M. Krahl, C. Hagel, M. Zarnkow, W. Back and S. Kreisz

Changes of the Content of Water-soluble Bioactive Compounds during the Malting Process of Spelt Wheat (*Triticum aestivum var. spelta*)

Spelt wheat (*Triticum aestivum var. spelta*), a hexaploid variety of the genus Triticum is a cereal closely related to common wheat (*Triticum aestivum*). The major non-starchy carbohydrates in spelt wheat are arabinoxylans and fructans. Furthermore spelt wheat is known to contain relatively high levels of thiamine and riboflavin, two vitamins from the B-group. In this work the influence of a standard malting process on the content of these four bioactive compounds was studied. The amount of water-extractable arabinoxylan increased significantly during the malting process, as well as the water-extractable riboflavin content. The fructan concentration in the analysed samples showed a slight increase and thiamine levels staid stable.

Descriptors: spelt wheat, Arabinoxylan, malting, Thiamine, Riboflavin, Fructan, dietary fibre

1 Introduction

Nowadays the worldwide nutrition is mainly based on only three cereals, wheat, rice and corn; they contribute over 75 % to the worlds starch production. At the same time diseases caused by wrong or unbalanced diet are becoming problematic in western countries. In this regard the enrichment or conservation of bioactive compounds in the malting process with the objective to provide their beneficial health effects to the consumer is a very important field.

Spelt (*Triticum aestivum var. spelta*), a hexaploid sub-species of common wheat [1], is an ancient bread cereal that is still grown in Europe and some other regions of the northern hemisphere [2]. The use of spelt in cooking and baking has been a tradition in Europe, particularly in parts of Germany, Switzerland and South Tyrol where it was widely used in many grain based foods [3]. The spelt wheat grain is a covered kernel cereal, the glumes are harvested with the kernel [2] and has to be peeled prior to further processing in the malting or food industry. Spelt wheat demonstrates a higher resistance to environmental factors like poorly drained, low-fertility soils than common wheat and can be cultivated in harsh ecological conditions, without the use of pesticides [4]. A fact which makes spelt wheat suitable for organic farming. During the 20th century the acreage of spelt wheat was continuously dropping. Due to a growing number of food intolerances against wheat, apart from celiac disease, spelt wheat is now being rediscovered in Europe and North America [5]. Formerly it has been suggested that spelt wheat may not trigger the gluten-intolerance reaction in patients suffering from celiac disease because it might lack gluten but several studies negated this suggestion [3] [6] [7] [8] [9]. Previous studies indicated that spelt wheat is higher in vitamin and mineral content than common wheat used for bread making [3]. The average protein content of spelt wheats has shown to be significantly higher than that of common wheats [6] [4].

The malting of spelt wheat especially for baking purposes has gained in importance in the last years in Germany. Additionally there has also been a developing market for beer made of spelt wheat. Consequently the relevance of malted spelt as a raw material for the food and beverage production is growing.

Arabinoxylans are a part of the natural occurring dietary fibres in the plant kingdom. The chemical structure of arabinoxylan is based on a chain of linear β(1-4)-D-xylopyranose units, which can be substituted with α-L-arabinofuranose in the O-2 or the O-3 position or both [10]. Arabinoxylans in the cell wall are partly cross-linked by diferulic acid bridges and possibly other condensation products of ferulic acid [11], which may complicate their solubilisation. The content of water-extractable arabinoxylan (WEAX) increases during the germination in the malting process, as the cell walls are being degraded [12]. The solubility of arabinoxylan also increases with a higher degree of arabinose substitution.

