Food Chemistry 110 (2008) 916–922

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Impact of parboiling conditions on Maillard precursors and indicators in long-grain rice cultivars

Lieve Lamberts *, Ine Rombouts, Kristof Brijs, Kurt Gebruers, Jan A. Delcour

Laboratory of Food Chemistry and Biochemistry, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium

ARTICLE INFO

Article history:
Received 8 November 2007
Received in revised form 28 December 2007
Accepted 27 February 2008
Available online xxxx

Keywords:
Rice
Parboiling
Maillard reaction
Colour
Sugars
Free lysine
Furosine
Free HMF

ABSTRACT

The effect of steaming conditions (mild, intermediate and severe) during parboiling of five different long-grain rice cultivars (brown rice cultivars Puntal, Cocodrie, XL8 and Jacinto, and a red rice) on rice colour, and Maillard precursors and indicators was investigated. Rice colour increased with severity of parboiling conditions. Redness increased more than yellowness when parboiling brown rice. Parboiling turned red rice black. It changed the levels of glucose, fructose, sucrose, and maltose. Losses of the non-reducing sugar, sucrose were caused by both leaching into the soaking water and enzymic conversion, rather than by thermal degradation during steaming. Concentrations of the reducing sugars, glucose and fructose, in intermediately parboiled rice were higher than those of mildly parboiled rice. After severe parboiling, glucose levels were lower than those of intermediately parboiled rice, while fructose levels were higher. These changes were ascribed to the sum of losses in the Maillard reaction (MR), formations as a result of starch degradation and isomerisation of glucose into fructose. It was clear that the ε-amino group of protein-bound lysine was more affected by parboiling conditions and loss in MRs, than that of free lysine. Low values of the MR indicators furosine and free 5-hydroxymethyl-2-furaldehyde (HMF) in processed brown and red rices were related to mild parboiling, whereas high furosine and low free HMF levels were indicative of rices being subjected to intermediate processing conditions. High furosine and high free HMF contents corresponded to severe hydrothermal treatments. The strong correlation ($r = 0.89$) between the free HMF levels and the increase in redness of parboiled brown rices suggested that Maillard browning was reflected more in the red than in the yellow colour.

1. Introduction

Rice parboiling is a hydrothermal treatment consisting of soaking, heating and drying. Parboiled rice has a higher nutritional value than its non-parboiled counterpart due to migration of bran components (e.g., vitamins, minerals) into the endosperm during the hydrothermal treatment (Bhattacharya, 2004). Parboiling changes physicochemical and organoleptic properties of the rice grain. It reduces rice stickiness, increases hardness, and darkens the colour. The typical European consumer prefers non-sticky, relatively hard, white rice. Thus, dark rice colour is a negative quality attribute. The literature explains colour changes during parboiling as caused by migration of husk and/or bran pigments, enzymic browning and non-enzymic browning of the Maillard type.

The level of bran pigments in brown rice decreases from outer bran layers to the endosperm (Itani, Tamaki, Arai, & Horino, 2002; Lamberts et al., 2007). Bran pigments determine parboiled rice colour since they leach out during soaking in excess water and diffuse into the endosperm during steaming (Lamberts, Brijs, Mohamed, Verhelst, & Delcour, 2006a; Lamberts et al., 2006b). The contribution of enzymic browning during soaking to rice colour is rather small. Lamberts et al. (2006a) recently confirmed the occurrence of Maillard browning during parboiling by measuring the Maillard indicator furosine, a component formed during acid hydrolysis of the Amadori component ε-*N*-(1-deoxy-D-fructosyl)-L-lysine in parboiled rice. An increased severity of parboiling for both brown and milled rice resulted in an increase of furosine contents which was an indication that Maillard browning occurs on the surface of brown rice as well as in the endosperm (Lamberts et al., 2006a).

Maillard reactions (MRs) occur during hydrothermal treatment and storage of food products (Villamiel, del Castillo, & Corzo, 2006). They involve carbonyl groups of reducing sugars and amino groups of amino acids (mainly lysine), peptides, or proteins and induce nutritional changes. Apart from their reactions with amino groups, carbohydrates can also undergo isomerisation and degradation reactions. Martins and van Boekel (2005) studied kinetics of the MR pathways in glucose–glycine model systems and observed that fructose formation increased with increasing temperature. In heat-processed cereals, the isomerisation of maltose to maltulose has frequently been observed (Garcia-Banos, Corzo, Sanz, & Olano, 2004; Morales, Olano, & Corzo, 2004). Furthermore, MRs cause

* Corresponding author. Tel.: +32 16 321582; fax: +32 16 321997.
E-mail address: Lieve.Lamberts@biw.kuleuven.be (L. Lamberts).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.02.080

destruction of essential amino acids or reduction of protein digestibility and amino acid availability. The loss of available lysine, which is a most unfavourable nutritional consequence of the MR, is particularly significant in cereals, where lysine is already a limiting amino acid (Fernandez-Artigas, Garcia-Villanova, & Guerra-Hernandez, 1999).

The MR can not only be monitored based on changes in levels of reducing sugars and amino groups, but also by studying the intermediates formed during the complex transformations. In the early stage of the MR, Amadori products formed can be monitored by measurement of furosine. Furosine concentration measurements have been used to evaluate the extent of the MR in different cereal-based products as the compound is generated upon acid hydrolysis of Amadori products (Guerra-Hernandez, Corzo, & Garcia-Villanova, 1999; Lamberts et al., 2006a; Rufian-Henares, Garcia-Villanova, & Guerra-Hernandez, 2004). During the intermediate stage of the MR, *inter alia* breakdown of Amadori products occurs, resulting in the regeneration of amino acids and the formation of, e.g., 5-hydroxymethyl-2-furaldehyde (HMF). HMF is frequently used as a heat-damage indicator in milk (Morales, Romero, & Jimenez-Perez, 1996; van Boekel, 1998) and cereals (Ramirez-Jimenez, Garcia-Villanova, & Guerra-Hernandez, 2000).

