

# Stability of Retinyl Palmitate During Cooking and Storage in Rice Fortified with Ultra Rice™ Fortification Technology

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**ABSTRACT:** Rice fortified with Ultra Rice™ (UR) containing retinyl palmitate (RP) was tested as a potential vehicle for vitamin A delivery. After UR was mixed with a long grain rice at the ratio of 1 to 99 (w/w), the stability of RP in the rice mixture was studied during cooking and storage for 6 mo. After cooking, the percent retention range of RP was 75 to 87, depending upon the cooking methods. The stability of RP in the rice stored at 2 different temperatures and relative humidities (RH) appeared to be more affected by temperature than RH. Therefore, under tropical conditions, rice fortified with RP might require special handling to avoid significant RP losses.

**Key Words:** Ultra Rice™, vitamin A, stability, fortification

## Introduction

RICE IS ONE OF THE WORLD'S MOST IMPORTANT CEREALS FOR human consumption. In the densely populated areas of Asia, rice has always been the most important staple food. As much as 80% of the daily human caloric intake in some countries is derived from rice (Luh 1991). Where rice is a staple food, nutritional problems including protein-energy malnutrition, nutritional anemia, particularly due to iron deficiency, iodine deficiency and vitamin A deficiency disorders are sometimes significant. These nutritional problems are not directly caused by the consumption of rice per se but reflect an overall impact of multiple causative factors including socio-economic, developmental, cultural, environmental and dietary factors. Among those factors, inadequate and unbalanced dietary intakes is the most important ones (Juliano 1993).

Vitamin A deficiency (VAD) is a common public health problem in many developing countries. VAD causes the death of about 500,000 children worldwide each year. Clinical manifestation of eye disease due to VAD affects 5 to 10 million children (Murphy and others 1992; Murphy 1996). In addition, VAD is associated with increased childhood morbidity and mortality due to a variety of infectious diseases including measles, malaria, tuberculosis, and respiratory and diarrheal diseases (Sommer and others 1983; Milton and others 1987; Gerster 1997; Nimmagadda and others 1998). However, this could be corrected and prevented by improving the vitamin A status. Muhilal and others (1988) reported that fortification of monosodium glutamate (MSG) with retinyl palmitate (RP) improved serum vitamin A levels of young children and the vitamin A content of breast milk of lactating women. They also found that the children receiving vitamin A-fortified MSG showed better linear growth, improved hemoglobin level, and reduced mortality rate compared to a control group.

In general, 3 main intervention strategies are currently in use to improve vitamin A status: (1) increasing the dietary intake of foods rich in vitamin A and provitamin A by changing dietary patterns, (2) periodic administration of large doses of vitamin A, and (3) fortification of vitamin A of one or more commonly consumed dietary items (Sommer 1995). Fortification of suitable foods with vitamin A is a well recognized approach to solve VAD problems in many parts of the world, and particularly so in the developing nations (Favaro and others 1991). Several foods in-

cluding sugar (Arroyave 1981), MSG (Solon and others 1985), soybean oil (Favaro and others 1991), and rice (Murphy and others 1992) have been explored as a potential vehicle for vitamin A fortification. Among them, rice is an ideal candidate for vitamin A fortification because rice is a staple food in countries where VAD is widespread.

Previous attempts at fortifying rice by powder enrichment has proven unsuccessful due to the typical washing and cooking methods employed in most countries, which result in the rinsing away of the enrichment (Hoffpauer 1992). A new technology has emerged, however, that overcomes this barrier. Broken rice grains, which typically comprise 20% to 30% of the harvest and are usually diverted to animal feed or brewing uses, can be milled into rice flour, combined with a binder and vitamin A and other fortificants, and reformed into rice grains with the same size, shape, and texture as the whole rice grains. The level of fortificant can be concentrated in these grains, such that they can be blended with virgin, unfortified rice at a ratio of 1:100 to 1:200 to yield a final product that can provide 100% of the daily vitamin A requirement for a child eating a typical daily quantity of rice. The Ultra Rice (UR) fortification technology was developed in the late 1980s by James Cox and Duffy Cox of Lynden, Wash., U.S.A. Formulation and process improvements have been made since that time to make the product resemble rice more closely, improve sensory characteristics, and to enhance the ability of the product to carry a variety of fortificants. The Coxes donated the patents regarding the vitamin-fortified rice to the Program for Appropriate Technology in Health (PATH) in 1997 (PATH 1999).

A clinical study with school children in Brazil has confirmed the efficacy of the fortified rice in improving the blood levels of vitamin A. Another study in Indonesia demonstrated the feasibility of blending the concentrated fortified rice with virgin rice at small, rural rice mills, and further showed that market acceptance of the resulting fortified product was excellent (PATH 1999). Recently, Garcia-Casal and others (1998) reported that the presence of vitamin A increased iron absorption from wheat, corn, and rice, suggesting that vitamin A prevented the inhibitory effect of dietary phytates on iron absorption. Thus, vitamin A fortification in rice could make a very important contribution to the problems of VAD and iron deficiency which are the two main deficiencies in many developing countries.

