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Liang, J., Tsuji, K., Nakano, K., Nout, M. J. R., & Hamer, R. J.

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1 Milling characteristics and distribution of phytic acid and zinc in long-, medium- and short-
2 grain rice

3

4 Jianfen Liang^{1,2}, Zaigui Li¹, Kouichi Tsuji³, Kazuhiko Nakano³, M.J. Robert Nout^{2*} and
5 Robert J. Hamer²

6

7 ¹College of Food Science and Nutritional Engineering, China Agricultural University, Beijing
8 100083, P.R. China

9 ²Department of Agrotechnology and Food Sciences, Wageningen University, Wageningen,
10 The Netherlands

11 ³ Department of Applied Chemistry, Graduate School of Engineering, Osaka City University,
12 Osaka 558-8585, Japan

13

14 *Corresponding author:

15 M.J.R. Nout

16 Laboratory of Food Microbiology,

17 Bomenweg 2,

18 6703 HD Wageningen,

19 The Netherlands.

20 Tel +31 317 482834

21 Fax +31 317 484978

22 Rob.Nout@wur.nl

23 **Abstract**

24

25 Milling and polishing are important operations during the production of white rice. The
26 degree of milling and polishing has a significant effect on the nutritional aspects of white rice,
27 especially on minerals, due to a non-uniform distribution of nutrients in the kernel.
28 Information on the distribution of nutrients in rice will greatly help to understand the effect of
29 milling and aid in designing procedures that improve technological and sensory properties of
30 rice while retaining its essential nutrients as much as possible. In this study, three kernel
31 shapes (short-, medium- and long-grain) of rice were selected for the study of milling
32 characteristics and distribution of zinc (Zn) and phytic acid using abrasive milling and X-ray
33 fluorescent microscope imaging approaches.

34 Milling characteristics differed with kernel shapes and cultivars. Mass loss (y, %)
35 correlated well with milling duration (x, s) and was fitted using a polynomial equation of $y =$
36 ax^2+bx+c ($R^2=0.99$). Different kernel shapes of rice resulted in different patterns. Breakage in
37 milling increased with longer duration of milling. The relation between breakage (y, %) and
38 milling duration (x, s) fitted the exponential equation $y = ae^{bx}$. Levels of phytic acid, as well
39 as Zn decreased with prolonged milling. Phytic acid decreased at a higher rate than Zn. The
40 analysis of different milling runs showed that the concentration of phytic acid decreased from
41 the surface region inward, whereas X-ray fluorescent images indicated that the highest
42 concentration of phosphorus was at the interface of embryo and perisperm.

43 Our results help to understand the milling characteristics of different rice cultivars.
44 Understanding these characteristics offers opportunities to optimize milling procedures for
45 maximum phytate removal, at minimum mineral losses and yield loss.

46

47 Key words: mass loss, breakage, rice kernel, distribution, abrasive methods, X-ray fluorescent
48 microscope imaging

49

50 Running title: distribution of Zn and phytic acid in rice kernels

51

52 Abbreviations:

53

54 B37: Bijing 37

55 G30: Ganwanxian 30

56 KSA: kernel surface area

57 PA: phytic acid

58 PC: protein content

59 RLW: ratio of length to width

60 TKW: thousand kernel weight

61 YBR: yield of brown rice

62 Z752: Zhongyou 752

63 Zn : zinc

64 Introduction

65

66 Rice is one of the important cereals in the world. It is commonly used as milled (white) rice
67 produced by removing the hull and bran layer of the rough rice kernel (paddy) (Perdon et al.,
68 2001). Brown rice (hulled rice) is composed of surface bran (6-7% by weight), endosperm (\approx
69 90%) and embryo (2-3%) (Chen et al., 1998). White rice is referred to as milled, polished or
70 whitened rice when 8-10% of mass (mainly bran) has been removed from brown rice
71 (Kennedy et al., 2002). During milling, brown rice is subjected to abrasive or friction pressure
72 to remove bran layers resulting in high, medium or low degrees of milling depending on the
73 amount of bran removed (Chen, Siebenmorgen, 1997; Chen et al., 1998). Milling brings about
74 considerable losses of nutrients and affects the edible properties of milled rice (Chen et al.,
75 1998; Doesthale et al., 1979). As most cereals, rice does not show a homogeneous structure
76 from its outer (surface) to inner (central) portions (Itani et al., 2002). As a consequence,
77 information on the distribution of nutrients will greatly help to understand the effect of
78 milling and aid in improving sensory properties of rice while retaining its essential nutrients
79 as much as possible.

80 Depending on the extent of milling, changes of some nutrients, such as surface lipids
81 (Chen et al., 1998; Perdon et al., 2001), protein (Chen et al., 1998; Heinemann et al., 2005),
82 physical properties such as rice paste viscosity (Perdon et al., 2001), and sensory quality of
83 milled rice, including taste (Park et al., 2001; Tran et al., 2004), have been reported. Effect of
84 milling on some macro- and micro-elements, e.g. iron, magnesium, phosphorus, phytic acid,
85 have also been studied (Bryant et al., 2005). Early studies described the effect of milling on
86 minerals or distribution of minerals according to approximate milling degrees, such as lightly
87 milled, reasonably milled and well milled, or as fractions I, II and III, respectively (Kennedy,
88 Schelstraete, 1975; Song et al., 1988; Tabekhia, Luh, 1979). These authors did not provide
89 detailed information about the distribution of these nutrients in rice kernels. Itani et al. (2002)
90 reported the distribution of some nutrients in more detail, although phytic acid and trace
91 minerals were not included. Studies on Indian rice indicated that the extent of milling had a
92 significant effect on losses of magnesium and calcium, but not on phosphorus and trace
93 minerals ($p < 0.05$) (Bajaj et al., 1989). Recently, X-ray fluorescent microscopy techniques
94 were developed and applied to map the distribution of minerals such as magnesium,
95 potassium, phosphorus, calcium and sulphur in quinoa seeds (Emoto et al., 2004; Konishi et
96 al., 2004).