This study reports on the effect of parboiling conditions on Maillard browning of five long-grain cultivars with different chemical composition. Colour parameters of the different non-parboiled and mildly, intermediately and severely parboiled rices were determined. Furthermore, the impact of severity of parboiling on mono- and disaccharide levels, free lysine levels, and on both furosine and free HMF contents was investigated for the first time. Determination of the Maillard indicators increased insights on the feasibility of using furosine or free HMF alone or in combination for hydrothermal treatment evaluation of parboiled rices. Finally, the effect of parboiling conditions on colour parameters changes, in relation to levels of Maillard indicators, was determined.

2. Materials and methods

2.1. Rice parboiling

Brown rice (*Oryza sativa* L., harvest 2003) from cultivars with brown pericarp Cocodrie (USA, 1000 kernel weight 21.3 g), Puntal (Spain, 1000 kernel weight 20.8 g), XL8 (USA, 1000 kernel weight 23.3 g) and Jacinto (Spain, 1000 kernel weight 19.2 g), and from a cultivar with red pericarp (unknown cultivar, Thailand, 1000 kernel weight 21.5 g) were obtained from Mars Belgium NV (Olen, Belgium). The unknown cultivar with red pericarp is further referred to as red rice, while the cultivars with brown pericarp are referred to as brown rice. In the classification of Juliano (1998), all cultivars were long-grain rices, since their kernel lengths exceeded 6.0 mm. To obtain parboiled rice, brown/red rice (1.0 kg) was soaked in excess water (2.0 l) for 30 min at 50 °C. The excess water was discarded and the soaked rice was heated by steaming. To obtain mildly parboiled rice, the soaked rice was heated by steaming using three standard steps (10 min at 80 °C, 2 min at 100 °C and 10 min at 105 °C). To obtain intermediately and severely parboiled rice, the three standard steps were followed by one additional steaming step (12 min at 115 °C, and 17 min at 125 °C, respectively). After parboiling, the pressure was reduced to atmospheric in three subsequent steps. The steamed rice was dried on trays for 48 h at room temperature, to obtain moisture levels of ca. 11%.

2.2. Rice milling

Brown/red rices were milled (50 s) with a TM05C testing mill (Satake, Bredbury, UK) and stored at room temperature until analysis. The degree of milling (DOM), i.e., the weight percentage of rice layers removed by milling, was calculated from the weight of rice before and after milling. Broken kernels were removed using a test rice grader. DOM ranged from 13.9% (Jacinto) to 15.9% (Cocodrie) for non-parboiled rices, and from 9.7% (Jacinto) to 12.4% (Cocodrie) for parboiled rices. There was no significant effect of parboiling conditions on DOM within the same cultivar. The brown/red and milled rice kernels were ground with a laboratory mill (M20 Universal Mill, Ika, Wilmington, NC) to pass a 250 μm sieve. Quality characteristics of the milled, intermediately parboiled rice of the cultivar Puntal (intermediate-amylose) were comparable to those of the commercial 10 min rice, i.e., non-sticky, relatively hard and light yellow-coloured rice. Mild and severe parboiling reduced quality: mildly parboiled rices contained white bellies, i.e., ungelatinised opaque core, and severely parboiled rices showed undesirable darkening.

2.3. Chemicals

All chemicals used were of at least analytical grade and obtained from Sigma (Bornem, Belgium) unless otherwise indicated.

2.4. Moisture content determination

Moisture contents of rices were analysed according to AACC method 44-15A (AACC International, 2000) and estimated from the mass loss of ca. 1.0 g accurately weighed rice flour when heating for 90 min at 130 °C. Analyses were performed in duplicate. All determinations (except for colour parameters) were expressed on dry matter basis.

2.5. Chemical composition

The apparent amylose contents were determined based on Derycke et al. (2005). Analyses were performed in triplicate. Protein contents were determined in duplicate, using an adaptation of the AOAC Official Method (AOAC, 1995) to an automated Dumas protein analysis system (EAS vario Max N/CN, Elt, Gouda, The Netherlands). A conversion factor of 5.95 (Shih, 2004) was used to calculate protein from nitrogen content.

2.6. Colour measurements

Colour measurements of rice kernels were performed in triplicate with a colorimeter (model Colourquest 45/0 LAV, CQ/UNI-1600, HunterLab, Reston, VA), as described by Lamberts et al. (2006b). The total colour differences (ΔE) and the chroma values (C^*) were calculated from the colour parameters L^* (brightness), a^* (redness) and b^* (yellowness). The non-parboiled brown/red or milled, non-parboiled rices were used as reference to calculate ΔE-values. The coefficient of variation (CV) for the determination of the colour parameters was below 10%.

