Journal of Applied Botany and Food Quality 81, 172 - 174 (2007)

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Free sugars in spelt wholemeal and flour

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(Received August 7, 2007)

Summary

Spelt (*Triticum aestivum* L. ssp. *spelta*) is experiencing a renaissance in Europe and North America, where it is used for baking, brewing, production of pasta, and self-supplied animal feed. One of the characteristics of spelt is that in comparison to modern wheat it is more resistant to harsh climatic and poor soil conditions. In contrast to wheat the hulls remain on the grain after threshing. Drawbacks are that spelt yields are quite low compared to modern wheat. The subject of the current study was to gain information about the composition of soluble sugars and their concentrations in spelt wholemeal and flour. High performance liquid chromatography (HPLC) was used for analysis. Concentrations of nine free sugars in spelt wholemeal and flour are reported. Flour cumulative free sugar concentrations were 63% lower than in wholemeal. For comparisons, we also analyzed wholemeal of wheat. The cumulative concentration of free sugars was 27% lower than in spelt wholemeal. However, when published data for sugar concentration ranges of wheat are taken into account, the total concentration of free sugar was not different between spelt and modern wheats. Low concentrations of xylose and stachyose were detected in spelt. Higher concentrations of fructans such as 1-kestose and kestotetraose were detected in spelt when compared with wheat. Generally, concentrations of free sugars in spelt were in the range of free sugar levels published for wheat, except for maltose which was higher in spelt.

Introduction

Spelt wheat (*Triticum aestivum* L. ssp. *spelta*) is one of the earliest wheat species cultivated and has been used as bread cereal since ancient times (Abdel Aal et al., 1998; Rösch, 1998). Later, it has been replaced by high-yielding cultivars of common bread wheat (*Triticum aestivum* L. ssp. *vulgare*). Drawbacks of spelt are that the hulls are not removed during threshing and that yield is quite low compared to modern wheat varieties. Modern wheat varieties intended for human consumption have compact ears, lose their hulls during threshing, and produce high yields. Spelt is more resistant to harsh climatic and poor soil conditions (Rösch et al., 1998), and better resists to pests and plant diseases. These features render spelt highly suitable for organic agriculture (Skrabanja et al., 2001). Moreover, spelt can be grown in sub-alpine regions as well as in low-input farming (Nesbitt and Samuel, 1996). For this reasons, spelt is experiencing a renaissance in Europe and North America (Mielke and Rodemann, 2007), where it is increasingly used for baking, brewing, and the production of pasta in a number of regional specialties such as the not fully matured grain ‘Grünkern’, i.e. not fully matured grain torrefied after harvest, of southern Germany. Another field of application is self-supplied animal feed. Today, a variety of spelt-based products are available including flour, bread, breakfast cereals, pasta and crackers.

It has been proposed that this cereal has valuable nutritional and/or physiological properties, an argument which has been used to help to promote the consumption of these products (Marques et al., 2007). It is a matter of fact that interest in alternative cereals and the demand for organically produced foods, creating a niche market for spelt, is growing. As a consequence, prices for spelt flour are up to 50% above the price of respective wheat flour in European countries (von Büren et al., 2001).

Carbohydrates such as starch, the cell wall compounds cellulose, hemicelluloses, and pentosans and soluble sugars are the most abundant constituents of the cereal grain, making up 80% of the total dry matter (Täufel et al., 1959). Because there is a lack of sound scientific data on the composition and concentration of nine soluble sugars in spelt, we determined these sugars by means of high performance liquid chromatography (HPLC). Taken together, we present results of spelt wholemeal and flour. We also compare the spelt data with published results of soluble sugar concentration ranges of modern wheats.

Materials and methods

Spelt wholemeals and flours

The spelt samples were purchased in grocery stores or from mill operating companies from the harvest of 2006. Four samples of each, wholemeal and flour (Type 630), were collected from the marketplace. Spelt wholemeals were obtained from Adler-Mühle, Bahlingen; Bronnmühle, Rottenburg; Aurora Hamburg; Diamant, Hamburg. Spelt flours (Type 630) were obtained from Pfälzische Mühlenwerke, Mannheim Aurora Hamburg; Rosenmühle Landshut; and Adler-Mühle, Bahlingen. Four wholemeal samples (bread wheat) were collected and analyzed.

