A mixture of the first two flours at equal parts was used for the baking trials. The naked barley flour was stored at 4 °C before use and allowed to equilibrate to room temperature before baking trials.

The baking experiments were accomplished with following ingredients: Baking margarine was obtained from Senna Nahrungsmittel (Austria), malt flour, pre-gelatinised flour and acidifier were from Stamag Stadlauer Malzfabrik GmbH (Austria). Salt was obtained from Gustosal Salinen (Austria) and dry yeast from Lesaffre Austria AG (Austria).

Enzyme samples as granulates (Table 4.1) were provided by Novozymes Switzerland AG: Fungamyl® 2500SG, an alpha-amylase, AMG® 1100BG, a gluco-amylase, Novamyl® 10000BG, a maltogenic-amylase, Pentopan® MonoBG and Pentopan® 500BG, (endo-1,4)-xylanases, Celluclast® BG, a cellulase, Gluzyme® 10000BG, a

glucoseoxidase, Laccase® NS27011, an experimental laccase, and Lipopan® F BG, a lipase.

4.3.2 Analysis

4.3.2.1 Analysis of flour characteristics

The moisture content which is defined as weightloss [%] during drying under specified conditions of the naked barley flour was determined according to ICC-standard method 110/1 (ICC, 1996).

4.3.2.2 Analysis of bread characteristics

The fresh bread was cooled at room temperature for one hour and subsequently stored for 24h ± 2h at 20°C and 50% rH (MMM, Medcenter Einrichtungen GmbH, Germany).

The loaf volume was measured via rapeseed method (Chopin, France) according to the AACC-Standard method 10-05 (AACC, 2000) and expressed on the basis of 1 kg flour. The breads circumference on the short and the long side was added to calculate the total circumference. The color of crumb and crust was measured in the CIELAB color space (Micro Color, Dr. Lange, Germany).

Bread crumb firmness was determined as the maximum compression force (40 % compression) by pressing a 36 mm diameter cylindrical plunger into the center of the bread cube (40 x 40 x 25 mm l x w x h) using a TA-XT2iR Texture Analyzer (Stable Micro SystemsTM, Great Britain) according to the AACC-Standard method 74-09 (AACC, 2000). Maximum Force „Fmax", Residual Force „Fres" and dedicated force after 25% compression „F25" were taken for statistical analysis (9 replications per bread). The quotient of Fres and Fmax is called relative elasticity „elasticityrel".

The sensory evaluation included the six variables “appearance”, “browning”, “consistency of the crust”, “consistency of the crumb”, “mouthfeel” and “smell & taste”. Four trained persons scored each attribute from 1 (poor) to 5 (optimum).

4.3.2.3 Bread making

The barley bread recipe based on previous experiments (Kinner et al. 2010) after adoption of the ICC-standard method 131 (ICC, 1996). The amount of flour was corrected according to the moisture content of the naked barley flour.

Barley flour, malt flour, pre-gelatinised flour and acidifier were mixed in the kneading machine (KM020 Titanium Major, Kenwood, UK) for 1 min. Afterwards the yeast, suspended in a sugar solution (35 °C, 72 mL H2O containing 52.63 gL-1), water, sugar-salt-solution and dissolved enzymes were added. The ingredients were kneaded to a homogenous dough for 3 min at level 2.5.

Then the dough was divided in three 400 g pieces and shortly rounded by hand. The pieces were fermented in the leaving cell (30°C, 85% rH) for 18 min. Subsequently, the dough was rolled out with a rolling pin, triple folded and formed to a cylinder. The three loaves were placed in small tins (135 x 95 x 70 mm, l x w x h) and finally proofed a second time at equal conditions for 10 min and baked (230 °C down to 180 °C) for 35 min.

4.3.2.4 Statistics

Dose related response of enzymes on bread quality

Each enzyme was tested at three equidistant concentrations as listed in Table 4.2. The resulting breads were analysed as described above. Analysis of the variance was performed by the general linear model procedure. Multiple mean comparison within the sample set were carried

out at the 5 % significance level using the LSD. All statistical analyses were performed using the software Statgraphics® Centurion XV (Statpoint Technologies Inc., Virginia, USA). Impact factors were adopted from Kinner et al (2010); the emphasis was set on sensory attractiveness.

Experimental design

Based on the results of the dose related response, M-AMYL, G-AMYL, XYL-5 and CEL were selected for further evaluation of their combined effects on bread properties. The coded levels of independent variables are given in Table 4.3. A factorial 24-experimental screening design was developed by using the software Statgraphics® Centurion XV (Statpoint Technologies Inc., Virginia, USA) with three replicates at the center point leading to 19 runs.

Multiple Response Optimization

The optimum values of M-AMYL, G-AMYL, XYL-5 and CEL were obtained following a Multiple Response Optimization. Again impact factors were adopted from Kinner et al (2010).

Statistical analysis was performed using the software Statgraphics® Centurion XV (Statpoint Technologies Inc., Virginia, USA). All differences were considered significant at $P \leq 0.05$.