2.7. Levels of mono- and disaccharides

Rice flour (0.100 g) was defatted with hexane (3.0 ml). After shaking (15 min), the suspension was centrifuged (3000*g*, 10 min), and the pellet was dried under a stream of nitrogen gas. It was then extracted with deionised water (5.0 ml) for 30 min at 6 °C. The suspension was centrifuged (3000*g*, 15 min). The levels of mono- and disaccharides in the diluted (10- and 5-fold for brown/red and milled rice, respectively) supernatants were determined by high performance anion-exchange chromatography with integrated pulsed amperometric detection. Analyses were performed in triplicate. A Dionex BioLC system (Sunnyvale, CA) consisting of a GS50 gradient pump with online degasser, an AS50 autosampler with a thermal compartment, and an ED50 electro-

chemical detector containing a gold working electrode and a pH reference electrode was used. Separation was performed using the AminoPac PA10 guard column (50 × 2 mm) and analytical column (250 × 2 mm) at a flow rate of 0.25 ml/min at 30 °C. The sample injection volume was 12 μl. To prepare the gradient mobile phases, 18 MΩ water (mobile phase A), dilute sodium hydroxide (0.250 M, mobile phase B; Baker, Deventer, The Netherlands) and 1.0 M sodium acetate (mobile phase C; Dionex Benelux, Amsterdam, The Netherlands) were used. All three mobile phases were degassed and kept under slight helium overpressure to prevent accumulation of atmospheric carbon dioxide. The chromatographic system control, data acquisition and data analysis were performed using Chromeleon Version 6.70 software (Dionex). The levels of mono- and disaccharides were determined in the diluted extracts using the gradient conditions based on Ding, Yu, and Mou (2002) with some modifications (Table 1). The detection waveform used for mono- and disaccharides was borrowed from Ding et al. (2002). Mono- and disaccharide levels of non-parboiled brown/red rice extracts were directly determined since enzymic conversion may occur, even during incubation at lower temperature (10 °C). The CV for the determination of the mono- and disaccharides was below 8%.

2.8. Free lysine content

Rice flour (0.300 g) was defatted with 6.0 ml hexane (*cfr. supra*). It was then extracted with sodium acetate buffer (25 mM, pH 4.0) for 30 min at 6 °C. The suspension was centrifuged (3000*g*, 15 min). Amino acid analysis was performed in duplicate using the easy-fast amino acid sample testing kit (EZ:faast™, Phenomenex, Aschaffenburg, Germany). After sample preparation by solid phase extraction, derivatisation and liquid/liquid extraction, free lysine was quantified by gas chromatography (Agilent 6890 series, Wilmington, DE) with flame ionisation detection. The derivatives were separated on a Zebron ZB-5 ms column (Phenomenex) using the following temperature profile: from 120 to 210 °C, at 20 °C/min and from 210 to 300 °C, at 32 °C/min. The carrier gas was helium and the internal standard was norvaline (Phenomenex). The difference between two measurements did not exceed 1.5 ppm for both parboiled brown/red and milled, parboiled rices.

2.9. Furosine content

The levels of furosine were measured according to Resmini, Pellegrino, and Battelli (1990). Following hydrolysis of rice flour (500.0 mg) with HCl solution (10.6 M, 6.0 ml), suspensions were saturated with nitrogen gas (2 min) and heated (25 h, 110 °C). The filtrates (0.50 ml) were applied to a Maxi-Clean cartridge (C18, 500 mg, Alltech, Laarne, Belgium) prewetted with water (5.0 ml), and eluted with HCl (3.0 M, 2.0 ml). Furosine was quantified by RP-HPLC (Shimadzu, Kyoto, Japan) on a furosine-dedicated column (C8, 250 × 4.6 mm, Metal-Free, Alltech). The furosine standard was obtained from Neosystem (Strasbourg, France). Analyses were performed in duplicate. The difference between two measurements did not exceed 10 and 5 ppm for parboiled brown/red and milled, parboiled rices, respectively.

Table 1
Gradient conditions used for separation of mono- and disaccharides (glucose and fructose, and sucrose) in rice extracts by high performance anion-exchange chromatography with integrated pulsed amperometric detection

Time (min)	250 mM NaOH (%)	1 M NaOAc (%)
0.0	4	
14.0	4	
14.1	24	
16.0	24	
24.0	36	
26.0	36	
26.1	70	
33.0	70	
35.0	20	40
38.0	20	40
40.0	16	70
59.0	16	70
59.1	80	
61.0	80	
61.1	4	
93.0	4	

2.10. Free 5-hydroxymethyl-2-furaldehyde content

Free HMF content was quantified using 2-thiobarbituric acid (TBA), based on the method of Keeney and Bassette (1959). Oxalic acid solution (0.15 M, 3.0 ml) was added to 300.0 mg defatted rice flour (*cfr. supra*). After shaking (30 min), the mixture was deproteinised with trichloroacetic acid solution (40% (w/v), 2.0 ml). Following centrifugation (3000*g*, 10 min), the supernatant (1.6 ml) was added to TBA solution (0.03 M, 0.9 ml), which forms a yellow complex. After incubation (40 min, 40 °C) the absorbance was measured (443 nm). The external HMF standard was obtained from Acros Organics (Geel, Belgium). Each rice was analysed in triplicate. The CV for the determination of free HMF was below 5% for both parboiled brown/red and milled, parboiled rices.

3. Results and discussion

3.1. Chemical composition of non-parboiled rice

Table 2 shows the apparent amylose and protein contents in non-parboiled rices. In the classification by Juliano (1998), Jacinto and red rice are low-amylose cultivars (17.6% and 15.2%, respectively), Puntal is an intermediate-amylose cultivar (23.8%), and Cocodrie and XL8 are high-amylose cultivars (30.5% and 28.7%, respectively). In contrast to amylose levels, protein contents were measured in the brown/red rices, since outer rice layers were richer in protein than endosperm (Lamberts et al., 2007). Protein levels of brown/red rices ranged from 7.2% (XL8) to 9.3% (Puntal). Thus, the rice cultivars used in this browning study varied in the levels of their major components.

