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Impact of processing conditions on the extractability and molecular weight distribution of proteins in parboiled brown rice

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ABSTRACT

Parboiling, a hydrothermal treatment of paddy or brown rice, impacts the texture and nutritional characteristics of cooked rice. We investigated the impact of parboiling conditions on the extractability and molecular weight (MW) distribution of proteins in brown rice. Brown rice was parboiled using different soaking and steaming conditions. The extractability and MW distribution of proteins extracted with sodium phosphate buffer (50 mmol/L; pH 6.8) containing (i) 2.0% sodium dodecyl sulfate (SDS), (ii) 2.0% SDS/1.0% dithiothreitol (DTT)/6.0 mol/L urea, (iii) 2.0% SDS/1.0% DTT, and (iv) 2.0% SDS/6.0 mol/L urea was examined by size exclusion-high performance liquid chromatography. Depending on the parboiling conditions, protein extractabilities in media (i), (ii), (iii), and (iv) ranged from 14 to 25%, 83 to 100%, 40 to 82%, and 19 to 37%, respectively. Unlike soaking conditions, steaming conditions had pronounced effects on the level of extractable protein. In general, more severe steaming conditions caused greater reductions in protein extractability, indicating a denser protein network. Apparent MW profiles revealed that especially glutelins polymerize upon severe steaming. Albumins, globulins and prolamins either polymerize through disulfide bonds and/or interact with one another through hydrogen bonds or hydrophobic interactions to form a separate protein network or become incorporated in the glutelin network.

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1. Introduction

Rice (*Oryza sativa* L.) is one of the leading food crops in the world and the staple food for more than half world's population. About 20% of rice produced worldwide is parboiled, *i.e.* "partially boiled". Parboiling, a hydrothermal treatment consisting of soaking, heating and drying of rice, is performed either on paddy or on brown rice. The parboiling process has considerable impact on the texture and nutritional characteristics of the consumable cooked rice. In particular, cooked parboiled rice is firmer, less sticky and has a higher nutritional value (more vitamin B_1) than raw rice, features preferred by most Western consumers. Furthermore, parboiling can reduce the level of kernel breakage during milling. Differences between parboiled and raw rice include the darker color of the former and its slightly different flavor (Bhattacharya, 2004; Buggenhout et al., 2013; Delcour and Hoseney, 2010). It is, however, not clear how structural changes in the major rice components during parboiling impact the parboiled end-product. A better understanding of these changes may lead to more optimal conditions during industrial production of parboiled rice. Most earlier studies in this field have focused on changes in the starch fraction during parboiling, *i.e.* gelatinization of starch, retrogradation of amylopectin, and formation of amylose–lipid complexes (Delcour et al., 2010; Derycke et al., 2005; Lamberts et al., 2009; Priestley, 1976). Limited research has been performed on changes in proteins as a result of parboiling.

Proteins typically make up 8.5% (on dry matter basis) of brown rice. Based on the Osborne fractionation scheme, these proteins are classified as water extractable albumins, dilute salt extractable globulins, alcoholic media extractable prolamins and dilute acid extractable glutelins. Globulin (about 12%) and glutelin (about 80%) are the two major rice protein fractions, whereas albumin (about 5%) and prolamin (about 3%) are the minor ones (Juliano, 1994). Rice albumins have a wide range of molecular weights (MWs), with

Abbreviations: DTT, dithiothreitol; HMW, high molecular weight; LMW, low molecular weight; MW, molecular weight; SDS, sodium dodecyl sulfate; SDSEP, level of extractable proteins in SDS containing medium; $SDSEP_{DTT}$, level of extractable proteins in SDS and DTT containing medium; $SDSEP_{tot}$, total level of extractable proteins; $SDSEP_{urea}$, level of extractable proteins in SDS and urea containing medium; SE-HPLC, size exclusion – high performance liquid chromatography; T, temperature.

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the major components having apparent MWs of 18–20 k (Houston and Mohammad, 1970). Globulins consist of α-, β-, γ- and δ-globulins with apparent MWs of 25.5 k, 15 k, 200 k and more than 200 k, respectively (Morita and Yoshida, 1968). Prolamins consist of cysteine-rich 10 k, 14 k and 16 k polypeptide subunits and a cysteine-poor 13 k subunit, which interact with each other and polymerize by disulfide bonds (Nagamine et al., 2011). Glutelins consist of an acidic (α) and a basic (β) subunit with apparent MWs of 30–39 k and 19–25 k (Kagawa et al., 1988; Kishimoto et al., 1999; Van Der Borght et al., 2006). These subunits are covalently linked to each other by an intermolecular disulfide bond. The (α-β)glutelin subunit pairs further polymerize by intermolecular disulfide bonds and are involved in hydrophobic interactions to form very large macromolecular complexes (Katsube-Tanaka et al., 2004; Sugimoto et al., 1986; Utsumi, 1992; Van Der Borght et al., 2006).

