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Full Length Article

Iron, Zinc and Total Antioxidant Capacity in Different Layers of Rice Grain among Different Varieties

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Abstract

In addition to being an important source of iron (Fe) and zinc (Zn) for rice consumers, rice also has a special value in its anti-oxidative capacity. This study explored how antioxidant capacity of the rice grain is associated with its Fe and Zn concentration, and how the relationship is affected by milling. Total antioxidant capacity and the concentration of Fe and Zn were determined in 5 rice genotypes in unpolished grain and after polishing for 15, 30, 45 or 60 s. Total antioxidant capacity of the unpolished grain was positively correlated with its Zn concentration both determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ($r = 0.60$, $p<0.05$) and Fe reducing antioxidant power (FRAP) methods ($r = 0.59$, $p<0.05$), while the correlation with its Fe concentration was found by FRAP method ($r= 0.62$, $p< 0.05$), suggesting an involvement of the nutrients in anti-oxidative activity. The concentration of Zn, Fe and total antioxidant capacity determined by DPPH method declined with longer polishing, while FRAP method was not detected the capacity after 15 s polishing. The total antioxidant capacity determined by DPPH method remained positively correlated with Zn concentration regardless of polishing time from 0 s to 60 s ($r = 0.62$, $p<0.001$), but not with Fe ($r = 0.12$, not significant at $p<0.05$) concentration. These results suggested a possible role of Zn in some oxygen radicals scavenging system that was constant through different layers of rice grain tissue, but the involvement of Fe was less clear.

Keywords: Rice; Total antioxidant capacity; Iron; Zinc; Grain layer

Introduction

Antioxidants in food offer protection against oxidative damages in living cells and tissues. These properties have been implicated in a range of diseases, including cancer and cardiovascular problems (Kehrer, 1993). Rice (*Oryza sativa* L) grain has been reported to contain several groups of antioxidants, including phenolic compounds, tocols and γ – oryzanol (Iqbal *et al*., 2005; Yu *et al*., 2007). These compounds have been especially rich in pigmented rice (black or red pericarp) (Kehrer, 1993). The main phenolic compound with an anti-oxidative capacity in pigmented rice has been identified as anthocyanin (Iqbal *et al*., 2005), specifically cyanidin-3-glucoside and peonidin-3-glucoside (Hu *et al*., 2003), malvidin, pelargonidin-3,5-diglucoside, cyanidin-3-glucoside and cyanidin-3,5-diglucoside (Zhang *et al*., 2006); cyanidin-3-glucoside, pelargonidin-3-glucoside (Yawadio *et al*., 2007).

On the other hand, Fe and Zn also act as an important role for antioxidant activity with its role in scavenging enzymes, such as superoxide dismutase (SOD) in the form of FeSOD and CuZnSOD. In the chloroplast, FeSOD is the typical isoenzyme of SOD (Kwiatowsky *et al*., 1985), but it may occur in the mitochondria and peroxisomes in the cytoplasm (Droillard and Paulin, 1990; Elstner, 1982). CuZnSOD is located in the mitochondria (Duke and Salin, 1985) and glyoxysomes (Sandalio and Del Rio, 1987), detoxifies superoxide radicals occurring from photosynthetic activities and physiological stress responses. This is an important process on mitigating their adverse effects on enzyme activity, integrity of polysaccharides, cell membrane and DNA and prevents cell death (Fridovich, 1983). Our previous investigation showed that the anti-oxidative capacity among different pigmented rice genotypes varied with Zn and anthocyanin concentration in a multiple regression (Rerkasem *et al*., 2015a).