3.2. Effect of parboiling and milling on rice colour

Table 3 shows the colour parameters L^* (brightness), a^* (redness) and b^* (yellowness), and the chroma (C^*) of non-parboiled and parboiled brown/red rices. It also lists the total colour difference (ΔE) between parboiled and non-parboiled rices. The brightness of non-parboiled brown rices ranged from 60.1 to 67.0, whereas redness ranged from 3.8 to 4.8 and yellowness from 22.2 to 23.6. These differences in colour parameters between the cultivars were readily observable by the human eye. The differences in redness and yellowness were also reflected in the values

Table 2
Apparent amylose levels in milled, non-parboiled rices and protein levels in non-parboiled brown/red rices

Cultivar	Milled rice	Brown/red rice
	Apparent amylose content (%)	Protein content (%)
Puntal	23.8	9.3
Cocodrie	30.5	8.8
XL8	28.7	7.2
Jacinto	17.6	8.6
Red rice	15.2	7.6

Table 3
Colour parameters L^* (brightness), a^* (redness) and b^* (yellowness), chroma (C^*) and total colour difference (ΔE) between non-parboiled (NPB) and mildly (M), intermediately (I) and severely (S) parboiled (PB) rices

Cultivar	Process	Brown/red rice					Milled rice				
		L^*	a^*	b^*	C^*	ΔE	L^*	a^*	b^*	C^*	ΔE
Puntal	NPB	60.1	4.7	23.7	24.2	–	75.9	-0.8	14.4	14.4	–
	MPB	53.7	6.2	25.0	25.7	6.7	73.3	0.8	19.8	19.8	6.2
	IPB	44.5	8.0	26.4	27.5	16.2	66.7	1.9	22.9	23.0	12.8
	SPB	40.5	9.5	25.9	27.6	20.3	62.4	4.8	25.4	25.9	18.3
Cocodrie	NPB	62.5	4.8	24.1	24.5	–	78.0	-0.7	14.0	14.0	–
	MPB	52.8	6.4	26.1	26.9	10.1	71.3	0.9	21.7	21.7	10.3
	IPB	45.5	7.7	26.7	27.8	17.5	66.6	2.1	24.0	24.1	15.4
	SPB	41.8	9.5	26.3	28.0	21.4	61.5	5.6	26.0	26.6	21.3
XL8	NPB	67.0	3.9	23.6	24.0	–	78.4	-1.4	11.4	11.5	–
	MPB	54.3	5.9	25.5	26.2	13.0	72.2	0.6	18.9	18.9	9.9
	IPB	nd[a]	nd	nd	nd	nd	69.9	0.9	21.4	21.4	13.3
	SPB	44.4	8.4	26.9	28.2	23.3	64.7	4.2	23.9	24.3	19.4
Jacinto	NPB	62.8	3.8	22.2	22.5	–	77.1	-0.9	13.1	13.1	–
	MPB	50.0	5.9	25.0	25.8	13.1	69.5	0.9	19.4	19.4	10.1
	IPB	45.9	7.3	26.1	27.2	19.0	67.0	1.9	21.3	21.4	13.3
	SPB	43.7	9.0	25.6	27.0	20.8	63.1	4.6	24.2	24.6	18.7
Red rice	NPB	31.2	13.0	16.1	20.7	–	72.2	2.1	15.0	15.1	–
	MPB	20.6	9.5	7.3	12.0	14.2	53.6	7.5	16.2	17.9	19.4
	IPB	17.6	7.1	5.1	8.7	18.5	50.7	7.3	16.7	18.3	22.2
	SPB	17.2	5.7	4.0	7.2	19.9	43.8	8.1	17.2	19.0	29.1

[a] nd: not determined.

of C^* (22.5–24.5). Non-parboiled red rice was less bright and contained more red and less yellow pigments than the brown cultivars.

Parboiling of brown rices (Puntal, Cocodrie, XL8 and Jacinto) increased darkness, yellowness and redness, as observed earlier (Lamberts et al., 2006a, 2006b). The effect of parboiling severity on colour parameters of brown rices was more pronounced for brightness and redness than for yellowness. Due to increased values of a^* and b^*, the chroma (C^*) of parboiled brown rices increased and ranged from 25.7 to 28.0. C^*-values increased with degree of parboiling, but the effect of parboiling conditions on colour was reflected more in total colour difference (ΔE), since the latter takes into account the changes in brightness. It seems that the effect of parboiling on values of ΔE depends on cultivar and steaming conditions. Mild parboiling resulted in small values of ΔE (6.7–13.1). Intermediately parboiled brown rices showed higher values of ΔE (16.2–19.0), and severe parboiling resulted in the largest values of ΔE (20.3–23.3). In contrast to what could be observed with brown rices, parboiling of red rice decreased redness, yellowness and hence chroma. Parboiled red rice was perceived by the human eye as black and its colour was translated in decreased chroma. Values of ΔE were comparable to the values for brown rices.

As expected, milling of non-parboiled and parboiled rices increased brightness and decreased redness, yellowness and chroma for all rice cultivars (Table 3). Although the DOM of red rice was in the range of values for the brown rice cultivars, milling here resulted in darker and more red and yellow rices than with the brown rice cultivars. The increased redness and yellowness of milled red rice can be ascribed to its larger (pigment-rich) bran fraction, which was not completely removed by milling for 50 s. Unlike what was measured on parboiled brown rice, the colour change caused by parboiling in milled rices was reflected more in yellowness than in redness. Also, milled, parboiled red rice contained more red and less yellow pigments than milled, parboiled brown rices. The differences in effect of parboiling on values of a^* and b^* in brown/red and milled rices were also reflected in a broader range of values of C^* (from 25.7 to 28.2 and from 18.9 to 26.6, for parboiled brown and milled, parboiled brown rices, respectively, and from 7.2 to 12.0 and 17.9 to 19.0, for parboiled red and milled, parboiled red rice, respectively). Values of ΔE of parboiled brown rices were higher than or matched those of the corresponding milled, parboiled rices.