Among plant carbohydrates, arabinoxylans are non-digestible ingredients, which are not degraded or absorbed in the stomach or in the small intestine and reach the colon intact [13]. There they are fermented to a large extend, especially the water-extractable part, by the large bowel microflora to lactic acid and short chain fatty acids (SCFA). These can be absorbed and metabolised by the host. Associated with this is the prebiotic effect of soluble dietary

Authors: M. Krahl, C. Hagel, M. Zarnkow, W. Back, Technische Universität München, Lehrstuhl für Technologie der Brauerei I, Weihenstephaner Steig 20, 85354 Freising-Weihenstephan, Germany; Moritz.Krahl@wzw.tum.de; S. Kreisz, Novozymes A/S, Bagsværd, Denmark

Tables see Appendix

fiber, which has been shown to result in enhanced concentrations of probiotics such as *Lactobacilii* and *Bifidobacteria* in the colon lumen [14]. SCFA like propionate, butyrate or lactate are the end-products of probiotic carbohydrate fermentation. They are used as an energy source by the colonic epithelial cells [15], as well as they have a beneficial effect on human liver cells. Especially butyrate is an important factor in maintaining normal functions in colonocytes and a protective agent against colon cancer [16]. By decreasing the pH of the colon contents SCFA also prevent the growth of pathogenic bacteria [15]. The lower pH in the colon in combination with the SCFA significantly increases the mineral absorption, especially of calcium and magnesium from the gut. The calcium absorption decreases when people are aging. So the relative increase in absorption induced by soluble dietary fiber gets more important for elder population groups [17]. This is of course of even more interest for people suffering from osteoporosis.

Fructans are β-D-linked polymers of fructose. Approximately 15 % of higher plants store fructans [18]. Fructans can be stored in plants in high concentrations as an alternative to starch. The substrate of fructan biosynthesis is sucrose. Chemically cereal fructans are a linear carbohydrate material consisting mainly, if not exclusively, of β(2-1)-fructosyl-fructose linkages. A starting α-D-glucose moiety is mostly present but not necessary [17]. Plant fructans generally show a degree of polymerisation (DP) less than 50, although some have been shown to exceed 200 [19]. Fructans are divided into inulin, fructooligosaccharides (FOS) and neosugars. Long-chained polymers with a DP greater than 30 are referred to as inulin. FOS have a DP of up to 10, and FOS with a DP of 3–5 are called neosugars [20]. These oligosaccharides are food products with interesting nutritional properties. The extent of resistance to enzymatic reactions occurring in the upper part of the gastrointestinal tract allows fructans to become colonic nutrients, as some bacterial species express specific hydrolases and are able to convert these sugars into short-chain fatty acids and/or gases by fermenting them [21].

Thiamine and riboflavin are two vitamins of the B-group. Thiamine or vitamin B1 is an essential coenzyme in the carbohydrate metabolism. It is a constituent of all living cells and acts as a cofactor for many other key enzymes as well [22]. Insufficient supply may lead to interferences in the citric acid cycle, glycolysis and pentose phosphate cycle for example [23]. The recommended daily uptake is 1.0–1.3 mg for adults. Riboflavin or vitamin B2 is an essential coenzyme in the protein metabolism. The recommended daily uptake for adults is 1.2–1.6 mg. The most important sources for vitamin B1 and B2 in the human nutrition are vegetables, legumes and whole-grain products [24].

The aim of this work was to study the changes of these bioactive compounds in the malting process with the objective to provide their health-beneficial attributes to consumers. The results of this study will give useful information for optimising their content in spelt wheat through malting. The focus of this work was the water-soluble amount arabinoxylan, riboflavin, thiamine and fructan in malted spelt wheat. These compounds could be found in wort and any beverages derived from it and thus contribute to the nutrition.

2 Materials and Methods

2.1 Samples

The spelt wheat sample has been obtained from a commercial organic farmer in Nördlingen, Germany harvested in 2007. The protein content of the used spelt wheat sample was 13.5 % (N x 6,25) dry mater, water content was 9.5 % and the germination energy was 96 %.

2.2 Malting

Malting was done in a pilot scale malting facility. The degree of steeping was 45 %, the germination temperature 15 °C and the germination time was 7 days.

Withering was done at 50 °C for 14 hours and kilning one hour at 60 °C, one hour at 70 °C and one hour at 80 °C.

Samples were taken each day after steeping during the malting process, freeze dried, milled and stored at –18 °C until the analyses were done.

