

Carotenoids in Raw and Parboiled Brown and Milled Rice

LIEVE LAMBERTS* AND JAN A. DELCOUR

Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium

Color measurements on flour of five raw rice cultivars with different degrees of milling (DOM) showed that red and brown pigments are concentrated in the outer rice layers, i.e. bran and outer endosperm (DOM < 15%). Extinction measurements (λ 450 nm) of rice extracts showed that yellow pigments are virtually absent in the middle and core endosperm (DOM > 15%). The relation between the extinction values and the yellow color parameter (b^*) showed that both are representative for the yellow pigment content of flour from rice with DOM lower than 9%. Determinations of the carotenoid levels in raw brown rice samples indicated that carotenoid levels in raw brown rice are lower than in common nonrice cereals. The major brown rice carotenoids are β -carotene and lutein (both ca. 100 ng/g), while zeaxanthin levels are lower (ca. 30 ng/g). Regression analyses indicated that yellowness, extinction values, and quantitative carotenoid data are related. b^* -Values and contents of total carotenoids ($r = 0.70$), β -carotene ($r = 0.84$), lutein ($r = 0.78$), and zeaxanthin ($r = 0.83$) were linearly related. However, extinction values (λ 450 nm) and contents of total carotenoids ($r = 0.92$), β -carotene ($r = 0.91$), lutein ($r = 0.89$), and zeaxanthin ($r = 0.84$) showed the best correlations. The three-step hydrothermal treatment parboiling reduces carotenoid contents of brown rice to trace levels. Consequently, pigments do not contribute to the final color of milled parboiled rice.

KEYWORDS: Brown rice; color; carotenoids; lutein; β -carotene; parboiling

INTRODUCTION

Fruits and vegetables are the main sources of carotenoids. Carotenoids are yellow-orange pigments, which are traditionally divided into carotenes and xanthophylls. Carotenes, e.g., α and β -carotenes, are hydrocarbons. Xanthophylls, e.g. lutein and zeaxanthin, are oxygenated carotenoids, containing hydroxyl, carbonyl, or other functional groups. β -Carotene, i.e. a provitamin A carotenoid, reduces the incidence of cancer and cardiovascular diseases (1). Lutein and zeaxanthin, nonprovitamin A carotenoids, are present in the eye and protect against eye diseases, such as age-related macular degeneration and cataract (2).

The outer layers of raw (nonparboiled) brown rice contain β -carotene, lutein, and/or lycopene (3–5). Tan et al. (4) showed that nonparboiled brown rice contains β -carotene and/or lutein, while no such carotenoids can be detected in milled rice. Although color is an important sensory characteristic of (parboiled) rice, the mechanism of color changes during parboiling is not well understood. In this context, Bhattacharya and Subba Rao (6) and Lamberts et al. (7, 8) suggested that husk and/or bran pigments diffuse into the endosperm during rice soaking and/or steaming, essential steps in rice parboiling. They hypothesized that these pigments contribute to the color

of milled parboiled rice, beside color changes caused by nonenzymic browning reactions of the Maillard type. However, rice pigments were, to the best of our knowledge, never identified or quantified in parboiled rice samples.

In contrast to what is the case for rice, data on carotenoid levels in nonrice cereals are well documented. Panfili et al. (9) determined carotenoids in nonrice cereals and their byproducts. The highest carotenoid levels were present in maize, which contained high levels of zeaxanthin (6.4 $\mu\text{g/g}$), β -cryptoxanthin (2.4 $\mu\text{g/g}$), and α - and β -carotene (1.4 $\mu\text{g/g}$). The main carotenoid component in oats, wheat, spelt, and barley was lutein (0.2 – 2.7 $\mu\text{g/g}$), which was equally distributed in the kernel. α - And β -carotenes and zeaxanthin were concentrated in the germ. The carotenoid levels in durum wheat find their origin in breeding programs to select genotypes with high pigment concentration, since yellow color is an important quality characteristic of semolina and pasta (10). Hentschel et al. (11) detected lutein and zeaxanthin in durum wheat. They represent at least 90% of the total yellow pigment content (12). Lutein is also the most abundant carotenoid in einkorn wheat (ca. 8.0 $\mu\text{g/g}$), and its level is higher than that in other wheats (13, 14). Furthermore, in wheat, barley, oats, spelt, and einkorn, free lutein levels (ca. 78% or more) were much higher than those of lutein esters (9, 11, 13).

Different procedures for pigment determinations have been described. The best procedures for estimating pigment levels

* Corresponding author. Tel: (+32)-16-321649. Fax: (+32)-16-321997. E-mail: Lieve.Lamberts@biw.kuleuven.be.

use HPLC separations and extinction measurements (λ 450 nm) of water-saturated butanol cereal extracts. Color measurements and b^* -values (yellowness) are less accurate and provide only relative values of pigment levels (15). Humphries et al. (16) found a relation between b^* -values and lutein concentrations in different (bread and durum) wheat types, which was strongest in the durum group.