4.4 Results and Discussion

Dose related response of enzymes on bread quality

The effect of the enzymes on bread texture and sensory attributes is listed in Table 4.2.

CEL and XYL-M had an effect on bread volume. High and medium concentrations of CEL and low concentrations of XYL-M enhanced bread volumes. CEL and XYL-M also softened the crumb and thus improved the bread texture. CEL also increased the circumference of naked barley bread at high concentration. At high concentration XYL-5 increased the circumference of naked barley breads compared to non-supplemented control samples. The effects of Xylanases and Cellulases in naked barley bread correspond to the findings in wheat bread (Haros et al., 2002; Si and Drost-Lustenberger, 2002).

M-AMYL softened the crumb and thus improved the texture. It positively affected the consistence of the crumb especially at medium and high concentrations leading to a uniform pore distribution and a soft crumb. Moreover; M-AMYL improved the mouthfeel of the bread, especially concerning chewing and swallowing. G-AMYL also improved bread texture making the crumb softer and increased the browning of the crust. Low concentration of A-AMYL positively affected the mouthfeel. In the end, the amylases A-AMYL, G-AMYL and M-AMYL did not significantly increase loaf volume as it is stated in literature (Caballero et al., 2007). However, the higher softness of the crumb suggests a pronounced gas generation. The insufficient impact on bread volume can be explained by the poor gas retention capacity of naked barley flour (Bhatty, 1986).

Medium and high concentration of LACC ameliorated the characteristics of the crumb and led to a texture typical for bread. It also caused a more intense browning of the crust, albeit this amelioration was

not significant. The improved crumb structure goes along with the findings in wheat bread (Flander et al., 2008). Again the low gas retention capacity of naked barley is a putative explanation for the diminished effect on bread volume.

Experimental design

As the four enzymes, M-AMYL, CEL, XYL-5 and G-AMYL improved the quality of naked barley bread; they were selected for further evaluation. In contrast to the dose related response a factorial design was used to explore the interaction of these four enzymes. Table 4.3 displays the results of physical and sensory analysis of all enzyme combinations and concentration levels. Table 4.4 gives a summary of regression analysis.

CEL increased bread volume and circumference of naked barley bread; moreover, it promoted a more intense browning of the crust and reduced Fmax and F25. CEL in combination with G-AMYL lightened the crumb. Sensory evaluation ascribed CEL a positive effect on crust consistency and it showed the same effect in combination with G-AMYL.

Supplementation of M-AMYL caused a darker crumb and a higher elasticityrel. Weaker but still significant effects on a higher elasticityrel were obtained by combination of M-AMYL with CEL. M-AMYL reduced Fmax and F25 and thus caused a softer crumb. In the sensory evaluation M-AMYL improved the mouthfeel. The combination of M-AMYL and CEL improved the consistence of the crumb, even though this effect was not significant.

A combination of G-AMYL and CEL exhibited a positive impact on crumb with physical measurement. Additionally, a positive effect was detected in the sensory evaluation: G-AMYL combined with CEL or CEL alone improved the crust.

XYL-5 diminished Fmax and F25 and thus increased the softness of the bread.

Multiple Response Optimization

The multiple response optimization calculated a naked barley bread that contained M-AMYL and CEL in high (+1) and G-AMYL and XYL-5 in low (-1) concentrations. Good accordance between the statistical predicted properties and the observed values for the investigated variables was found. The deviations between predicted and observed values of the volume / kg flour, circumference, L*crust, L*crumb, elasticityrel, Fmax and F25 of predicted and observed values were 7.1, 1.7, 1.1, 0.2, 2.6, 7.3 and 6.3 %, respectively. Comparison of sensory evaluation showed that the ratings for consistence of the crumb and consistence of the crust were better than computed. The values for appearance, browning and mouthfeel hit the target but smell and taste were scored worse.

Table 4.5 shows the improvement of the bread baked with the optimized recipe (MRO) compared to the recently published (KINNER et al., 2010) control bread without enzymes. Enzyme addition strongly improved the sensory and physical characteristics of naked barley bread. In the sensory evaluation five of six parameters were rated better. The optimized bread reached full marks for the consistence of the crumb and the mouthfeel, whereas the bread without enzymes had previously achieved two of five scores for these parameters. The improvement in the physical determination of the bread characteristics is shown by the decrease of Fmax and F25 (40 and 47 %, respectively) which concomitantly lead to perceptible softer crumb. Volume, L*crust and circumference improved by 11, 8, 4 %, respectively.

4.5 Conclusion

This work is part of an optimization process for pure naked barley bread. The effects of nine commercially available enzymes on the quality

characteristics of pure naked barley bread were tested. Amylases, Cellulase and Xylase improved the naked barly bread; a combination of those enzymes further improved the bread. The application of maltogenic-amylase and cellulase in high concentration (+1) combined with gluco-amylase and xylanase in low concentration (-1) softened the crumb. The effects were detectable with physical texture measurements as well as with sensory evaluation. Thus, the addition of enzymes in naked barely bread can help to improve bread quality and promote its status in human nutrition. The impact of enzymes on shelf live and the applicability of different naked barley varieties for food production need further research.