2.3 Carbohydrate Analysis

The separation of the carbohydrates Arabinose (Roth, Germany), fructose, glucose and xylose (Sigma-Aldrich, Germany) was done on a high-performance anion-exchange chromatography system with pulsed amperometric detection (HPAEC/PAD) from Dionex. The analysed samples were injected using an AS 50 autosampler, the pump system used was a GP 40 Pump. Separation of monosaccharides was done using a CarboPac 10 analytical column and detection by an ED 50 detector, all from Dionex.

Eluent A was 250 mM NaOH (Baker, Germany) and eluent B was water. The flow-rate was 0.25 ml/min and the injection volume 2.5 μl. Monosaccharides were separated by isocratic elution (20 min, 5 % A). Adjacent polysaccharides and starch was removed from the column (35 min, 80 % A). The column was re-equilibrated subsequently (20 min, 5 % A). The potential during the detection of the monosaccharides was programmed from +0.1 (t = 0–0.4 s) to –2 (t = 0.41–0.42 s) to 0.6 (t = 0.43–0.44 s) and finally –0.1 (t = 0.44–0.5 s). Integration of the resulting signal was done from t = 0.2–0.4 s

2.4 Determination of total (AX) and water-extractable (WEAX) arabinoxylan

The analysis of AX and WEAX have been carried out similar to the method presented by *Houben et al* [25], with slight modifications. Due to a better separation of the monosaccharides glucose and xylose by the CarboPack 10 column, the treatment with glucoseoxidase was not necessary in the modified method. For the determination of the total AX content the samples were milled in a laboratory hammer mill (Laboratory Mill 3100, Danfoss). Then 0.1 g of the sample was dispersed in 4.0 mL of distilled water and 4.0 mL of 4 M HCl and hydrolysed for 60 minutes in boiling water in a normal laboratory screw-cap test tube. The samples were cooled to room temperature and 4.0 mL of 4.0 M NaOH was

added to neutralise the samples. Subsequently the concentration of monosaccharides was measured by HPAEC/PAD.

The amount of water-extractable arabinoxylan was determined by adding 1.5 mL of water to 0.1 g of milled sample. After stirring at 65° C for approximately 60 minutes and subsequent centrifugation, 1.0 mL of the supernatant was removed, hydrolysed and neutralised as described above.

Prior to analysis by HPAEC/PAD the chloride ions were removed using OnGuard II Ag cartridges from Dionex. The contents of AX and WEAX were calculated as the sum of the pentose sugars arabinose and xylose multiplied by 0.88 to correct for anhydro monosaccharides [25]. All analysis were carried out in duplicate. The coefficient of variation was less than 2 %.

2.5 Determination of fructan

The analysis of the fructan content of the samples was done according to the AOAC Method 999.03 with the modifications presented by *Andersen et al.* [26] and with some further slight modifications. For the extraction of fructan 0.1 g finely ground grist of each sample were mixed with 1.5 mL of water and stirred at 80 °C for about 60 minutes. Following centrifugation the amount of monosaccharides was measured.

In the enzymatic step 0.1 mL of Inulinase (EC 3.2.1.7, Novozymes, Denmark) was added to 0.2 mL of the supernatant and stirred at 37 °C for 60 minutes. Subsequently the resulting monosaccharides were measured.

The amount of fructan in the sample was calculated by subtracting the amount of fructose and the calculated amount of fructose formerly bound in sucrose measured before the enzymatic treatment from the total fructose analysed after the enzymatic step. In this study the possibly present final glucose moieties were not taken into account because fructans were calculated as dietary fibre and the final glucose lacks prebiotic properties. All analysis were carried out in duplicate. The coefficient of variation was less than 2 %.

2.6 Determination of thiamine and riboflavin

The determination of thiamine and riboflavin was done using the whole-blood test kits from Chromsystems (Germany). These kits proved to be suitable for beer, wort and aqueous extracts from malt because no interference with other substances occurred during the analysis.

The chromatographic separation of the samples was done on an Agilent 1100 system equipped with Chemstation software.

Thiamine and Riboflavin for the calibration of the system were obtained from Sigma-Aldrich (Germany).

