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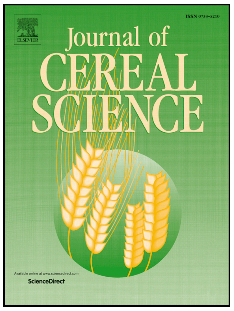

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PII: S0733-5210(16)30253-3
DOI: 10.1016/j.jcs.2016.09.009
Reference: YJCRS 2217

To appear in: *Journal of Cereal Science*

Received Date: 7 August 2016
Revised Date: 5 September 2016
Accepted Date: 13 September 2016

Please cite this article as: Nawaz, M.A., Fukai, S., Bhandari, B., Effect of alkali treatment on the milled grain surface protein and physicochemical properties of two contrasting rice varieties, *Journal of Cereal Science* (2016), doi: 10.1016/j.jcs.2016.09.009.

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

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Abstract

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 hour. 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_o), 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.

Keywords

Surface proteins, Alkali treatment, Adhesiveness, Pasting properties, Retrogradation

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 sec
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

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 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 (Lai et al. 2002), 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 hour with a rice to solution ratio of 1:8. After 1 hour the treated rice grains were washed with deionised water until completely neutralised (pH = 7.0 approx.). These concentrations corresponded to 7.0, 11.0, 11.7, 12.0 and 12.7 pH, respectively. The treated samples were spread on blotting paper and kept in fume hood at room temperature (22±1°C, RH~50 %) for 72 hours to reduce the moisture contents to 14 %. One control (C_c) sample without any treatment was also kept for comparison.

2.2. Colour estimation of alkali treated rice grains

A Konica Minolta Chroma Meter CR-400 (Tokyo, Japan) was used for all colour measurements. Prior to colour measurements, the colour meter was calibrated with a white tile. Colour measurements were made at least in three folds on samples placed in a clear petri dish. The colour was measured in CIE*L*a*b** colour space. *L** is a measurement of brightness from black (0) to white (100). Parameter *a** refers red-green colour with positive *a**-values indicating redness and negative *a**-values indicating greenness. Whereas, parameter *b** refers yellow-blue colour with positive *b**-values indicating yellowness and negative *b**-values indicating blueness (Good, 2002).

2.3. Confocal laser scanning microscopy (CLSM)

Alkali treated rice grains were dyed with a mixture (1:1) of 0.01 % (w/v in water) Rhodamine B (Sigma R6626) and 0.02 % (w/v in poly (ethylene glycol) 200 (Fluka 81150)) Nile Red (Sigma 72485) for labelling protein and lipid, respectively. The samples were treated with dyes in dark with intermittent shaking. After 10 min dye-labelled samples were washed with deionised water until supernatant became clear. The microstructure of rice grains was observed by using LSM 700 confocal laser scanning microscope (CLSM, Zeiss, Germany).

2.4. Crude protein analysis

Rice samples were ground to flour using a disc mill (Good Friends of the Guangzhou Machinery Co. Ltd., Guangzhou, China). The flour particles were sieved through 500 µm sieve to attain particle size ≤ 500 µm. Crude protein content in rice flour was determined by the semi-micro-Kjeldahl method using a Kjeltec 2300 Autoanalyser (Foss AB, Sweden). A nitrogen conversion factor of 5.95 was used to compute the protein value.

2.5. Textural profile analysis

Rice grains (5 g) were added in 15 mL of MilliQ water (rice:water ratio =1:3) in 50 mL glass beaker. The beaker was placed in water bath at 95±1°C. Cooking was continued until there was no ungelatinized white belly observed in rice kernel cross section (data not shown). Analysis of textural attributes was performed on a TA-XT*plus* Texture Analyser (Stable Microsystems, UK) using 35-mm circular probe. Three cooked grains were on the flat stage, and the texture determined. The texture analyser settings were as follows: pre-test speed, 2.00 mm/sec; post-test speed, 2.00 mm/sec; distance, 2.00 mm; time, 10.00 sec; (auto) trigger force, 0.05 N. From the force-time curve obtained, textural attributes of hardness (height of the force peak on cycle 1, N) and adhesiveness (negative force area of the first cycle, Nsec) were computed using the EXPONENT Stable Micro Systems software supplied with instrument. The TPA values reported are the averages of 3 different determinations.

2.6. Pasting properties

Pasting properties of rice flour (particle size $\leq$ 500 μm) were determined according to the AACC International Method 61-02.01 using a Rapid Visco Analyser (RVA-4 model Thermocline Windows Control and analysis software, Version 1.2 (New Port Scientific, Sydney, Australia)). Rice flour (3.01 g, 12.4 % moisture basis) was mixed with 25.0 g MilliQ water in the RVA canister. A programmed heating and cooling cycle was used, the samples were held at 50°C for 1 min, heated to 95°C in 3.45 min, held at 95°C for 2.7 min before cooling to 50°C in 3.91 min and holding at 50°C for 1.24 min. Pasting temperature (P_{temp}), Peak viscosity (V_p), Trough viscosity (V_t), Breakdown (BD), Final viscosity (V_f) and Setback (SB) were recorded.

There was no peak viscosity found in DG viscographs. Therefore, Point of inflection (pi) was calculated using 1st derivative for every 30 sec (F´ 30s) as shown in supplementary section Fig. S1. Viscosity at point of inflection (V_{pi}) was estimated by using pi.