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4.6 Tables

Table 4.1 Levels of enzymes chosen for the study

Enzyme species	Specific activity	Sphere of action [ppm]
A-AMYL	2500 FAU-F/g	2-10
CEL	3500 EGU/g	50-250
G-AMYL	1100 AGU/g	50-400
GO	10000 GODU/g	5-40
LACC	experimental sample	100
LIP	25 KLU/g	20-40
M-AMYL	10000 MANU/g	50-150
XYL-M	2500 FXU-W/g	30-75
XYL-5	2700 FXU-W/g	40-90

A-AMYL … Alpha-Amylase with FAU-F … Fungal Alpha Amylase Units, CEL … Cellulase with EGU … Endoglucanase Units, G-AMYL … Gluco-Amylase with AGU … Amyloglucosidase Units, GO … Glucoseoxidase with GODU … Glucose Oxidase Units, LACC … Laccase, LIP … Lipase with KLU … Lipase Units, M-AMYL … Maltogenic-Amylase with MANU … Maltogenase Units XYL-M and XYL-5 … (Endo-1,4-)Xylanase with FXU-W … Fungal Xylanase Wheat Units

Table 4.2 Effect of enzyme levels on the physical and sensory properties of bread.

Enzyme	concentration level	[ppm]	bread texture		sensory attributes	
			a	b	c	d
A-AMYL	low	2.0	-	+	+	+
	medium	6.0	-	+	-	-
	high	10.0	-	-	-	-
CEL	low	50.0	+	+	-	-
	medium	150.0	+	+	~	~
	high	250.0	+	+	-	-
G-AMYL	low	170.0	+	+	-	-
	medium	290.0	-	+	-	~
	high	410.0	+	+	~	+
GO	low	5.0	+	+	~	-
	medium	22.5	+	+	-	-
	high	40.0	~	-	-	-
LACC	low	50.0	+	+	+	-
	medium	100.0	+	-	~	~
	high	150.0	~	+	+	-
LIP	low	5.0	+	+	-	-
	medium	20.0	-	+	~	-
	high	35.0	-	+	-	-
M-AMYL	low	25.0	-	+	~	~
	medium	75.0	+	+	+	+
	high	125.0	-	+	+	+
XYL-M	low	30.0	+	+	-	-
	medium	52.5	+	-	-	-
	high	75.0	+	+	-	-
XYL-5	low	40.0	+	-	-	-
	medium	65.0	+	+	-	~
	high	90.0	-	+	-	~

a: volume/kg flour; b: $elasticity_{rel}$; c: mouthfeel; d: smell & taste; + positive effect; ~ no effect; - negative effect

Table 4.3 Factorial 24-experimental design with enzyme levels and observed values for physical and sensory attributes. Concentration levels of low (-1) to medium (0) and high (+1) are given according to the respective enzyme in Table 2.

	Enzymes				Bread texture							Sensory attributes					
Run	**A**	**B**	**C**	**D**	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**	**m**
01	0	0	0	0	2531	268	56.7	65.5	0.40	37.14	30.40	3	4	3	4	3	4
02	-1	-1	-1	-1	2531	265	59.1	66.7	0.39	45.86	39.38	3	3	3	2	3	3
03	+1	-1	-1	-1	2572	264	56.6	65.6	0.41	40.94	33.24	2	4	3	3	2	3
04	-1	+1	-1	-1	2531	268	56.5	66.9	0.39	46.77	40.63	4	3	3	3	1	2
05	+1	+1	-1	-1	2490	262	59.7	64.8	0.41	42.15	33.09	3	3	4	3	2	2
06	-1	-1	+1	-1	2586	265	59.4	66.8	0.39	42.36	38.25	3	3	2	2	2	4
07	+1	-1	+1	-1	2366	268	56.2	65.9	0.41	37.92	30.59	2	3	2	3	3	3
08	-1	+1	+1	-1	2655	265	58.6	66.5	0.40	42.61	37.61	3	3	3	2	2	2
09	+1	+1	+1	-1	2531	264	59.4	64.9	0.40	36.86	29.91	2	3	2	4	2	2
10	0	0	0	0	2669	266	56.3	66.0	0.41	40.65	32.98	3	4	3	3	3	3
11	-1	-1	-1	+1	2614	271	57.5	66.1	0.39	38.99	34.05	3	3	2	2	1	3
12	+1	-1	-1	+1	2738	269	55.4	64.7	0.41	35.94	28.31	3	4	3	5	4	3
13	-1	+1	-1	+1	2600	268	57.9	66.3	0.39	37.63	32.72	3	3	2	1	2	4
14	+1	+1	-1	+1	2834	269	53.5	65.4	0.43	37.98	28.46	2	3	4	3	3	4
15	-1	-1	+1	+1	2738	272	54.8	66.0	0.39	38.61	33.25	2	3	2	3	2	3
16	+1	-1	+1	+1	2751	274	53.8	64.4	0.42	36.33	27.33	2	3	3	4	3	4
17	-1	+1	+1	+1	2779	273	55.0	66.8	0.39	38.33	33.51	3	4	2	2	2	3
18	+1	+1	+1	1+	2614	268	58.0	65.1	0.41	31.78	24.83	3	3	3	3	3	3
19	0	0	0	0	2861	272	55.8	65.0	0.40	39.33	31.92	4	4	4	4	5	4

