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Effect of alkali treatment on the milled grain surface protein and physicochemical properties of two contrasting rice varieties

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A B S T R A C T

A systematic study was conducted to explore the effect of grain surface proteins on the physicochemical properties (pasting, retrogradation and textural quality) of rice. Milled rice grains of two selected glutinous (Thadokkham-8 (TDK8)) and non-glutinous (Doongara (DG)) varieties were treated with different concentrations (0%, 0.004%, 0.02%, 0.04%, and 0.2% w/v) of NaOH solution for 1 h. After surface protein removal, the cooked rice grains showed a significant ($P < 0.05$) increase in adhesiveness. Similarly, protein removal showed a significant ($P < 0.05$) decrease in the final viscosity (V_f) of rice flours. Furthermore, NaOH treatment at a concentration above 0.04% induced yellow colour development in grains. Differential calorimetric study showed that alkali treatment resulted in increased onset ($T_\circ$), peak (T_p), conclusion (T_c) temperatures and enthalpy (ΔH) of both rice varieties. No significant ($P > 0.05$) effect of alkali treatment was observed on the retrogradation thermal temperatures ($T_{o(r)}$, $T_{p(r)}$, and $T_{c(r)}$), but the amount of retrograded starch (as indicated by reduction in $\Delta H_{(r)}$) was decreased significantly ($P < 0.05$) in both varieties. These findings suggest a good potential of applying alkali pre-treatments in the processing of rice to alter the hardness and stickiness properties of rice.

1. Introduction

An increasing trend in the consumption pattern of rice has been observed due to rising interest in gluten free products. Rice can be broadly divided into two distinctive types based on the native starch type present in the endosperm; glutinous rice cultivars primarily containing branched amylopectin and non-glutinous rice cultivars containing linear chain amylose as well as amylopectin (Yu et al., 2015). The textural attributes of cooked glutinous and non-glutinous rice are quite different from each other due to this compositional difference. Good quality glutinous rice should be very sticky and vice versa for non-glutinous rice (Nawaz et al., 2016a). However, ageing induces functional changes in the stored glutinous rice (Nawaz et al., 2016b) making it less sticky. The mechanism of reduction in the cooked rice stickiness is still an area of research interest.

The functional attributes of rice have long been ascribed to starch composition and property. Many studies to date have focused on the role of amylose content (Lu et al., 2013; Syahariza et al., 2013), fine structures of amylopectin (Syahariza et al., 2013), solubility of amylose (Fu et al., 2015), the gelatinization and melting temperatures of amorphous and crystalline regions of amylopectin (Zeng et al., 2014), and the amount of native structures remaining in starch granules after heating (Klaovhanpong et al., 2015). Extensive consideration of investigation on only starch is not surprising considering that starch accounts for 92–95% of the dry matter in a milled rice grain. However, it has now been realised that starch may not be the only factor affecting the cooking/eating attributes of rice grains (Yadav et al., 2013).

Protein is the second most abundant macromolecule in rice endosperm after starch. Rice generally contains 6–8% protein and does not fluctuate widely from this level (Yadav et al., 2013). Proteins in a rice kernel are present in the form of round discrete protein bodies (PBs). The estimated size of PB is usually around 4–5 μm. There are two types of PBs; Protein body I and protein body II (Han and Hamaker, 2002). PBs in subaleurone layer are not similar to those present in endosperm (Baxter et al., 2004). Subaleurone PBs are rich in glutelin (alkali soluble) and albumin (water soluble). While endospermic PBs are rich in prolamin (alcohol soluble) (Baxter et al., 2004).

Various studies have been conducted in the past to find out the effect of protein (Yadav et al., 2013; Xie et al., 2008) and shown a