For the vitamin extraction from the samples, 100 mg of finely ground spelt grist were weighed accurately into Eppendorf vials, extracted with 1.5 ml water at 45 °C for 1 hour, centrifuged and analysed following the instructions for the whole blood analysis. All analysis were carried out in duplicate. The coefficient of variation was less than 4 % for both methods.

3. Results and discussion

3.1 Arabinoxylan

The total arabinoxylan content of the spelt wheat used in this study was 10.05 % in the raw material. During the malting process only a slight increase up to 10.24 % could be observed. As the embryo is not producing arabinoxylans during germination but breaking down the cell wall constituents and catabolising the non-starchy cell wall polysaccharides and considering the variation coefficient, this slight increase is of no statistical significance. Water-extractable arabinoxylan in the unmalted grains was 0.51 % dry mater or 5.1 % of the total arabinoxylan content. During malting the water-extractable arabinoxylan content increased up to 1.01 % of the total kernel weight equivalent to 9.7 % of the total arabinoxylans. The results for each day of the malting process are shown in Table 1.

In comparison to wheat the percentage of water-extractable arabinoxylan in the spelt-wheat kernel and malt was relatively low although the increase of about 100 % during the malting process is similar to the increase observed in wheat and rye [12].

3.2 Fructan

The fructan content in the spelt wheat grains prior to the malting process was 1.11 %. During the germination the fructan content decreased at the beginning of the pregermination phase and increased again in the following days. The amount of fructan in the kilned malt reached 1.75 % or 140 % of the original value. The fructan content increased 40 % during the malting process. The results for each day of the malting process are shown in Table 2.

A possible explanation for this development could be the fact that plants store fructans as a reserve carbohydrate which helps them during cold and harsh weather conditions. For example a high fructan content in plant storage carbohydrates is associated with better freezing tolerance [27]. In the pregermination phase the embryo could use the easily accessible fructans and possibly rebuild them later when the energy supply is kept up by hydrolysed starch.

3.3 Thiamine

The development of the thiamine concentration during the malting process is shown in Table 3. The concentration of thiamine after kilning is 395 μg/100 g and thus 16 % lower than the concentration in the unmalted grains. The thiamine content in spelt wheat found in this study was 470 μg/100 g and is comparable to previously measured values which varied from 330 μg/100g to 520 μg/100g [3] [28] [5]. The losses during the malting process might be caused by the essential role of thiamine in the energy metabolism of carbohydrates. Thiamine seems to be used by the embryo during growth. Previous studies obtained similar results for beans, lentils and peas [29].

3.4 Riboflavin

The Riboflavin concentration in the unmalted spelt wheat was significantly lower than those previously reported [3] [28] [5]. The development of the B2 content in the kernels during the malting process is shown in table 3. The amount of riboflavin measured after kilning was 340 % of the original measured vitamin content. The concentrations augmented from the beginning of the germination. In contrast to the human metabolism plants apparently do not need riboflavin during their respiration and carbohydrate metabolism or to build up significant levels during respiration. During the germination of rapeseed, beans, lentils and peas an increase of the riboflavin levels was reported earlier [29] [30].

4 Conclusion

This work shows that malting may be an effective way to enrich and preserve water-soluble bioactive compounds in spelt wheat. During malting of spelt wheat the amount of fructan increased by 40 %, the water-extractable arabinoxylans by 100 % and the riboflavin content by 240 %. In this study only the level of thiamine decreased by 16 %. Through optimised malting conditions it might even be possible to obtain better results for the enrichment of these and possibly other health beneficial natural substances. The resulting malts can be used as raw materials for several purposes in the food and beverage industry as for example for the production of beer, bread, breakfast cereals and many more.

5 Acknowledgement

This work was supported by the Fond InBev-Baillet Latour. The spelt wheat samples were donated by Mr. Rau, Nördlingen, Germany.

6 References

1. Belitz, H. D.; Seilmeier, W. and Wieser, H.: The Proteins of (Triticum-Spelta). Zeitschrift für Lebensmittel-Untersuchung und -Forschung 1989, **189**, (1), pp. 1-5.