A: M-AMYL; B: G-AMYL; C: XYL-5; D: CEL; a: volume/kg flour, b: circumference/kg flour; c: L*crust; d: L*crumb; e: $elasticity_{rel}$; f: F_{max}; g: F_{25}; h: appearence; i: browning; j: consistence crumb; k: mouthfeel; l: smell & taste; m: consistence crust

Table 4.4 Summary of Regression analysis of factorial screening design for prediction of significant variables. Significant positive effects are marked with pos. and negative ones with neg., both are shown with the P-Value at the 95% confidence level.

		factor				factorial interaction					
observed parameter	R^2	A	B	C	D	AB	AC	AD	BC	BD	CD
bread texture											
volume / kg flour	66.43				pos. 0.0132						
circumference / kg flour	80.36				pos. 0.0012						
L* crust	68.38				neg. 0.0153						
L* crumb	88.02	neg. 0.0001								pos. 0.0310	
elasticity $_{rel}$	89.47	pos. 0.0001						pos. 0.0221			
F $_{max}$	92.66	neg. 0.0006		neg. 0.0059	neg. 0.0001						
F 25	97.02	neg. 0.0000		neg. 0.0095	neg. 0.0000						
sensory											
appearance	50.64										
browning	51.76										
consistence crumb	72.34										
mouthfeel	71.55	pos. 0.0076									
smell & taste	39.58										
consistence crust	71.51				pos. 0.0347					pos. 0.0347	

A: M-AMYL; B: G-AMYL; C: XYL-5; D: CEL; a: volume/kg flour, b: circumference/kg flour; c: L*crust; d: L*crumb; e: elasticity $_{rel}$; f: F_{max}; g: F_{25}; h: appearance; i: browning; j: consistence crumb; k: mouthfeel; l: smell & taste; m: consistence crust

Table 4.5 Comparison of pure naked barley bread with (MRO) and without (control) enzyme addition.

	MRO	control	% improvement
volume / kg flour [cm³]	2600	2339	11.2
circumference / kg flour [cm]	265	255	3.8
L*crust	54	59	8.4
L*crumb	65	68	-5.2
elasticity$_{rel}$	0.41	0.38	7.4
F_{max} [N]	39	65	39.5
F_{25} [N]	30	57	47.2
browning	4	3	33.3
consistence crumb	5	2	150
mouthfeel	5	2	150
smell & taste	3	2	50
consistence crust	4	4	0
appearance	3	4	-25.0

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5 Pure Naked Barley Flour – relating rheology, analysis and bread making properties[3]

5.1 Abstract

Naked barley is an underutilized crop with many health beneficial properties. Considerable amounts of β-glucans are responsible for the blood cholesterol, short-time blood sugar and weight-regulating effects. However, the use of new crops in food production demands to question the applicability of standard methods that are commonly tailored to wheat. The verification or modification of traditional methods is indispensable to reliably describe naked barley flours and predict their baking qualities. Physical dough tests are commonly used to estimate the baking qualities of wheat flours but they can hardly predict bread quality. In contrast, baking experiments allow meaningful statements but they are time and raw material intensive. The establishment of quick and meaningful methods could promote the breeding of new cultivars for food production and subsequently increase the implementation of naked barley flour in staple foods.

The aim of the present work was to predict the baking qualities of naked barley flour with a quick and raw material saving method. It was studied whether the Micro-Z-Arm was a suitable method to compare the rheological properties of naked barley doughs. Furthermore, correlations between the rheological curves, chemical analyses and bread quality were studied.

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It was shown that the Micro-Z-Arm could picture differences between naked barley doughs. Furthermore, the rhological curves correlated with bread quality when they were combined with analyses of protein, starch, β-glucans and ash-content. The results were first promising steps to establish a quick method to compare naked barley flours and therefore facilitate the breeding of new crop lines.