Most of the anti-oxidative compounds in rice have been found in the 'bran' section, surface layers of the de-husked caryopsis (pericarp, the aleurone and some sub-aleurone cells) plus the embryo, are removed by polishing in

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the milling process (Ichiyanagi *et al*., 2001; Park *et al*., 2008; Boonsit *et al*., 2010; Yodmanee *et al*., 2011). Removal of the bran by polishing or buffing to produce the 'white rice' generally preferred by rice consumers has been clearly shown to depress Fe and Zn concentration (Prom-u-thai *et al*., 2007b; Saenchai *et al*., 2012). Our previous study showed that the anthocyanin, Fe and Zn were distributed differently in successive layers of the pigmented rice grain removed by polishing (Rerkasem *et al*., 2015b). The distribution of Fe and Zn may influence differently on anti-oxidative capacity in different grain layers, but its effect on antiradical scavenging enzyme would be difficult to observe when dealing with pigmented rice with a large contribution of anthocyanin on antioxidant activity. This study evaluated the influence of Fe and Zn on anti-oxidative capacity in different grain tissue layers of 4 off-white pericarp color genotypes in comparison with a black pericarp color genotypes.

Materials and Methods

Plant Culture

Four ordinary rice genotypes with off-white pericarp (Khao Dak Mali 105, KDML 105; Chainat 1, CNT 1; Patumthani 1, PTT 1; RD 21) and one black pericarp genotype (Kam Doi Saket, KDK) were grown together in the field in the wet season (June-November) of 2012, at Chiang Mai University, Thailand. The soil was a sandy loam of Sansai series. Rice seeds were soaked in water overnight and incubated moist until germinated and grown for 30 days in a seedbed. Single seedlings were transplanted into hills at 25 × 25 cm spacing. Nitrogen fertilizer was applied at the rate of 75 kg N ha^{-1}, half at maximum tillering and half at flowering. The field was kept flooded under 0.1 – 0.2 m of water until maturity. Rice seed was harvested at maturity.

Sample Preparation

The rice seed was de-husked to produce unpolished rice (0 s) with a laboratory husker (Model P-1 from Ngek Seng Huat Co. Ltd., Thailand). Three replicates of 50 g sub-samples of unpolished rice of each genotype were milled separately at 15, 30, 45 and 60 s with a laboratory polisher (Model K-1 from Ngek Seng Huat Co. Ltd., Thailand). The metal parts of both de-husker and polisher were carefully cleaned to avoid iron and zinc contamination between samples (Prom-u-thai *et al*., 2007a). Weight of rice samples were recorded at each polishing period. Degree of milling (DOM), which measured the loss of grain mass by polishing, was calculated by dividing grain weight of polished grains with unpolished ones. Translucency was evaluated in a milling meter (Satake, Japan). The image of aleurone and pericarp layers on the dorsal sides of pigmented and non-pigmented genotypes were obtained using thin sections under incident UV light to observe autofluorescence (Olympus BX 51).

Chemical Analysis

Samples were oven dried at 75°C for 72 h before Fe and Zn was determined using a Hitachi Z-8230 atomic absorption spectrophotometer (Zarcinas *et al*., 1987). The Trolox equivalent antioxidant capacity (TEAC) was performed using the DPPH free radical scavenging method (Yue and Xu, 2008). The DPPH reagent (0.39 g) was dissolved in 1000 mL of methanol for preparing the DPPH reagent solution. About 0.1 g of ground samples were extracted with methanol and filtered with 0.5 µm nylon membrane before measuring for the DPPH free radical scavenging test. The 0.5 mL of DPPH solution and 1.6 mL methanol were added into the sample solution and transferred to a spectrophotometer cuvette. The reaction solution was carried out in the dark room at 25°C for 20 min. Then the absorbance of the reaction mixture was monitored at 517 nm using a UV-visible spectrophotometer. The percentage of radical scavenging activity was calculated using the following equation:

DPPH scavenging activity (%) = [(Abs of control-Abs of sample)/Abs of control] × 100

The DPPH scavenging activity percentage of the absorbance of DPPH was plotted against each quantity of the extracting to produce a regression line. Trolox (0.4 mM) in methanol was used as a standard to convert the inhibition capability of the extractant to the trolox equivalent antioxidant activity. The ratio of the slopes of the regression lines of the extract solution and the trolox solution was defined as the trolox equivalent antioxidant capacity. Then, it was converted to µmol of trolox equivalent (TE)/g of rice.