2. Oplinger, E. S.; Oelke, E. A.; Kaminski, A. R.; Kelling, K. A.; Doll, J. D.; Durgan, B. R. and Schuler, R. T.: Alternative Field Crops Manual. University of Wisconsin, Madison, WI: 1990.

3. Ranhotra, G. S.; Gelroth, J. A.; Glaser, B. K. and Lorenz, K. J.: Baking and Nutritional Qualities of a Spelt Wheat Sample. Food Science and Technology-Lebensmittel-Wissenschaft & Technologie 1995, **28**, (1), pp. 118-122.

4. Bonafaccia, G.; Galli, V.; Francisci, R.; Mair, V.; Skrabanja, V. and Kreft, I.: Characteristics of spelt wheat products and nutritional value of spelt wheat-based bread. Food Chemistry 2000, **68**, (4), pp. 437-441.

5. Abdelaal, E. S. M.; Hucl, P. and Sosulski, F. W.: Compositional and Nutritional Characteristics of Spring Einkorn and Spelt Wheats. Cereal Chemistry 1995, **72**, (6), pp. 621-624.

6. Ranhotra, G. S.; Gelroth, J. A.; Glaser, B. K. and Stallknecht, G. F.: Nutritional profile of three spelt wheat cultivars grown at five different locations. Cereal Chemistry 1996, **73**, (5), pp. 533-535.

7. Kasarda, D. D. and D'Ovidio, R.: Deduced amino acid sequence of an alpha-gliadin gene from spelt wheat (spelta) includes sequences active in celiac disease. Cereal Chemistry 1999, **76**, (4), pp. 548-551.

8. Forsell, F. and Wieser, H.: Dinkel und Zöliakie. Zeitschrift für Lebensmittel-Untersuchung und -Forschung 1995, **201**, (1), pp. 33-39.

9. Vincentini, O.; Maialetti, F.; Gazza, L.; Silano, M.; Dessi, M.; De Vincenzi, M. and Pogna, N. E.: Environmental factors of celiac disease: Cytotoxicity of hulled wheat species Triticum monococcum, T-turgidum ssp dicoccum and T-aestivum ssp spelta. Journal of Gastroenterology and Hepatology 2007, **22**, (11), pp. 1816-1822.

10. Hartmann, G.; Piber, M. and Koehler, P.: Isolation and chemical characterisation of water-extractable arabinoxylans from wheat and rye during breadmaking. European Food Research and Technology 2005, **221**, (3-4), pp. 487-492.

11. Vinkx, C. J. A. and Delcour, J. A.: Rye (Secale cereale L) arabinoxylans: A critical review. Journal of Cereal Science 1996, **24**, (1), pp. 1-14.

12. Krahl, M.; Back, W. and Kreisz, S.: In Fructan and Arabinoxylan in Malting and Brewing of Wheat and Rye, 31st EBC Congress, Venice, 2007; Fachverlag Hans Carl, Nürnberg, Germany: Venice, 2007; pp. 1216-1223.

13. Corradini, C.; Bianchi, F.; Matteuzzi, D.; Amoretti, A.; Rossi, M. and Zanoni, S.: High-performance anion-exchange chromatography coupled with pulsed amperometric detection and capillary zone electrophoresis with indirect ultra violet detection as powerful tools to evaluate prebiotic properties of fructooligosaccharides and inulin. Journal of Chromatography A 2004, **1054**, (1-2), pp. 165-173.

14. Pool-Zobel, B. L.: Inulin-type fructans and reduction in colon cancer risk: review of experimental and human data. British Journal of Nutrition 2005, **93**, Suppl. 1, pp. S73-S90.

15. Grasten, S.; Liukkonen, K. H.; Chrevatidis, A.; El-Nezami, H.; Poutanen, K. and Mykkanen, H.: Effects of wheat pentosan and inulin on the metabolic activity of fecal microbiota and on bowel function in healthy humans. Nutrition Research 2003, **23**, (11), pp. 1503-1514.

16. Topping, D. L. and Clifton, P. M.: Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. Physiological Reviews 2001, **81**, (3), pp. 1031-1064.