Keywords: Hordeum vulgare, hull-less barley, bread, multiple response optimization, beta-glucan, micro-Z-arm

5.2 Introduction

Naked barley is an underutilized crop that is a putative source for functional foods for many reasons. First of all it is a good source of soluble (SDF) and insoluble dietary fibre (Bhatty, 1999; Izydorczyk et al., 2000). The respective dietary fibre content underlies a natural fluctuation depending on the variety and pre- and post-harvesting conditions. The soluble dietary fibre fractions that are mainly mixed-linkage (1-3),(1-4)-β-d-glucans (henceforth named β-glucans) are quantitatively more important. The level of β-glucans in high-amylose and waxy naked barleys is stated about 8% whereas regular naked barleys exhibit significantly less (Gao et al., 2009; Tiwari and Cummins, 2008; (Xue et al., 1997)). Arabinoxylans are a fraction of partly soluble nonstarch polysaccharides (NSP) occurring in the cell walls. Both fibre fractions are present in naked barley and have great impact on cereal processing and product properties. The positive effects of β-glucans are mainly attributed to their high viscosity in aqueous solution and thus increasing the viscosity of the contents within the intestinal tract (Jalili et al., 2000). Other studies underline its short time blood sugar regulating effects (Cavallero et al., 2002). β-Glucans also offer health benefits concerning the cardiovascular system and weight management; numerous scientific studies have

demonstrated that β-glucans can help to maintain cholesterol levels that are already within the normal range. In Europe barley- and oat-containing products will be allowed to claim the cholesterol lowering properties of β-glucans (EFSA, 2009). The US Food and Drug Administration (FDA) approved the health claim for β-glucan-containing food to lower the risk of coronary heart diseases (21CFR101.81). Beside the positive effects that are associated to the dietary fibre content coloured naked barley varieties exhibit anticancerogenic flavonoids (Andersson et al., 2003).

In contrast to hulled barley naked barley (Hordeum vulgare var. nudum) is free threshing and requires no further dehulling because the expression of the naked caryopsis gene (nud) prevents the intergrowth of husks and caryopsis. The removal of the fibre-rich hull proportionally increases the levels of protein, starch and nutrients in naked barleys (McGuire and Hockett, 1981; Newman and Newman, 2005).

A further advantage in context with dietary fibre intake is the distribution of β-glucan within the barley grain. Considerable amounts of β-glucan are located in the endosperm; consequently, also refined flour contains high amounts of dietary fibre. In average the intake of white bread exceeds the intake of wholemeal bread. The population of Austria eats on average 100 g white bread but only 16 g of wholemeal bread per day (Elmadfa et al., 2009). Thus, pure naked barley bread that resembles conventional white bread could increase the β-glucan intake of the average consumer.

Despite the numerous advantages of naked barley it is not yet established in human consumption in Western civilisation. Previous studies have shown that it was possible to bake pure naked barley bread with good sensory properties and a β-glucan content high enough to meet the demands of the stated health claims (Kinner et al., 2010). To commercially use naked barley as a raw material for staple food it is inevitable to facilitate the breeding of appropriate varieties and adapt the associated technological processes as good as possible. Two problems that have to be faced in this regard are the economic selection of new varieties

and the verification or modification of analysis methods that are tailored to wheat. Several studies suggest that dough rheological methods are useful to qualitatively compare wheat flours but that they cannot satisfactorily predict the performance of doughs in the baking process. The statistical selection of several parameters was shown to give more meaningful information about bread quality (Bettge et al., 1989; Dobraszczyk et al., 2005). The use of dough rheological methods has limited informative value for wheat; consequently, the results obtained with other cereals must be critically questioned even more. There are studies that confirm the applicability of dough rheological tests on other cereals (Cuevas and Puche, 1986). Mixograms of wheat flour and naked barley flour at various mixing ratios revealed the poor dough building capabilities of naked barley flour. Baking trials confirmed these findings but no regression models were created (Bhatty, 1986; 1997).

The aim of the present work was to predict the baking qualities of naked barley flour with Micro-Z-Arm data and chemical analysis to substitute raw material and time intensive baking experiments.

5.3 Materials & Methods

5.3.1 Plant material

Seven spring naked barley varieties (Table 5.1) were grown in 2008 in Raasdorf (16°35‘ E, 48°13‘ N), Austria, on fields of the BOKU experimental station Groß-Enzersdorf.

5.3.2 Chemical analysis

All chemical analyses were done in three replicates. Dry substance was determined according to ICC standard method 110/1 (ICC, 2006) and ash content was performed according to Approved Method 08-01 (AACC,

2000). Crude protein content was determined with the Dumas combustion according to ICC standard method 167 (ICC, 2006) using a CN-2000 (Leco Instrumente GmbH, Mönchengladbach, Germany). In the CN analyzer the sample was combusted in an oxygen-rich environment at about 1000°C to give oxides of nitrogen which were catalytically reduced to nitrogen. Nitrogen gas was measured with a thermal conductivity detector. Total nitrogen was calculated from the detector response. The nitrogen content was transferred into protein content by multiplication with the conversion factor 5.7. Total starch (AOAC method 996.11) as well as β-glucan (AOAC method 995.16) was determined enzymatically (Megazyme, Bray, Ireland).

5.3.3 Milling

Water content varied in natural ranges and is listed for each variety in Table 5.2. Milling was accomplished with a MLU 202 roller mill (Bühler, Switzerland). All six flour fractions (B1-B3, C1-C3) were combined to one flour sample and used for baking trials, bran and shorts fractions were collected separately.