97 Considering world wide deficiencies of iron and Zn, our ultimate aim is to improve
98 the bioavailability of these minerals by reducing insoluble mineral-phytate complexes, and
99 fortification if desired. As the Zn-phytate complex is very stable (Vasca et al., 2002), we
100 focussed on Zn as a target mineral. Our previous study indicated that Zn and phytate contents
101 in Chinese rice cultivars cover a broad range (Liang et al., 2007). The purpose of the present
102 study was to compare the milling characteristics and the distribution of Zn and phytic acid in
103 long-, medium- and short-grain kernels of rice from China, with a view to optimize for
104 maximum phytate removal at minimum losses of Zn. Precision abrasive milling was used to
105 obtain a range of milling degrees, and X-ray imaging methods to map the distribution of

106 different minerals. The degree of milling for specific rice cultivars could be optimized for
107 maximum removal of phytic acid, maximum retention of Zn, and appropriate whiteness to
108 satisfy consumer expectations for white rice.

109

110 Materials and methods

111

112 *Paddy rice and characteristics*

113 Based on our survey of the variation of phytic acid and minerals in rice cultivars cultivated in
114 China (Liang et al., 2007), three cultivars namely Ganwanxian 30 (G30), Zhongyou 752
115 (Z752) and Bijing 37 (B37) having different levels of phytic acid and minerals were selected.
116 According to the industrial standard of China, the three cultivars were classified as long-grain,
117 medium-grain and short-grain, respectively (CHISA, 2002). G30 and Z752 were obtained
118 from the Jiangxi Seeds Company, and B37 from the Academy of Agricultural Science of
119 Guizhou. All paddies were harvested during the autumn of 2003 and were stored dry and cool
120 (~15°C) less than 90 days before processing and analysis. General characteristics, including
121 crude protein content (PC), yield of brown rice (YBR), breakage from hulling, shape i.e. ratio
122 of length to width (RLW), kernel surface area (KSA), and thousand-kernel weight (TKW), are
123 presented in table 1.

124

125 *Hulling and Milling*

126 Paddy was dehusked with a lab-scale hulling machine (THU-35C, Satake, Japan). Each
127 cultivar was assessed in triplicates for thousand-kernel weight (TKW), yield of brown rice
128 (YBR) and breakage from hulling.

129 Only intact brown rice kernels were used for subsequent milling experiments. About 30
130 (± 1) g of brown rice were milled for the duration of 6, 10, 20, 30, 45, 60, 90, 180 and 300 s,
131 respectively, with a lab-scale milling machine (TM 05C, Satake, Japan) to obtain rice milled
132 to different degrees. Each milling treatment (duration) was performed in triplicates. Yields of
133 white rice and breakage from milling were measured. After milling, whole milled rice kernels
134 (head rice) were separated, and then ground with grinder (HY-04B, Beijing Xinhuanya,
135 China) to pass a 1 mm sieve, and dried at 100°C till constant weight. Dried rice flour was kept
136 in sealed plastic bags at 4°C until chemical analysis.

137 *Zinc (Zn)*

138 Samples of 0.5 g (accuracy 0.1 mg) dried rice flour were digested using a microwave
139 laboratory system (Milestone, Italy) with nitric acid (HNO₃, reagent grade) and hydrogen
140 peroxide (H₂O₂, analytical reagent, Beijing Chemical Works, China) as described by D'Ilio et
141 al. (2002). Contents of Zn in solutions were measured with a Vario 6 Atomic Absorption
142 System (Analytik Jena, Germany). Each sample was digested and measured in triplicates.

143

144 *Phytic acid*

145 Phytic acid levels in brown rice and milled rice were determined after extraction in 100g L⁻¹
146 Na₂SO₄-HCl (1.2 %), concentration on an anion exchange column, and were analysed
147 spectrophotometrically at 500 nm after reacting with a 0.03% FeCl₃ solution containing 0.3%
148 sulfosalicylic acid, according to Ma et al. (2005). All materials were analysed in triplicates.

149

150

151 *Sample preparation for SEM*

152 Rice kernels were longitudinally mounted in a brass cylindrical sample holder with carbon
153 conductive glue (Leit- C, Neubauer Chemicalien, Germany). The samples were placed in a
154 sample holder in a ultra microtome (Reichert Ultracut E/FC4D) and cut. These samples were
155 first planed with a glass knife, after which the surface was planed with a diamond knife (Histo
156 no trough, 8 mm 45°C, Drukker International, The Netherlands). This method is based on
157 (Nijssse, Van Aelst, 1999).

158

159 *X-ray fluorescent imaging*

160 X-ray elemental maps were obtained with a micro-X-ray fluorescence instrument developed
161 at Osaka City University (Emoto et al., 2004). The X-ray tube (MCBM 50-0.6B, rtw,
162 Germany) with Mo target was operated at 50 kV and 0.45 mA. The tube was installed into an
163 X-ray tube shield holder equipped with an X-Y-Z positional device, where the polycapillary
164 X-ray lens was attached. The polycapillary lens was designed and manufactured at Beijing
165 Normal University. The length, input focal distance, and output focal distance were designed
166 to be 50 mm, 34 mm, and 16 mm, respectively. A spot size of about 40 mm was obtained at a
167 focal point. A silicon drift X-ray detector (SDD, X-Flash Detector, Type 1201, Rontec,
168 Germany; sensitive area: 10 mm², energy resolution: <150 eV at 5.9 keV) was suspended
169 using a down-looking geometry. The sample stage was placed on the X-Y-Z stage [YA05A-
170 R1 (X-Y stage) and ZA07AR3S (Z stage); Kohzu precision, Japan], which was controlled by
171 stepping motors driven by a computer. To control the sample stage, motor drivers and a motor
172 controller (NT2400, Laboratory Equipment Co., Japan) were applied. An SDD signal was
173 analysed by a multi-channel analyser (NT2400/MCA, Laboratory Equipment Co., Japan). To
174 confirm the position of the sample, a visible CCD camera was also installed.

175

176 *Statistical analysis*

177 Where appropriate, data were presented as means with standard deviation, or by error bars.
178 Significance of differences was tested by two-tailed t-tests.

179 Results and discussion

180

181 *Milling characteristics*

182 For the miller, the main quality characteristic of rice is related to the amount of material that
183 needs to be removed to obtain white rice. During the abrasion of rice, not only the outer layers
184 of the kernels are removed, but also kernels are broken. These are also considered a loss. We
185 therefore defined milling characteristics as the mass loss due to bran removal, and breakage
186 during milling. The milling characteristics for the rice cultivars used in this study are shown
187 in figure 1a and 1b.