2.7. Gelatinisation and retrogradation properties

Differential Scanning Calorimeter (DSC) (Mettler Toledo, Schwerzenbach, Switzerland) with internal coolant and nitrogen/air purge gas was used to determine the gelatinisation characteristics of rice flours. The DSC was calibrated for the heat flow using indium as standard. Rice flour (4 mg, dry weight basis) was accurately weighed into aluminium pan and 6 µL MilliQ water was added. The pan was hermetically sealed and equilibrated at room temperature for 30 min, then scanned at the heating rate of 15°C/min from 0 to 100°C with empty sealed pan as reference. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures, and enthalpy (ΔH) of gelatinisation were determined by Star^e Software Version 9.1 (Mettler Toledo).

After cooling the scanned samples pans were placed in a refrigerator at 4±1°C for 7 days. Retrogradation properties were measured by rescanning these samples at the rate of 15°C/min from 0 to 100°C. The onset ($T_{o(r)}$), peak ($T_{p(r)}$) and conclusion ($T_{c(r)}$) temperatures, and enthalpy of retrograded starch ($\Delta H_{(r)}$) were determined. The percentage of retrogradation (R %) was calculated as $\Delta H_{(r)}/\Delta H \times 100$.

2.8. Statistical analysis

All treatments were replicated three times to obtain mean values. The reported data for the CIE*L*a*b** colour space, crude protein, textural profile analysis, pasting properties, and gelatinisation and retrogradation for each variety was analysed separately by analysis of variance using Minitab R17 (Minitab® for Windows Release 17, Minitab Inc, Chicago) in order to determine significant differences. The data was then analysed using Tukeys pair-wise comparison, at 5 % level of significance, to compare the results between different treatments.

3. Results and discussion

3.1. Colour estimation of alkali treated rice grains

The colour parameters of the raw and cooked grains of control and alkali treated TDK8 and DG are shown in Table 1. Significant ($P<0.05$) decrease in the brightness (L^*) of raw TDK8 kernels was observed when treated with 0.2 % of NaOH. However, no change in L^* was observed in raw DG rice kernels. From 0.02 % to 0.2 % of NaOH concentration induced a significant ($P<0.05$) increase in greenness ($-a^*$) in raw TDK8 rice; whereas, only 0.2 % of NaOH induced a significant ($P<0.05$) greenness in raw DG rice grains. For raw TDK8 rice grains, yellowness (b^*) increased significantly with 0.2 % of NaOH. Raw DG rice grains were susceptible to alkali yellowness (b^*) and significant ($P<0.05$) increase in yellowness (b^*) was observed with increase in alkali concentration. In cooked TDK8 rice kernels significant ($P<0.05$) decrease in the brightness (L^*) at 0.004 % of NaOH concentration was observed. However, no significant ($P>0.05$) decrease in L^* was observed with further increase in NaOH concentration. Moreover, NaOH concentration of 0.04 % induced significant ($P<0.05$) increase in L^* of cooked DG rice kernels. NaOH concentration of 0.02 % induced a significant ($P<0.05$) increase in greenness ($-a^*$) in cooked TDK8 rice. Further increase in NaOH concentration had no significant ($P>0.05$) effect on a^* of cooked TDK8 rice kernels. Whereas, NaOH concentration of 0.04 % induced a significant ($P<0.05$) increase in greenness ($-a^*$) in cooked DG rice kernels. From 0.02 % to 0.2 % of NaOH concentration induced a significant ($P<0.05$) increase in yellowness (b^*) in both cooked TDK8 and DG rice kernels. Previous studies on the effect of alkali treatment on cereal products also reported the induced yellowness in sodium hydroxide treated grains and flour (Lai et al. 2004; Nadiha et al. 2010). The induced yellow colour in alkali treated rice grains may be attributable to the naturally occurring flavonoids (such as apigenin-*C*-diglycosides) present in cereal aleurone and sub-aleurone layers. These compounds are colourless at acidic or neutral pH but turn yellow at basic pH (Asenstorfer et al. 2006). The scanned images showing colour differences of raw and cooked grains of control and alkali treated TDK8 and DG are presented in supplementary section in Fig. S2.

3.2. *Confocal laser scanning microscopy (CLSM)*

The CLSM images of control and alkali treated rice kernels are shown in Fig. 1. The surface of C_c and C_{c0} in both rice varieties (TDK8 and DG) showed a layer of lipids (labelled as red with Nile red) and protein (labelled as green with Rhodamine B). Reduction of surface proteins and lipids was observed with increase in NaOH concentration from 0.004 % to 0.2 %, showing washing of surface proteins (possibly mostly glutelin) and lipids. In addition, besides surface proteins some of the proteins located internally might have been removed. Although CLSM was unable to detect this, as the dyes (Rhodamine B and Nile red) were unable to penetrate the interior of the kernel.

3.3. *Mass loss during alkali treatment*

Less than 3 % and 4.5 % of mass loss was recorded in the water and maximum alkali (0.2 % NaOH w/v) treated samples (data not shown). Soaking of milled rice kernels with alkali solution not only washed surface proteins and saponify surface lipids but also removed intact dust and bran residues. No doubt there might be loss of water soluble components from grains which were not analysed in the present study and needs further investigation.