Rice proteins undergo changes as a result of the heat-moisture conditions applied during parboiling, as shown by their altered extractability in different media. In a study of Dimopoulos and Muller (1972), protein extractability in a 3% straight-chain sodium alkyl benzene sulfonate containing medium (pH 4.6) decreases as a result of parboiling from 73.4% to 20.1–31.1% and thus by 42.3–53.1 percentage points, depending on the severity of steaming. When proteins were extracted from the same samples with alkaline detergent medium (pH 10.8) containing bisulfite, approximately 94.7% of the proteins could be extracted. Raghavendra Rao and Juliano (1970) and Kato et al. (1983) showed that the extractability of albumins, globulins, prolamins and glutelins (extracted according to the classical Osborne fractionation scheme) decreases by about 45% as a result of parboiling. They attributed this to heat denaturation of proteins, a greater adhesion/cohesion of proteins to/with starch granules and/or other components. Devi et al. (1997) used alternative solvents in the Osborne fractionation scheme. In contrast with results of Raghavendra Rao and Juliano (1970) and Kato et al. (1983), the solubility of the glutelin-like fraction extracted with borate buffer (pH 10.0) containing the reducing agent β-mercaptoethanol increased as a result of parboiling, while that of the glutelin fraction extracted with borate buffer (pH 10.0) containing β-mercaptoethanol in combination with sodium dodecyl sulfate (SDS) decreased. Furthermore, the solubility of the prolamin fraction (extractable with 70% propanol) and the cross-linked prolamin fraction (extractable with 70% propanol containing β-mercaptoethanol) decreased.

The aim of the present study was to investigate the impact of parboiling on the properties of rice proteins, and, more in particular, to examine the relative contribution of non-covalent interactions and covalent bonds within and between the different proteins. Brown rice was parboiled using different soaking and steaming conditions. The protein aggregation behavior was monitored by evaluating its extractability in SDS containing medium (SDSEP) using size exclusion – high performance liquid chromatography (SE-HPLC). A decreased level of SDSEP indicates an increased aggregation level (Hayta and Schofield, 2004). The chaotropic agent urea and/or the reducing agent dithiothreitol (DTT) were added to the SDS containing medium to better understand the relative contribution of interactions and reactions. The apparent MW distributions of the different protein extracts were examined in detail to understand the contribution of the individual protein fractions to the protein network formed upon parboiling.

2. Material and methods

2.1. Materials

Brown rice from the long-grain cultivar Puntal (Spain, harvest 2010) was obtained from Mars NV (Olen, Belgium). Puntal is a typical rice cultivar used in industrial practices. The rice was dehulled in the country of harvesting. All solvents, chemicals and reagents were purchased from VWR (Leuven, Belgium) unless specified otherwise, and were at least of analytical grade.

2.2. Parboiling conditions

Brown rice was parboiled on laboratory scale applying the soaking and steaming conditions outlined in Table 1. These conditions were chosen based on lab experience and close contacts with industry. A sample (650.0 g) was soaked in excess water at 40 °C for 60 min, 55 °C for 30 min and 65 °C for 60 min. Rice grain moisture contents at the end of soaking were 29.4% (±0.2%), 30.0% (±0.1%) and 33.6% (±0.1%), respectively. Soaking was performed in triplicate. After soaking, excess water was discarded, and the rice was rested for 20–35 min. The soaked rice was then steamed in two steps in a cylindrical container in a Lagarde (Maltaverne, France) autoclave Type RA250. The first step was at 106 °C for 15 min. The temperature (T) and time of the second step were 106, 120 or 130 °C and 15 or 20 min, respectively. After steaming, the pressure was released and the samples were mildly dried on trays for 62 h at 27 °C (60% relative humidity) in a Vötsch VC 4060 climatic testing chamber (Vötsch Industrietechnik, Germany). The rice grain moisture content averaged 14.0%. The parboiled brown rice samples were stored in sealed plastic bags at 5 °C. As all steaming conditions were preceded by the same fixed steaming step at 106 °C for 15 min, only the impact of the second steaming step is discussed.

2.3. Composition of rice flour

Raw and parboiled brown rice samples were frozen using solid carbon dioxide. They were then freeze dried, ground into flour with a universal mill (IKA labortechnik, Staufen, Germany) to pass a 250 μm sieve. The moisture content of lyophilized rice flour was determined according to AACC-method 44-15.02. Protein content was determined using an adaptation of the AOAC Official Method 990.03 (AOAC, 1995) to an automated Dumas protein analysis system (EAS, varioMax N/CN, Elt, Gouda, The Netherlands), using 5.95 as the nitrogen to protein conversion factor.

2.4. Determination of protein extractability and MW distribution

Samples containing 1.0 mg dry matter protein were extracted with 1.0 ml sodium phosphate buffer (0.05 mol/L; pH 6.8) containing (i) 2.0% (*w/v*) SDS, hereafter referred to as SDS buffer, (ii) 2.0% (*w/v*) SDS/1.0% (*w/v*) DTT (Acros Organics, Geel, Belgium)/6.0 mol/L urea, (iii) 2.0% (*w/v*) SDS/1.0% (*w/v*) DTT, and (iv) 2.0% (*w/*

Table 1
Soaking and steaming conditions used during brown rice parboiling. The steaming conditions listed in the table, were proceeded by a fixed steaming step at 106 °C for 15 min. The corresponding sample codes consist of the soaking and steaming temperatures.

Sample code	Soaking conditions		Steaming conditions	
	Temperature (°C)	Time (min)	Temperature (°C)	Time (min)
40 – 106	40	60	106	15
40 – 120	40	60	120	15
40 – 130	40	60	130	20
55 – 106	55	30	106	15
55 – 120	55	30	120	15
55 – 130	55	30	130	20
65 – 106	65	60	106	15
65 – 120	65	60	120	15
65 – 130	65	60	130	20

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