Analytical procedures

Soluble sugars from 4 g of each flour and wholemeal sample were extracted with 50 mL of 80% ethanol at 60° C for 30 min. The extraction was repeated once (Munzquiz et al., 1992). The two extracts were combined and centrifuged for 10 min at 1500 *g* at room temperature. The supernatant was concentrated by rotary evaporation from 100 mbar to 30 mbar and the dry residue was resuspended in 8 mL water (double destilled) and sonicated for 3 min in an ultra sonic bath. The suspension was centrifuged for 10 min at 20° C and 1500 *g*. The supernatant was filtrated through a Strata™ X 60 mg cartridge (Phenomenex, Germany) which was previously equilibrated with 2 mL methanol and 2 mL water, subsequently. To 4 mL of the filtrate 6 mL acetonitrile was added. After centrifugation (see above), 50 µL of the supernatant was applied to the high performance liquid chromatography system (HPLC) for sugar separation.

Analysis was done by HPLC according to Munzquiz et al. (1992) with a modified mobile phase (see below). The chromatographic equipment consisted of a Kontron HPLC system, a Geminyx data module (Spectro BioNova, Germany), and an RI Detector 7515A (ERC, Germany). A stainless-steel analytical column CC 250/4.6 Nucleosil 100-5 NH_2 (Macherey & Nagel, Germany) was used together with a precolumn CC 8/4 Nucleosil 100-5 NH_2 (Macherey

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& Nagel, Germany). The mobile phase, was degassed (acetonitrile-water 6:4, w/w), and the flow-rate was set to 0.5 mL/min. The column temperature was maintained at 30° C. Quantification was done on the basis of peak heights. External standards were used for calibration. A linear response was obtained in the range of 0.0 - 4.0 mg mL^{-1} with a correlation coefficient of 0.99.

Statistical analyses and accuracy

Results were expressed as means obtained for four samples from wholemeal or flour of spelt, respectively. Each sample was extracted and analyzed at least two times. Standard errors of the mean (SE) were calculated. The reproducibility of the extraction of soluble sugars was calculated from three independent extractions and the standard error of the mean ranged between 0.1% and 5.6%. The recovery of sugars tested were 70% for fructose, 95% for glucose, 90% for sucrose, 70% for maltose, 81% for 1-kestose, 85% for raffinose, 98% for kestotetraose and 78% for stachyose.

Results

The overlay of two chromatograms derived from two separate HPLC runs from flour and from wholemeal of spelt is shown in Fig. 1. In total, nine free sugars could be detected and quantified. The non-identified peaks were relatively small. Differences in sugar concentrations between flour and wholemeal from spelt were quantitative. No qualitative difference was found (Fig. 1). Mean values and standard errors of the mean from all four samples of wholemeal and flour (type 630) from spelt are shown in Fig. 2. In spelt wholemeal the concentrations of sucrose, 1-kestose, raffinose, and maltose were between 2 and 7.5 mg g^{-1} DW and were three- to two-fold higher than in spelt flour. Concentrations of kestotetraose, fructose, glucose, xylose, and stachyose were below 0.6 mg g^{-1} DW in wholemeal. In flour, the concentrations of these sugars were approximately half of those in wholemeal (Tab. 1). The concentrations of free sugars in spelt were in the following order: sucrose > 1-kestose > raffinose > maltose >> kestotetraose > fructose = glucose = xylose > stachyose (Fig. 2).

Four wheat wholemeal samples were analyzed for soluble sugars. The comparison of free sugar concentrations in wholemeal of spelt and wheat revealed higher sugar concentrations in spelt except for raffinose, which was higher in wheat. Neither xylose nor stachyose were detected in wholemeal from wheat. In total 17.1 mg and 6.3 mg of free sugar g^{-1} DW were detected in wholemeal and flour of spelt, respectively. In wheat wholemeal 12.5 mg free sugar g^{-1} DW was found in total (Tab. 1). These results nicely match published data of sugar concentrations in wheat (summarized by POMERANZ, 1988) shown in Tab. 1.