In this study, the stability of retinyl palmitate (RP) in UR forti-

fied with RP was studied during cooking and under various conditions of storage for a 6-mo period.

## Results and Discussion

### Validation of retinyl palmitate assay

In order to ensure that the direct solvent extraction procedure efficiently extracted RP from the beadlet matrix, the extraction was compared to saponification. Saponification, because of its severity, was assumed to be an adequate extraction to break the beadlet matrix. Retinol, determined after saponification, was calculated as RP. The analytical values obtained from the two extraction methods are presented in Table 1. The values from the direct solvent extraction were slightly higher than those from saponification in both the beadlet (Type 500) and UR, but not significantly different ( $p < 0.05$ ). The slightly lower analytical values from saponification are probably due to the severe nature of the extraction, resulting in some degradation and/or isomerization from trans to cis forms. We prefer to use direct solvent extraction for RP analysis of fortified foods if the values obtained, as in this study, are equivalent to those obtained by saponification of the food. Direct solvent extraction saves time and solvents and allows assay of the analyte as the more stable retinyl palmitate compared to retinol. All reported analytical values were determined using the direct solvent extraction procedure. RP levels found in the Type 500 beadlet and UR were 83% and 82% of the label and product target levels, respectively. Analytical method validation parameters including accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and specificity were calculated to ensure the overall method's validity. The HPLC

**Table 1—Assay values of retinyl palmitate in Palmabeads and Ultra Rice**

| Sample     | Retinyl palmitate (IU/g, wet weight basis) |                |                     |
|------------|--|----------------|---------------------|
|            | Direct solvent extraction                  | Saponification | Target level        |
| Beadlet    | 414822 ± 14109 <sup>1</sup>                | 404750 ± 39422 | 500000 <sup>2</sup> |
| Ultra Rice | 2056 ± 214                                 | 1846 ± 118     | 2500                |

<sup>1</sup> Mean ± standard deviation (n = 5)

<sup>2</sup> Label value

**Table 2—Precision and accuracy of assay.**

| Precision                    | Parameter           | Retinyl palmitate |                       |
|------------------------------|---------------------|-------------------|-----------------------|
|                              |                     | IU/g              | Accuracy <sup>1</sup> |
| Repeatability <sup>2</sup>   | Mean <sup>4</sup>   | 19.8397.60        |                       |
|                              | S.D. <sup>5</sup>   | 1.32              | 2.58                  |
|                              | CV <sup>6</sup> , % | 6.64              | 2.64                  |
| Reproducibility <sup>3</sup> | Mean                | 19.32             | 98.80                 |
|                              | S.D.                | 0.70              | 1.72                  |
|                              | CV, %               | 3.64              | 1.74                  |

<sup>1</sup> Accuracy is a measure of the closeness of the analytical result to the true value evaluated by analyzing a spiked sample (Recovery, %).

<sup>2</sup> Repeatability refers to the results of independent determinations carried out on a sample by analyzing five replicates of the sample on same day.

<sup>3</sup> Reproducibility refers to the results of independent determinations carried out on a sample by analyzing five replicates of the sample at different periods of time.

<sup>4</sup> n = 5.

<sup>5</sup> Standard deviation.

<sup>6</sup> Coefficient of variation.

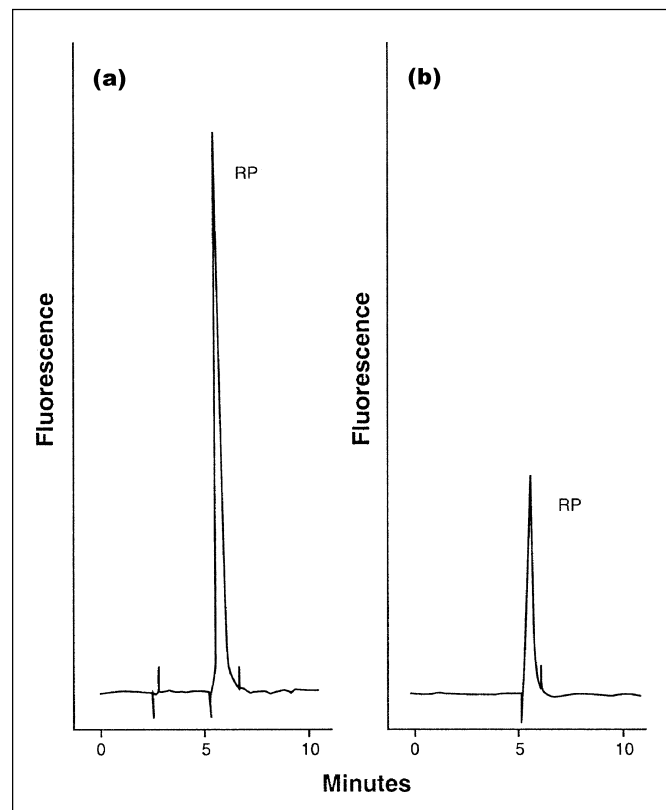
**Table 3—Specificity (peak purity test)<sup>a,b</sup>**

| Excitation wavelength (nm) | Peak response ratio |              |
|----------------------------|---------------------|--------------|
|                            | Standard            | Rice mixture |
| RP <sup>c</sup>            |                     |              |
| 315/325                    | 1.14                | 1.17         |
| 335/325                    | 0.73                | 0.74         |
| 315/335                    | 1.55                | 1.57         |

<sup>a</sup>Emission wavelength was constant (470 nm).

