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Fractionation of cereal flour by sedimentation in non-aqueous systems. I. Development of the method and chemical characterisation of the fractions

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Abstract

A method for the fractionation of wheat, rye, and barley flours without using aqueous solvents was developed. The separation of protein and starch was based on differences in their densities. Therefore, ball-milled flour was suspended in a mixture of inert solvents (toluene/tetrachoroethene) with a density of 1.47 g/cm^3 and centrifuged. Owing to its higher density, the starch fraction was obtained as sediment whereas the protein fraction (PF) formed a layer on the surface of the solvent because of its lower density. The PF was enriched in a solvent mixture with a density of 1.355 g/cm^3 yielding a middle fraction (sediment) and the enriched PF (upper layer). The latter was then defatted with toluene (0.87 g/cm) providing a lipid fraction in addition. The influence of ball milling under air or in the sedimentation solvent on the yield and the purity of the fractions was studied. Three varieties of wheat, and one rye and barley variety were fractionated by the optimised method and the obtained fractions were characterised by chemical methods e.g. gel permeation chromatography, SDS electrophoresis, and a combined extraction/HPLC method.

Keywords: Non-aqueous fractionation; Cereals; Flour; Protein

1. Introduction

To study the contribution of flour components to dough properties and baking performance, wheat flour can be fractionated and the fractions may be recombined in a suitable manner to give a reconstituted flour. As compared with the direct addition of fractions to native flour, the advantage of a reconstituted flour is that the desired composition can be adjusted. The classical methods of wheat flour fractionation used aqueous systems and provided the four fractions lipids, solubles, gluten, and starch (Bechtel and Meisner, 1954; De Stefanis and Ponte, 1969; Finney, 1943; Grassberger et al., 2003; MacRitchie, 1985; Ponte and De Stefanis, 1969; Sollars, 1969; Udy, 1957; Walden and McConnell, 1955). However, as described in a previous study (Grassberger et al., 2003), the baking performance of reconstituted flours from aqueously isolated fractions differs from native flour. A possible explanation of this phenomenon is that wheat gluten proteins might undergo irreversible changes after they have been in contact with water (Chakraborty and Khan, 1988; Grassberger et al., 2003). However, other papers report that functionality can be preserved in aqueous media (MacRitchie, 1985; Skerritt et al., 1996). Therefore, approaches for the non-aqueous fractionation of wheat flour have been designed to better maintain the properties of native gluten proteins. This can be achieved by a physical separation of starch and protein in an inert solvent due to the difference of their densities.

In the 1950s, Hess was the first to develop methods for the non-aqueous fractionation of wheat flour (Hess, 1954,

Abbreviations: BM, ball milled; xhBM, flour which was x hours milled in a ball mill; xhBMsolv, flour which was x hours milled in a ball mill with addition of solvent; SF, starch fraction; MF, middle fraction; PF, protein fraction; LF, lipid fraction; SE-HPLC, high-performance-size-exclusion chromatography

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1955; Hess and Hille, 1961). He found that it was necessary to crush the flour particles to a small size (approximately 30 μm) prior to fractionation. Ball milling under air for 14 h was used in this step, however, the impact of ball milling on chemical parameters of the flour was not studied at all. For fractionation, mixtures of benzene and tetrachloromethane with densities between 1.30 and 1.50 g/cm^3 were used as solvents and the resulting fractions were called wedge-space protein and adhesive protein/starch, respectively. Microscopic, radiographic (Hess, 1954), and potentiometric analyses (Hess, 1955) of the fractions were made and the specific surface (Hess, 1954) as well as the behaviour during mixing were determined (Hess, 1955). These investigations led to the assumption that wedge-space protein and adhesive protein, which had been separated by the density fractionation method, were totally different proteins. Later on, the fractionation method of Hess was modified (Finley, 1976; Rohrlich et al., 1969, 1972; Rohrlich and Mueller, 1969; Rohrlich and Niederauer, 1963, 1965) and the obtained fractions were further analysed by chemical methods. These studies supported the conclusion of Hess (1954, 1955) that wedge-space and adhesive proteins were different.

