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Effects of glutelin and globulin on the physicochemical properties of rice starch and flour

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ABSTRACT

The effect of two rice endosperm proteins, glutelin and globulin, on the physicochemical properties of rice starch and flour was investigated. Albumin, globulin, prolamin and glutelin were sequentially extracted from defatted rice flour with de-ionised water, 1.5 M NaCl, propan-2-ol and 0.1 M NaOH, respectively, followed by dialysis and lyophilisation. Globulin and glutelin were then added to pure rice starch at various concentrations, separately and together, and the pasting and textural properties of mixtures were analysed by the Rapid Visco Analyser (RVA) and TA-XT2 textural analyser, respectively. The presence of glutelin in rice starch caused an increase in pasting temperature but a decrease in the viscosity parameters of the starch paste. The concentration of glutelin was also positively correlated with the hardness and adhesive properties of the starch gel. The presence of globulin, on the other hand, resulted in a decrease in all the pasting and textural parameters except gel hardness and the changes were linearly correlated with the concentration of the protein for most of the physical parameters. When the two proteins were added to rice starch together, the outcomes in pasting and textural properties were generally dependent upon the relative concentrations of the two proteins, but were also influenced by the presence of the other two protein fractions, albumin and prolamin. The presence of globulin initially accelerated the rate of water absorption by starch during cooking while the presence of glutelin slowed it down, but in both cases, the ultimate amount of water absorbed was significantly lower than that by pure starch. The contrasting effects of the different protein fractions mean that it might be possible to manipulate the textural properties of rice starch and flour to achieve desirable sensory outcomes by varying the proportions of the protein fractions in product formulations.

1. Introduction

Rice starch and flour are common ingredients for many foods including batters, sauces, bakery products and rice noodles. Rice starch has a unique combination of several desirable characteristics, including hypoallergenicity, digestibility, smooth texture and bland flavour, which make makes it especially suitable for infant foods and pharmaceutical products (Mitchell, 2009; Wani et al., 2012). For most of these products, the pasting and textural properties of rice starch and flour have a significant impact on their processing, storage and sensory qualities. Therefore, many studies have been conducted to investigate the physicochemical properties of rice starch and flour and the factors affecting them. Early studies are mainly focused on the effect of the composition and structure of starch, which is not surprising as starch is the most predominant component, accounting for about 90% of the dry matter of rice flour (Champagne, 1996; Wani et al., 2012). These studies have generally concluded that the starch composition (amylose/amylopectin ratio) and structure play a dominant role in determining the physicochemical properties of rice flour (Sandhya Rani and Bhattacharya, 1989; Reddy et al., 1993; Remesh et al., 1999). Later, a number of studies have investigated the effect of external factors such as pH, salt and the presence of other components on the physicochemical properties of rice starch and flour (Wang et al., 2000; Zhou et al., 2007; Yu et al., 2012). Curiously, studies on the impact of rice protein, the second large component of rice, on these properties have been relatively few.

Martin and Fitzgerald (2002) reported that when protein was removed from rice flour by treatment with a protease, an overall

Abbreviations: RVA, rapid visco-analyser; ANOVA, analysis of variance; RVU, rapid visco-analyser unit.

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lowering of the RVA (Rapid Visco Analyser) profile occurred with viscosity declining along all the points of the curve. This was confirmed by Derycke et al. (2005) and Xie et al. (2008), although the latter study found the effect of protein on pasting temperature differing between waxy and non–non waxy rice cultivars. Furthermore, protein content has generally been found to be negatively correlated with the adhesiveness of cooked rice (Lyon at al., 2000; Derycke et al., 2005), but has a positive impact on hardness (Derycke et al., 2005). While these studies have established a link between total protein and the physicochemical properties of rice, the role of individual protein fractions on the pasting and rheological properties of rice remains to be elucidated.

Some early studies have shown that glutelin and the 60 kDa starch granule bound starch synthase protein are related to adhesiveness and other textural characteristics (Chrastil, 1992; Hamaker and Griffin, 1993). We have previously reported that prolamin and albumin can significantly influence the pasting and textural properties of rice starch and flour (Baxter et al., 2004, 2010). Specifically, higher prolamin content is linked with significantly higher breakdown viscosity but lower hardness, adhesiveness and gumminess of the gel produced from the flour, while albumin is positively correlated with the pasting, breakdown and final viscosities and gel hardness, but negatively related to gel adhesiveness. However, little is known about the effect of glutelin and globulin on the physicochemical properties of rice starch and flour.

Glutelin, which is a storage protein soluble in dilute alkaline and acid solutions, is the major rice protein, accounting for 70–80% of total rice endosperm protein (Shabbir et al., 2011). The shear dominance of this protein fraction means that it is likely to have a significant impact on the rheological properties of rice starch and flour. Globulin is the protein fraction that is soluble in salt solutions and is a relatively small component compared with glutelin. However, its solubility in salt solutions may make it easier to interact with, and compete with starch for, water during gelatinisation and retrogradation, thus affecting its pasting and textural properties.

