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Fatty acids and all-*trans*-β-carotene are correlated in differently colored rice landraces

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Abstract: A set of 54 rice landrace samples was compiled from various Asian countries, including six red/brownish and eight black/purple varieties. Brown rice samples were analyzed for lipid content and fatty acid profile, as well as all-*trans*-β-carotene content. Black/purple varieties were found to be higher in crude lipid content than the red/brownish and colorless varieties. They also had a higher β-carotene content than the other two color classes. The highest β-carotene content determined was 0.22 mg kg^{-1}. Black/purple varieties tended to have a higher proportion of saturated fatty acids in their lipid fraction and a lower proportion of unsaturated fatty acids. The differences were statistically significant ($P < 0.05$) for oleic acid, which accounted for 42.1% of the lipid fraction in black/purple varieties and for 45.3% and 46.3% in red/brownish and colorless varieties, respectively. β-Carotene content showed a significantly positive correlation with the crude lipid content ($P < 0.001$) and the content of saturated fatty acids ($P < 0.001$) on a dry matter basis. However, it was not correlated with the unsaturated fatty acids content on a dry matter basis. Within the total lipid extract, β-carotene showed a significantly positive correlation with the proportion of saturated fatty acids ($P < 0.01$), especially palmitic acid ($P < 0.01$), and a significantly negative correlation with unsaturated fatty acids ($P < 0.001$), especially oleic acid ($P < 0.01$).

Keywords: brown rice; rice landraces; nutritional value, β-carotene; vitamin A deficiency; fatty acids

INTRODUCTION

Rice serves as a staple food for around half of the world's population. It supplies approximately 20% of dietary energy worldwide, and in Asia the proportion is even higher with an average share of around 30% in 2000.[1] Rice is commonly consumed as milled rice, which consists mostly of starch and around 6–10% protein.[2] Other essential nutrients, namely lipids, vitamins and essential minerals, are hardly found in the milled rice fraction.[3] The rice bran fraction, which is relatively rich in lipids, minerals and vitamins (especially B vitamins),[3] is removed from the grain during the milling process and is for the most part used as an animal feed. In many Asian countries, the adoption of rice milling technology was accompanied by the spread of micronutrient deficiency diseases, particularly vitamin B deficiency (beriberi).[4]

Today the problem of micronutrient malnutrition in rice-consuming countries is widely recognized and it has led to tremendous efforts in improving rice micronutrient density. Currently, iron and vitamin A deficiency have been identified as two major health hazards associated with a rice-based diet, and they are being tackled by rice scientists throughout the world.[5,6] Biotechnology, especially genetic engineering, is widely considered the most promising approach to improving rice nutritional value. Transgenic rice varieties have reportedly yielded iron content up to 38 mg kg^{-1} (brown rice).[7] The most spectacular success in genetic engineering, however, was the introduction of the β-carotene biosynthesis metabolism into the rice endosperm. Carotenoid levels between 0.3 and 1.6 mg kg^{-1} have reportedly been attained in the rice endosperm, thus being characterized by a yellowish color.[8–10] Such transgenic rice varieties are believed to have potential in combating vitamin A deficiency in developing countries, because β-carotene is a vitamin A precursor that can be converted in the human digestive tract.

Alternatively, favorable nutritional characteristics associated with certain conventional rice varieties offer scope for enhancement of rice-based diets. This may particularly be true for the genetically diverse pool of rice landraces. Such varieties are often characterized by a high protein and lipid content,[11] but some are also promising with regard to the micronutrient level. For example, traditional rice varieties in the Philippines had a 2.5 times higher iron level as compared with commonly grown high-yielding varieties.[12] Certain colored rice varieties were reported to contain up to

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63.5 mg kg^{-1} of iron in brown rice,[12] which is higher than genetically manipulated grains. Black upland rice samples from the Philippines had β-carotene values up to 0.13 mg kg^{-1}, which was significantly higher than in red, brown or colorless varieties.[11] Black and red rice varieties were likewise demonstrated to have a beneficial anti-oxidative and anti-inflammatory effect in rabbits *in vivo*,[13,14] although the mechanisms behind this were not entirely elucidated.