The aim of the present study was to optimise the fractionation of wheat flour with non-aqueous solvents based on the method of Hess, to replace the toxic solvents benzene and tetrachloromethane, to use the method for other cereal species i.e. rye and barley and to determine the chemical properties of the isolated fractions. The rheological and baking properties will be the subject of a separate paper. The focus of the present study was to verify Hess's assumption about the distinction between wedge-space and adhesive protein.

2. Experimental

2.1. Materials

Wheat, rye, and barley, conditioned to 14.0% moisture (wheat cv. Astron, 2003 harvest; Strube Saatzucht, Soellingen, Germany; wheat cv. Glockner, 2000 harvest; Saatzucht Engelen, Büchling/Oberschmeiding, Germany; wheat cv. Contra, 2004 harvest, Saatzucht Josef Breun, Herzogenaurach, Germany; rye cv. Nikita, 2001 harvest, Lochow Petkus GmbH, Bergen, Germany; barley cv. Auriga, 2004 harvest, Saatzucht Dr. J. Ackermann & Co., Irlbach, Germany) were milled into white flours on a Brabender Quadrumat Junior mill (Duisburg, Germany) and passed through a 0.2 mm test sieve (DIN 4188; Retsch, Haan, Germany). The protein content was calculated from the nitrogen content (protein = nitrogen × 5.7), determined by the method of Dumas using an FP 328 nitrogen analyser (Leco, Kirchheim, Germany). The ash content (Table 1) was measured according to ICC method 104 (1978). Solvents and all other chemicals were from VWR International (formerly Merck, Darmstadt, Germany). The quality of the solvents was "extra pure" or better.

Table 1
Ash and protein content of flours (in dry mass)

Flour	Ash content (g/100 g)	Protein content (g/100 g)
Wheat cv. Astron	0.47 ± 0.01	13.85 ± 0.04
Wheat cv. Contra	0.44 ± 0.01	10.64 ± 0.02
Wheat cv. Glockner	0.58 ± 0.02	14.44 ± 0.03
Rye cv. Nikita	0.72 ± 0.02	6.33 ± 0.03
Barley cv. Auriga	0.73 ± 0.02	6.85 ± 0.03

2.2. Ball milling of flour

The flour was lyophilised before ball milling. Dried flour (130 g) was put into a 500 mL grinding jar and 100 sintered aluminium oxide balls (diameter 10 mm) were added. The flour was then milled for one (1 hBM) or 10 h (10 hBM) in a centrifugal ball mill Type S 1 (Retsch, Haan, Germany), respectively. For wet grinding, a mixture of toluene and tetrachloroethene (200 mL; 50 + 50, v + v) was added to the flour. To remove oxygen, the grinding jar was put into a desiccator, evacuated four times and ventilated with nitrogen prior to grinding. The flour/solvent mixture was then milled for one (1hBMsolv) and 10 h (10 hBMsolv), respectively. After 30 min, grinding was interrupted for 30 min and then continued to prevent the flour from being heated by the input of mechanical energy.

2.3. Microscopy

Flour was suspended in anhydrous glycerine and examined in a light microscope (Carl Zeiss, Unterkochen, Germany) with a 10-fold magnification objective (Phaco1, 10/0.25; Leitz, Wetzlar, Germany).

2.4. Determination of reactive thiol groups with Ellman's reagent

The determination of the reactive thiol content was carried out according to Bak et al. (2000) with some modifications. Sample (60 mg) was mixed with buffer 1 (50 μL; 0.2 mol/L Tris, 3 mmol/L EDTA, pH 8.0, 10 mmol/L 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB, Ellman's reagent)) and buffer 2 (1.8 mL, 0.2 mol/L Tris, 3 mmol/L EDTA, pH 8.0). The suspension was mixed and reacted for 60 min with magnetic stirring, centrifuged for 20 min at 45.000*g*, and the supernatant was measured spectrophotometrically at 412 nm ($d = 1$ cm). For calibration, reduced glutathione (5–30 μg) was used and treated as described above.

2.5. Fractionation procedure for ball-milled flour

Fractionation of ball-milled flour was carried out according to Rohrlich et al. (1972) with some modifications. A schematical representation of the procedure is given in Fig. 1. Ball-milled flour (60 g) was suspended in one of the solvent mixtures given in Table 2 (300 mL) with