188 Figures 1a and 1b show the loss of mass, and breakage, with increasing milling time.
189 For the three cultivars tested, mass losses (figure 1a) were similar: with increasing milling
190 duration more of the outer layers is removed. Loss rates become less at longer milling times.
191 The relation of mass loss (y, %) vs duration of milling (x, s) fitted a polynomial equation of y
192 = ax^2+bx+c ($R^2=0.99$), as was observed in other studies (Perdon et al., 2001; Singh Gujral et
193 al., 2002). Different kernel shape of rice resulted in different patterns. Z752 had a higher mass
194 loss (figure 1a) at each milling time. To achieve a mass loss of 2%, it took less than 10 s for
195 Z752, and about 20 s for B37 and G30, respectively. Generally, in order to obtain white rice,
196 about 10-15% of mass is removed from the outer layers. In this study, Z752 required the
197 shortest duration of milling to obtain white rice, followed by B37 and G30. The differences
198 among mass loss patterns of the three cultivars might be related to the removal of the embryo.
199 Visual inspection to check the removal of the embryo from brown rice during milling
200 revealed a large variation between the different rice cultivars. Under our experimental milling
201 conditions, one third of the kernels of Z752 and B37 had lost their embryo after 30-45 s
202 milling, corresponding to a mass loss of 3.5-4.5%, whereas more than 90% of the kernels had
203 lost their embryo after 90 s milling, at mass loss of 8.5-9.5%. For G30, about 30% of kernels
204 had lost their embryo at 2% mass loss (20 s milling), whereas after 45 s milling, most kernels
205 had lost their embryo at a mass loss of 5%.

206 Rice breakage was quantified as the weight of broken rice expressed as a percentage
207 of the total weight of milled rice (Chen et al., 1998). Breakage of the three cultivars was
208 similar (figure 1b), increasing with longer duration of milling. The relation between breakage
209 (y, %) and milling duration (x, s) fitted the exponential equation $y = ae^{bx}$. The different
210 cultivars had different a and b values with a R^2 ranging from 0.76 to 0.95. Of the three
211 cultivars, Z752 had the highest breakage values, and B37 had the lowest at each time interval.
212 After 90 s milling, breakage of Z752 had reached 30%, while breakage of B37 was still low
213 (about 5%). The amount of broken kernels of long-grain in the present study was of a similar
214 magnitude as of long-grain rice reported elsewhere (Chen et al., 1998).

215

216 *Phytic acid and Zn levels*

217 Phytic acid and Zn levels in rice after increasing degrees of milling are presented in table 2.

218 Levels of phytic acid and Zn decreased with prolonged milling. Although it has been
219 observed earlier (Itani et al., 2002) that all minerals (including phosphorus) decrease from the
220 outermost fraction, it appears here that phytic acid levels decrease at a higher rate than those
221 of Zn.

222 Phytic acid levels in brown rice of B37, Z752 and G30 were 8.9, 7.8 and 11.1 g kg⁻¹,
223 respectively. These values differ from our previous results (Liang et al., 2007) probably due
224 to differences of cultivating environments and agricultural practice (Liu et al., 2005a). G30
225 had the highest phytic acid content in raw brown rice and this decreased quickly during
226 milling. After 30 s milling, phytic acid levels in milled rice of B37 and Z752 were at the same
227 level (7 g kg⁻¹) although they started at different initial values, and it was 8 g kg⁻¹ in G30 after
228 the same milling time. After 120 s milling (duration considered to be optimum in commercial
229 milling of white rice), phytic acid in G30 and Z752 were still at the level of 3.2 g kg⁻¹, higher
230 than in B37 (2.0 g kg⁻¹). After 300 s milling, phytic acid in all cultivars were at the level of
231 0.2 g kg⁻¹. Phytic acid levels in brown rice decreased at a similar rate as reported elsewhere
232 (Doesthale et al., 1979) for phosphorus.

233 The Zn levels in the brown rice cultivars studies did not decrease significantly (22.1,
234 22.8 and 19.3 mg kg⁻¹, respectively), and even after 30 s milling (corresponding to a mass loss
235 of about 5%), the Zn levels in all cultivars of milled rice were still at the same level of brown
236 rice. The biggest lost of Zn in Z752 occurred after 45 s milling, and in B37 after 120 s milling.
237 However, in G30, up to 120 s milling did not affect its Zn level, a phenomenon that has been
238 previously reported (Juliano, 1972; Villareal et al., 1991). With some milled rice samples
239 even higher Zn levels were reported than in the initial brown rice (Heinemann et al., 2005). In
240 the three cultivars studied here, Zn levels after 300 s milling were 4-38% lower than that of
241 initial values.

242

243 *Location and distribution of phytic acid and Zn in brown rice*

244 In order to visualize the distribution of phytic acid and Zn in brown rice, X-ray fluorescent
245 microscope imaging techniques were used. Images of location of phytic acid (indicated as
246 phosphorus, P) and Zn obtained with X-ray fluorescent scanning, as well as the distribution of
247 phytic acid and Zn in brown rice kernels obtained by abrasive milling are shown in figures 2a,
248 2b and 2c and demonstrate the location of P and Zn in rice kernels.

249 In all three cultivars, the density of phosphorus decreased from the surface region
250 inward. This agrees with data from abrasive milling experiments (Bryant et al., 2005). The
251 peripheral embryo region did not show high phosphorus intensities, whereas much higher
252 densities were observed near or at the interface of embryo and endosperm. We observed that
253 whereas in B37 (figure 2a) and G30 (figure 2c), the distribution of phosphorus was similar;
254 the distribution in Z752 (figure 2b) was different, having no distinct layer with higher
255 phosphorus concentration. Notably, at the side of the embryo, we could not observe the high
256 density of phosphorus as observed in figures 2a and 2c. The distribution of phosphorus in the
257 rice kernels suggested that at least the outer layer should be removed if we want to
258 significantly decrease phytic acid in milled rice, since 70-85% of phosphorus occurs as phytic
259 acid in rice.

260 The location of Zn in the three cultivars was similar. All three cultivars had the highest
261 density of Zn in the embryo whereas Zn was relatively evenly distributed in the other regions.
262 This helps us to understand earlier reports, that milling degrees higher than 10% had little
263 effect on Zn levels in milled rice (Bryant et al., 2005). The location of Zn indicates that it may
264 be beneficial to retain more embryo to obtain higher final levels of Zn.