Against this background, the current study aimed at increased insight in both the distribution of color-determining components in brown rice and the impact of processing on carotenoid levels of brown rice. To that end, the distribution and composition of carotenoids of five rice cultivars with brown pericarp and with different DOMs (10 fractions) were determined. Apart from color measurements and colorimetric determinations, carotenoids were identified and quantified by HPLC. Additionally and for the first time, the impact of soaking and different steaming treatments (mild, intermediate and severe) on carotenoid levels was investigated.

MATERIALS AND METHODS

Rice Samples. Brown rice (*Oryza sativa* L.) from cultivars Koshihikari (Japanese harvest 2004), Basmati (Indian harvest 2006), Loto and SisR215 (Italian harvest 2006), and Puntal (Spanish harvest 2003 and 2006) were obtained from Mars NV (Olen, Belgium). All samples were Indica cultivars. Dimensions of the rice kernels were determined by a 312 GrainCheck Analyzer (Foss Tecator AB, UK). In the classification of Juliano (17), Koshihikari is a medium-grain rice cultivar (5.2 mm < length < 6.0 mm), and Basmati, Loto, SisR215, and Puntal are long-grain rice cultivars (length > 6.0 mm).

Nonparboiled Rice Milling and Grinding. All nonparboiled rice samples (200.0 g), i.e. all samples of cultivars harvested in 2004 or 2006, were milled abrasively for different times (0–105 s) with a TM05C testing mill (Satake, Bredbury, UK) to obtain rice with different DOM (0–27%). The 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. The rice kernels with different DOM were stored in the dark at 6 °C until analysis. In line with Wadsworth (18), Resurreccion et al. (19), and Lamberts et al. (20), the fractions obtained after 0–9%, 9–15%, and 15–25% rice fraction removal were designated as bran, outer endosperm, and middle endosperm, respectively. Rice samples with a DOM > 25% were considered to contain only core endosperm. The rice kernels of variable DOM were ground with a laboratory grinder to pass through a 250 μ m sieve.

Rice Parboiling and Milling. Brown rice of the cultivar Puntal (Spanish harvest 2003) was mildly, intermediately, and severely parboiled by soaking brown rice in excess water, steaming the soaked rice kernels using three standard steps. To obtain intermediately and severely parboiled rice, one additional steaming step was applied. The time and temperature combinations used during soaking and steaming, and brown rice milling were described earlier in more detail by Lamberts et al. (8). Following steaming, rice samples were dried for two days at room temperature. The parboiled rice samples were stored in the dark at 6 °C until analysis.

Chemicals. Zeaxanthin was from Extrasynthèse (Genay, France). β -Carotene, β -apo-8'-carotenal, and lutein were from Sigma (Bornem, Belgium), as were all other chemicals and reagents used (at least analytical grade).

Chemical Composition. Moisture content (MC) determination of rice samples was according to AACC method 44–15A (21). Analyses were performed in duplicate. The apparent amylose content was determined on the basis of Derycke et al. (22). Protein and mineral contents were determined according to Lamberts et al. (20). All analyses were performed in triplicate, and the results are expressed on a dry matter basis.

Level of Gelatinized Starch. Differential scanning calorimetry analyses of the milled (parboiled) rice samples (Puntal, Spanish harvest 2003) were according to Lamberts et al. (8) and performed in triplicate.

Table 1. Apparent Amylose, Protein, and Mineral Contents of Brown Rice (DOM 0%)^a

cultivar	apparent amylose content (% \pm SD)	protein content (% \pm SD)	mineral content (% \pm SD)
Loto	12.4 \pm 0.1c	8.3 \pm 0.0b	1.5 \pm 0.2a
SisR215	11.7 \pm 0.3b	7.5 \pm 0.0a	1.8 \pm 0.0b
Puntal	14.3 \pm 0.3e	8.7 \pm 0.1c	1.7 \pm 0.0a
Koshihikari	9.4 \pm 0.2a	8.3 \pm 0.0b	1.6 \pm 0.0a
Basmati	13.6 \pm 0.1d	11.1 \pm 0.0d	1.7 \pm 0.0a

^a The same letters within the same column are not significantly different ($P < 0.05$).

Color and Pigment Measurements. Color measurements of rice flour samples were determined corresponding to Lamberts et al. (20) and were performed in triplicate. Pigment extraction from flour of rice with different DOM was based on AACC method 14–50 (23). Water-saturated butanol (5.0 mL) was added to rice flour (1.50 g) to extract total carotenoids. Extinction values were monitored at 450 nm. Analyses were performed in triplicate. The coefficient of variation for the extinction readings was below 6.0%.