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List of abbreviations

AAC	Apparent amylose content
AACC	American Association for Cereal Chemist
approx.	Approximately
ACIAR	Australian Centre for International Agricultural Research
BD	Breakdown
C_c	Control (without any treatment)
C_{c0}	0% alkali (NaOH) concentration
$C_{0.004}$	0.004% alkali (NaOH) concentration
$C_{0.02}$	0.02% alkali (NaOH) concentration
$C_{0.04}$	0.04% alkali (NaOH) concentration
$C_{0.2}$	0.2% alkali (NaOH) concentration
CLSM	Confocal Laser Scanning Microscope/Micrograph
DSC	Differential Scanning Calorimeter
DG	Doongara
F' 30s	1st derivative for every 30 s
g	gram/Grams
Jg^{-1}	Joules per gram
min	minute/minutes
mL	Millilitre
mm	Millimetre
mPa-s	millipascal-second
N	Newton
n	number of independent replicates
NaOH	Sodium hydroxide
NAFRI	National Agriculture and Forestry Research Institute, Lao PDR
Na_2CO_3	Sodium carbonate
Nsec	Newton second
NSW DPI	New South Wales Department of Primary Industries
PB	Protein body
PBs	Protein bodies
pi	Point of inflection
RH	Relative humidity
PDR	Peoples' Democratic Republic
P_{temp}	Pasting temperature
r	Correlation
RRAPL	Rice Research Australia Pty Ltd
RVA	Rapid Visco Analyser
R %	Percentage of retrogradation
sec	Second/Seconds
SB	Setback
SD	Standard deviation
TDK8	Thadokkham-8
TPA	Texture Profile Analysis
T_o	Onset temperature of gelatinization
T_p	Peak temperature of gelatinization
T_c	Conclusion temperature of gelatinization
$T_{o(r)}$	Onset temperature of retrogradation
$T_{p(r)}$	Peak temperature of retrogradation
$T_{c(r)}$	Conclusion temperature of retrogradation
V_f	Final viscosity
V_p	Peak viscosity
V_{pi}	Viscosity at point of inflection
V_t	Trough viscosity
w/v	Weight by volume
XPS	X-ray Photoelectron Spectroscopy
ΔH	Enthalpy of starch gelatinization
$\Delta H_{(r)}$	Enthalpy of retrograded starch
µL	Microliter
µm	Micrometre

weak correlation between the gross protein content and the texture of cooked rice, higher protein content rice being harder than low protein content rice (Baxter et al., 2004). Moreover, in a recent study the surface analysis of rice kernels using X-ray Photoelectron Spectroscopy (XPS) and Confocal Laser Scanning Microscopy (CLSM) showed an over-expression of proteins and lipids and an under-expression of starch on the surface of rice endosperm compared to the bulk composition of endosperm (Nawaz et al., 2016c). Alkali extraction has been used in recent studies to extract protein from cereal flours, especially in rice (Souza et al., 2016). Alkaline treatment by agents such as lye or sodium hydroxide is widely used in the production of many value-added food products from cereals, including tortillas, waxy rice dumplings, and various extruded products such as instant noodles and yellow alkaline noodles (Nadiha et al., 2010). It is assumed that dilute alkali treatment to the whole rice grains may be a useful technique to remove surface protein residues resulting in more starch on the surface. An increase in stickiness/adhesiveness in stored rice may be improved by removing surface proteins, as starch is stickier than protein (Hamaker et al., 1991). Alkali treatment may also wash surface lipids by saponification. However, alkali application to food products especially cereals should be employed carefully as steeping with higher concentration of alkali (such as 0.4% NaOH) for longer time (7–14 days) can lead to structural changes in rice starches (Cai et al., 2014), resulting in changes in functional properties such as swelling power, water binding capacity, gelatinization and pasting attributes (Karim et al., 2008; Wang and Copeland, 2012). Our study has avoided the inappropriate alkali steeping by using lower NaOH concentration for shorter period of time. The objective of the present study is to investigate if the removal of the protein bodies from the surface of the grain alters the stickiness of the cooked grain. For this the milled rice grains of two contrasting rice varieties (waxy and non-waxy, respectively) were treated with various concentrations of sodium hydroxide solution to wash surface proteins and lipids. This washing was expected to lead to increase in stickiness of cooked rice grains which is one of the most important quality attributes of waxy rice.

2. Materials and methods

One *Oryza sativa* indica cultivar of glutinous rice from Lao PDR (Thadokkham-8 (TDK8) having 3.77% apparent amylose contents (AAC)) and one *O. sativa* japonica non-glutinous rice from Australia (Doongara (DG), 19.71% (AAC)) were used in this study. The milled TDK8 was provided by National Agriculture and Forestry Research Institute (NAFRI), Lao PDR, while Doongara were provided by Rice Research Australia Pty Ltd (RRAPL), Mackay, Qld, Australia.

2.1. Alkali treatment

The milled rice grains of selected varieties were soaked in various concentrations of NaOH solution ($C_{c0} \simeq 0\%$, $C_{0.004} \simeq 0.004\%$, $C_{0.02} \simeq 0.02\%$, $C_{0.04} \simeq 0.04\%$, and $C_{0.2} \simeq 0.2\%$) at 40 °C for 1 h with a rice to solution ratio of 1:8. After 1 h the treated rice grains were washed with deionised water until completely neutralised (pH = 7.0 approx.). These concentrations corresponded to 7.0, 11.0,