265 We observed an inverse relation: $y = a-bx$ ($a=7.7-8.3$, $b=0.03$, $R^2=0.82-0.91$) between
266 phytic acid levels (y , mg g^{-1}) and milling duration (x , s) (figure 2a, 2b, 2c). This relation
267 shows some similarity with that between milling degree and surface lipid, and phenolic acids
268 observed elsewhere (Perdon et al., 2001; Zhou, 2003). In B37 and Z752, about 23 to 33% of
269 total phytic acid was located in the surface outer layer of kernel (2-3% weight percent of
270 brown rice). In all cultivars, about 23-25% of total phytic acid was located in the sub-surface
271 layer, which accounted for 3.4-4.5% of total weight. Less than 2% of total phytic acid was
272 located in the 75-80% of central portion of kernel. The remaining 40-50% of phytic acid was
273 located in the peripheral layers of brown rice, representing 13-15% of kernel weight. The
274 distribution of phytic acid observed from milling experiments is very well supported by
275 images obtained from X-ray scanning. Differences in the distribution of phytic acid location
276 in cultivars mainly occurred in the outermost layer. In this region, distribution of phytic acid
277 in B37 and Z752 was similar, and relatively even at the outermost surface with a steep
278 decrease inward. However, the distribution in G30 was quite different, showing a steep
279 decrease already at the outermost surface layer, followed by a relatively even distribution.
280 This distribution pattern was similar to the distribution of phosphorus in other rice cultivars
281 (Bajaj et al., 1989). The perisperm is another layer of the kernel, removed at the interval of
282 milling duration from 60 s to 120 s. Phytic acid located here varied by 20-40%, with weight
283 percent at 4-6% in different cultivars.

284 The distribution of Zn in the three cultivars was different. For B37, distribution of Zn
285 was relatively even in the layer occupied 30% of the total kernel weight, with a steep decrease
286 in next region, and followed with another even distribution in the central part. In contrast, in
287 Z752, a steep decrease of Zn occurred at sub-surface layer, at the milling interval from 30 to
288 60 s (occupied about 4% of total weight of kernel), and with an even distribution in other
289 parts. For G30, the distribution of Zn was relatively even from the surface to the central part
290 of the kernel. The highest decrease occurred at the interval of 60-120 s. Further analysis of
291 regression showed there was no correlation between Zn contents and milling duration. Figures
292 2 also showed that more than 60-70% of total phytic acid was located in the 10% of surface
293 layer, and less than 40% of Zn was in the same layer. The different distribution patterns of
294 phosphorus and Zn should enable an optimized milling, removing as much as possible phytate
295 while retaining relevant levels (at least 50%) of Zn.

296 Molar ratios of phytic acid to Zn varied with regions in kernel and cultivars. For all
297 cultivars, only when more than 20% of outer layer was removed, molar ratios of phytic acid to
298 Zn could decrease to less than 1. This was achieved only after 300 s milling, which is
299 considerably longer than the standard commercial practice.

300

301 Discussion

302

303 Milling consequences, such as mass loss and breakage, could be affected by intrinsic factors
304 (e.g., cultivar, kernel shape) as well as extrinsic factors (e.g., milling equipment). Under
305 identical processing conditions, rice can display different processing properties. These can be
306 caused by cultivar, maturity, and cultivating conditions, and can influence mass loss and
307 breakage because of different shape, hardness of kernels, and thickness of the aleurone layer
308 (Juliano, 1972; Zhou, 2003). Different bran loss rates by milling have been attributed to shape
309 and hardness of grains, as well as pericarp thickness, oil bodies, cellulose, and hemicelluloses
310 in bran layers (Juliano, 1972; Mohapatra, Bal, 2004; Singh Gujral et al., 2002; Singh et al.,
311 2000). These would contribute to the differences observed for the three cultivars and the
312 higher mass loss rate after short milling periods. Cultivars differ in thickness of the aleurone
313 layer and in hardness distribution in the endosperm. *Japonica* (bold or coarse short-grain)
314 kernels tend to have more cell layers than *indica* (slender long- or medium- grain) kernels.
315 The central core and the mesocarp in *indica* and *japonica*, respectively are hard, and kernel
316 hardness is negatively correlated to length-to-breadth ratio (Juliano, 1972). Combined effects
317 of such factors may have caused the relatively high mass loss of Z752 from milling.

318 In addition to the effect of equipment and process conditions, the composition,
319 structure and thickness of rice kernels also affect the extent of breakage from milling
320 (Siebenmorgen, Qin, 2005; Zhou, 2003). Whereas breakage could not be related to kernel
321 width or length (Siebenmorgen, Qin, 2005), the susceptibility to relative humidity and
322 fissuring could play a role in breakage (Lloyd, Siebenmorgen, 1999). Further investigation is
323 required to help understand the mechanism for the high extent of breakage from milling in
324 Z752.

325 Phytic acid is an important storage of phosphorus and minerals present in seeds. It
326 usually occurs as a mixed salt of potassium and magnesium, and may also contain calcium,
327 Zn and/or iron. Phytate has a different accumulation pattern from protein reserves which are
328 mainly deposited within the numerous protein bodies in seed storage cells (Liu et al., 2004;
329 Liu et al., 2005b). Studies on *japonica* rice indicated that phytic acid levels were not related to
330 protein levels, which were significantly influenced by genetic and environmental factors
331 (Juliano, 1972; Liu et al., 2005a; Liu et al., 2005b). Our milling experiment indicated that
332 about 25% of the phytic acid is located in the perisperm of the kernel, which differs from
333 earlier findings that phytic acid was only present in the aleurone layer after embryo removal
334 (Liu et al., 2004). This difference suggests that although phytic acid is approximately located
335 in the outer layer of kernel, the precise distribution might differ among rice cultivars. In order
336 to obtain milled rice with minimum weight losses but maximum removal of phytic acid, the
337 distribution of phytic acid in the kernel should be established first, and this should form the
338 basis to determine the appropriate milling treatment. Our X-ray microscope images indicate
339 that in absolute terms, very little phosphorus is located in the embryo itself, which differs
340 from the observation made earlier (Liu et al., 2004) that phosphorus concentration in embryos
341 was about 5 times higher than in whole kernels. Both abrasive milling experiments and X-ray
342 images indicate that Zn was not mainly located in rice bran, unlike total ash or other minerals
343 (Dikeman et al., 1980; Doesthale et al., 1979; Kennedy et al., 2002; Resurreccion et al., 1979).