Determination of Pigments by HPLC. Carotenoid extraction from nonparboiled rice samples with different DOM and parboiled rice samples was performed in triplicate and based on Panfilii et al. (9) with some modifications. Carotenoids were released from flour of (parboiled) rice (3.0 g) and durum wheat semolina (100.0 mg) in a screw-capped tube by adding ascorbic acid (0.300 g) as antioxidant, ethanol (95%, 15.0 mL), and sodium chloride solution (0.01 g/mL, 3.0 mL). After vortex mixing of the suspension, it was placed in a water bath (85 °C) for 5 min. Potassium hydroxide solution (0.6 g/mL, 3.0 mL) was added to the heated mixture to saponify potentially interfering oils and/or esterified carotenoids (9, 16). Following mixing, the samples were returned to the 85 °C water bath for 20 min with an additional mixing after 8 and 15 min. After saponification, the tubes were cooled in an ice bath and cold sodium chloride solution (10 g/L, 15.0 mL) was added. β -Apo-8'-carotenal solution (6.0 mg/L, 15 μ L) was used as internal standard. The suspension was extracted twice with 12.0 mL portions of *n*-hexane/ethyl acetate (9:1 v/v). The combined organic layers were washed with deionized water (10.0 mL). Following separation of the organic layers, the aqueous layer was discarded. The organic phase was dried under nitrogen gas at 40 °C and dissolved in methanol:acetonitrile (40:60 v/v; 0.5 mL) for chromatography. Carotenoid analysis was based on Hentschel et al. (11). The carotenoid extracts (100 μ L) were separated and quantified by reversed-phase high performance liquid chromatography using a LC-10ADvp liquid chromatograph (Shimadzu, Kyoto, Japan) consisting of a gradient pump with online degasser, a SIL-10ADvp autosampler, a CTO-10ACvp column oven, and a SPD-20A multichannel UV/vis detector. Extinction values were monitored at 450 nm. The column was a 250 \times 3.0 mm, 5 μ m Vydac 201TP53 C18 reverse phase (Grace, Belgium). A column temperature of 9 °C, a flow rate of 0.450 mL/min, and a mobile phase consisting of methanol:acetonitrile:2-propanol (40:58:2) were used. β -Carotene, lutein, and zeaxanthin were used as external standards, and carotenoid levels in the rice samples were calculated from the peak areas.

Statistical Analyses. For statistical analyses, the *t*-test (PROC ANOVA) was used (significance level $P < 0.05$). Pearson's correlation coefficient analyses were determined (significance level $P < 0.05$). Statistical analyses were conducted using the Statistical Analysis System software 8.1 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Chemical Composition of Rice. Table 1 lists the chemical composition (on dry matter basis) of the different rice samples. The apparent amylose contents of the dehulled rice samples (DOM 0%) ranged from 9.4% (Koshihikari) to 14.3% (Puntal). All samples were from nonwaxy rice cultivars, when using the classification of Juliano (17). The protein contents ranged from 7.5% (SisR215) to 11.1% (Basmati). The mineral contents ranged from 1.5% (Loto) to 1.8% (SisR215).

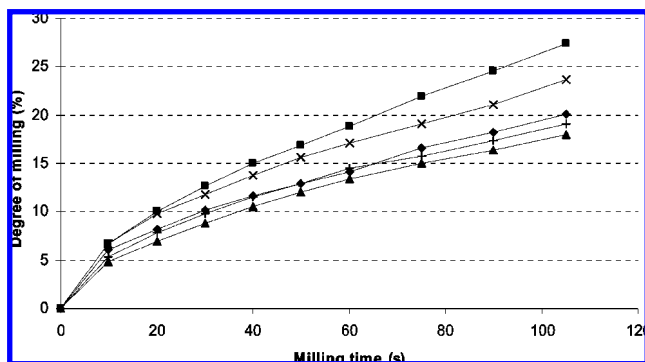


Figure 1. Effect of milling time on the degree of milling (DOM) of rice kernels of different cultivars [Loto (■), SisR215 (×), Puntal (harvest 2006; ◆), Koshihikari (+), Basmati (▲)].

Level of Gelatinized Starch of Rice. The enthalpy of gelatinization of the milled nonparboiled rice sample was ca. 9.3 J/g. From the gelatinization enthalpies of the different parboiled samples, we estimated that approximately 93% and 98% of the starch was gelatinized for mildly and intermediately parboiled rice samples, respectively. No residual endotherm was observed for severely parboiled rice samples, as described earlier (14).