3.4. *Crude protein of control and alkali treated rice samples*

The crude protein content of control and alkali treated TDK8 and DG are presented in Fig. 2a and 2b, respectively. Results showed that DG may have more alkali soluble protein (such as glutelin) content than TDK8. There was no significant ($P>0.05$) reduction of total crude protein content of TDK8 up to 0.04 % of NaOH when compared to C_c (control). However, significant ($P<0.05$) reduction of total protein content was found in DG even at 0.004 % of NaOH.

3.5. *Textural profile analysis*

Textural profiles of cooked control and alkali treated TDK8 and DG rice grains are shown in Fig. 3a and 3b, respectively. Cooked TDK8 rice hardness values ranged from 2.12±0.004 N (C_{c0}) to 7.84±0.011 N ($C_{0.2}$) and from 8.03±0.058 N (C_{c0}) to 18.56±0.157 N ($C_{0.2}$) for cooked DG rice. The rice samples treated with 0 % water showed the least hardness in both rice varieties. Results showed a significant ($P<0.05$) increase in cooked rice hardness with increase in NaOH concentration from $C_{0.004}$ to $C_{0.2}$ in both rice varieties. Baxter et al. (2004) reported that removal of prolamin by 100 % propan-2-ol resulted in significant ($P<0.05$) increase in hardness which is similar to our results where we removed the water soluble proteins by treating with alkaline conditions.

The effect of protein removal through alkali (NaOH) treatment on the adhesiveness/stickiness of cooked rice grains of selected rice varieties TDK8 and DG is shown in Fig. 3a and 3b, respectively. Cooked rice stickiness increased significantly ($P<0.05$) with decrease in grain surface protein contents, as indicated in CLSM images of control and alkali treated rice grains of selected varieties (Fig. 1). Similar to the current findings, Chrastil (1990) also reported that the adhesiveness/stickiness of rice could be increased with the reduced amount of glutelin it contained. In addition, there was significant ($P<0.05$) correlation found between hardness and adhesiveness of alkali treated rice grains TDK8 and DG as shown in Fig. 3b and 3c, respectively. Cooking is a complex process due to several physicochemical changes taking place simultaneously. The surface layers, cells and granules disintegrate and components leach out from the cells (Tamura et al. 2014). This disintegration allows starch granules to interact with protein bodies, mostly amylopectin with glutelin (Chrastil, 1990; Baxter et al. 2014). This starch-protein interaction affects the overall stickiness of the cooked rice, higher the interaction greater will be the stickiness. Previous studies reported the reduced starch-protein interaction in aged rice resulted in decreased stickiness (Chrastil, 1990; Derycke et al. 2005; Baxter et al. 2014), probably due to a thin layer of non-interacted protein (glutelin) bodies on the rice endosperm surface. This explanation supports the hypothesis and findings of present study; washing of surface proteins led to increase in stickiness of cooked rice.

3.6. Pasting properties

The pasting properties of control and alkali treated TDK8 and DG rice flours are shown in Table 2. Results showed diverse behaviour of samples due to alkali treatment. Increase in pasting temperature (P_{temp}) with increase in surface proteins removal was observed for TDK8. However, slight decrease in P_{temp} was found in DG with increase in surface proteins removal. Increase in surface proteins removal restricted the swelling of starch granules in TDK8 flour, resulting in significant ($P<0.05$) decrease in peak viscosity (V_p). However, protein removal had no effect on viscosity at point of inflection (V_{pi}) in case of DG. Probably, sodium hydroxide (NaOH) treatment had masked the effect of protein on V_p. It was also found that increase in alkali concentration also significantly ($P<0.05$) reduced the V_p. Interestingly, these results are not in agreement with the findings of Lai et al. (2004), who reported significant ($P<0.05$) increase in V_p of native cereal starches in sodium carbonate (Na_2CO_3) and NaOH solutions. However, similar results of restricted V_p of alkali treated sago starch were reported by Karim et al. (2008). The possible reason of variation in the pasting behaviours may be the difference in the sample preparation. In this study and the study carried out by Karim et al. (2008), cereal samples were treated with alkali and subsequently washed with deionised water for 15 min to neutralise the pH prior to examining the pasting behaviour. However, Lai et al. (2004) carried out pasting studies on starch samples suspended in alkali solutions such as 1 % Na_2CO_3 and 1 % NaOH. As the sample preparation in the present study is similar to that of Karim et al. (2008), the reduced V_p could be due to the disrupted amorphous regions and weakened granular rigidity of alkali treated starch samples.

In general, protein removal resulted in decreased Breakdown (BD) and Setback (SB) of TDK8 flour and Final viscosity (V_f) of both TDK8 and DG flours. Although there was no BD and Trough viscosity (V_t) observed in DG, increase in alkali concentration reduced the rate of increase in viscosity during holding at 95°C as shown in supplementary section S3. The results are at par with the findings of Baxter and co-workers (2004), who studied the effect of prolamin on the pasting properties of rice flour. They reported that extraction of approximately 95 % of total prolamin fractions in rice flours resulted in significant ($P<0.05$) reduction in BD and V_f and slight reduction in SB. But this study did not investigate the effect of surface protein removal from the rice flour or kernels.