5.3.4 Micro Z-arm

Dough properties of each sample were recorded with a micro Z-arm mixer (Metefem, Hungary). The Approved Method 54-21 (AACC, 2000) following the constant flour weight procedure was adjusted to the micro Z-arm; consequently, only 4g flour were necessary. Water absorption was determined in preliminary runs. Time to breakdown (QN) was taken for interpretation and is defined as space of time from the beginning of the increase of the centre line till the point where the centre line reaches 50 units under maximum of the curve.

5.3.5 Bread making

Bread baking was conducted according to Kinner et al. (2010). Three tin breads of each flour were produced. The recipe included 914 g naked barley flour and the following ingredients (% flour weight): 1.3% dry yeast, 2% malt flour, 0.08% pre-gelatinised flour, 0.01% acidifier, 1.64% sugar, 1.64% salt and 1.05% margarine. Water adsorption from the micro-Z-arm curves was not directly transferred to the baking trials since the doughs contained further ingredients. Therefore, water addition was individually adapted for each dough to optimize dough performance. Hora, Taiga, Ae13, Digersano, ICARDA Black Naked and Washonubet contained 48.69%, 52.29%, 53.43%, 49.61%, 55.22% and 62.80% water, respectively. The dough was kneaded for 3 min (KM020 Titanium Major, Kenwood, UK) at speed level 2.5. After proofing (30°C and 85% rH) for 17.5 min in a model G66W proofing chamber (Manz Backtechnik Gmbh, Creglingen, Germany) 400 g were filled into baking tins and proofed again at equal conditions for 10 min. Baking started at 230°C with top and bottom heat for 35 min in a BS60/3W baking station (Manz Backtechnik Gmbh, Creglingen, Germany). The oven was steamed once after loading at the maximum level and the temperature was subsequently reduced to 180°C.

5.3.6 Bread characteristics

Breads were cooled after baking for one hour at room temperature and subsequently stored for 24 h ± 2 h at 20°C and 50% rH (MMM, Medcenter Einrichtungen GmbH, Germany).

Bread volume (V) was determined according to ICC-Standard method 131 (ICC, 2006) with the rapeseed method (Chopin, France) and expressed on the basis of 1 kg flour. Color of crumb (L*) was measured in the CIELAB color space (Micro Color, Dr. Lange, Germany). Bread firmness (F25) was collected after AACC Method 74-09 (AACC, 2000) as follows:

bread cubes (40×40×25 mm l×w×h) with removed crust were compressed using the TA-XT2iR Texture Analyzer (Stable Micro Systems™, Great Britain) with a 25kg cell. The force [N] at 25% compression was taken for statistical analysis (10 replications per bread).

5.3.7 Statistical analysis

Statistic was calculated with STATGRAPHICS Centurion® XV after eliminating outliers using box and whisker diagrams. In a first step simple regression was computed with V, F25 and L* as dependent variables and QN, ash, β-glucan, protein and starch as independent variables. Multiple regressions were performed for V, F25 and L*. All chemical analyses (ash, β-glucan, protein, starch) were combined to one set of independent data (a). Multiple regressions were performed with the set of chemical data extended to include the QN value (a+QN).

5.4 Results & Discussion

5.4.1 Milling

The milling trials with the MLU 202 showed that it was possible to mill naked barley with wheat milling technology with the difference of a lower flour yield (Table 5.2) which goes along with other literature (Andersson et al., 2003). Reasons therefore can be the softer kernel texture as well as the composition of the endosperm; e.g. the waxy variety Washonubet had the lowest flour yield. In general the flour seemed to be sticky which is reflected in the high amounts of shorts which contained obviously a big bulk of flour fraction. Hence a way to raise flour yield could be the use of a bigger sifter or purification steps as for example bran centrifuges. In general yellow varieties had higher flour yields followed closely by the black grained ones.

5.4.2 Micro-Z-arm

Naked barley flour has sufficient dough building characteristics with low values of water absorption. Yellow and black varieties have a similar water absorption that ranges from 50 to 55%; the waxy Washonubet had a higher water absorption with 64%. The micro-Z-arm curves were comparable to common farinograms; hence, the curve strongly ascended in the dough building area and descended in the dough stability area; albeit, the decrease was more distinct compared to wheat. The curves illustrated the good dough building capabilities of naked barley flours; even though dough stability was weaker compared to wheat. This observation goes along with literature (Bhatty, 1986). The kernel color seemed to have no impact on the curves. The QN values are listed in Table 5.3. The yellow grained variety Hora and the black Ae 14 dunkel had the highest QN compared to the other samples which ranged between 0.6 and 1.35 min for QN. These results suggest that the higher content of secondary plant metabolites in black varieties that goes along with additional health benefits has no negative impact on the dough characteristics compared to yellow varieties. The waxy variety Washonubet had the lowest QN which goes along with findings in wheat (Hung et al., 2006).