Relation between Milling Time and Degree of Milling of Raw Rice. Figure 1 shows DOM as a function of milling time for the raw rice cultivars Koshihikari, Basmati, Loto, SisR215, and Puntal (harvest 2006). Similar milling times did not necessarily result in equal percentages of rice layer removal for the different cultivars. Outer layer removal was highest for Loto and lowest for Basmati (DOM 27.4 and 18.0%, respectively, after milling for 105 s). These differences in milling responses are mainly to be ascribed to differences in hardness between the cultivars, since morphology (except for Koshihikari, which is a medium-grain cultivar) and moisture levels (typically 11%, results not shown) of the different cultivars were quite similar. Furthermore, as mentioned earlier by Wadsworth (18) and Lamberts et al. (20), an increase in milling time did not necessarily result in a linear increase in DOM, and the change in the rate of material removal (slope) during the milling process depended on rice cultivar.

Color Parameters (L^* , a^* , and b^*) of Flour of Raw Rice As a Function of DOM. The color parameters of the rice flours of the different raw rice cultivars Koshihikari, Basmati, Loto, SisR215, and Puntal (harvest 2006) were determined (Figure 2A–C) as a function of DOM. The brightness (L^*) increased, and redness (a^*) and yellowness (b^*) decreased until reaching a DOM of ca. 15%. Once the bran and outer endosperm (DOM > 15%) were removed, the color parameters hardly changed. Hence, the concentrations of color-determining components of brown rice decreased from the surface to the outer endosperm fractions. Within the same cultivar, color parameters of flours containing middle and/or core endosperm material did not change. Hence, these samples showed similar physical appearances. Comparison of the color parameters of the flours containing residual bran and/or outer endosperm (DOM < 15%) indicated that the medium-grain cultivar Koshihikari was the brightest and the least red. Loto was also a relatively bright cultivar. The yellowness was highest for Basmati, followed by that of Koshihikari. Loto, SisR215, and Puntal showed similar yellow color intensities. Color parameters of the flours of rice kernels with DOM exceeding 15% were similar, except for Basmati. The low brightness and high yellowness of Basmati probably suggest that factors other than the here measured pigments determine rice flour color.

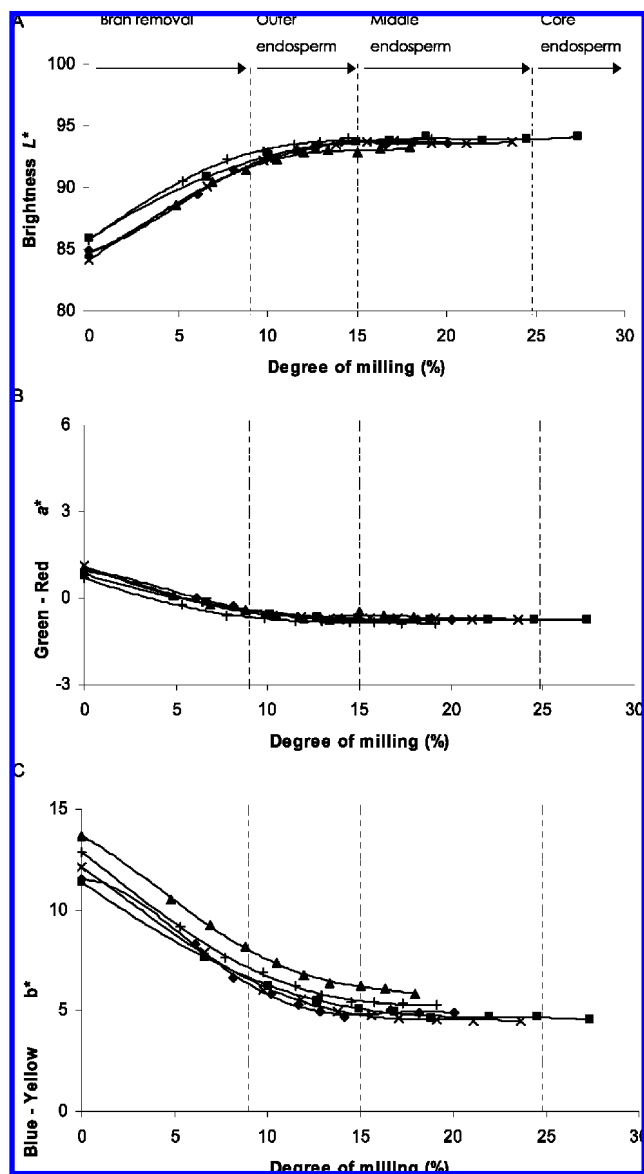


Figure 2. Color parameters L^* (brightness), a^* (redness), and b^* (yellowness) of rice flours [Loto (■), SisR215 (×), Puntal (harvest 2006; ◆), Koshihikari (+), Basmati (▲)] as a function of degree of milling (DOM).