3.3. Effect of parboiling and milling on mono- and disaccharide levels

Table 4 presents the levels of sucrose, glucose and fructose in non-parboiled and parboiled brown/red rices. Sucrose levels (11,100–21,200 ppm) were higher than those of glucose (190–290 ppm) and fructose (60–250 ppm). Maltose contents ranged from 170 to 930 ppm (results not shown). These levels were in agreement with earlier data of Ali and Bhattacharya (1980). These authors found sucrose levels of 9300 ppm and reducing sugar levels of 800 ppm for non-parboiled brown rice. Furthermore, our analyses indicate that the rices contained raffinose, the level of which decreased as a result of parboiling (results not shown). This non-reducing sugar could not be quantified with the gradient conditions used here, since raffinose coeluted with the amino acids isoleucine and leucine. Parboiling reduced maltose contents to undetectable levels. It also decreased the levels of the non-reducing sugar sucrose. The decrease was highest for Puntal and Cocodrie (more than 20%) and lowest for XL8, Jacinto and red rice (less than 20%) and was not affected by the severity of parboiling (with the exception of Jacinto). As discussed earlier by Ali and Bhattacharya (1980), and Lamberts et al. (2006a), the loss of sucrose caused by parboiling can be explained by enzymic conversion of sucrose into glucose and fructose during soaking, and by leaching of sugars from the bran layers into the soaking water. Furthermore, the observation that sucrose levels did not decrease with severity of steaming suggested that it was not thermally degraded during steaming. Parboiling increased the levels of the reducing sugars glucose and fructose for most parboiled brown/red rices, and the reducing sugar levels depended on the severity of parboiling conditions. Mildly parboiled rice showed the lowest glucose and fructose levels. The additional heating step (12 min at 115 °C) to obtain intermediately parboiled rices increased glucose and fructose contents. The additional heating step (17 min at 125 °C) to obtain severely parboiled rice also increased reducing sugar levels. However, the increase in glucose content was smaller in severely

Table 4
Levels of mono- and disaccharides (glucose and fructose, and sucrose), and levels of free lysine in non-parboiled (NPB), mildly (M), intermediately (I) and severely (S) parboiled (PB) rices

Cultivar	Process	Brown/red rice				Milled rice			
		Sucrose content (ppm)	Glucose content (ppm)	Fructose content (ppm)	Free lysine content (ppm)	Sucrose content (ppm)	Glucose content (ppm)	Fructose content (ppm)	Free lysine content (ppm)
Puntal	NPB	15,800	190	140	14.0	200	–	–	–
	MPB	9800	240	120	12.5	7600	220	140	7.0
	IPB	9800	480	190	15.5	7600	470	190	9.0
	SPB	9700	430	280	16.0	7800	440	290	8.5
Cocodrie	NPB	21,200	290	250	27.5	100	–	–	–
	MPB	10,300	270	200	28.5	7100	290	130	12.5
	IPB	9900	570	260	27.0	7200	260	150	13.0
	SPB	10,100	490	390	27.5	7300	240	270	13.5
XL8	NPB	14,000	200	170	11.5	100	–	–	–
	MPB	7800	210	100	13.5	6400	190	50	7.5
	IPB	nd[a]	nd	nd	nd	6900	240	70	7.0
	SPB	7600	410	180	14.0	6900	210	140	8.0
Jacinto	NPB	11,100	270	150	16.5	100	–	–	–
	MPB	7600	320	140	20.5	6400	300	120	9.5
	IPB	7100	620	230	21.0	6300	590	180	12.0
	SPB	8500	350	210	18.0	7500	320	180	12.5
Red rice	NPB	12,700	240	60	11.5	200	–	–	–
	MPB	7300	330	130	15.0	6200	340	110	9.5
	IPB	7100	770	200	14.0	6000	750	180	9.0
	SPB	7000	690	300	11.0	6100	680	270	9.0

[a] nd: not determined.

parboiled rice than in intermediately parboiled rice, while the increase in fructose was more pronounced (except for Jacinto). When explaining the changes in reducing sugar levels, only steaming conditions should be taken into account, since soaking conditions were similar for mild, intermediate and severe parboiling. Differences in glucose and fructose levels caused by parboiling were the result of losses in Maillard, glucose–fructose isomerisation reactions and degradation reactions during heating on the one hand, and formation during thermal degradation of starch, on the other hand. Our results indicate that glucose and fructose formation were the lowest for mild parboiling conditions. The increased glucose and fructose levels of intermediately and severely parboiled rice demonstrated that the formation of glucose during severe parboiling was compensated more by its loss in MRs and/or isomerisation into fructose than for intermediately parboiled rices (except for severely parboiled Jacinto).

Milling of non-parboiled rices decreased the levels of the different sugars to contents lower than or equal to 200 ppm for sucrose and to undetectable levels for glucose, fructose (Table 4) and maltose (results not shown). Milling of parboiled rices resulted in decreased sucrose levels for all cultivars, and in glucose and fructose levels that were similar or lower than those of their non-milled counterparts. The effect of parboiling conditions on glucose and fructose levels was similar for most of the cultivars (except for intermediately and severely parboiled Cocodrie and severely parboiled XL8, which showed large losses in reducing sugars). These observations indicated that sucrose distribution depends on rice cultivar and that the level decreased from rice surface to the centre of the rice kernel. They also showed that reducing sugars are more concentrated in the outer layers than in endosperm of non-parboiled rices. The highest reducing sugar levels of parboiled red rices after milling support the hypothesis that bran layers were partly retained after red rice milling, as suggested by the increase in redness and yellowness.