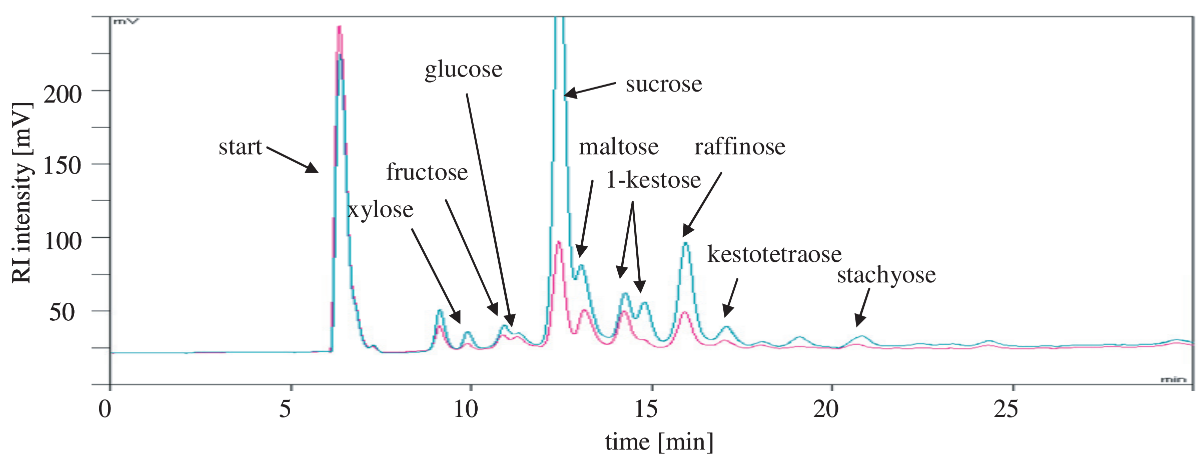

Fig. 1: Overlay of HPLC chromatograms of free sugars from spelt wholemeal and spelt flour (Type 630). The upper chromatogram represents wholemeal, the lower chromatogram flour.

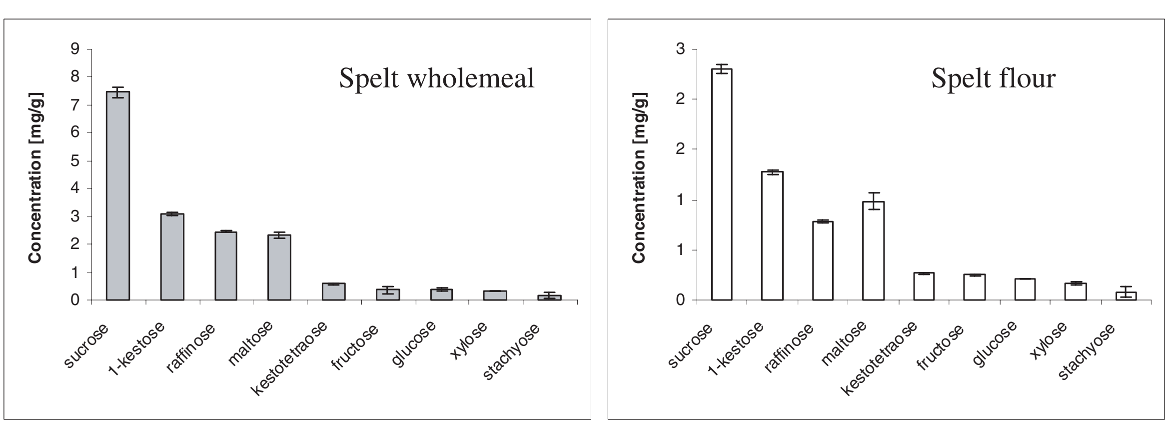

Fig. 2: Concentrations of free sugars in spelt wholemeal (left) and flour (right). were from four different companies each. Sugar concentrations were determined by HPLC and are given as mg g^{-1} dry weight. Standard errors of the mean (± SE) were calculated from four independent samples. Note the different scales!