3.7. Gelatinisation and retrogradation properties by DSC

The gelatinisation and retrogradation properties of control and alkali treated TDK8 and DG rice flours are shown in Table 3. Protein removal via alkali treatment resulted in increased onset (T_o), peak (T_p), conclusion (T_c) temperatures and enthalpy (ΔH) during gelatinization of both rice varieties. The rise in gelatinization transition temperatures and enthalpy with increase in NaOH concentration may be attributed to the starch granule stability, possibly through electrostatic interactions between hydroxyl groups of starch and Na^+ ions. Starch exhibits Donnan-potential in the presence of water due to its weak acidic ion-exchanging behaviour (Oosten, 1990). The starch particles have negative charge; therefore, penetration of Na^+ into the amorphous regions of starch granules is promoted. Moreover, under alkaline conditions, hydroxyl groups of starch might have greater tendency to ionise and create even more binding sites for cations. It is hypothesized by Oosten (1990) that anions might tend to destabilise starch granules by breaking hydrogen bonds. However, such destabilising effects of anions might be much weaker than the stabilising effects of cations. Several researchers have reported similar stabilising effects of sodium salts such as sodium chloride (Abd Ghani et al. 1999; Evans and Haisman, 1982), sodium acetate (Evans and Haisman, 1982), sodium sulphate (Evans and Haisman, 1982), and sodium carbonate (Lai et al. 2002).

On the other hand, no significant (*P>0.05*) effect of protein removal was observed on the retrogradation thermal temperatures ($T_{o(r)}$, $T_{p(r)}$, and $T_{c(r)}$), but enthalpy of retrograded starch ($\Delta H_{(r)}$) was decreased significantly (*P<0.05*) in the flour samples of both varieties. This indicates that higher concentration of alkali treatment on starch restricts retrogradation. This was a highest amount of decrease in retrogradation at highest concentration (0.2% w/v) of alkali treatment. In fact, $\Delta H_{(r)}$ provides an overall measure of the energy requirement for melting or uncoiling of double helices of recrystallized amylopectin (Russell, 1987). Significant (*P<0.05*) decrease in $\Delta H_{(r)}$ with increase in alkali treatment may be indicative of structural changes in amylopectin, possibly alkali-induced depolymerisation. This will need further investigation.

4. Conclusion

This study showed that the protein surface layer from rice kernel or rice flour could be washed by using very dilute (as low as 0.002 %) alkali (NaOH) solutions. It was found that NaOH treatment at a concentration above 0.04 % induced yellow colour development in grains. Moreover, it was also observed that textural, pasting, thermal attributes and retrogradation properties were also affected by alkali solution washing. Stickiness of cooked glutinous and non-glutinous (TDK8 and DG, respectively) rice could be significantly increased by washing with dilute NaOH solutions. The contrasting effects of washing of surface proteins and NaOH concentration mean that it might be promising to manipulate the textural properties of glutinous and non-glutinous rice kernels to achieve desirable sensory outcomes by varying the proportions of the surface proteins in milled rice kernels.

Acknowledgements

This work was financially supported by the Australian Centre for International Agricultural Research (ACIAR). Thanks also to Dr Jaquie Mitchell (Research Fellow, The University of Queensland), Andrew Barfield (Farmer), Russell Ford (Research Manager, Sunrice, RRAPL), Peter Snell (Rice Breeder, NSW DPI) and NAFRI (Lao PDR) for providing the rice paddy samples used in this research.

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Table 1

CIEL*a*b* colour space of control and alkali treated rice grains of Thadokkham-8 (TDK8) and Doongara (DG).*