3.4. Effect of parboiling and milling on free lysine levels

Table 4 presents free lysine contents in non-parboiled and parboiled brown/red rices. The free lysine content ranged from 11.5 (XL8) to 27.5 ppm (Cocodrie). Parboiled Puntal and Cocodrie had free lysine levels comparable to those of their non-parboiled counterparts. Jacinto, XL8 and red rice showed slightly increased free lysine levels after parboiling. Furthermore, free lysine levels were not related to the severity of parboiling. The increase in free lysine in Jacinto, XL8 and red rice can be ascribed to the release of free amino acids by proteolytic activity during soaking.

Milling decreased free lysine levels in non-parboiled rices to undetectable levels (Table 4). The decrease in free lysine in parboiled brown rices exceeded 40%, while the decrease in red rices was less than 40%. Thus, free amino acids are concentrated in the outer bran layers, in agreement with Saikusa, Horino, and Mori (1994).

3.5. Effect of parboiling and milling on furosine levels

Fig. 1 shows the furosine levels in parboiled brown/red, and milled, parboiled rices. Furosine contents formed in parboiled red rices (except for severely parboiled rice) were highest, while those in XL8 were the lowest (Fig. 1A). Furthermore, furosine levels formed in mildly parboiled rices ranged from ca. 70 to 120 ppm. Intermediate parboiling increased furosine contents (ca. 170 to 210 ppm, value for XL8 not determined). Severe parboiling led to lower or similar furosine levels (ca. 90 to 210 ppm). The presence of furosine precursors in parboiled rices indicated that lysine blockage in the early stages of the MR occurred during parboiling. From the protein levels in the brown/red rices (7.2–9.3%) and from the mean amino acid composition of brown rice protein (ca. 4.0% lysine in proteins; Shih, 2004) it was clear that protein-bound lysine levels were much higher (2800–3700 ppm) than free lysine levels (11.5–27.5 ppm). Consequently, losses in lysine during parboiling must not be attributed to the reaction of free lysine in MR, but to involvement of the ε-NH_2 group of protein-bound lysine in MRs as well as in intra- and intermolecular crosslinking reactions in proteins.

In contrast to earlier observations (Lamberts et al., 2006a), it seems that the parboiling conditions used here did not increase furosine precursor levels to an extent related to the severity of parboiling conditions. As explained earlier, Maillard browning covers a

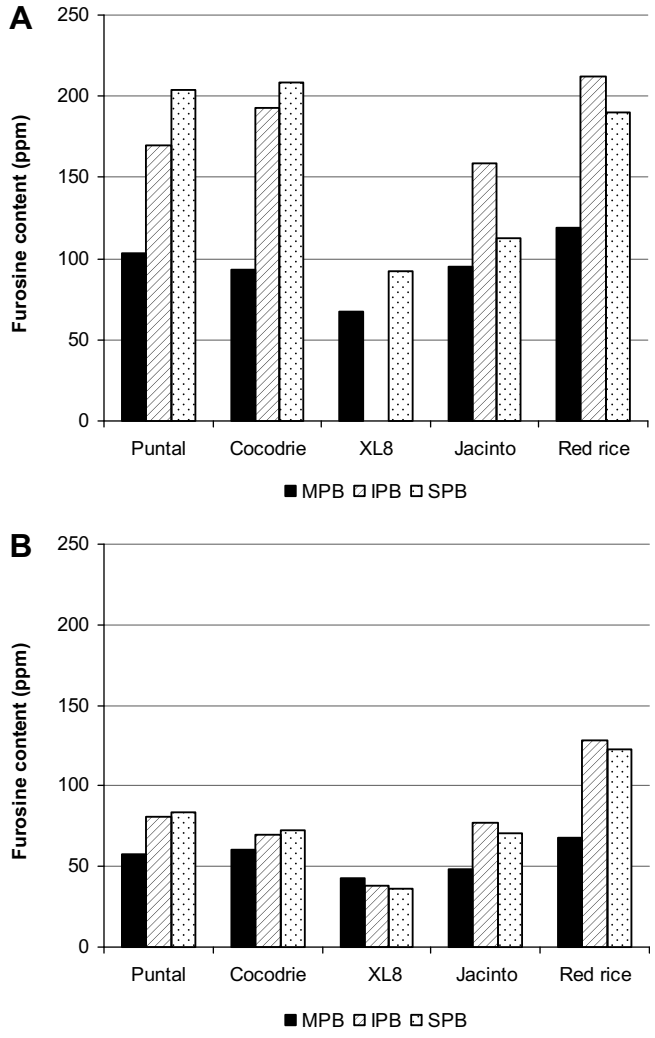

Fig. 1. Levels of furosine in parboiled brown/red (A), and milled, parboiled (B) rices (Mild (M), Intermediate (I), and Severe (S) parboiling (PB) conditions).

complex set of reactions. From furosine determinations, it was clear that the early stage of the MRs were favoured during mild and intermediate parboiling conditions, since furosine levels increased from mild to intermediate parboiling conditions. The observation that furosine content did not increase further significantly for severely parboiled rices suggested that severe parboiling conditions favour the Amadori pathway and the formation of advanced MRPs. Studies on the effect of drying conditions on furosine precursors in pasta also indicated that furosine yields decreased for samples dried at 110 °C for drying periods exceeding 50 min (Anese, Nicoli, Massini, & Lerici, 1999). Hence, as earlier described by van Boekel (2001), the levels of the early stage Maillard indicator can only be used for mild heating processes.

Fig. 1B presents furosine levels formed from milled, parboiled rices. As investigated earlier, milling reduced furosine yields (by more than 35%), indicating that the extent of MRs decreased from the outer bran layers to the endosperm.

