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Fractionation-reconstitution studies to determine the functional properties of rye flour constituents

Isabel Grossmann, Peter Koehler*

Deutsche Forschungsanstalt für Lebensmittelchemie, Leibniz Institut, Lise-Meitner-Straße 34, 85354, Freising, Germany

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ABSTRACT

Rye flour was fractionated into starch, protein, water-extractable (WE), and water-unextractable (WU) arabinoxylans (AX). For protein isolation a novel method was developed consisting of extraction of rye flour at pH 9.5 and 50 °C followed by dialysis under slightly acidic conditions. Flours containing different amounts of these fractions were recombined; doughs were mixed and analyzed for their rheological behavior in order to get insight into the role of the different fractions on the dough properties. WEAX decreased the elastic properties of the doughs compared to the control dough, whereas WUAX led to an increase. In doughs containing both AX fractions the effects compensated each other. The onset of starch gelatinization in the reconstituted doughs and the difference to the temperature of complete gelatinization was strongly affected by the composition of the doughs. However, the role of the protein fraction on the elastic properties of the dough and on starch gelatinization remained unclear.

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1. Introduction

The influence of different constituents of rye flour such as starch, protein, and non-starch-polysaccharides on the dough properties and the baking performance can be studied by means of two different approaches. One is to add a distinct flour fraction to base flour and to assess the functional effect. However, this increases the content of the flour fraction under study and comparison with the base flour is not possible. In rye flour, this approach was pursued by Buksa et al. (2013, 2015) who found that an addition of water-extractable (WE) arabinoxylans (AX) to rye flour resulted in breads with a higher volume and softer crumb. Weipert and Zwingelberg (1979) mixed milling streams with different pentosan content and prepared bread with the modified flours. They found that a pentosan to starch ratio of about 1:16 gave the best results regarding bread volume and crumb structure.

Another powerful tool to investigate the effects of different constituents on flour and dough properties is to fractionate and reconstitute the flour. For rye so far, only Meuser and Suckow (1986a) as well as Kuehn and Grosch (1985, 1988, 1989) used this approach. The focus of these studies was to investigate the influence of the non-starch-polysaccharides and enzymatic modifications thereof and of the starch on the baking performance of rye flour. For this purpose, Kuehn and Grosch fractionated rye flour into three water-soluble and three water-insoluble fractions, one of them starch. Kuehn and Grosch showed that their reconstituted flour had the same properties as the original flour and that different combinations of the fractions gave breads with higher or lower bread volumes compared to the control. Also the crumb firmness varied depending on how the fractions had been recombined. Enzymatic modification of the non-starch-polysaccharide fractions decreased crumb firmness and resulted in a flattened bread shape (Kuehn and Grosch, 1985, 1988, 1989). However, the baking properties not only depended on the total amount of AX but also on the type of AX. WEAX had a positive influence on the baking properties, i. e. the crumb was soft with fine pores and the volume increased compared to the control, whereas the water unextractable (WU) AX were detrimental, i.e. the crumb was hard and chewy with an unpleasant and bitter taste (Meuser and Suckow, 1986a, 1986b).

In none of these studies a distinct protein fraction was isolated. The protein was distributed among all fractions in varying amounts. The isolation of rye protein differs from that of wheat protein because rye protein is not able to form a continuous network such as gluten in wheat and, therefore, cannot be separated from starch by washing rye dough with water as it is possible with wheat. Functional rye protein can be isolated by sedimentation in a non-aqueous system (Hartmann and Koehler, 2008), but this method

* Corresponding author.
E-mail address: peter.koehler@tum.de (P. Koehler).

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is time consuming and difficult to perform. To the best of our knowledge, effective methods for isolating rye protein in an aqueous system are not available up to now.

Fundamental rheology has the benefit that exact, defined measurements with results in absolute physical SI units can be conducted where the elastic and viscous components of the complex properties of the material are recorded separately (Weipert, 1990). With a rheometer it is also possible to perform continuous measurements throughout a simulated baking process (Weipert, 1990). Thus, the influence of flour constituents in a model system on the consistency and structural changes in dough during bread making and in a bread-like matrix can be monitored and the results transferred to the bread production process. Moreover, only small amounts of sample are required (Weipert, 1990). This is especially important for reconstituted flours that are made from fractions or isolates only available in limited amounts.

Therefore, the aim of this study was to determine the functional effects of rye flour constituents by an approach using fractionation and reconstitution. Rye flour was fractionated into the four fractions starch, protein, WEAX, and WUAX, and special focus was laid in the isolation of a functional rye protein fraction by using alkaline extraction of rye flour. The fractions were then recombined in different ratios to give nineteen different remix flours. Doughs were prepared from the reconstituted flours and characterized by means of fundamental rheology including simulation of baking by heating the dough in the rheometer.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich (Steinheim, Germany) or VWR (Darmstadt, Germany) at analytical or higher grade. Water was deionized by an Arium 611VF water purification system (Sartorius, Göttingen, Germany).