The Fe reducing antioxidant power (FRAP) was performed according to methods described by (Benzie and Strain, 1999). Briefly, freshly prepared FRAP reagent consisted of 0.1 M acetate buffer (pH 4.0), 0.5% (w/v) 1, 10 phenanthroline in 10% methanol and 0.3 mM $FeCl_3$ in a ratio of 1:1:1 (v/v/v). The 2 mL of rice extract was mixed with 0.5 mL of the phenanthroline and FRAP reagent and after 20 min of incubation at 37°C, absorption was measured at 510 using a spectrophotometer. Aqueous or methanolic solutions of known Fe (II) concentration are used for calibration of the FRAP assay. FRAP values, expressed as µmol of Fe (II) equivalent per 100 g rice, was obtained by comparing the absorption change in the test mixture with doses obtained from Fe (II) standard concentrations curve.

Data Analysis

The data were subjected to analysis of variance (ANOVA) and means that were significantly different were separated at $p < 0.05$ by the least significant difference (LSD) test. Certain sets of data were also subjected to correlation and regression analysis.

Result

In unpolished (0 s) grain, there was significant variation in total antioxidant activities as well as in Fe and Zn concentrations among the 5 rice genotypes (Fig. 1). These rice genotypes ranged from low to high Fe and Zn concentration, with KDK being the highest, while the lowest were CNT 1 and PTT 1 and KDML 105 and RD 21 were moderate in both Fe and Zn. The total antioxidant capacity determined by the DPPH method was observed in all rice genotypes compared with those determined by FRAP, which were not detected in some genotypes. In unpolished grain, the total antioxidant capacity determined by the DPPH method varied among genotypes, with the highest in KDK, which was double to triple than in PTT 1, KDML 105, and CNT 1, and more than 5 times of RD 21. While, total antioxidant capacity determined by the FRAP method was only detected in KDK but not in the other non-pigmented genotypes (data not shown). Total antioxidant capacity of the unpolished grain determined by DPPH method correlated significantly with its Zn ($r=0.60$, $p<0.05$), but not for Fe. On the other hand, total antioxidant capacity by FRAP method correlated significantly with both Zn ($r=0.59$, $p< 0.05$) and Fe ($r=0.57$, $p< 0.05$) (Table 1).

Prolonged polishing increased degree of milling (DOM) and translucency to different extent among the non-pigmented rice genotypes and the pigmented KDK (Fig. 2). The DOM increased to about the same extent with longer polishing in the 4 non-pigmented genotypes, losing 12–16% of their unpolished weight after 60 s. The pigmented KDK lost weight much more rapidly, with the DOM of 12% after 15 s, and 28% after 60 s. Grain translucency, an important quality characteristic of ordinary rice was substantially increased after polishing at 15 s in all 5 genotypes, but the change was much smaller in KDK, which has opaque endosperm of waxy grain. However, there was almost no change of grain translucency after longer polishing from 30 s to 60 s. Transverse section of unpolished grain of the pigmented genotype KDK showed thicker pericarp and aleurone than the non-pigmented genotype CNT 1 (Fig. 3).

Polishing depressed Zn and Fe concentration as well as total antioxidant capacity of the grain in the different rice genotypes (Fig. 4). Concentration of Zn and Fe both declined with longer polishing in all rice genotypes, but the effect on Zn was much milder than on Fe. Without significant difference among the genotypes ($P < 0.05$), the biggest Zn depression was 15% after 15 s, followed by another 7% decline after 30 s, but there was little further effect of longer polishing. At 60 s the grain Zn concentration still averaged three quarters of that in unpolished grain. Iron concentration was depressed more strongly, with significantly sharper decline over polishing time in the high Fe genotype KDK, which was brought to the same level or even lower as that in some of the other 4 genotypes at 30 s. After 60 s only one-fifth of the original Fe was retained in KDK, while 32 to 46% was retained in the other 4 genotypes. Almost no change in total antioxidant capacity determined by DPPH method was observed in the 4 non-pigmented genotypes after polishing for up to 60 s, with the highest total antioxidant capacity observed in PTT1, followed by CNT1, KDML 105 and RD 21, in that order. Depression of total antioxidant capacity in pigmented KDK, which had the highest total antioxidant capacity in unpolished rice with 14 mg trolox equivalent per g, was also significant only after 15 s polishing, but with a much sharper drop of 43%. The total antioxidant capacity