3.6. Effect of parboiling and milling on free 5-hydroxymethyl-2-furaldehyde levels

Fig. 2 shows the levels of free HMF, an indicator of the advanced stages of MRs, in parboiled brown/red and milled, parboiled rices. From the literature, it was already clear that high furosine levels are associated with detectable advanced MRPs from the second stage (Villamiel et al., 2006). Free HMF levels in parboiled brown/red rices ranged from ca. 28 (mildly parboiled XL8) to 58 ppm (severely parboiled Jacinto) and increased with severity of parboiling conditions (Fig. 2A). Additionally, rice with the highest free HMF, i.e., severely parboiled Jacinto, had the lowest glucose content. In contrast to severely parboiled Puntal, Cocodrie, XL8 and red rices, severely parboiled Jacinto showed a decreased fructose level. The large increase in free HMF could probably be explained by higher fructose degradation and its transformation to HMF. The transformation was not compensated by fructose formation from glucose isomerisation reactions, since the glucose level was limited in Jacinto. The increase in free HMF contrasted with the effect of parboiling conditions on furosine levels. The differences in effect of parboiling conditions on Maillard indicators were attributed to the type of Maillard indicator that was determined. While furosine is an indicator of the early MR stage, free HMF is the result of degradation of Amadori components and, hence, a marker of the advanced MR stages. The increase in HMF content with severity of parboiling conditions confirmed that the advanced MR stage was favoured when using severe parboiling conditions. Hence, our determinations suggest that, for more severe parboiling processes, free HMF is a better marker of the extent of the MR than furosine. Additionally, free HMF contents of parboiled brown rices are re-

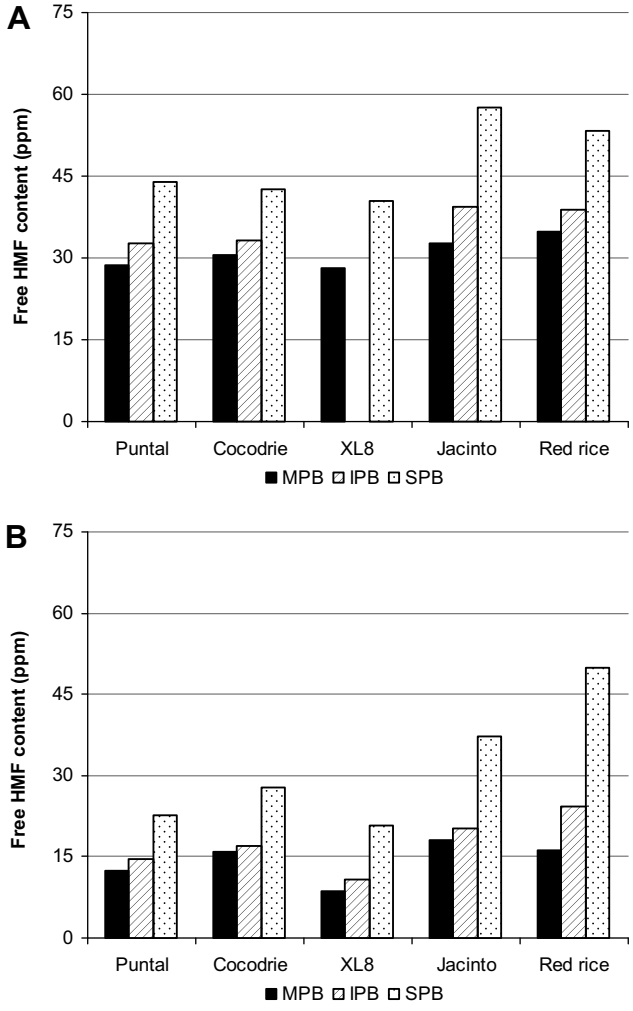

Fig. 2. Levels of free 5-hydroxymethyl-2-furaldehyde (HMF) in parboiled brown/red (A), and milled, parboiled (B) rices (Mild (M), Intermediate (I), and Severe (S) parboiling (PB) conditions).

lated to the increase in redness, i.e., Δa^* ($r = 0.89$), indicating that Maillard browning is more related to changes in red colour than in brightness or yellowness for brown rice.

In line with the effect of milling on furosine yields of parboiled rices, milling decreased free HMF levels (more than 35%, with the exception of the severely parboiled red rice), confirming that MRs are more intense at the brown/red rice surface than in the endosperm (Fig. 2B). In contrast to observations for parboiled brown rice, the correlation between the increase in redness and free HMF no longer holds for milled, parboiled rice. This can be explained by the fact that milled, parboiled rice colour was not mainly determined by Maillard browning as observed for parboiled brown rice. Earlier determinations showed that diffused bran pigments also contribute to milled, parboiled rice colour and degree of pigmentation depended on severity of parboiling (Lamberts et al., 2006a).

4. Conclusions

Parboiling increased browning of brown and milled rices. Parboiling of red rice resulted in black coloured rice. Sucrose was not thermally degraded during steaming. The levels of the Maillard precursors glucose and fructose were mainly determined by their losses in Maillard reactions, glucose formation as a result of thermal starch degradation and isomerisation of glucose into fructose during steaming. Free lysine levels were not affected by parboiling. To determine the extent of Maillard browning in parboiled rice, furosine measurements can be used for mild parboiling conditions, while HMF is a better indicator for mild and severe parboiling conditions. The Maillard precursors and indicators were more abundant in outer bran layers than in endosperm. The strong correlation between free HMF and increased redness led us to conclude that Maillard browning was reflected most in the red colour of parboiled brown rice.