2.2. Flour

Commercial rye flour type 1150 (mean ash content 1.15% in dry mass) was obtained from a commercial mill (Rosenmühle GmbH, Ergolding, Germany). The water content of the flour was 12.7%, the crude protein content was 9.1% in dry mass (Dumas method; N × 5.7), the starch content was 63.3% with 23.2% amylose content in dry mass and the non-starch polysaccharide content was 14.7% in dry mass.

2.3. Isolation of rye starch

Rye starch was isolated according to the method described by Kuehn and Grosch (1985) with some modifications. Rye flour (50 g) and water were mixed at a ratio of 1:10 (w/v) and stirred for 60 min at room temperature (RT ≈ 20 °C). The slurry was centrifuged (3500 × *g*, 30 min, RT; Multifuge 3 L-R, Fisher Scientific, Waltham MA, USA) and the supernatant discarded. The upper greyish layer of the residue was scraped off with a spatula and discarded. The remaining residue was separated with a spatula into a light brown and a dark brown fraction. These fractions were put into two 50 mL centrifuge tubes and water (40 mL) was added to each tube. The tubes were vigorously shaken at a multi vortex shaker for 15 min and subsequently centrifuged (3500 × *g*, 15 min, RT). The supernatant and the upper greyish layer of the residues were discarded. The residues were separated into the upper brownish layer and the lower white layer, which was the starch. The brownish and the white fractions were combined and extracted with water as described above. This step was repeated until the starch fraction was completely white. The starch fraction was dried at room temperature for three days, ground with a mortar and pestle and sieved through a 0.2 mm screen.

2.4. Isolation of rye protein

The method for the preparation of a rye protein isolate was developed based on a method for the isolation of protein from oat bran (Guan and Yao, 2008) and a method for the isolation of proteins from wheat germ (Zhu et al., 2006). Rye protein with the highest protein content was obtained with the following method: Flour (50 g) was suspended in water (500 mL), stirred for 60 min at RT and centrifuged (3500 × *g*, 30 min, RT). The supernatant containing the WEAX and the water-soluble albumins was discarded. The residue containing the globulins, prolamins, and glutelins was resuspended in water (150 mL) and the pH was brought to 9.5 with NaOH (1 mol/L) to assist in protein solubilization (negative net charge of proteins). This slurry was incubated for 30 min at 50 °C to further assist in solubilizing the proteins. The warm suspension was centrifuged (3500 × *g*, 30 min, RT) and the supernatant containing the proteins was dialyzed against water containing 0.1% (v/v) acetic acid as a preservative to avoid microbial growth for 48 h and subsequently against deionized water to remove residual acetic acid for 3 h in dialysis tubes with a molecular cut-off of 12,000–14,000. The dialyzed suspension was lyophilized and the protein isolate stored at −24 °C until use.

2.5. Isolation of WEAX

WEAX were extracted from rye flour following a method described by Ragaee et al. (2001) with some modifications. Rye flour (500 g) was heated at 130 °C for 90 min to inactivate endogenous enzymes. Heat-treated flour (50 g) was extracted with deionized water (500 mL) for 90 min at RT under continuous stirring. The slurry was centrifuged (3500 × *g*, 30 min, RT) and phosphate buffer (10 mL; 0.05 mol/L, pH 6.9) was added to the supernatant. To remove remaining starch, the supernatant was incubated with thermostable α-amylase (from *Bacillus licheniformis*; 3000 U; Sigma A 4551) at 95 °C for 60 min. The mixture was cooled to RT, centrifuged (3500 × *g*, 30 min, RT), acetate buffer (5 mL; 1 mol/L, pH 5.0) and amyloglucosidase (from *Aspergillus niger*; 100 U; Sigma 10115) were added to the supernatant, and the solution was incubated at 60 °C overnight. The mixture was again centrifuged (3500 × *g*, 30 min, RT) and the supernatant was brought to pH 3 with hydrochloric acid (1 mol/L). To remove proteins, the supernatant was stirred with Montmorillonite (10 g; Sigma 69904) for 30 min at RT. Before centrifugation (3500 × *g*, 30 min, RT), the slurry was adjusted to pH 7 with sodium hydroxide (2 mol/L). The supernatant was diafiltrated through a membrane with a molecular cut-off of 2000 (Sartocon Slice 200, Hydrosart) on a Sartorius Benchtop Crossflow System (Sartoflow Slice 200). Seven diafitration steps were performed, and the resulting solution was lyophilized. The pH-value was checked after addition of each buffer.

2.6. Isolation of WUAX

The method of Moers et al. (2005) for the isolation of WUAX from wheat flour was adopted for rye flour. Heat-treated rye flour (50 g) was suspended in water (500 mL), stirred for 90 min at RT and centrifuged (3500 × *g*, 30 min, RT). The supernatant was discarded and the upper light layer of the residue was scraped of the darker layer with a spatula. The darker fraction was resuspended in water at a ratio of 1:10 (v/v), centrifuged (3500 × *g*, 30 min, RT) and the light layer scraped off. This step was repeated twice. The remaining dark fraction was suspended in water (200 mL; final pH

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