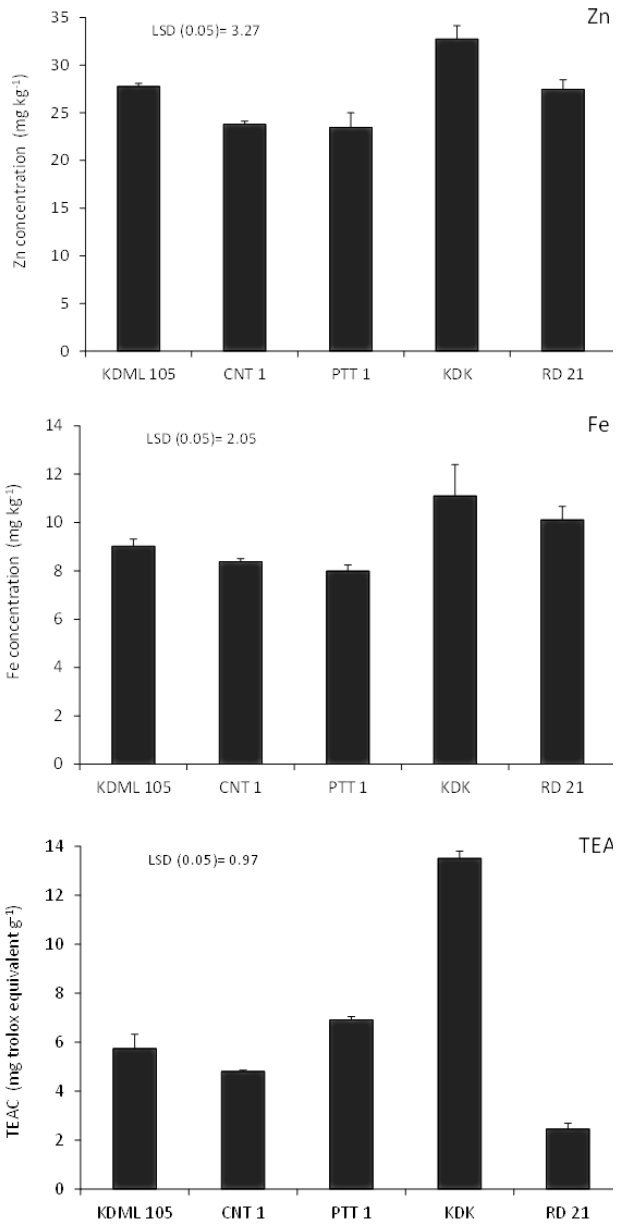

Fig. 1: Concentration of iron, zinc and total antioxidant capacity in unpolished rice of 5 genotypes. The bars represent standard error of the means

Table 1: Relationship between total antioxidant capacity of rice grain after various polishing time and their iron and zinc concentration determined by DPPH and FRAP methods