5.4.3 Baking trials

All doughs of the different naked barley varieties were processable and showed good leavening capabilities. The breads differed in their crust and crumb formation but all varieties exhibited cracks in the crust. The bread crumb of Washonubet which had the highest water absorption was too humid and hence had an almost flat crust. This problem is attributed to the strong water binding capacity of β-glucans and the different starch composition of waxy varieties. Since the other breads had rather dry crumbs a combination of different varieties might lead to naked barley

breads with a soft and spongy crumb. Furthermore the blending of flour fractions could increase the β-glucan content.

Interestingly the yellow and black varieties Hora and Ae 13 dunkel, with the highest QN (which came close to the QN levels of wheat) had very low bread volumes. Naked black and Digersano which had mean values for QN exhibited the highest bread volumes in the baking experiments. Compared to wheat the QN values were very low. The results underline the need to find analysis methods appropriate for naked barley to predict its baking properties.

5.4.4 Simple regression

Table 5.4 lists the significant correlations of the simple regression. Volume and starch correlated with no other parameter. Protein, β-glucan and ash content had a meaningful impact on L*. Protein and β-glucan darkened the crumb by increasing the water binding capacity with a higher effect of protein. β-Glucan had the highest impact on F25 followed by protein and ash. β-Glucan and protein enhanced the water absorption and thus softened the crumb. Ash had the same effect because it weakened the gluten network by dilution.

5.4.5 Multiple regression

Multiple linear regression models for the three bread quality parameters baking volume (V), crumb firmness (F25) and crumb colour (L*) are given in Table 5.5 with their P-values and the coefficient of determination R^2. R^2 adjusted is also given which allows a comparison between regression models with different amounts of factors. Two different models (a, a+QN) illustrate the influence of the measured parameters on the correlation. The correlation models that included the values of the flour analysis (a) gave significant correlations. Coefficients of determination for these regression models ranged from 0.46 for V up to

0.96 for L*. The combination of QN and the four values of chemical analysis (a+QN) gave significant regression models for all three quality parameters. The combination of both parameters ended up in higher coefficients of determination.

QN meaningfully raises R^2 to 0.61% in the regression model for V; the impact on the other two bread quality parameters was less distinct. The existing correlation of V, QN and the four chemical parameters can help to estimate bread volume without baking experiments.

5.5 Conclusions

Rheological dough tests, chemical analyses and baking trials were performed to predict and differentiate the bread making qualities of seven naked barley flours. Bread properties underlay variations within the naked barley varieties. The micro-Z-arm is a method commonly used for wheat could picture differences between the seven naked barley varieties. However, there was no correlation with bread quality parameters. The differences within the naked barley varieties were also pictured with chemical analyses. Ash content, β-glucan and protein correlated with the softness and color of the crumb. Bread volume became predictable by combining micro-Z-arm data with the results of chemical analyses in a multiple regression.

The results might help breeders to predict bread quality without time and raw material intensive baking trials and thus accelerate the selection process with a multiple regression model based on chemical analysis and micro-Z-arm data. Both, the baking properties and the interpretation of micro-Z-arm curves of pure naked barley flour need further research to comprehensively reveal the technological aspects of naked barley and thus use its full potential.

5.6 Tables

Table 5.1 Ear characteristics and origin of the investigated barley genotypes.

Genotype	Origin	Donor	Ear morphology	Pericarp colour
Hora (BVAL350010)	DE	AGES, Linz, AT	2-row	*blx*
Taiga (BVAL350017)	DE	AGES, Linz, AT	2-row	*blx*
Ae 13 dunkel (BVAL358117)	ET	AGES, Linz, AT	6	*Blp*
Naked Black (BVAL358163)	ET	AGES, Linz, AT	2	*Blp*
Digersano	IT	ISC, Fiorenzuola d'Arda, IT	2-row	*blx*
ICARDA Black Naked	SY	IFA, Tulln, AT	6-row	*Blp*
Washonubet (waxy)	US	VUKROM, Kromeriz, CZ	2-row	*blx*

Country codes: AT, Austria; CZ, Czech Republic; DE, Germany; ET, Ethiopia; IT, Italy; SY, Syria; US, United States of America

Pericarp colour: *Blp*, black pericarp; *blx*, white/yellow pericarp

Table 5.2 Kernel Moisture (%) and flour yield (%) from seven naked barley varieties milled with a Bühler MLU 202

	Hora	Taiga	Ae 13 dunkel	Naked Black	Digersano	ICARDA Black Naked	Washonubet
moisture (%)	13,65	13,75	13,29	13,41	13,77	12,77	13,30
flour fraction							
B1	5,7	4,9	3,9	3,7	5,0	4,6	1,6
B2	5,2	4,3	3,8	4,1	5,4	2,8	3,5
B3	1,7	1,5	1,1	0,9	1,8	1,1	1,2
B total	*12,5*	*10,8*	*8,7*	*8,7*	*12,2*	*8,5*	*6,3*
C1	9,9	7,0	6,3	5,2	9,1	6,0	4,8
C2	2,4	2,3	2,3	1,8	2,9	2,2	1,9
C3	1,4	1,5	1,7	1,5	2,6	1,3	1,3
C total	*13,7*	*10,8*	*10,4*	*8,5*	*14,6*	*9,5*	*8,0*
flour total	*26,2*	*21,6*	*19,1*	*17,2*	*26,8*	*18,1*	*14,3*
bran	29,6	28,2	27,0	30,3	24,2	21,4	39,8
shorts	44,2	50,2	53,9	52,5	49,0	60,5	45,9