Discussion

Because there is a lack of a sound scientific basis of spelt based data were determined. Concentrations of nine free sugars in wholemeal and flour of spelt were determined by HPLC (Tab. 1). The main advantage of HPLC by comparison to enzymatic methods lies in the reliable simultaneous detection of a set of sugars from one extract with a relatively low detection limit. The 63% lower cumulative free sugar concentrations flour by comparison with wholemeal from spelt, which reflects the removal of free sugars during the milling process. Fructose amounts to only 1.5% of total free sugars in spelt. Sucrose, 1-kestose, raffinose, and maltose show the largest reduction by milling, with kestotetraose, fructose, glucose, xylose and stachyose being less affected (Fig. 2). FRETZDORFF and WELGE (2003) reported higher concentrations of kestotrioses compared to larger fructo-oligosaccharides.

The second issue of our investigation was to compare the concentrations of free sugars from spelt and wheat. The concentration of cumulative free sugars from spelt was 27% higher than that of wholemeal of wheat analyzed in this work (Tab. 1). However, when many published data are considered (summarized by POMERANZ, 1988), sugar concentrations in spelt are within the range observed for wheats (Tab. 1). Xylose and stachyose could not be detected at all in wheat in our work while other workers found low concentration (Tab. 1), indicating a slight difference in sugar composition in wheat and spelt. Sucrose was found the sugar most abundant in both, spelt and wheat, the sucrose concentration in spelt being significantly higher (Tab. 1). Additionally, higher concentrations of fructans such as 1-kestose and kestotetraose were detected in spelt compared to wheat (Tab. 1). In our work using HPLC, arabinose was not detected in spelt and wheat. In contrast, BARRON et al. (2007) reported arabinose in wheat, although little.

Tab. 1: Comparison of mean values and ± SE of free sugar concentrations from wholemeal and flour (Type 630) from four different spelt wholemeal and flour (*Triticum aestivum* L. ssp. *spelta*) samples and from four different samples of wholemeal wheat (*Triticum aestivum* L. ssp. *vulgare*). Sugar concentrations are given in mg g^{-1} dry weight. (± SE), Standard error of the mean.

	Spelt		Wheat	
	Wholemeal	Flour	Wholemeal	Range*
Sucrose	7.47 ± 0.20	2.30 ± 0.05	5.91 ± 0.13	5.4 - 15.5[a]
1-Kestose	3.08 ± 0.05	1.28 ± 0.02	2.00 ± 0.04	2.6 - 4.1[a]
Raffinose	2.46 ± 0.03	0.79 ± 0.01	2.61 ± 0.04	1.9 - 6.8[a]
Maltose	2.35 ± 0.12	0.99 ± 0.08	1.37 ± 0.1	0.0 - 1.8[a]
Kestotetraose	0.58 ± 0.01	0.27 ± 0.01	0.33 ± 0.01	n.a.
Fructose	0.36 ± 0.12	0.25 ± 0.01	0.17 ± 0.01	0.6 - 0.8[a]
Glucose	0.36 ± 0.05	0.21 ± 0.01	0.15 ± 0.01	0.3 - 0.9[a]
Xylose	0.32 ± 0.01	0.17 ± 0.01	n.d.	n.a.
Stachyose	0.16 ± 0.12	0.08 ± 0.05	n.d.	0.06[b]
Σ Sugars	17.14	6.34	12.54	

n.d., concentration not detectable; n.a., data not available; *concentration ranges according to [a] POMERANZ (1988); [b] HENRY and SAINI (1989)

Generally, concentrations of free sugars in spelt were in the range of free sugar levels published for wheat, except for maltose which was higher in spelt (Tab. 1).

Acknowledgements

The excellent assistance of Annette Meyer-Wieneke during the sample preparation and analysis is gratefully acknowledged.

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