Rice variety	Treatment	CIEL*a*b* colour space		
		L*	a*	b*
Raw TDK8 rice grains	C_c	$97.4^{ab}\pm0.18$	$-0.41^{a}\pm0.04$	$7.56^{a}\pm0.09$
	C_{c0}	$97.6^{a}\pm0.01$	$-0.38^{a}\pm0.05$	$7.99^{a}\pm0.12$
	$C_{0.004}$	$97.2^{ab}\pm0.41$	$-0.41^{a}\pm0.02$	$7.82^{a}\pm0.28$
	$C_{0.02}$	$97.7^{a}\pm0.04$	$-0.59^{b}\pm0.04$	$9.00^{a}\pm0.29$
	$C_{0.04}$	$97.6^{a}\pm0.52$	$-0.86^{c}\pm0.04$	$9.27^{a}\pm0.95$
	$C_{0.2}$	$96.4^{b}\pm0.09$	$-1.88^{d}\pm0.03$	$13.21^{b}\pm0.20$
Cooked TDK8 rice grains	C_c	$96.8^{ab}\pm0.35$	$-0.96^{a}\pm0.02$	$0.55^{a}\pm0.01$
	C_{c0}	$97.7^{a}\pm0.37$	$-0.68^{a}\pm0.32$	$0.37^{a}\pm0.01$
	$C_{0.004}$	$95.6^{c}\pm0.21$	$-0.84^{a}\pm0.05$	$0.65^{a}\pm0.04$
	$C_{0.02}$	$95.4^{cd}\pm0.57$	$-1.69^{b}\pm0.02$	$2.54^{b}\pm0.06$
	$C_{0.04}$	$94.8^{cd}\pm0.14$	$-1.79^{b}\pm0.01$	$3.55^{c}\pm0.10$
	$C_{0.2}$	$94.2^{d}\pm0.05$	$-1.85^{b}\pm0.04$	$4.35^{d}\pm0.32$
Raw DG rice grains	C_c	$94.1^{a}\pm0.23$	$0.43^{a}\pm0.04$	$8.87^{a}\pm0.08$
	C_{c0}	$94.6^{a}\pm0.71$	$0.38^{a}\pm0.02$	$9.69^{b}\pm0.03$
	$C_{0.004}$	$93.9^{a}\pm1.38$	$0.34^{a}\pm0.04$	$9.97^{bc}\pm0.18$
	$C_{0.02}$	$93.9^{a}\pm0.60$	$0.35^{a}\pm0.01$	$10.34^{d}\pm0.18$
	$C_{0.04}$	$94.3^{a}\pm0.55$	$0.39^{a}\pm0.02$	$10.30^{bc}\pm0.03$
	$C_{0.2}$	$94.1^{a}\pm0.18$	$-0.55^{b}\pm0.10$	$13.10^{e}\pm0.05$
Cooked DG rice grains	C_c	$93.1^{a}\pm0.26$	$1.20^{a}\pm0.28$	$7.25^{ab}\pm0.35$
	C_{c0}	$94.3^{ab}\pm0.35$	$0.73^{ab}\pm0.04$	$6.45^{a}\pm0.35$
	$C_{0.004}$	$93.4^{a}\pm0.21$	$0.65^{b}\pm0.04$	$7.55^{ab}\pm0.21$
	$C_{0.02}$	$93.8^{a}\pm0.57$	$0.39^{b}\pm0.01$	$8.55^{b}\pm0.21$
	$C_{0.04}$	$95.2^{bc}\pm0.07$	$-0.13^{c}\pm0.04$	$10.34^{c}\pm0.09$
	$C_{0.2}$	$95.5^{c}\pm0.19$	$-0.35^{c}\pm0.07$	$14.10^{d}\pm0.71$

*Means ± SD (n = 3). For a particular rice variety, means with different letters in same column denote significant difference at 5 % probability level within each rice variety.

Table 2

Pasting properties of control and alkali treated flour of Thadokkham-8 (TDK8) and Doongara (DG).*

Rice variety	Treatment	Pasting properties					
		P_{temp} (°C)	V_p (mPa-s)	V_t (mPa-s)	BD (mPa-s)	V_f (mPa-s)	SB (mPa-s)
TDK8 flour	C_c	74.7^{a}±0.67	2417^{a}±27.58	2121^{a}±46.67	296^{ab}±74.25	2527^{a}±43.13	406^{a}±3.54
	C_{c0}	73.3^{a}±0.78	2784^{b}±108.19	2183^{a}±61.52	601^{a}±169.71	2584^{a}±45.96	401^{a}±15.56
	$C_{0.004}$	73.9^{a}±0.49	2692^{b}±25.46	2063^{ab}±41.01	629^{a}±15.59	2473^{a}±17.68	410^{a}±23.33
	$C_{0.02}$	73.8^{a}±0.64	2357^{a}±70.00	1966^{b}±7.78	391^{ab}±77.78	2244^{b}±22.63	279^{b}±14.85
	$C_{0.04}$	74.1^{a}±0.28	2184^{a}±62.23	1722^{c}±12.73	462^{a}±74.95	2029^{c}±52.33	307^{b}±39.60
	$C_{0.2}$	79.7^{b}±0.46	1535^{c}±14.14	1481^{d}±16.26	255^{b}±2.12	1751^{d}±14.14	271^{b}±2.12
Sample	Treatment	P_{temp} (°C)	V_{pi} (mPa-s)	V_f (mPa-s)			
DG flour	C_c	83.3^{a}±0.60	304^{a}±44.55	3469^{a}±151.32			
	C_{c0}	82.5^{a}±1.73	297^{a}±87.68	3014^{b}±177.48			
	$C_{0.004}$	82.1^{a}±0.78	341^{a}±50.91	3037^{b}±62.93			
	$C_{0.02}$	80.8^{a}±0.49	363^{a}±87.68	2848^{bc}±75.66			
	$C_{0.04}$	80.7^{a}±0.18	316^{a}±3.54	2466^{c}±3.54			
	$C_{0.2}$	79.9^{a}±0.35	225^{a}±31.82	2594^{c}±11.31			

*Means ± SD (n = 3). For a particular rice variety, means with different letters in same column denote significant difference at 5 % probability level within each variety.

Table 3

Gelatinisation and retrogradation properties of control and alkali treated flour of Thadokkham-8 (TDK8) and Doongara (DG).