17. Roberfroid, M. B.: Introducing inulin-type fructans. British Journal of Nutrition 2005, **93**, pp. S13-S25.

18. Karppinen, S.; Myllymaki, O.; Forssell, P. and Poutanen, K.: Fructan content of rye and rye products. Cereal Chemistry 2003, **80**, (2), pp. 168-171.

19. Chalmers, J.; Lidgett, A.; Cummings, N.; Cao, Y. Y. and Forster, J.:Spangenberg, G.: Molecular genetics of fructan metabolism in perennial ryegrass. Plant Biotechnology Journal 2005, **3**, (5), pp. 459-474.

20. Korakli, M.; Hinrichs, C.; Ehrmann, M. A. and Vogel, R. F.: Enzymatic determination of inulin and fructooligosaccharides in food. European Food Research and Technology 2003, **217**, (6), pp. 530-534.

21. Delzenne, N. M.: Oligosaccharides: state of the art. Proceedings of the Nutrition Society 2003, **62**, (1), pp. 177-182.

22. Golda, A.; Szyniarowski, P.; Ostrowska, K.; Kozik, A. and Rapala-Kozik, M.: Thiamine binding and metabolism in germinating seeds of selected cereals and legumes. Plant Physiology and Biochemistry 2004, **42**, (3), pp. 187-195.

23. Friedrich, W.: Handbuch der Vitamine. Urban&Schwarzenberg: München, Wien, Baltimore, 1987.

24. Prodanov, M.; Sierra, I. and Vidal-Valverde, C.: Influence of soaking and cooking on the thiamin, riboflavin and niacin contents of legumes. Food Chemistry 2004, **84**, (2), pp. 271-277.

25. Houben, R.; deRuijter, C. F. and Brunt, K.: Determination of the pentosan content of wheat products by hydrolysis, glucose oxidase treatment and analysis by HPAEC/PAD. Journal of Cereal Science 1997, **26**, (1), pp. 37-46.

26. Andersen, R. and Sorensen, A.: An enzymatic method for the determination of fructans in foods and food products – Comparison of the results by high performance anion exchange chromatography with pulsed amperemetric detection. European Food Research and Technology 1999, **210**, (2), pp. 148-152.

27. Crafts-Brandner, S. J.: Fructans and freezing tolerance. New Phytologist 2005, **166**, (3), pp. 708-709.

28. Ranhotra, G. S.; Gelroth, J. A.; Glaser, B. K. and Lorenz, K. J.: Nutrient Composition of Spelt Wheat. Journal of Food Composition and Analysis 1996, **9**, (1), pp. 81-84.

29. Vidal-Valverde, C.; Frias, J.; Sierra, I.; Blazquez, I.; Lambein, F.; Kuo, Y. H.: New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. European Food Research and Technology 2002, **215**, (6), pp. 472-477.

30. Zielinski, H.; Frias, J.; Piskula, M. K.; Kozlowska, H.; Vidal-Valverde, C.: The effect of germination process on the superoxide dismutase-like activity and thiamine, riboflavin and mineral contents of rapeseeds. Food Chemistry 2006, **99**, (3), pp. 516-520.

Received 11 September, 2008, accepted 08 October, 2008

Appendix

Table 1 Arabinoxylans during the malting of spelt wheat

Sample	Arabinoxylan [% DM]	WEAX [% DM]
Unmalted spelt wheat	10.05	0.51
3rd day	10.24	0.66
4th day	10.00	0.75
5th day	10.14	0.92
6th day	10.39	1.01
Malt	10.24	1.01

Table 2 Fructan during the malting of spelt wheat

Sample	Fructan [% DM]
Unmalted spelt wheat	1.11
3rd day	0.99
4th day	1.22
5th day	1.31
6th day	1.75
Malt	1.84

Table 3 Thiamine and riboflavin during the malting of spelt wheat

Sample	Thiamine [μg/100 g DM]	Riboflavin [μg/100 g DM]
Unmalted spelt wheat	419.7	45.9
3rd day	465.5	63.7
4th day	464.0	89.7
5th day	448.0	110.8
6th day	445.4	126.8
Malt	395.2	152.2