MEDIUM RICE GRAIN ENDOSPERM PROTEIN COMPOSITION ASSOCIATED WITH BROKEN GRAINS AND VISCOSITY PROPERTIES

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Introduction

Rice consumers prefer wholegrain rice (Saleh and Meullenet, 2013) with a particular texture when cooked (Rousset et al., 1999). Hence, there is a persistent need for breeders to develop rice germplasm of high quality that suits the needs of the end user-consumer while also attaining the best possible milling yield.

Rice milling yield is affected by multiple sub-component traits controlled by numerous genes such as grain dimension, grain hardness, and bran thickness (Kepiro et al. 2008). Rice starch is the main component of the rice endosperm and two genes, the *Waxy/waxy* and *Alk/alk* genes, are the primary controllers of rice starch structure as it affects rice grain eating quality (Tian et al., 2009; Kharabian-Masouleh et al., 2013). Although many studies have investigated the relationship between starch and its structure in the context of rice eating quality, starch does not explain all variation in rice grain quality across all rice germplasm (Kharabian-Masouleh et al. 2013).

Protein is the second most abundant component of the rice endosperm after starch and it too influences rice grain texture (Martin and Fitzgerald 2002; Derycke et al 2005; Xie et al 2008). However, the extent to which natural variation in rice grain protein composition influences rice grain textural properties is not well understood. The relationship between rice grain protein composition and rice grain texture was assessed using rapid visco analyser (RVA) and high pressure liquid chromatography (HPLC). Differences in protein composition between broken and unbroken/ wholegrain medium rice grains are also presented.

Materials and methods

Rice samples

Endosperm proteins were extracted from the broken and unbroken grains of 20 medium grain (MG) rice lines obtained from Yanco Agricultural Institute (YAI), Yanco, NSW, Australia, 2013 Quality Evaluation Program (QEP) and 80 MG rice lines from the 2014 QEP. The amylose content of all samples were from 17% to 20%.

Extraction and characterisation of the rice grain protein fractions

Prolamins, glutelins, globulins and albumins were extracted from duplicate 250 mg sub-samples of rice flour with 60% n-propanol, 5 M acetic acid, 1M NaCl and Milli-Q water, respectively, following the protocol of Balindong et al. (2016). HPLC analysis was carried out using an Agilent 1260 HPLC System equipped with a vacuum degasser, quaternary pump, auto-injector, and diode array detector. Column temperature was set at 50ºC and absorbance was monitored at 280 nm (Balindong et al 2016).

The HPLC gradient for prolamins and albumins was as follows: 25% aqueous acetonitrile (ACN) at 0 min, 40% at 5 min, 45% at 15 min, 60% at 25 min, concluding at 95% at 26 and 27 min and returning to 25% at 28 to 33 min (Balindong et al. 2016). For glutelins and globulins, the HPLC gradient commenced at 25% ACN, increased to 35% at 5 min, 40% at 10 and 15 min, 50% at 25 min and reaching a maximum of 95% at 26 and 27

min before returning to 25% between 28 and 33 min (Balindong et al. 2016). Both HPLC methods utilised a C8-5 column.

Data analysis

Protein peaks were identified and analysed using ChemStation software B.04.03 and correlated with millout and RVA data. Data were compiled with Microsoft Excel 2013 and imported into GenStat 64-bit Release 17.1 for statistical analysis.

Results and Discussion

A. Endosperm proteins in the broken medium rice grains

The protein content of the 20 broken MG rice lines was lower compared to the corresponding unbroken MG samples, but the reduction was not uniform across all fractions. Prolamin and glutelin in the broken grain were reduced by 21.0% and 38.7%, respectively, while globulin did not differ between broken and unbroken grain. Wholegrain total prolamin and globulin content was positively correlated with wholegrain millout (r = 0.60 – 0.75). Although glutelin is the main storage protein in rice and exhibited the largest reduction, it seems to have a minor role in maintaining rice grain structure. Prolamin and globulin may be the dominant protein fractions associated with maintaining wholegrain medium rice integrity (Fig. 1).