The objective of the present study is to investigate the influence of glutelin and globulin on the pasting and textural properties of rice flour using a similar methodology that we have developed for studying the role of prolamin and albumin in the same context (Baxter et al., 2004, 2010), thus completing our investigations on the contribution of individual rice protein fractions to the physicochemical properties of rice starch and flour.

2. Materials and methods

2.1. Materials

The non-waxy rice cultivar, Opus, was mainly used in this study. Two other non-waxy cultivars, Langi and Amaroo, were also used for confirmation of the trends obtained from Opus. The rice samples were kindly provided by the Yanco Agricultural Institute, Yanco, NSW, Australia. Rice grains with an average of 12% moisture (w/w) were dehulled (THU35A Test Husker, Satake) and then milled (McGill No. 2 Mill) for 60 s. Broken grains were separated from whole grains by weight differentials and only whole grains were used for the study. Milled grains were ground to pass through a 0.12 mm screen (Retsch model, Zm100). Rice starch was obtained from Sigma–Aldrich Pty Ltd (Product No. S7260) and was of non-waxy type that contained 11.6% moisture, 0.14% ash and 0.55% protein (data from Sigma–Aldrich Pty Ltd).

2.2. Extraction of rice proteins

Flour samples were defatted before protein extraction to minimise lipid contamination in the protein extracts. Defatting was carried out following the same procedure described previously (Baxter et al., 2004).

Albumin was extracted from rice flour using de-ionised water. In order to maximise the efficiency for extracting the remaining protein fractions, several different solvents and solvent concentrations were trialled. For globulin, extraction with NaCl concentrations ranging from 0.25 to 3.0 M was investigated. For prolamin, the solutions of propanol (100%, 50%), ethanol (100%, 50%) and propanol (100%, 50%) with the addition of 1% 1, 4 Dithio-DL-Threitol (DTT) were tested. In the case of glutelin, 1% and 2% lactic acid with 1 mM EDTA and NaOH ranging in concentration from 0.01 to 0.15 M were trialled. These preliminary experiments established that 1.5 M NaCl, 100% propanol, and 0.1 M NaOH were the most efficient solvents for extracting globulin, prolamin and glutelin, respectively, and they were used in subsequent experiments for extracting these protein fractions. The extractions were performed sequentially with albumin being removed first, followed by globulin, prolamin and glutelin. For each extraction, rice flour samples were mixed with three volumes of the respective solvent and the suspensions were mixed thoroughly. The suspensions were allowed to stand for 30 min, mixed thoroughly again and centrifuged at 10,000 g for 10 min at 15 °C. The extraction procedures were repeated a total of four times for each solvent. The globulin, prolamin and glutelin extracts were dialysed against de-ionised water overnight at room temperature, and the dialysed protein solutions, together with the aqueous extract of albumin, lyophilised (Christ-Alpha 2-4 LD plus freeze dryer, Biotech International, Germany). The lyophilised extracts were weighed and the total nitrogen determined using a LECO FP-2000 analyser (Leco Corp., St Jeseph, MI, USA). Protein content (purity) of the lyophilised protein fractions was calculated by multiplying the percentage nitrogen found by the factor 5.95 (Chrastil, 1992) and the protein purity was found to be greater than 95% for all protein fractions. Each protein assay was conducted in triplicate.

2.3. RVA and textural analysis

Pasting characteristics of rice starch were determined using a Rapid-Visco Analyser (Newport Scientific model 3D, Warriewood, Australia) following the procedure described previously (Baxter et al., 2010). RVA analyses were performed on commercial rice starch alone and on starch samples to which globulin and glutelin were added at concentrations of 5–50 and 5–200 mg/g starch, respectively. The protein added to rice starch was extracted from defatted rice flour as described previously. Each RVA canister contained 3 g of starch or starch plus protein and was made up to 28 g with de-ionised water. Pasting temperature, peak viscosity, hot paste viscosity, final viscosity and breakdown (peak viscosity - hot paste viscosity) were recorded. Each analysis was performed at least twice. Textural properties of the rice starch gels formed 24 h after RVA analyses were determined with a TA XT2 texture analyser (Stable Microsystems, Surrey, Great Britain), following the procedure described elsewhere (Baxter et al., 2010). From the force–time curve obtained, the textural parameters of hardness (height of the force peak on cycle 1, g) and adhesiveness (negative force area of the first cycle, −gs) were computed using the Texture Expert software supplied with the instrument. RVA and textual analyses were conducted in triplicate.

2.4. Measurement of water absorption

Water absorption of rice starch during cooking was measured using a variation of the method by Konik-Rose et al. (2001). The detailed procedure was described elsewhere (Baxter et al., 2010). Briefly, rice starch (100 mg) was accurately weighed into a 1.5 ml