Polishing time (s)	Linear regression of total antioxidant capacity (mg Trolox equivalent 100 g^{-1}, y) determined by DPPH method on	
	Fe concentration (mg Fe kg^{-1}, x)	Zn concentration (mg Zn kg^{-1}, x)
0	y = 1.04x – 3.00 (NS, p<0.05)	y = 0.62x – 9.97 (p<0.01)
15	y = –0.10x + 5.42 (NS, p<0.05)	y = 0.35x – 3.31 (p<0.001)
30	y = –0.88x + 8.30 (p<0.01)	y = 0.27x – 0.97 (p<0.01)
45	y = –0.83x + 8.18 (p<0.01)	y = 0.37x – 3.13 (p<0.05)
60	y = –1.29x + 9.07 (p<0.05)	y = 0.30x – 1.52 (p<0.05)
0-60	y = 0.11x + 7.20 (NS, p<0.05)	y = 0.37x – 1.95 (p<0.001)
15-60	y = –0.58x + 7.20 (p<0.001)	y = 0.31x – 1.95 (p<0.001)
Polishing time (s)	Linear regression of total antioxidant capacity (mg Trolox equivalent 100 g^{-1}, y) determined by FRAP method on	
	Fe concentration (mg Fe kg^{-1}, x)	Zn concentration (mg Zn kg^{-1}, x)
0	y = 48.17x – 394.03 (p<0.05)	y = 20.04x – 487.04 (p<0.05)
15	y = –18.04x + 132.73 (NS, p<0.05)	y = 19.25x – 398.89 (p<0.001)
0-15	y = 0.36x + 3.29 (NS, p<0.05)	y = 0.48x – 6.22 (p<0.001)

determined by FRAP method was observed in KDK genotype only after polishing for up to 15 s, it was not detected with the longer polishing (data not shown).

Polishing time had different effects on the relationship between the grain total antioxidant capacities determined by both DPPH and FRAP methods and its Fe and Zn concentration (Table 1). Total antioxidant capacity determined by DPPH method, the positive relationship between Zn concentration and total antioxidant capacity found in unpolished rice was held relatively constant after polishing for 15 s to 60 s. With combined dataset from 0, 15, 30, 45 and 60 s polishing, total antioxidant capacity increased linearly with Zn concentration ($y = 0.367x – 3.226$, $p<0.001$). On the other hand, total antioxidant capacity was not associated with Fe concentration after 15 s polishing, it declined consistently with increasing Fe concentration after further polishing, with combined dataset from 30, 45 and 60 s polishing showing antioxidant capacity decreasing linearly with increasing Fe concentration ($y = –0.577x + 7.198$, $p<0.001$). Total antioxidant capacity determined by FRAP method, the positive relationship between Zn concentration and total antioxidant capacity found in unpolished rice was held relatively constant after polishing for 15 s. With combined dataset from 0 and 15 s polishing, total antioxidant capacity increased linearly with Zn concentration ($y = 0.48x – 6.22$, $p<0.001$). On the other hand, total antioxidant capacity was not associated with Fe concentration after 15 s polishing and with combined dataset from 0 and 15 s polishing.

Discussion

This study has shown differential association between total anti-oxidative capacity in rice and its Fe and Zn concentration. Antioxidants are considered important nutraceuticals with many health benefits (Sharma and Bhat, 2009). Plant compounds with antioxidant capacity include polyphenolics, flavonoids, phytic acid, γ-oryzanol and vitamin E (Kong and Lee, 2010). Pigmented rice grain has been reported to contain suites of compounds with anti-oxidative property (Kehrer, 1993). Reports of anti-oxidative activities in rice have focused on the bran fraction of pigmented genotypes which contains the pericarp, aleurone, sub-aleurone cells and embryo, and thus pigmented compounds such as anthocyanins and phenols (Ichiyanagi *et al*., 2001; Park *et al*., 2008; Boonsit *et al*., 2010; Yodmanee *et al*., 2011). Anthocyanin is a major phenolic compound in pigmented rice not found in non-pigmented rice (Iqbal *et al*., 2005). The higher antioxidant capacity in unpolished grain of the pigmented KDK, in this study thus could be attributed to its anthocyanin content. The positive correlation between total antioxidative activity of unpolished grain and its Zn concentration therefore suggested some promotional roles of the nutrient. Indeed, our previous investigation found that total antioxidant capacity in 9 pigmented rice genotypes varied in a multiple regression with anthocyanin and Zn concentration (Rerkasem *et al*., 2015a). This study also suggested DPPH method as the proper procedure to determine total antioxidative capacity among rice genotypes as FRAP method showed a limit detection of total antioxidant capacity in non-pigmented rice genotypes and in the pigmented genotype was not detected after 15 s polishing of rice grain due to very low amount of antioxidative compounds in non-pigmented genotypes in the bran layers.