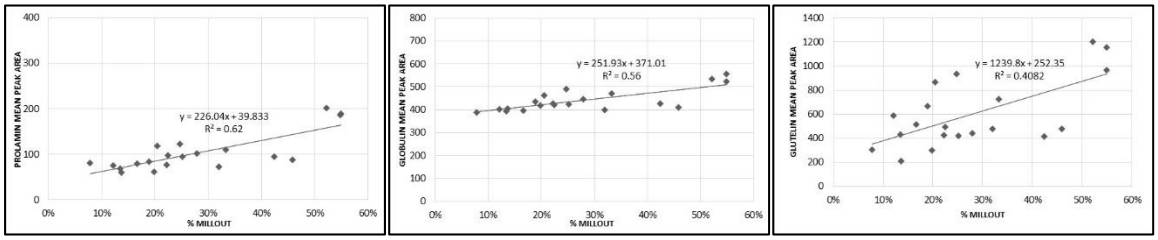

Fig. 1. Regression analyses of mean peak area of prolamin, globulin and glutelin in wholegrain medium rice grain vs. millout.

B. Endosperm proteins and rice grain texture

Analysis of 80 2014 MG samples identified two HPLC profiles; Profile 1 (67 samples) and Profile 2 (13 samples). Correlation analysis of HPLC Profile 1 found individual prolamin peaks and total prolamin (r = 0.66) were most highly correlated with RVA parameters including setback while total globulin (r= 0.16) and total glutelin (r = -0.47) exhibited weaker correlations. Setback is the difference between final and peak RVA viscosity and predicts the firmness of cooked rice. Apparent amylose content (R^2 = 0) and total protein content (R^2 = 0.18) explains very little variation in setback. The ratio between the total prolamin: total prolamin + total glutelin (R^2 = 0.67) explains a significant proportion of the variation in setback (Fig. 2). Correlation analysis of HPLC Profile 2 also found individual prolamin peaks and total prolamin were highly correlated with RVA parameters, however, total protein was more highly correlated in all cases.

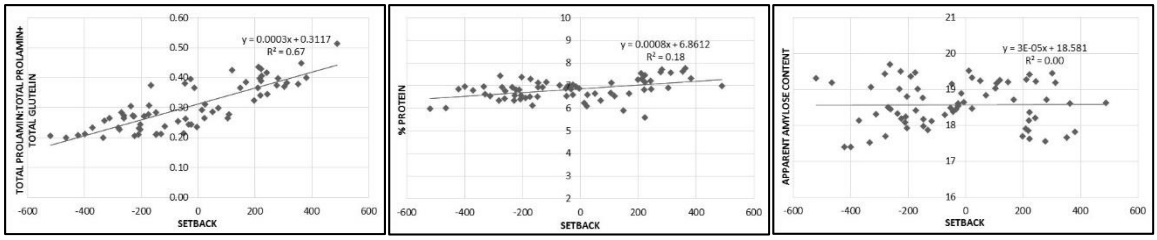

Fig. 2. Scatter diagram and regression analyses of apparent amylose content, % protein and total prolamin:sum of total prolamin and total glutelin ratio vs setback of medium rice grain.

Although the textural properties of rice are complex, these data suggest low prolamin rice has a softer texture and consideration of rice grain protein composition could contribute to breeding high quality rice. Prolamins are poorly digested by humans (Resurreccion and Juliano, 1981), thus breeding for low prolamin content rice could also result in more nutritious rice.

Prolamin and globulin seem to play a role in maintaining wholegrain integrity. Breeding for soft-textured rice varieties requires reducing prolamin content, which may have the negative effect of increasing grain breakage, however, it is possible this adverse effect may be balanced by increasing globulin content.

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