Information on the interrelation of nutritionally relevant components in rice is relatively scarce. As carotenoids are extremely lipophilic compounds,[15] an interaction between the lipid components and the β-carotene content is conceivable. The objective of the current study was to investigate such interrelations in a genetically highly diverse set of 54 rice landrace samples. This was done by analyzing the samples for lipid and fatty acid fractions and correlating these values with the β-carotene content. As both fractions, ie lipids and carotenoids, are located predominantly in the bran fraction in conventional rice varieties, the analyses were carried out on finely ground brown rice.

MATERIALS AND METHODS

Samples

A set of 54 rice (*Oryza sativa* L) landrace samples was compiled from various South and Southeast Asian countries. Most of the samples (45) were traditional rice landraces from the Province of Aklan in the Philippines that are predominantly cultivated under extensive upland conditions. These samples were collected directly from upland farms during two excursions in 2000. Five samples originated from Vietnam (Hanoi), two from Malaysia (Kuala Lumpur) and two from Thailand (Chiang Mai), and these samples were purchased from local markets in the respective cities. The rice samples were de-hulled mechanically and kept in a refrigerator (4 °C) until analysis. The compilation contained short and long grain varieties, with the thousand kernel weight ranging from 8 to 27 g. They were classified visually into different color categories based on the pericarp color. The three color categories were black/purple (8 samples), red/brownish (6 samples) and colorless (40 samples).

Proximate composition analysis

These analyses were carried out in duplicate on de-hulled brown rice in accordance with AOAC standards[16] and the results were expressed as a proportion of the dry matter. Dry matter content was derived by first grinding the sample and then drying it overnight at a temperature of 105 °C. Crude ash values were determined after burning the samples in a muffle furnace for 6 h at a temperature of 500 °C in order to remove all organic components. Protein contents were obtained by the Kjeldahl method with copper as a digestion catalyst. Ammonia was distilled in a Büchi distillation unit (Büchi, Flawil, Switzerland) and nitrogen was titrated with a model 7195 Titrino Metrohm-type titration unit (Metrohm AG, Herisau, Switzerland). Nitrogen content was converted into protein content by multiplying by the factor 6.25. Crude lipid content was determined with a Soxhlet apparatus (Soxtec HT 1043 Tecator; Foss A/S, Hillerod, Denmark), following extraction with petroleum ether as solvent. The solvent was distilled off and the residue was dried for 1 h at 105 °C.

Fatty acid analysis

Rice oil was extracted from ground samples according to the procedure described earlier, using petroleum ether as solvent. Methyl esters were prepared from the crude lipids using BF_3 reagent.[16] Analyses were performed using a Shimadzu GC 14A gas chromatograph (Shimadzu Deutschland, Duisburg, Germany), equipped with a Varian Chrompack capillary column (CP-Wax 52 CB; 50 m × 0.25 mm inner diameter, 0.2 µm film) (Varian Deutschland, Darmstadt, Germany) and a Shimadzu AOC-20i auto-injector, C-R4AX Chromatopac integrator and flame ionization detector (FID). A column temperature gradient ranging from 160 to 250 °C with declining ramp rate was run as follows: 160–198 °C at 2.5 °C min^{-1}; temperature was held for 5 min; 198–218 °C at 2 °C min^{-1}; temperature was held for 15 min; 218–240 °C at 1.5 °C min^{-1}; temperature was held for 10 min; 240–250 °C at 1 °C min^{-1}; temperature was held for 2 min; 250–160 °C at 40 °C min^{-1}. The injector temperature was 230 °C and detector temperature 250 °C, and the carrier gas was nitrogen with a pressure of 1.3 kg cm^{-3}. A standard fatty acid methyl ester mixture was run and retention times were used in identifying the sample peaks. The proportion of each fatty acid was determined from the integrated peak areas.