344 From our X-ray images, it can be observed that the embryo has the highest concentration of
345 Zn. It however represents a very small fraction of the total grain and in absolute terms, does
346 not contribute very much to the total Zn in milled rice.

347 The different distributions of phytic acid and Zn in rice kernels confirmed earlier
348 statements that phytic acid is primarily present in the potassium or magnesium form instead of
349 the Zn form (Dikeman et al., 1980). Further research is required to assess the effects of other
350 factors such as environmental conditions and agricultural practice, on the distribution of
351 phytic acid and minerals in rice.

352

353 Conclusion

354

355 From this study we conclude that milling characteristics, including mass loss and breakage,
356 varied among the rice cultivars having different kernel shapes. This indicates an opportunity
357 for optimized milling, dedicated to improve the quality of white rice.

358 In the cultivars studied, we observed that whereas the distribution of phytic acid
359 differed, most of it was located in the outermost layer. In contrast, Zn distribution in the 3
360 cultivars was quite similar, characterized by an even distribution throughout the kernel with
361 the exception of a higher concentration in the embryo. The results give us the possibility to
362 process brown rice to obtain low phytic acid contents at a relatively high Zn content.

363

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365

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368 assistance of Dr Adriaan van Aelst with the preparation of rice kernels for microscopy, and of
369 Dr Zengling Yang with the digestion of samples and AAS analysis.

370 Table 1

371

Cultivar	Collection	PC (g 100g ⁻¹) ^a	Length (mm)	RLW ^b	KSA (mm ²) ^c	TKW (g) ^d	YBR (%) ^e	Breakage (%) ^f
<i>Bijing 37</i> (Yu et al., 2001)	Guizhou	8.8	5.2	1.8	68	25.5	80.6	3.7
<i>Zhongyou 752</i> (Centre of China Crop Science and Technology, 2006)	Jiangxi	10.9	6.4	2.8	50	26.5	71.9	16.2
<i>Gangwanxian30</i> (Information of Jiangxi foodstuff-oil and soil-fertilizer, 2006)	Jiangxi	8.5	7.6	3.4	52	27.6	73.6	28.0

372

373 Table 2

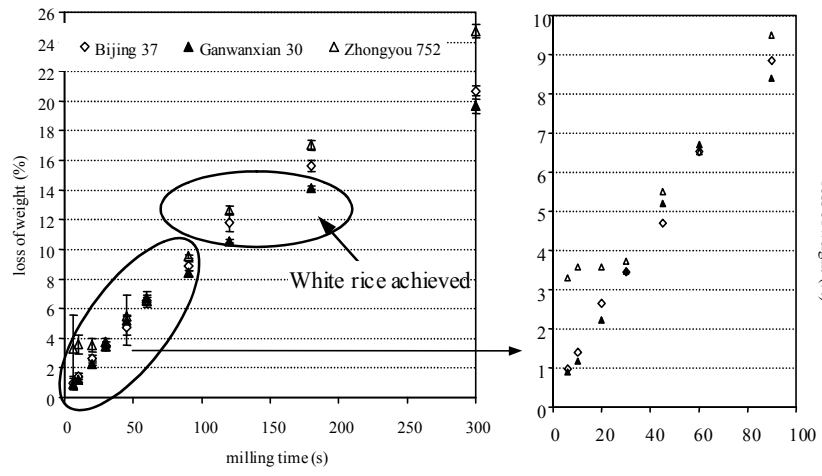
374

Milling time (s)	Bijing 37		Zhongyou 752		Ganwanxian 30	
	PA* (g kg ⁻¹)	Zn (mg kg ⁻¹)	PA (g kg ⁻¹)	Zn (mg kg ⁻¹)	PA (g kg ⁻¹)	Zn (mg kg ⁻¹)
0	8.9 ± 0.1 ^a	22.1 ± 0.7 ^a	7.8 ± 0.1 ^a	22.8 ± 1.0 ^a	11.1 ± 0.1 ^a	19.3 ± 4.9 ^a
6	9.0 ± 0.3 ^a	20.8 ± 2.5 ^a	7.6 ± 0.1 ^a	20.7 ± 0.1 ^a	8.1 ± 0.3 ^b	20.3 ± 4.8 ^a
10	8.9 ± 0.2 ^a	20.0 ± 0.6 ^a	7.9 ± 0.2 ^a	22.6 ± 0.3 ^a	7.5 ± 0.3 ^b	20.0 ± 3.6 ^a
20	6.9 ± 0.3 ^b	19.8 ± 0.5 ^a	8.7 ± 0.3 ^a	22.6 ± 0.8 ^a	7.5 ± 0.4 ^b	19.0 ± 2.9 ^a
30	6.5 ± 0.2 ^b	21.7 ± 1.3 ^a	6.8 ± 0.2 ^b	21.5 ± 1.2 ^a	8.0 ± 0.3 ^b	22.7 ± 1.4 ^a
45	6.3 ± 0.2 ^b	22.3 ± 2.6 ^a	6.5 ± 0.3 ^b	18.1 ± 0.8 ^{ab}	5.9 ± 0.6 ^c	21.9 ± 0.7 ^a
60	4.8 ± 0.1 ^c	21.2 ± 1.3 ^a	6.3 ± 0.5 ^b	17.7 ± 0.9 ^{bc}	5.3 ± 0.6 ^{cd}	21.3 ± 2.2 ^a
90	3.1 ± 0.1 ^d	20.1 ± 1.8 ^a	4.7 ± 0.2 ^c	16.3 ± 0.2 ^c	4.9 ± 0.2 ^d	25.8 ± 4.0 ^a
120	2.1 ± 0.1 ^e	15.0 ± 1.0 ^b	3.2 ± 0.4 ^d	16.4 ± 0.2 ^c	3.3 ± 0.1 ^e	20.5 ± 1.1 ^a
180	0.7 ± 0.0 ^f	14.8 ± 0.7 ^b	0.9 ± 0.0 ^e	15.9 ± 0.3 ^c	1.2 ± 0.1 ^f	21.2 ± 0.3 ^a
300	0.2 ± 0.0 ^g	13.7 ± 1.7 ^b	0.1 ± 0.0 ^f	16.7 ± 1.1 ^c	0.2 ± 0.1 ^g	18.5 ± 2.8 ^a