References

AACC International (2000). *Approved methods of the American Association of Cereal Chemists* (10th ed.). St. Paul, MN: The Association [Method 44-15A].

Ali, S. Z., & Bhattacharya, K. R. (1980). Changes in sugars and amino acids during parboiling of rice. *Journal of Food Biochemistry, 4*, 169–179.

Anese, M., Nicoli, M. C., Massini, R., & Lerici, C. R. (1999). Effects of drying processing on the Maillard reaction in pasta. *Food Research International, 32*, 193–199.

AOAC (1995). *Official methods of analysis of the association of official analytical chemists* (16th ed.). Washington, DC: The Association [Method 990.03].

Bhattacharya, K. R. (2004). In E. T. Champagne (Ed.), *Rice: Chemistry and technology* (3rd ed., pp. 329–404). USA: The American Association of Cereal Chemists, Inc.

Derycke, V., Veraverbeke, W. S., Vandeputte, G. E., De Man, W., Hoseney, R. C., & Delcour, J. A. (2005). Impact of proteins on pasting and cooking properties of non-parboiled and parboiled rice. *Cereal Chemistry, 82*, 468–474.

Ding, Y., Yu, H., & Mou, S. (2002). Direct determination of free amino acids and sugars in green tea by anion-exchange chromatography with integrated pulsed amperometric detection. *Journal of Chromatography A, 982*, 237–244.

Fernandez-Artigas, P., Garcia-Villanova, B., & Guerra-Hernandez, E. (1999). Blockage of available lysine at different stages of infant cereal production. *Journal of the Science of Food and Agriculture, 79*, 851–854.

Garcia-Banos, J. L., Corzo, N., Sanz, M. L., & Olano, A. (2004). Maltulose and furosine as indicators of quality of pasta products. *Food Chemistry, 88*, 35–38.

Guerra-Hernandez, E., Corzo, N., & Garcia-Villanova, B. (1999). Maillard reaction evaluation by furosine determination during infant cereal processing. *Journal of Cereal Science, 29*, 171–176.

Itani, T., Tamaki, M., Arai, E., & Horino, T. (2002). Distribution of amylose, nitrogen, and minerals in rice kernels with various characters. *Journal of Agricultural and Food Chemistry, 50*, 5326–5332.

Juliano, B. O. (1998). Varietal impact on rice quality. *Cereal Foods World, 43*, 207–222.

Keeney, M., & Bassette, R. (1959). Detection of intermediate compounds in the early stages of browning reactions in milk products. *Journal of Dairy Science, 6*, 945–960.

Lamberts, L., Brijs, K., Mohamed, R., Verhelst, N., & Delcour, J. A. (2006a). Impact of browning reactions and bran pigments on colour of parboiled rice. *Journal of Agricultural and Food Chemistry, 54*, 9924–9929.

Lamberts, L., De Bie, E., Derycke, V., Veraverbeke, W. S., De Man, W., & Delcour, J. A. (2006b). Effect of processing conditions on colour change of brown and milled parboiled rice. *Cereal Chemistry, 83*, 80–85.

Lamberts, L., De Bie, E., Vandeputte, G. E., Veraverbeke, W. S., Derycke, V., De Man, W., et al. (2007). Effect of milling on colour and nutritional properties of rice. *Food Chemistry, 100*, 1496–1503.

Martins, S. I. F. S., & van Boekel, M. A. J. S. (2005). A kinetic model for the glucose/glycine Maillard reaction pathways. *Food Chemistry, 90*, 257–269.

Morales, V., Olano, A., & Corzo, N. (2004). Ratio of maltose to maltulose and furosine as quality parameters for infant formula. *Journal of Agricultural and Food Chemistry, 52*, 6732–6736.

Morales, F. J., Romero, C., & Jimenez-Perez, S. (1996). Evaluation of heat-induced changes in Spanish commercial milk: Hydroxymethylfural and available lysine content. *International Journal of the Food Science and Technology, 31*, 411–418.

Ramirez-Jimenez, A., Garcia-Villanova, B., & Guerra-Hernandez, E. (2000). Hydroxymethylfural and methylfural content of selected bakery products. *Food Research International, 33*, 833–838.

Resmini, P., Pellegrino, L., & Battelli, G. (1990). Accurate quantification of furosine in milk and dairy products by a direct HPLC method. *Italian Journal of Food Science, 3*, 173–183.

Rufian-Henares, J. A., Garcia-Villanova, B., & Guerra-Hernandez, E. (2004). Generation of furosine and colour in infant/enteral formula-resembling systems. *Journal of Agricultural and Food Chemistry, 52*, 5354–5358.

Saikusa, T., Horino, T., & Mori, Y. (1994). Distribution of free amino acids in the rice kernel and kernel fraction and the effect of water soaking on the distribution. *Journal of Agricultural and Food Chemistry, 42*, 1122–1125.

Shih, F. F. (2004). Rice proteins. In E. T. Champagne (Ed.), *Rice: Chemistry and technology* (3rd ed., pp. 143–162). USA: The American Association of Cereal Chemists, Inc.

van Boekel, M. A. (1998). Effect of heating on Maillard reactions in milk. *Food Chemistry, 62*, 403–414.

van Boekel, M. A. J. S. (2001). Kinetic aspects on the Maillard reaction: A critical review. *Nahrung/Food*, 150–159.

Villamiel, M., del Castillo, D., & Corzo, N. (2006). Browning reactions. In Y. H. Hui, W.-K. Nip, L. M. L. Nollet, G. Paliyath, & B. K. Simpson (Eds.), *Food Biochemistry and Food Processing* (1st ed., pp. 71–100). Oxford, UK: Blackwell Publishing Ltd.