β-Carotene analysis

All-*trans*-β-carotene was determined by carrying out high-performance liquid chromatography (HPLC).[17] The HPLC instrument was equipped with a Rainin (Woburn, MA, USA) Dynamax AI200 autosampling injection module, a Varian ProStar 210 solvent delivery system (Varian Deutschland), a Spherisorb column (ODS2, 4 × 250 mm) (Waters, Milford, MA, USA) and a Waters 2487 DAD. Prior to the extraction, samples were finely ground using mortar and pestle. Carotenoids were extracted from the ground samples until the residue was colorless, using a 1:1 mixture of dichloromethane and isopropanol as extraction solvent. After centrifugation, 20 µL were injected into the chromatograph. The mobile phase consisted of 82% acetonitrile, 15% dioxane and 3% methanol with 100 mmol L^{-1} ammonium acetate and 0.1% triethylamine. Retention time for all-*trans*-β-carotene was approximately 8 min, and it was detected at 450 nm. Quantification was made by multi-point external calibration.

Statistics and graphics

Mean values were compared by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test at a significance level of $P < 0.05$. The software used was Statistica version 5 for MS Windows (Statsoft Inc, Tulsa, OK, USA). Bravais–Pearson correlation coefficients (r) were calculated using the same software. Graphics were prepared using Graphpad Prism version 3 for MS Windows (Graphpad Software Inc, San Diego, CA, USA).

RESULTS

Table 1 compares the various color categories of rice samples with regard to the chemical fractions determined. Crude protein and crude ash values showed no statistically significant differences between the color classes. The crude lipid values, however, were significantly higher in the black/purple samples than in the red/brownish and the colorless varieties. The same applied to the saturated fatty acids fractions. The unsaturated fatty acids fraction in black/purple samples differed significantly only from the red samples but not from the colorless samples. β-Carotene values were significantly higher in the black/purple category as well, with values reaching up to $0.22\,\text{mg}\,\text{kg}^{-1}$. In red/brownish and colorless varieties, the β-carotene level was generally low, reaching only up to $0.01\,\text{mg}\,\text{kg}^{-1}$ and $0.02\,\text{mg}\,\text{kg}^{-1}$, respectively.

The fatty acids profile of the three color categories is shown in Table 2 on a total lipid basis. Unsaturated fatty acids accounted for the largest proportion of the lipid fraction, with the major unsaturated fatty acids being oleic and linoleic acid. The major saturated fatty acid was palmitic acid in all three color classes. Black/purple varieties tended to have a higher proportion of saturated fatty acids and a lower proportion of unsaturated fatty acids than the other samples. Statistically significant differences occurred only in the proportion of oleic acid, an unsaturated fatty acid, which was significantly lower in the black/purple varieties.

Correlation coefficients ($n = 54$) between β-carotene content and selected lipid fractions for the total sample set are illustrated in Fig 1. Correlation was significant ($r = 0.46$, $P < 0.001$) with the dry matter crude lipid content and even higher for the dry matter saturated fatty acid content ($r = 0.63$, $P < 0.001$). In contrast, the correlation with the dry matter unsaturated fatty acid content was only slightly positive ($r = 0.17$) and not statistically significant. Interestingly, β-carotene was positively correlated with the proportion of saturated fatty acids on a lipid basis ($r = 0.42$, $P < 0.01$), whereas it was negatively correlated with the proportion of unsaturated fatty acids ($r = -0.48$, $P < 0.001$). A closer look at individual major fatty acids revealed a positive correlation with the proportion (lipid basis) of palmitic acid ($r = 0.40$,

Table 1. Nutrient content (dry matter basis) of rice landraces (brown rice) as classified by the pericarp color

	Average ± SD($\text{g}\,\text{kg}^{-1}$)			Range ($\text{g}\,\text{kg}^{-1}$)		
Chemical fraction	Black/purple ($n = 8$)	Red/brownish ($n = 6$)	Colorless ($n = 40$)	Black/purple ($n = 8$)	Red/brownish ($n = 6$)	Colorless ($n = 40$)
Crude ash (CA)	10 ± 4^{a}	12 ± 4^{a}	9 ± 4^{a}	5–16	7–17	2–17
Crude protein (CP)	102 ± 10^{a}	97 ± 5^{a}	95 ± 11^{a}	83–118	88–102	70–122
Crude lipid (CL)	27 ± 4^{a}	21 ± 2^{b}	22 ± 4^{b}	22–35	20–25	7–30
Saturated fatty acids (SFA)	7 ± 2^{a}	5 ± 1^{b}	5 ± 1^{b}	5–11	4–6	2–7
Unsaturated fatty acids (USFA)	19 ± 2^{a}	16 ± 1^{b}	17 ± 3^{ab}	17–22	15–20	4–24
β-Carotene ($\text{mg}\,\text{kg}^{-1}$)	0.08 ± 0.07^{a}	0.00 ± 0.00^{b}	0.01 ± 0.01^{b}	0–0.22	0–0.01	0–0.02