375

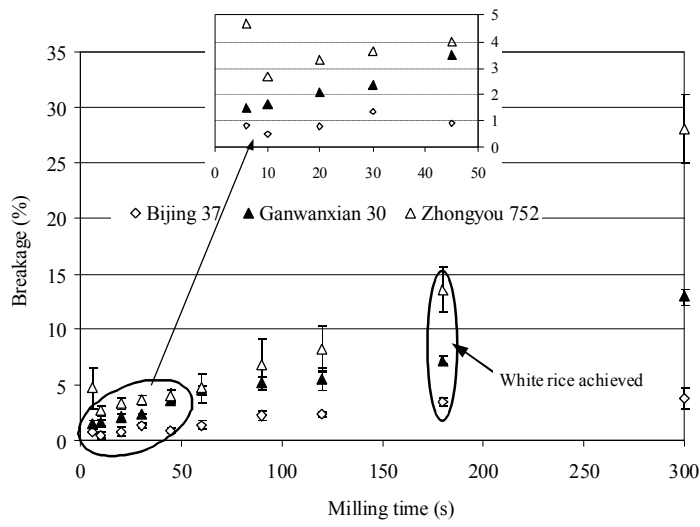
376 Figure 1a

377



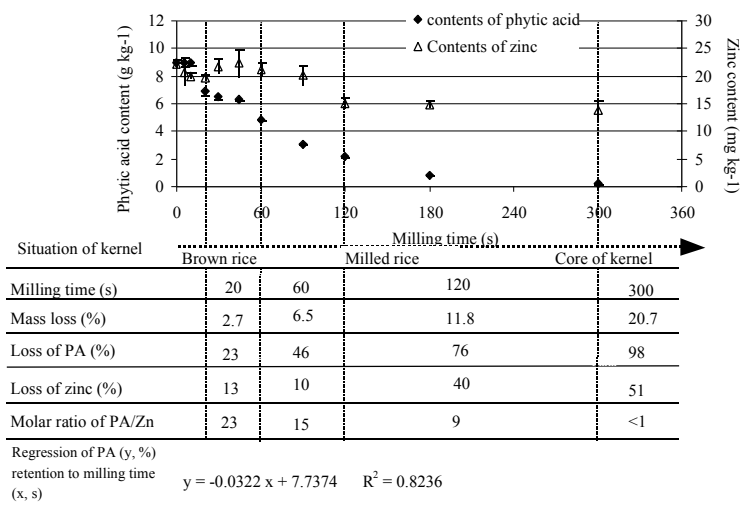
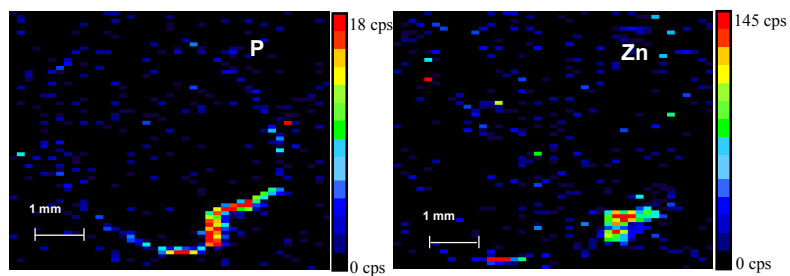
378

379 Figure 1b



380

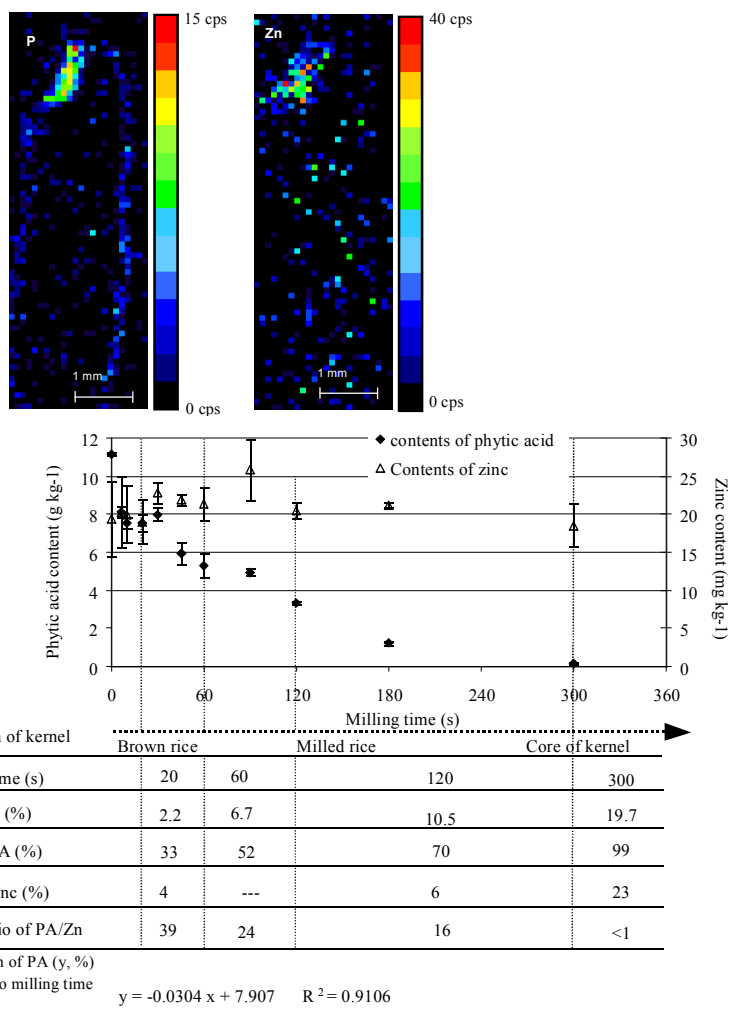
381 Figure 2a



Situation of kernel	Milling time (s)			
	Brown rice	Brown rice	Milled rice	Core of kernel
Milling time (s)	20	60	120	300
Mass loss (%)	2.7	6.5	11.8	20.7
Loss of PA (%)	23	46	76	98
Loss of zinc (%)	13	10	40	51
Molar ratio of PA/Zn	23	15	9	<1