Levels of Total Yellow Pigments of Raw Rice Flour in Relation to Yellowness (b^*). For all rice flours obtained from the rice cultivars (Loto, SisR215, Puntal, Koshihikari, and Basmati) with different DOMs, the relative levels of total yellow pigments were determined by extinction measurements at 450 nm in water-saturated butanol extracts. Figure 3 shows the extinction values at 450 nm as a function of the yellowness (b^*) of the rice flours. A distinction was made between the flour of rice kernels still containing some bran (DOM < 9%), outer endosperm (9% < DOM < 15%), or middle and core endosperm (DOM > 15%). The levels of yellow pigments of flours with residual bran (DOM < 9%) were linearly related ($r = 0.92$) with the b^* -values. In contrast, after bran layer removal (DOM > 9%), yellow pigment levels changed only slightly ($0.001 < \text{extinction at } 450 \text{ nm} < 0.010$), while the b^* -values ranged from 4.5 to 7.4. These observations confirm that yellow pigments are mainly present in the bran fraction and that the color parameter b^* is representative for the yellow pigment content of flour from rice with DOM lower than 9%. Pigment levels were low in rice flours containing only outer, middle, and/or

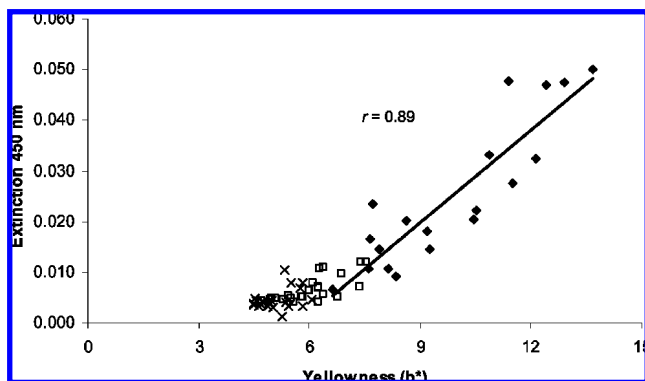


Figure 3. Extinction values (450 nm) of rice flour extracts as a function of yellowness (b^*) of rice flours. Flour samples were obtained by grinding rice samples previously submitted to different degrees of milling (DOM), and a distinction was made between flours of rice kernels with DOM < 9% (\blacklozenge), 9% < DOM < 15% (\square), DOM > 15% (\times).

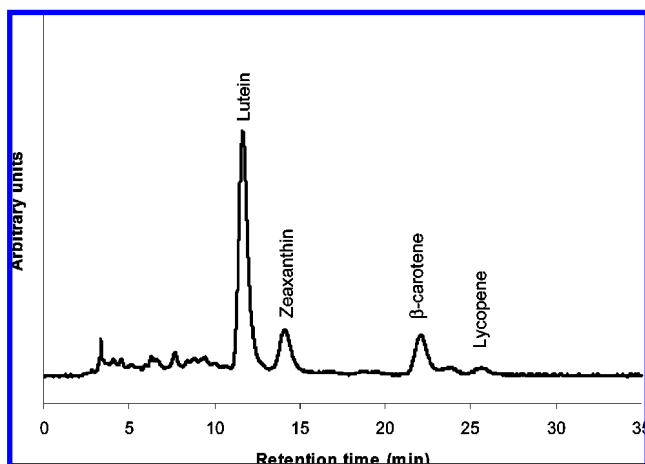


Figure 4. Chromatogram of carotenoids extracted from brown rice flour (DOM 0%) of the cultivar Koshihikari. Detection was at 450 nm.

core endosperm (DOM > 9%). However, the relatively large differences in b^* -values of these flours may indicate that not only the pigments extractable under the used experimental conditions determine the color of these rice flours, as suggested earlier. This was also described by Fratianni et al. (15) for whole meal from durum wheat samples.

Identification and Quantification of Carotenoids in Raw Rice. Figure 4 shows a typical HPLC chromatogram of a brown rice sample extract (DOM 0%). β -Carotene and lutein are the main carotenoid components in brown rice, followed by zeaxanthin. The molar extinction coefficient (at 450 nm) of lutein and zeaxanthin are comparable, whereas that of β -carotene is significantly lower. Some samples showed trace levels of lycopene, which was not quantified. There was also a small peak of an unidentified component eluting after ca. 24 min.

Table 2 presents β -carotene, lutein, and zeaxanthin levels of the different samples. The levels were determined in five cultivars (Loto, SisR215, Puntal, Koshihikari, and Basmati) after milling for 0, 10, 20, and 30 s. These samples differed in DOM and contained residual bran and/or outer endosperm. Flours of rice kernels milled for times exceeding 30 s were not determined since their carotenoid levels were quite low. β -Carotene, lutein, and zeaxanthin levels in the brown rice samples (DOM 0%) ranged from 66 to 150, from 36 to 109, and from 14 to 37 ng/g, respectively. Outer layer removal by ca. 5% reduced β -carotene and lutein levels by more than 50 and 20% (except for Loto), respectively. Zeaxanthin levels also decreased with