Rice variety	Treatment	Gelatinization				Retrogradation				
		T_o (^{o}C)	T_p (^{o}C)	T_c (^{o}C)	ΔH (Jg^{-1})	$T_{o(r)}$ (^{o}C)	$T_{p(r)}$ (^{o}C)	$T_{c(r)}$ (^{o}C)	$\Delta H_{(r)}$ (Jg^{-1})	R (%)
TDK8 flour	C_c	$64.4^{a}\pm0.40$	$71.6^{a}\pm0.01$	$84.6^{a}\pm0.47$	$10.4^{ab}\pm0.23$	$41.1^{a}\pm0.18$	$51.9^{a}\pm0.33$	$60.0^{a}\pm0.45$	$4.4^{ab}\pm0.26$	$42.2^{ab}\pm3.44$
	C_{c0}	$64.9^{a}\pm0.45$	$72.5^{b}\pm0.16$	$87.8^{b}\pm0.81$	$9.3^{a}\pm0.78$	$41.4^{a}\pm1.22$	$52.2^{a}\pm1.01$	$60.3^{a}\pm0.23$	$4.5^{a}\pm0.14$	$48.2^{a}\pm2.53$
	$C_{0.004}$	$65.1^{a}\pm0.75$	$72.5^{b}\pm0.22$	$86.3^{ab}\pm0.18$	$9.3^{a}\pm0.19$	$41.7^{a}\pm1.28$	$52.2^{a}\pm0.34$	$59.8^{a}\pm0.16$	$3.5^{c}\pm0.06$	$38.0^{b}\pm0.10$
	$C_{0.02}$	$64.1^{a}\pm0.71$	$72.5^{b}\pm0.19$	$87.8^{ab}\pm0.27$	$10.6^{ab}\pm0.21$	$41.5^{a}\pm1.97$	$52.3^{a}\pm0.86$	$60.3^{a}\pm0.18$	$3.8^{c}\pm0.09$	$35.9^{b}\pm1.56$
	$C_{0.04}$	$64.8^{a}\pm0.16$	$72.9^{b}\pm0.43$	$88.7^{b}\pm0.13$	$11.0^{b}\pm0.08$	$42.0^{a}\pm0.23$	$52.7^{a}\pm0.34$	$60.2^{a}\pm0.25$	$3.9^{bc}\pm0.02$	$35.5^{b}\pm0.47$
	$C_{0.2}$	$68.2^{b}\pm1.24$	$75.1^{c}\pm0.03$	$92.6^{c}\pm1.74$	$11.1^{b}\pm0.02$	$41.8^{a}\pm0.02$	$52.7^{a}\pm0.33$	$60.5^{a}\pm0.04$	$3.9^{bc}\pm0.04$	$35.0^{b}\pm0.39$
DG flour	C_c	$70.3^{a}\pm0.45$	$76.1^{a}\pm0.49$	$83.3^{a}\pm0.73$	$6.9^{ab}\pm0.35$	$39.2^{a}\pm2.17$	$53.4^{a}\pm0.01$	$62.4^{a}\pm0.11$	$4.1^{ab}\pm0.12$	$58.6^{b}\pm1.26$
	C_{c0}	$70.7^{ab}\pm0.08$	$76.3^{a}\pm0.43$	$83.8^{a}\pm0.34$	$6.0^{a}\pm0.12$	$40.2^{a}\pm0.54$	$53.7^{a}\pm1.00$	$62.7^{a}\pm0.30$	$4.2^{b}\pm0.06$	$70.2^{a}\pm2.47$
	$C_{0.004}$	$70.9^{ab}\pm0.16$	$76.6^{a}\pm0.23$	$84.1^{a}\pm0.17$	$6.2^{a}\pm0.17$	$41.3^{a}\pm0.52$	$53.6^{a}\pm0.54$	$63.1^{a}\pm0.07$	$3.5^{bc}\pm0.02$	$56.6^{b}\pm1.21$
	$C_{0.02}$	$70.9^{ab}\pm0.02$	$76.5^{a}\pm0.25$	$83.7^{a}\pm0.62$	$6.2^{a}\pm0.06$	$42.0^{a}\pm1.10$	$54.1^{a}\pm0.66$	$62.8^{a}\pm0.37$	$3.2^{c}\pm0.05$	$50.9^{bc}\pm1.26$
	$C_{0.04}$	$70.6^{ab}\pm0.16$	$76.3^{a}\pm0.22$	$83.1^{a}\pm0.04$	$7.4^{b}\pm0.40$	$42.2^{a}\pm0.68$	$54.6^{a}\pm0.01$	$62.3^{a}\pm0.13$	$3.3^{c}\pm0.07$	$45.1^{c}\pm3.36$
	$C_{0.2}$	$71.5^{b}\pm0.47$	$78.2^{b}\pm0.15$	$88.3^{b}\pm1.67$	$8.4^{c}\pm0.09$	$42.3^{a}\pm0.62$	$54.8^{a}\pm0.52$	$62.8^{a}\pm1.46$	$2.4^{d}\pm0.36$	$29.0^{d}\pm3.97$

*Means ± SD (n = 3). For a particular rice variety, means with different letters in same column denote significant difference at 5 % probability level within each variety.