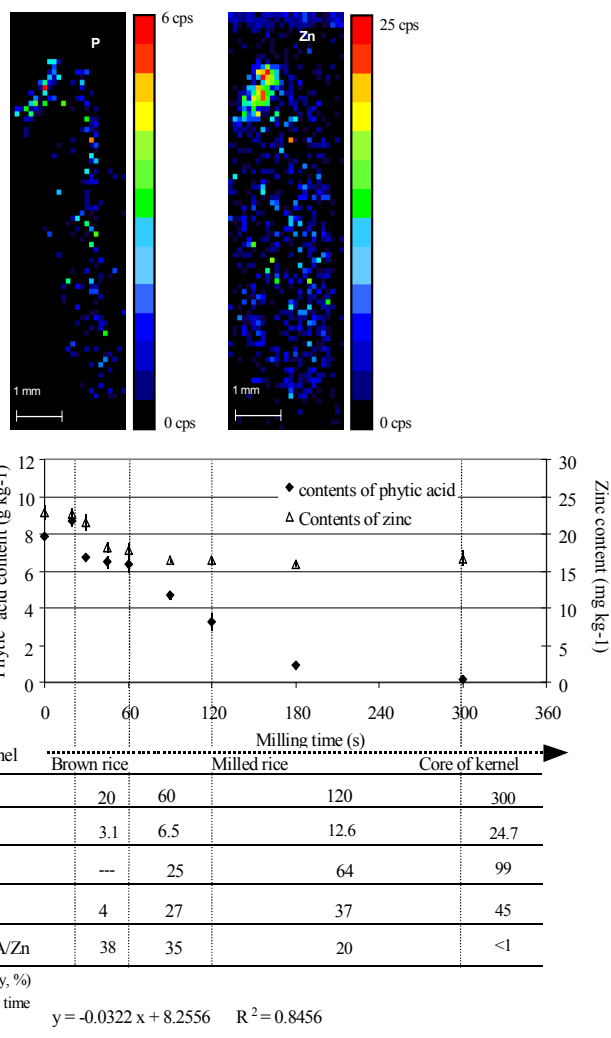
382

385 Figure 2c



386

387



388 **TITLES AND LEGENDS FOR TABLES AND FIGURES**

389 Table 1 General characteristics of brown rice samples

390 a: PC: protein contents (provided by supplier, see reference)

391 b: RLW: ratios of length to width (analysed in this study)

392 c: KSA: kernel surface area, assumed that the shape of integral kernels were separated into
393 two semi-sphere and a cylinder. Surface area was calculated with $4 * (\text{width}/2)^2 * \pi + 0.9 * \pi * \text{width} * \text{length}$ (analysed in this study)

395 d: TKW: thousand-kernel weight (provided by supplier, see reference)

396 e: YBR: yield of brown rice, on wet mass basis.

397 f: Breakage caused by dehulling: percentage of broken brown rice weight to total brown rice
398 weight (analysed in this study)

399

400 Table 2 Contents of phytic acid and zinc in milled rice*

401 * All data are based on dry mass weight and are presented as average \pm standard deviations
402 (n=3). Within columns, different superscripts indicate significant differences ($P < 0.05$, two-
403 tailed t-test).

404

405 Figure 1a Mass loss during milling

406

407 Figure 1b Breakage during milling

408

409 Figure 2a Distribution of phytic acid and Zn in Bijing 37 (short-grain)

410 Figure 2b Distribution of phytic acid and Zn in Zhongyou 752 (medium-grain)

411 Figure 2c Distribution of phytic acid and Zn in Ganwanxian 30 (long-grain)

412

413 REFERENCES

414

415 Bajaj, M., Arora, C.L., Chhibba, I.M., Sidhu, J.S., 1989. Extended milling of Indian rice. III.
416 Effect on mineral composition. *Chemie Mikrobiologie Technologie der Lebensmittel* 12, 58-
417 60.

418 Bryant, R.J., Dorsch, J.A., Rutger, J.N., Roboy, V., 2005. Phosphorus and mineral
419 concentration in whole grain and milled low phytic acid (lpa) 1-1 rice. *Cereal Chemistry* 82,
420 517-522.

421 Centre of China Crop Science and Technology, 2006.
422 <http://www.cropchina.com/old/food/zy751.htm> (visited Sept 2006).

423 Chen, H., Siebenmorgen, T.J., 1997. Effect of rice thickness in degree of milling and
424 associated optical measurements. *Cereal Chemistry* 74, 821-825.

425 Chen, H., Siebenmorgen, T.J., Griffin, K., 1998. Quality characteristics of long-grain rice
426 milled in two commercial systems. *Cereal Chemistry* 75, 560-565.

427 CHISA, 2002. China Industrial Standards Authority: Rice, China Industrial Standard
428 (Agriculture) NY/T 593-2002.

429 D'Ilio, S., Alessandrelli, M., Cresti, R., Forte, G., Caroli, S., 2002. Arsenic contents of various
430 types of rice as determined by plasma based techniques. *Microchemical Journal* 73, 195-201.

431 Dikeman, E., Bechtel, D.B., Pomeranz, Y., 1980. Distribution of elements in the rice kernel
432 determined by X-ray analysis and atomic absorption spectroscopy. *Cereal Chemistry* 58, 148-
433 152.

434 Doesthale, Y.G., Devara, S., Rao, S., Belavady, B., 1979. Effect of milling on mineral and
435 trace element composition of raw and parboiled rice. *Journal of the Science of Food and*
436 *Agriculture* 30, 40-46.

437 Emoto, T., Sato, Y., Konishi, Y., Ding, X., Tsuji, K., 2004. Development and applications of
438 grazing exit micro X-ray fluorescence instrument using a polycapillary X-ray lens.
439 *Spectrochimica Acta Part B* 59, 1291-1294.

440 Heinemann, R.J.B., Fagundes, P.L., Pinto, E.A., Pentead, M.V.C., Lanfer-Marquez, U.M.,
441 2005. Comparative study of nutrient composition of commercial brown, parboiled and milled
442 rice from Brazil. *Journal of Food Composition and Analysis* 18, 287-296.