Table 2. Carotenoid Levels in Flours of Brown Rice Milled for Different Times^a

cultivar	milling time (s)	DOM (%)	carotenoids (ng/g) \pm SD		
			β -carotene	lutein	zeaxanthin
Loto	0	0.0	107 \pm 11a	98 \pm 10a	16 \pm 4a
	10	6.7	80 \pm 7b	75 \pm 8b	16 \pm 3a
	20	10.1	28 \pm 5c	34 \pm 9c	10 \pm 1b
	30	12.7	13 \pm 6d	12 \pm 5d	<10c
SisR215	0	0.0	66 \pm 4a	36 \pm 5a	14 \pm 4a
	10	6.7	14 \pm 8b	22 \pm 3b	<10b
	20	9.8	<10c	17 \pm 4c	<10b
	30	11.8	<10c	17 \pm 4c	<10b
Puntal	0	0.0	72 \pm 15a	47 \pm 7a	16 \pm 2a
	10	6.1	20 \pm 6b	27 \pm 11b	<10b
	20	8.2	<10c	16 \pm 4b	<10b
	30	10.2	<10c	<10c	<10b
Koshihikari	0	0.0	85 \pm 8a	91 \pm 10a	28 \pm 5a
	10	5.3	24 \pm 8b	31 \pm 5b	18 \pm 4a
	20	7.8	11 \pm 3c	20 \pm 4b	13 \pm 2ab
	30	9.8	<10c	18 \pm 5bc	13 \pm 3ab
Basmati	0	0.0	150 \pm 15a	109 \pm 12a	37 \pm 4a
	10	4.8	75 \pm 8b	66 \pm 7b	13 \pm 3b
	20	6.9	17 \pm 2c	27 \pm 6c	14 \pm 2b
	30	8.8	<10d	14 \pm 1d	<10bc

^a The same letters within the same column for the same cultivar are not significantly different ($P < 0.05$).

increasing DOM. Bran layer removal (DOM > 9%) resulted in lutein, β -carotene, and zeaxanthin levels that are lower than 20 ng/g (except for Loto). Determination of the carotenoid contents of different durum wheat semolina samples indicated that lutein is the major carotenoid in semolina, followed by zeaxanthin and β -carotene (results not shown). These results were in agreement with Fratianni et al. (15). Lutein levels in the semolina samples were 40 times higher (ca. 4.2 μ g/g) than those of brown rice (DOM 0%).

Screening of black/purple, red/brownish, and colorless rice cultivars for β -carotene indicated that black/purple cultivars had higher β -carotene levels (ca. 0.08 μ g/g) than the other two groups (ca. 0.01 μ g/g) (3). Determination of β -carotene and lycopene in brown rice bran fractions indicated that these carotenoids are most abundant in the outer bran fractions (from 0.54 to 2.04 and from 0.04 to 0.17 μ g/g for β -carotene and lycopene, respectively) and that their levels decrease from outer to inner bran layers (5). However, to the best of our knowledge, earlier literature data has not quantitatively described the presence of both lutein and zeaxanthin in brown rice. The β -carotene levels in the presently studied rice samples exceed those found by Frei and Becker (3). Furthermore, our determinations evidently confirmed the qualitative observations of Tan et al. (4) that lutein and/or β -carotene are present in brown rice. Compared to nonrice cereals, the total carotenoid levels, i.e., the sum of β -carotene, lutein, and zeaxanthin levels, in brown rice were low (<300 ng/g). Additionally, β -carotene and lutein contents in the studied brown rices were comparable, whereas the major component in nonrice cereals was lutein, followed by zeaxanthin and β -carotene (9, 13).

Carotenoid Levels in Raw Rice in Relation to Yellowness (b^*) and Pigment Levels. Regression analyses were performed on the samples used for HPLC determination (milling times \leq 30 s). Yellowness, extinction values (450 nm), and data obtained by HPLC analyses were related. b^* -Values and contents of total carotenoids ($r = 0.70$), β -carotene ($r = 0.84$), lutein ($r = 0.78$), and zeaxanthin ($r = 0.83$) showed a linear correlation. However, the best correlations were found between extinction values (450 nm) and contents of total carotenoids ($r = 0.92$), β -carotene ($r = 0.91$), lutein ($r = 0.89$), and zeaxanthin

($r = 0.84$). The stronger relationship between extinction values and HPLC data illustrated that color measurements on flours are not only affected by the measured pigments, as discussed earlier.

From these observations, it is clear that b^* -values can be used to compare the pigment levels of rice samples containing bran layers (DOM < 9%). Extinction measurements at 450 nm of rice extracts suffice for estimating relative amounts of yellow pigments in brown rice samples with varying DOM.

Carotenoid Levels in Parboiled Brown and Milled Rice.