SD = standard deviation.
Different superscript letters within one line denote statistically significant differences ($P < 0.05$) by ANOVA and Duncan's multiple range test.

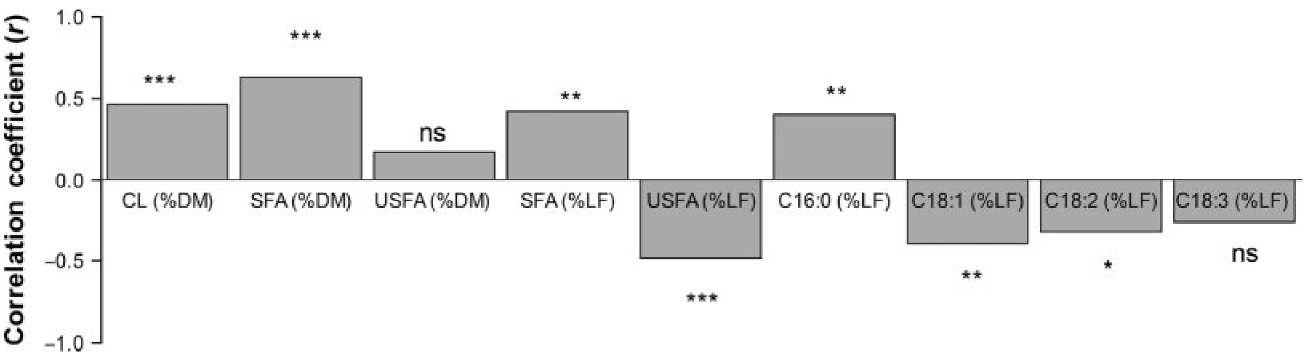

Figure 1. Bravais–Pearson correlation coefficients ($n = 54$) of β-carotene with various lipid components. Correlations are statistically significant at $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$; ns = not significant. CL = crude lipid; DM = dry matter; SFA = saturated fatty acids; LF = lipid fraction; USFA = unsaturated fatty acids; C16:0 = palmitic acid; C18:1 = oleic acid; C18:2 = linoleic acid; C18:3 = linolenic acid.

Table 2. Fatty acid composition of rice landraces (brown rice) on a total lipid basis

	Lipid fraction ± SD (%)		
Fatty acid	Black/purple ($n = 8$)	Red/brownish ($n = 6$)	Colorless ($n = 40$)
Saturated fatty acids (SFA)	25.4 ± 4.3	22.8 ± 3.1	22.3 ± 4.0
Unsaturated fatty acids (USFA)	73.4 ± 6.5	76.9 ± 4.9	77.9 ± 5.0
Myristic acid C14:0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Palmitic acid C16:0	20.3 ± 4.2	17.9 ± 2.7	17.8 ± 3.6
Stearic acid C18:0	2.6 ± 0.3	2.5 ± 0.3	2.3 ± 0.4
Oleic acid C18:1	42.1 ± 4.2^b	45.3 ± 2.3^a	46.3 ± 2.8^a
Linoleic acid C18:2	29.3 ± 4.8	29.5 ± 4.0	29.4 ± 3.7
Linolenic acid C18:3	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
Arachidic acid C20:0	0.8 ± 0.2	0.9 ± 0.1	0.8 ± 0.1
Eicosenoic acid C20:1	0.6 ± 0.3	0.5 ± 0.1	0.5 ± 0.1
Behenic acid C22:0	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Lignoceric acid C24:0	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.2

SD = standard deviation.
Different superscript letters within one line denote statistically significant differences ($P < 0.05$) by ANOVA and Duncan's multiple range test.