443 Information of Jiangxi foodstuff-oil and soil-fertilizer, 2006.
444 <http://lytf.jxagri.gov.cn/ShowDetailZD.Asp?ID=7150> (visited Sept 2006).

445 Itani, T., Tamaki, M., Arai, E., Horino, T., 2002. Distribution of amylose, nitrogen, and
446 minerals in rice kernels with various characters. *Journal of Agricultural and Food Chemistry*
447 50, 5326-5332.

448 Juliano, B.O., 1972. The rice caryopsis and its composition. In: Houston, D.F. (Ed.), *Rice*
449 *Chemistry and Technology*. Monograph Series. American Association of Cereal Chemists, St.
450 Paul, MIN, pp. 16-74.

- 451 Kennedy, B.M., Schelstraete, M., 1975. Chemical, physical and nutritional properties of high-
452 protein flours and residual kernel from the overmilling of uncoated milled rice. *Cereal*
453 *Chemistry* 52, 173-182
- 454 Kennedy, G., Burlingame, B., Nguyen, N., 2002. Nutrient impact assessment of rice in major
455 rice-consuming countries. *International Rice Commission Newsletter* 51, 33-41.
- 456 Konishi, Y., Hirano, S., Tsuboi, H., Wada, M., 2004. Distribution of minerals in quinoa
457 (*Chenopodium quinoa* Willd.) seeds. *Bioscience, Biotechnology and Biochemistry* 68, 231-
458 234.
- 459 Liang, J., Han, B.-Z., Han, L., Nout, M.J.R., Hamer, R.J., 2007. Iron, zinc, and phytic acid
460 content of selected rice varieties from China. *Journal of the Science of Food and Agriculture*
461 87, 504-510.
- 462 Liu, J.C., Ockenden, I., Truax, M., Lott, J.N.A., 2004. Phytic acid-phosphorus and other
463 nutritionally important mineral nutrient elements in grains of wild-type and low phytic acid
464 (*lpa1-1*) rice. *Seed Science Research* 14, 109-116.
- 465 Liu, Z., Cheng, F., Zhang, G., 2005a. Grain phytic acid content in japonica rice as affected by
466 cultivar and environment and its relation to protein content. *Food Chemistry* 89, 49-52.
- 467 Liu, Z.H., Cheng, F.M., Cheng, W.D., Zhang, G.P., 2005b. Positional variations in phytic acid
468 and protein content within a panicle of japonica rice. *Journal of Cereal Science* 41, 297-303.
- 469 Lloyd, B.J., Siebenmorgen, T.J., 1999. Environmental conditions causing milled rice kernel
470 breakage in medium-grain varieties. *Cereal Chemistry* 76, 426-427.
- 471 Ma, G., Jin, Y., Piao, J., Kok, F., Bonnema, G., Jacobsen, E., 2005. Phytate, calcium, iron,
472 and zinc contents and their molar ratios in food commonly consumed in China. *Journal of*
473 *Agricultural and Food Chemistry* 53, 10285-10290.
- 474 Mohapatra, D., Bal, S., 2004. Wear of rice in an abrasive milling operation, part 1: prediction
475 of degree of milling. *Biosystems Engineering* 88, 337-342.
- 476 Nijssen, J., Van Aelst, A.C., 1999. Cryo-planing for cryo-scanning electron microscopy.
477 *Scanning* 21, 372-378.
- 478 Park, J.K., Kim, S.S., Kim, K.O., 2001. Effect of milling ratio on sensory properties of
479 cooked rice and on physicochemical properties of milled and cooked rice. *Cereal Chemistry*
480 78, 151-156.
- 481 Perdon, A.A., Siebenmorgen, T.J., Mauromoustakos, A., Griffin, V.K., Johnson, E.R., 2001.
482 Degree of milling effect on rice pasting properties. *Cereal Chemistry* 78, 205-209.
- 483 Resurreccion, A.P., Juliano, B.O., Tanaka, Y., 1979. Nutrient content and distribution in
484 milling fractions of rice grain. *Journal of the Science of Food and Agriculture* 30, 475-481.
- 485 Siebenmorgen, T.J., Qin, G., 2005. Relating rice kernel breaking force distributions to milling
486 quality. *Transactions of the ASAE* 48, 223-228.

- 487 Singh Gujral, H., Singh, J., Singh Sidhu, J., Singh, N., 2002. Effect of milling variables on the
488 degree of milling of unparboiled and parboiled rice. *International Journal of Food Science and*
489 *Technology* 5, 193-2-4.
- 490 Singh, N., Singh, H., Kaur, K., Singh Bakshi, M., 2000. Relationship between the degree of
491 milling, ash distribution pattern and conductivity in brown rice. *Food Chemistry* 69, 147-151.
- 492 Song, B.H., Kim, D.Y., Kim, S.K., Kim, Y.D., Choi, K.S., 1988. Distribution of minerals
493 within the degermed brown rice kernel. *Journal of Korean Agricultural Chemistry* 31, 162-
494 168.
- 495 Tabekhia, M.M., Luh, B.S., 1979. Effect of milling on macro and micro minerals and phytate
496 of rice. *Deutsche Lebensmittel-Rundschau* 75, 57-62.
- 497 Tran, T.U., Suzuki, K., Okadome, H., Homma, S., Ohtsubo, K., 2004. Analysis of the tastes
498 of brown rice and milled rice with different milling yields using a taste sensing system. *Food*
499 *Chemistry* 88, 557-566.
- 500 Vasca, E., Materazzi, S., Caruso, T., Milano, O., Fontanella, C., Manfredi, C., 2002. Complex
501 formation between phytic acid and divalent metal ions: a solution equilibria and solid state
502 investigation. *Analytical and Bioanalytical Chemistry* 374, 173-178.
- 503 Villareal, C.P., Maranville, J.W., Juliano, B.O., 1991. Nutrient content and retention during
504 milling of brown rices from the International Rice Research Institute. *Cereal Chemistry* 68,
505 437-439.
- 506 Yu, B., Zhang, S., Gu, S., Ye, Y., Luo, H., 2001. Selection and application research of high-
507 yielding new japonica rice variety - Bijing 37. *Review of China Agricultural Science and*
508 *Technology* 3, 16-19.
- 509 Zhou, H., 2003. *Cereals Science Principle* (in Chinese). China Light Industrial Press, Beijing,
510 pp. 1-3, 196-217.
511
512