Removal of the bran by polishing to produce the ‘white rice’ generally preferred by rice consumers depresses Fe and Zn concentration depending on DOM (Prom-u-thai *et al*., 2007b; Saenchai *et al*., 2012). In this study, the DOM of the pigmented KDK was higher than the non-pigmented genotypes. This was partly accounted for by its thicker pericarp and aleurone layer in pigmented rice, which was associated with the drop in total antioxidant capacity after 15s polishing. The constant total antioxidant capacity over increasing DOM in the non-pigmented genotypes as well as in pigmented KDK after 15 s polishing, on the other hand, suggested oxygen radicals scavenging systems in the pigment-free rice endosperm that was associated positively with Zn, but negatively with Fe. Both Fe and Zn have been found to be involved in free radicals scavenging enzyme

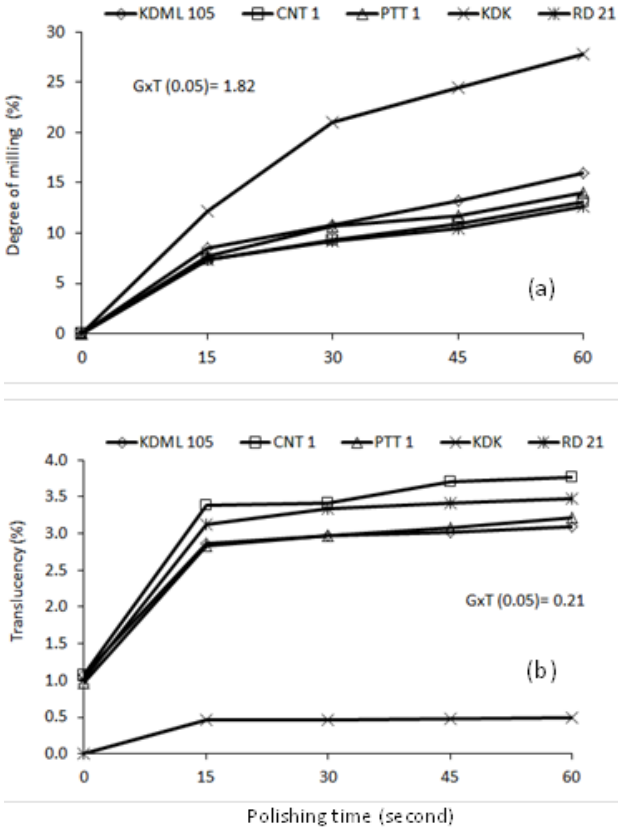

Fig. 2: Degree of milling (DOM) (a) and translucency (b) of 5 rice genotypes after polishing at 0, 15, 30, 45 and 60 s

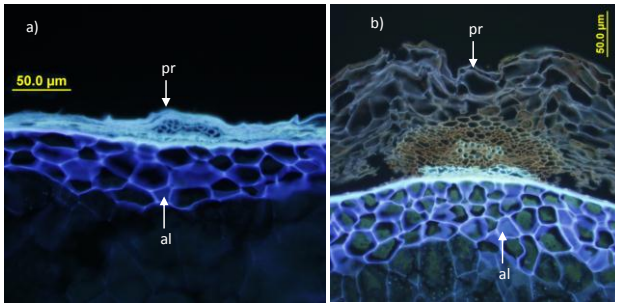

Fig. 3: Transverse section of brown rice grain of non-pigmented (CNT1, a) and pigmented (KDK, b) genotypes under fluorescence microscope (40x), pr = pericarp and al = aleurone layer

systems in rice, e.g., as components of the superoxide dismutases (Kaminaka *et al*., 1999; Dehury *et al*., 2013). The actual roles of Zn in anti-oxidative activity in rice as well as the adverse effect of Fe remain to be explored. The positive association between Zn and total antioxidant capacity, if confirmed in a large number of the other rice genotypes that are especially enriched with Zn, would be highly beneficial. Even with an antioxidant capacity that is