The lutein level in raw brown rice of the cultivar Puntal (harvest 2003) was 50 ng/g, and zeaxanthin and β -carotene levels were significantly lower (results not shown). Brown rice parboiling decreased carotenoid levels to a level below the limit of quantification (results not shown). This decrease was most likely a result of the loss of soluble solids in the soaking medium, oxidation of carotenoids during soaking, destruction of carotenoids during steaming, and/or differences in extraction efficiency caused by the hydrothermal treatment of brown rice. Even after milling, the chromatograms of the different parboiled rice samples showed very small carotenoid peaks, which could not be quantified.

Carotenoid Levels in Parboiled Brown and Milled Rice in Relation to Yellowness (b^*). The color parameters of the rice flours of the nonparboiled and mildly, intermediately, and severely parboiled brown and milled rice samples were described earlier (8). From these results it was clear that the yellow color intensities (b^*) of flour from parboiled rice (average values of 18.1 and 12.2 for brown and milled samples, respectively) were increased in comparison with flour samples from nonparboiled rice (12.4 and 5.9 for brown and milled samples, respectively). Furthermore, it was hypothesized that the increase in yellowness with severity of steaming conditions could partly be ascribed to increased bran pigment diffusion during steaming. However, the trace levels of carotenoids in both brown and milled parboiled rice samples indicated that brown rice soaking in excess water followed by steaming did not result in a significant color contribution by pigments diffused into the endosperm. Thus, components and/or phenomena other than carotenoids are mainly responsible for the color of milled parboiled rice. From the changes in levels of reducing sugar, a Maillard precursor, and furosine, an early stage Maillard indicator, and from the increase in redness and levels of free 5-hydroxymethyl-2-furaldehyde, an intermediate stage Maillard indicator, it was concluded that color changes during parboiling were (partly) ascribed to Maillard reactions (7, 8, 24). Furthermore, Miskelly (25) and Barnes (26) indicated that the reflectance of wheat flour pastes, i.e. intermediate products in the noodle production process, are affected by levels of damaged starch and proteins, respectively. Hence, it seems reasonable to assume the physicochemical changes caused by parboiling, as described earlier by Mujoo and Ali (27), probably affect the yellowness of rice flour. From these observations, it was suggested that variations in the relative proportions of (damaged) starch and proteins determine the color of flour of hydrothermally treated cereal products.

In conclusion, analyses of the color of flour of rice samples with different DOM demonstrated that yellow and red pigments are concentrated in the bran and outer endosperm. Consequently, all color-determining components were removed once a DOM of 15% was reached. Further investigation of the identity of the rice pigments present in the fractions of different rice cultivars indicated that the carotenoids β -carotene, lutein, and zeaxanthin are the color-determining components in brown rice.

Quantitative HPLC data show that β -carotene and lutein are the major carotenoids and their levels depend on rice cultivar. While these components are present in comparable levels (ca. 100 ng/g) in brown rice (DOM 0%), zeaxanthin levels are lower (ca. 30 ng/g). Carotenoid levels of brown rice are lower than those in common nonrice cereals. Color measurements as well as determination of extinction values (450 nm) allow determining relative levels of yellow pigments. However, once the bran fraction has been removed (DOM > 9%), other components than the here measured pigments significantly contribute to the visual appearance and, hence, the b^* -values of rice. Brown rice parboiling reduced carotenoid levels to trace levels. Hence, carotenoids do not contribute to milled parboiled rice color. Thus, the decreased brightness and increased red and yellow color intensities of parboiled rice are ascribed to Maillard reactions and/or physicochemical changes of different rice components occurring during parboiling.

ABBREVIATIONS USED

DOM, degree(s) of milling; MC, moisture content.