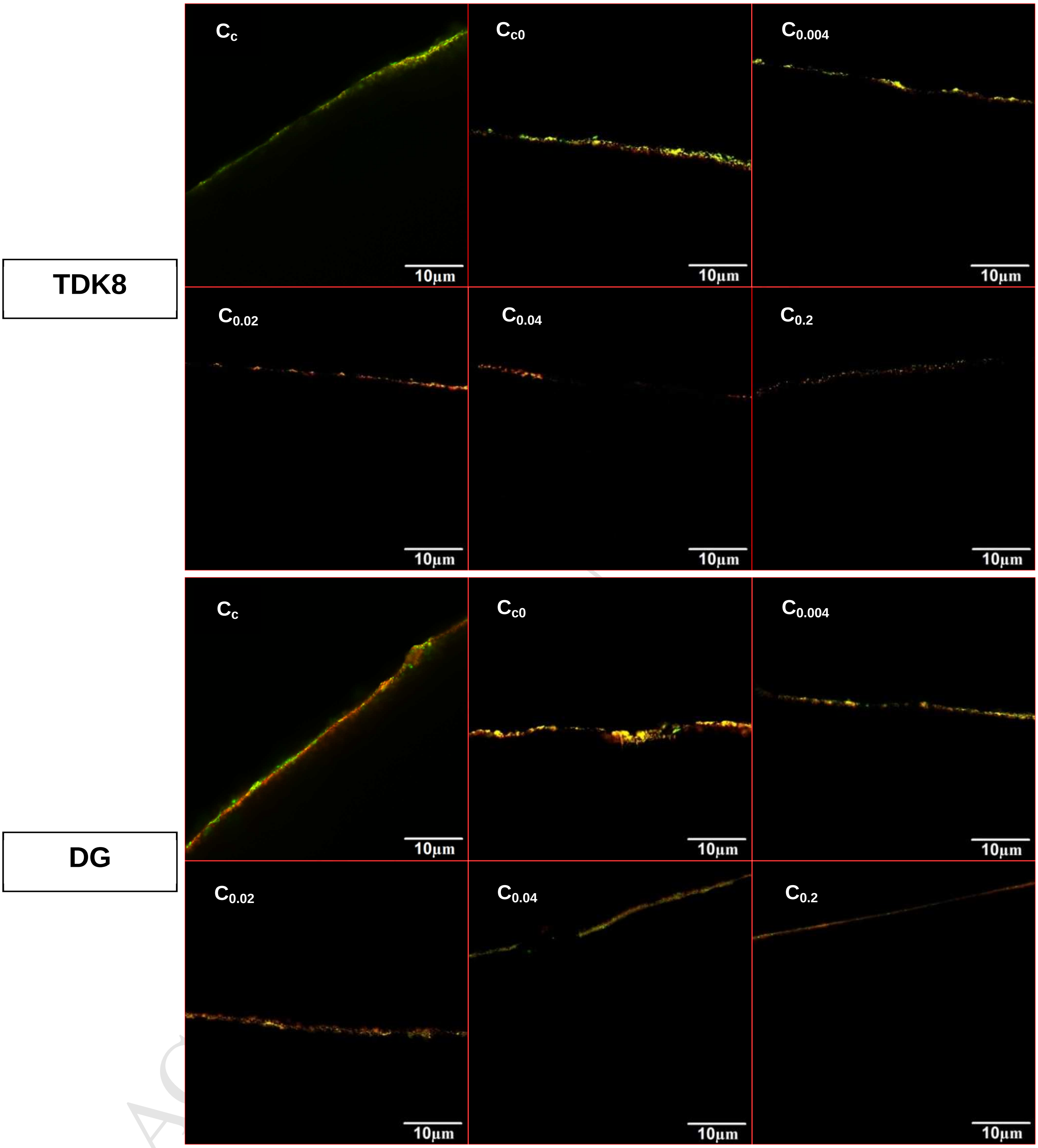

Fig. 1. Confocal laser scanning micrographs of control and alkali treated rice grains of Thadokkham-8 (TDK8) and Doongara (DG). Lipids and proteins are labelled in red and green, respectively.

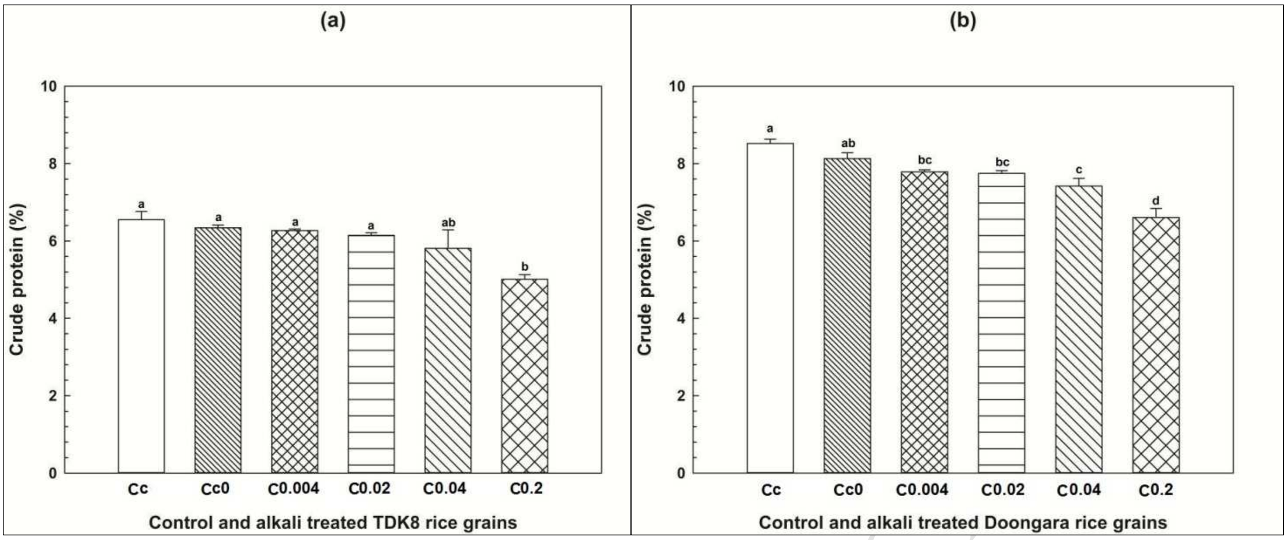

Fig. 2. Crude protein content of control and alkali treated rice grains; (a) Thadokkham-8 (TDK8), and (b) Doongara (DG).*

*Means ± SD (n = 3). Within figure, different letters denote significant difference at 5 % probability level.