Table 5.3 Results of the chemical analyses of the naked barley flours based on dry matter; QN obtained with the micro-Z-arm and physical bread characteristics.

	chemical analysis				micro-Z-arm	bread characteristics		
genotype	**ash [%]**	**β-glucan [%]**	**protein [%]**	**starch [%]**	**QN [min]**	**volume / kg flour [cm³]**	**F25 [N]**	**L* crumb**
Hora	0,71	1,48	7,77	76,14	12,80	4839	65,15	77
Taiga	0,85	1,77	7,83	76,80	1,10	4820	64,88	76
Ae13 dunkel	0,89	1,81	9,31	41,37	6,40	4922	51,79	63
Naked Black	1,18	1,94	9,79	73,97	1,35	5165	49,37	62
Digersano	0,66	1,38	7,64	76,16	1,10	5215	55,12	77
ICARDA Black Naked	1,72	1,90	9,83	75,62	0,90	4908	50,25	59
Washonubet	1,19	2,28	9,29	72,99	0,60	4849	38,57	68

Table 5.4 Results of simple regression for crumb firmness (F25) and lightness of the crumb (L*) with the three regressors ash, β-glucan and protein, respectively.

	ash			β-glucan			protein		
	p-value	**r**	**R²**	**p-value**	**r**	**R²**	**p-value**	**r**	**R²**
F25	0.0248	-0.4879	0.2381	0.0008	-0.6734	0.4537	0.0014	-0.6493	0.4216
L*	0.0000	-0.8160	0.6659	0.0030	-0.6156	0.3790	0.0000	-0.9565	0.9149

Table 5.5 Results of multiple regression for bread volume (V), crumb firmness (F25) and lightness of the crumb (L*). Regressors for statistical calculation were taken as follows: a –results of the analysis of ash, β-glucan, protein and starch; a+QN – combination of the data of a and QN.

	regressors	multiple linear regression model	p-value	R^2	R^2 adj.
V	a	V = 3062.82 -513.54*ash -446.85*β-glucan +295.18*protein +9.01*starch	0.0321	0.4641	0.3301
	a+QN	V = 3438.43 -569.05*ash -554.75* β-glucan +301.15*protein +7.41*starch -18.70*QN	0.0086	0.6115	0.4820
F25	a	F25 = 142.84 +10.26*ash -14.89*β-glucan -7.10*protein -0.15*starch	0.0111	0.5375	0.4219
	a+QN	F25 = 134.05 +11.56*ash –12.37*β-glucan -7.24*protein –0.11*starch +0.44*QN	0.0183	0.5652	0.4203
L*	a	L* = 109.32 -7.94*ash +4.25*β-glucan -5.55*protein +0.13*starch	0.0000	0.9615	0.9515
	a+QN	L* = 108.51 -7.82*ash +4.48*β-glucan -5.56*protein +0.13*starch +0.04*QN	0.0000	0.9620	0.9493

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6 Conclusion

Even though naked barley is a crop rich in β-glucans and several secondary plant metabolites its poor baking qualities have impeded a more pronounced use in human nutrition. The aim of the present work was to develop sensorily attractive naked barley bread and thus promote the use of naked barley. To estimate the potential of naked barley concerning bread making the characteristics of different naked barley varieties were compared with the help of an optimized recipe.

Naked barley flour requires high amounts of water during dough building and has a rather short dough stability. These characteristics demand a reduced kneading and leavening time to maintain bread volume. Naked barley breads have a dry and crumbly crumb; however, the addition of malt flour, margarine, pregelatinized flour, acidifier and higher amounts of water improve the qualities and make pure naked barley bread sensorily attractive. The level of β-glucans in the bread is high enough to meet the requirements of the EFSA Health Claim without substitution of extracts.

A further optimization of the existing recipe was accomplished with the use of various enzymes. The application of maltogenic amylase and cellulase as well as gluco amylase and xylanase contributed to a further improvement of naked barley bread. The amelioration of crumb texture was detectable with physical measurements and with sensory analysis.

Baking experiments are the most meaningful way to examine the baking qualities of flour. However they are time-, raw material- and labor intensive and thus make the selection of new varieties more difficult. Consequently, the potential of many colored naked barleys with additional health benefits that are linked to their high content of secondary plant metabolites are yet unknown. To economize research and promote the establishment of new naked barley varieties a further aim was to

substitute baking trials with a more time and raw material saving method. For this purpose the applicability of a micro rheological method was tested. The results of the rheological tests illustrated the differences between various kinds of naked barley flours and allowed an estimation of the baking qualities. When these results were combined with data of chemical analyses (ash, β-glucan, protein and starch) the computed multiple regression model could be used to predict bread quality.