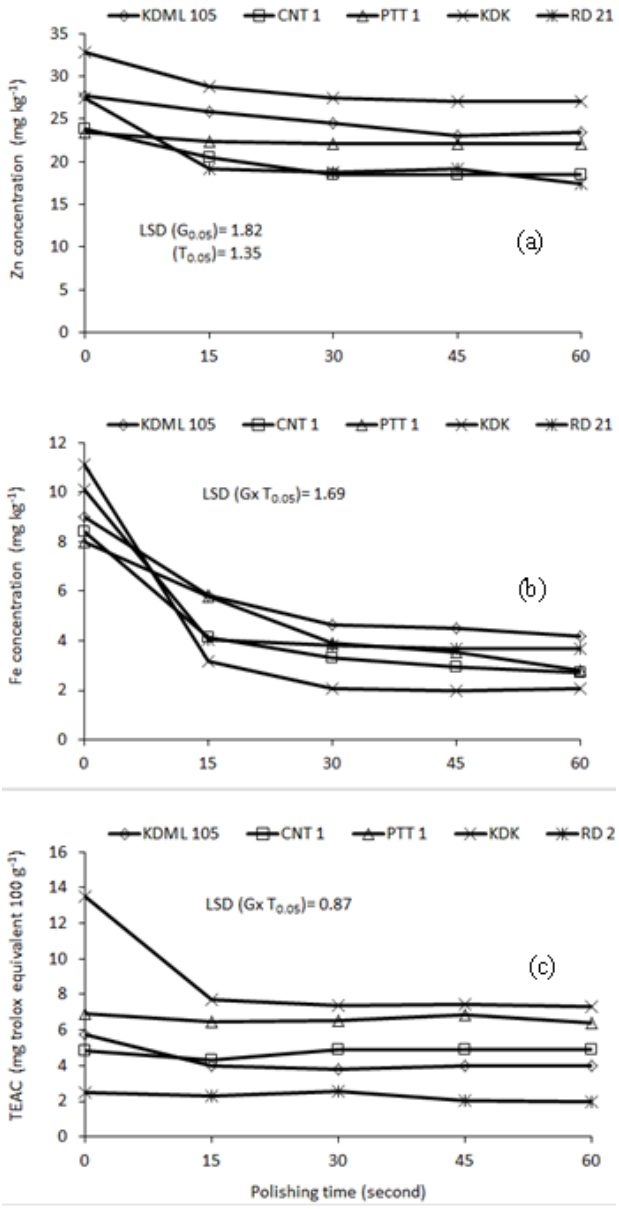

Fig. 4: Concentration of Zn (a), Fe (b) and total antioxidant capacity (c) of 5 rice genotypes after polishing at 0, 15, 30, 45 and 60 s

only a fraction of that in the pigmented grain fraction or other pigmented foods, the total volume consumed daily would make the rice endosperm a primary source for antioxidants along with the Zn for rice consumers.

Conclusion

This study has shown consistent positive association between Zn and total antioxidant capacity in rice regardless of the loss of the grain surface by prolonged polishing. If confirmed over larger sets of germplasm, this would mean

an added potential benefit for rice consumers from efforts to enrich rice with Zn. On the other hand, the negative correlation between high concentration of Fe and total antioxidant capacity in the rice endosperm could be problematic for rice especially enriched with Fe as both are significantly benefit to human health. However, this finding can be further confirmed by adding number of both non-pigmented and pigmented genotypes.

Acknowledgements

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