$P < 0.01$ and a negative correlation with the proportion of oleic acid ($r = -0.39$, $P < 0.01$), and linoleic acid ($r = -0.32$, $P < 0.05$). The correlation with linolenic acid was not statistically significant.

DISCUSSION

It is well known that rice can vary widely in its chemical composition.[12] Apart from environmental factors, the genotype is a major factor determining the grain's nutritional quality. The protein and mineral contents are quite sensitive to site–variety interactions, especially to nitrogen and mineral availability, management practices and climate.[3,18] Conversely, the variations in lipids and β-carotene—which are discussed here—may be attributed to the genotype rather than to environmental factors.[3] The highest genetic variability in rice is usually found in unfavorable environments that are not suitable for the production of high-yielding varieties, for example uplands. Quite often the cultivation of traditional landraces has persisted in such environments. Upland rice varieties have been found to be characterized by substantial morphological variability and favorable nutritional characteristic in previous studies.[11,19]

The current results suggest an interrelation between morphological characteristics of rice, namely the color, and nutrient density. The black/purple, ie heavily pigmented varieties, showed pronounced differences from the rest of the samples. An antioxidative effect of black rice varieties *in vivo* was reported in previous studies,[13,14] but could not be linked to a particular chemical constituent. The relatively high β-carotene level in black varieties detected in our study may help to explain these previous results, because of the high reactivity of carotenoids with electrophilic compounds.[15] With the β-carotene level detected, black rice can contribute moderately to the retinol supply in deficient diets. The highest value ($0.22\,\mathrm{mg\,kg^{-1}}$) comes close to the range of total carotenoid levels reported for transgenic 'golden rice' ($0.3-1.6\,\mathrm{mg\,kg^{-1}}$).[8–10] It should be mentioned that our values refer to all-*trans*-β-carotene, which is characterized by the most efficient conversion into vitamin A among the carotenoids.[20]

A further observation in our study was the correlation of β-carotene with certain fatty acids, which was the most pronounced with palmitic acid, the major saturated fatty acid in rice. In other words, varieties with a high β-carotene content had a higher proportion of saturated fatty acids in their lipid fraction and a lower proportion of unsaturated fatty acids. To the best of our knowledge, such correlations have not been investigated previously, and they remain difficult to interpret with the data at hand. The relation may point to an affinity of β-carotene to palmitic acid in the lipophilic grain matrices, possibly due to its specific polarity. It is known that palmitic acid is the predominant fatty acid in the formation of esters with the carotenoid zeaxanthin in plant tissues.[21] However, this relation cannot directly be transferred to β-carotene as it has a different chemical structure with no reactive oxygen function. The biosynthesis and deposition of β-carotene in lipophilic matrices merit further scientific study, especially in view of current efforts to further increase the provitamin A level in the rice endosperm by genetic engineering.

From a nutritional point of view, the demonstrated correlations between lipid content and β-carotene appear particularly favorable. Dietary lipids are a necessary component for the absorption of β-carotene into intestinal cells.[22] Moreover, the activity of the retinol carrier-protein CRBP-II is known to be supported by a high level of fatty acids in the diet.[22] Both factors point to potentially quite efficient conversion of black rice β-carotene into vitamin A owing to the lipids present. Such high lipid rice varieties can play an important role in rural populations in developing countries, which often rely on a mostly plant-based high-carbohydrate/low-fat diet.[11,23,24] However, it should be noted that black rice does not have the potential to act as a sole source of provitamin A to meet the daily retinol requirement of around 0.8–1 mg per day for a child.[25] This would require the ingestion of almost 30 kg of rice per day, assuming an optimistic conversion factor of 6:1 from β-carotene to vitamin A.

CONCLUSION

Certain rice landraces can add moderate amounts of β-carotene to the diet, and especially provide some essential lipids necessary for the absorption and conversion of β-carotene. Taking into account further favorable characteristics which such varieties tend to have, such as high protein content, they can play a vital role in providing a balanced diet in rural areas. As a prerequisite, rice has to be consumed as unmilled brown rice, which is urgently recommended.

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