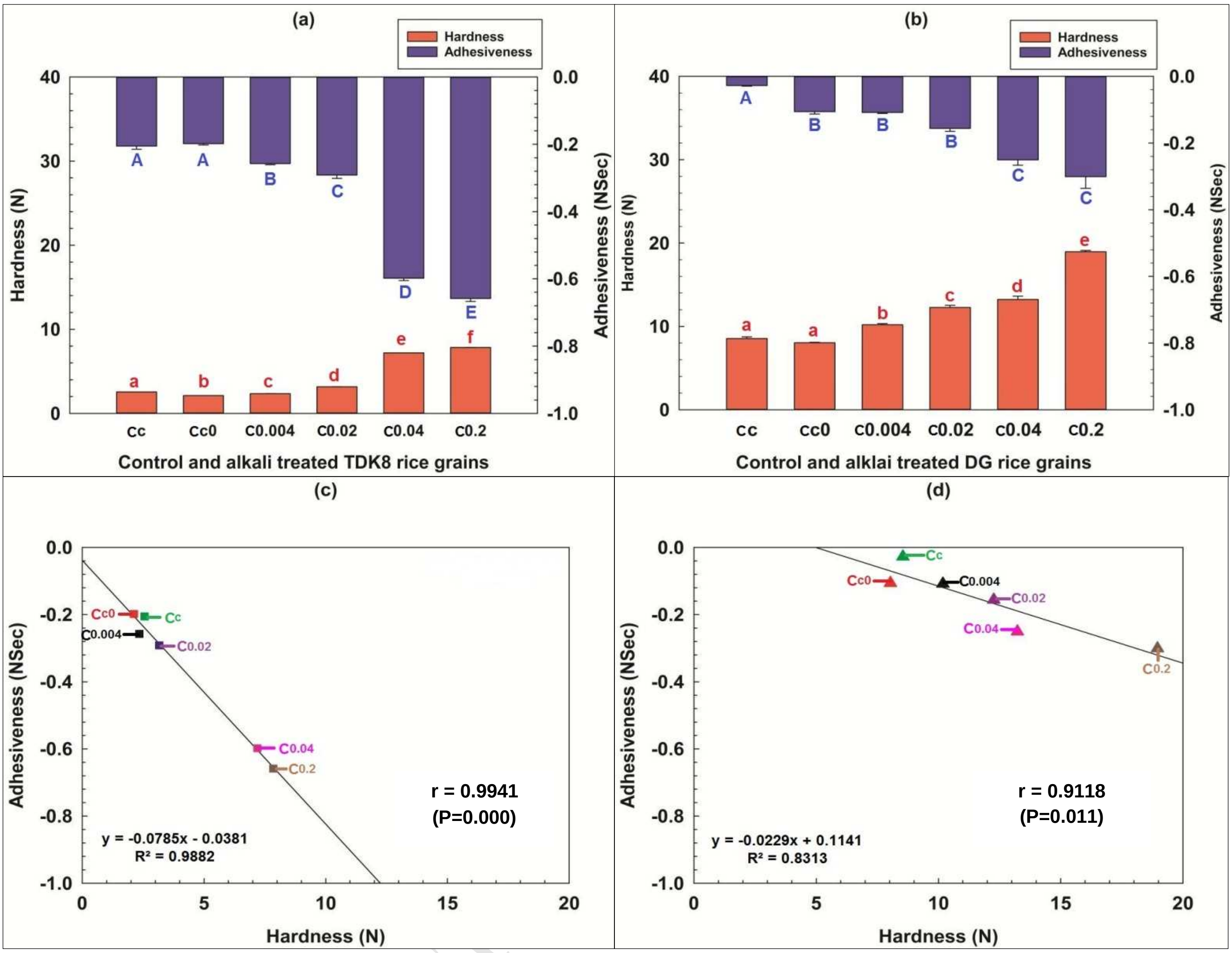

Fig. 3. Textural profile analysis of control and alkali treated rice grains (a) Thadokkham-8 (TDK8), and (b) Doongara (DG).* Correlation (r) between hardness and adhesiveness of control and alkali treated rice grains; (c) Thadokkham-8 (TDK8), and (d) Doongara (DG).[+]

*Means ± SD (n = 3). Within figure, significant differences are denoted by lowercase letters for hardness and uppercase letters for adhesiveness at 5 % probability level.

[+] The negative sign associated with adhesiveness was ignored while calculating the correlation (r) due to more adhesiveness associated with greater negative value.

Highlights

- Removal of surface proteins on rice grains by NaOH solution was revealed through CLS micrographs.
- Significant (*$P<0.05$*) increase in adhesiveness was found in alkali treated rice grains.
- NaOH treatment at a concentration above 0.04 % induced yellowness in grains.