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LITERATURE CITED

- (1) Perera, C. O.; Yen, G. M. Functional properties of carotenoids in human health. *Int. J. Food Prop.* **2007**, *10*, 201–230.
- (2) Johnson, E. J. The role of carotenoids in human health. *Nutr. Clin. Care.* **2002**, *5*, 56–65.
- (3) Frei, M.; Becker, K. Fatty acids and *all-trans*-beta-carotene are correlated in differently colored rice landraces. *J. Sci. Food Agric.* **2005**, *85*, 2380–2384.
- (4) Tan, J.; Baisakh, N.; Oliva, N.; Parkhi, V.; Rai, M.; Torrizo, L.; Datta, K.; Datta, S. K. The screening of rice germplasm, including those transgenic rice lines which accumulate beta-carotene in their polished seeds, for their carotenoid profile. *Int. J. Food Sci. Technol.* **2005**, *40*, 563–569.
- (5) Abdul-Hamid, A.; Sulaiman, R. R. R.; Osman, A.; Saari, N. Preliminary study of the chemical composition of rice milling fractions stabilized by microwave heating. *J. Food Compos. Anal.* **2007**, *20*, 627–637.
- (6) Bhattacharya, K. R.; Subba Rao, P. V. Effect of processing conditions on quality of parboiled rice. *J. Agric. Food Chem.* **1966**, *14*, 476–479.
- (7) Lamberts, L.; De Bie, E.; Derycke, V.; Veraverbeke, W. S.; De Man, W.; Delcour, J. A. Effect of processing conditions on color change of brown and milled parboiled rice. *Cereal Chem.* **2006**, *83*, 80–85.
- (8) Lamberts, L.; Brijs, K.; Mohamed, R.; Verhelst, N.; Delcour, J. A. Impact of browning reactions and bran pigments on color of parboiled rice. *J. Agric. Food Chem.* **2006**, *54*, 9924–9929.
- (9) Panfili, G.; Fratianni, A.; Irano, M. Improved normal-phase high-performance liquid chromatography procedure for the determination of carotenoids in cereals. *J. Agric. Food Chem.* **2004**, *52*, 6373–6377.
- (10) Johnston, R. A.; Quick, J. S.; Donnelly, B. J. Note on comparison of pigment extraction and reflectance colorimeter methods for evaluating semolina color. *Cereal Chem.* **1980**, *57*, 447–448.
- (11) Hentschel, V.; Kranl, K.; Hollmann, J.; Lindhauer, M. G.; Bohm, V.; Bitsch, R. Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. *J. Agric. Food Chem.* **2002**, *50*, 6663–6668.
- (12) Burkhardt, S.; Bohm, V. Development of a new method for the complete extraction of carotenoids from cereals with special

- reference to durum wheat (*Triticum durum* Desf.). *J. Agric. Food Chem.* **2007**, *55*, 8295–8301.
- (13) Abdel-Aal, E. S. M.; Young, J. C.; Rabalski, I.; Hucl, P.; Fregeau-Reid, J. Identification and quantification of seed carotenoids in selected wheat species. *J. Agric. Food Chem.* **2007**, *55*, 787–794.
- (14) Hidalgo, A.; Brandolini, A.; Pompei, C.; Piscozzi, R. Carotenoids and tocopherols of einkorn wheat (*Triticum monococcum* ssp. *monococcum* L.). *J. Cereal Sci.* **2006**, *44*, 182–193.
- (15) Fratianni, A.; Irano, M.; Panfili, G.; Acquistucci, R. Estimation of color of durum wheat. Comparison of WSB, HPLC, and reflectance colorimeter measurements. *J. Agric. Food Chem.* **2005**, *53*, 2373–2378.
- (16) Humphries, J. M.; Graham, R. D.; Mares, D. J. Application of reflectance colour measurement to the estimation of carotene and lutein content in wheat and triticale. *J. Cereal Sci.* **2004**, *40*, 151–159.
- (17) Juliano, B. O. Varietal impact on rice quality. *Cereal Foods World.* **1998**, *43*, 207–222.
- (18) Wadsworth, J. I. Degree of milling. In *Rice: Science and Technology*; Marshall, W. E., Wadsworth, J. I., Eds.; Marcel Dekker: New York, 1994; pp 139–176.
- (19) Resurreccion, A. P.; Juliano, B. O.; Tanaka, Y. Nutrient content and distribution in milling fractions of rice grain. *J. Sci. Food Agric.* **1979**, *30*, 475–481.
- (20) Lamberts, L.; De Bie, E.; Vandeputte, G. E.; Veraverbeke, W. S.; Derycke, V.; De Man, W.; Delcour, J. A. Effect of milling on colour and nutritional properties of rice. *Food Chem.* **2007**, *100*, 1496–1503.
- (21) American Association of Cereal Chemists AACC Method 44–15A. *Approved Methods of the AACC*, 10th ed.; AACC: St. Paul, MN, 2000.
- (22) Derycke, V.; Veraverbeke, W. S.; Vandeputte, G. E.; De Man, W.; Hosney, R. C.; Delcour, J. A. Impact of proteins on pasting and cooking properties of nonparboiled and parboiled rice. *Cereal Chem.* **2005**, *82*, 468–474.
- (23) American Association of Cereal Chemists AACC Method 14–50. *Approved Methods of the AACC*, 10th ed.; AACC: St. Paul, MN, 2000.
- (24) Lamberts, L.; Rombouts, I.; Brijs, K.; Gebruers, K.; Delcour, J. A. Impact of parboiling conditions on Maillard precursors and indicators in long-grain rice cultivars. *Food Chem.* **2008**, *110*, 916–922.
- (25) Miskelly, D. M. Flour components affecting paste and noodle color. *J. Sci. Food Agric.* **1984**, *35*, 463–471.
- (26) Barnes, P. J. The influence of wheat endosperm on flour colour grade. *J. Cereal Sci.* **1986**, *4*, 143–155.
- (27) Mujoo, R.; Ali, S. Z. Changes in physico-chemical and rheological properties of rice during flaking. *Int. J. Food Prop.* **2000**, *3*, 117–135.

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