<sup>b</sup>Fluorescence ratios shown were calculated by dividing the values for the two peak heights for each analyte obtained from separate chromatographic runs at two different excitation wavelengths.

<sup>c</sup>Retinyl palmitate.



**Fig. 1—Normal-phase LC chromatogram of retinyl palmitate (RP) using fluorescence detection (Ex 325 nm, Em 470 nm), flow rate of 0.5 mL/min, injection volume of 20  $\mu$ L, and mobile phase of 0.9 % isopropanol in hexane. 1A is the standard and 1B is the extract of the rice mixture**

chromatograms of RP from standard and rice fortified with UR are presented in Fig. 1. The linearity test for quantification was carried out over the range of 0.0 to 58.3 ng/20  $\mu$ L injected for RP. Regression analysis showed an excellent linear relationship ( $r^2 = 0.999$ ).

Repeatability and reproducibility of the assay were assessed for the samples of rice fortified with UR and stored at 23 °C and 80 % RH for 24 wk (Table 2). The repeatability and reproducibility (% CV) were 6.64 and 3.64, respectively. The accuracy was evaluated by analyzing 5 samples of the rice mixture to which known concentrations of RP were added prior to extraction. Table 2 shows recovery data based on 5 trials for RP. The % mean recovery ± S.D. (n = 5) was 97.6 ± 2.58 and 98.8 ± 1.72 from the repeatability and reproducibility studies, respectively.

The LOD and LOQ were determined based on the detector's signal-to-noise (S/N) ratio. The standard deviation of the S/N ratio was calculated and multiplied by a factor of 3; then this value was added to the average of the S/N ratio to obtain the LOD. For LOQ, 10 was chosen as a factor (Food Chemicals Codex 1996). The LOD and LOQ of RP in ng/20  $\mu$ L (injection volume) were 0.04 and 0.09, respectively. Sample levels of RP were approximately 20 times higher than the LOQ.

The peak purity (specificity) was determined by the procedure described by Haroon and others (1986) for fluorescence response. Peak heights of RP were determined at excitation wavelengths of 315, 325, and 335 nm, while keeping the emission wavelength constant at 470 nm. Then the ratios of the peak heights from samples were compared with the peak ratio of the

**Table 4—The stability of retinyl palmitate in rice fortified with Ultra Rice during cooking**

| Cooking method <sup>2</sup>   | Retinyl palmitate µg/g (IU/g) <sup>1</sup> , wet weight basis |                 |                    |
|-------------------------------|---|-----------------|--------------------|
|                               | in cooked rice  | in excess water | % retention        |
| Before cooking a <sup>3</sup> | 11.35 ± 1.18 (20.61 ± 2.10)                                   |                 | 100.00             |
| A b                           | AVG   | 8.78 (15.95)    | N.A. <sup>4</sup>  |
|                               | SD  | 0.39 (0.74)     | N.A.               |
|                               | CV,%  | 4.47 (4.65)     | N.A.               |
| B ab                          | AVG   | 9.86 (18.14)    | 86.88              |
|                               | SD  | 0.83 (1.35)     | 7.30               |
|                               | CV,%  | 8.39 (7.44)     | 8.40               |
| C b                           | AVG   | 8.55 (15.56)    | 75.35 <sup>5</sup> |
|                               | SD  | 0.51 (0.93)     | 4.52               |
|                               | CV,%  | 5.98 (6.00)     | 6.00               |

<sup>1</sup> Recoveries were 104 % (n = 4).<sup>2</sup> A = Rice cooker; B = Boil until water is absorbed; C = Boil in excess water and pour off.<sup>3</sup> Values in the same column that are followed by the same letter are not significantly different (p < 0.05).<sup>4</sup> Not available.<sup>5</sup> The amount of retinyl palmitate in excess water was not included to calculate % retention.

\* Zero time sample (n = 5):

11.35 µg/g ± 1.18 (10.39, CV%)

20.61 IU/g ± 2.10 (10.21, CV%), wet weight basis.

**Table 5—Stability of retinyl palmitate in rice fortified with Ultra Rice during storage for 24 wk**

| Storage conditions       | Retinyl palmitate (IU/g, dry weight basis)              |                               |                   |                  |
|--------------------------|---|-------------------------------|-------------------|------------------|
|                          | 6 wk  | 12 wk                         | 18 wk             | 24 wk            |
| a 0 °C, N <sub>2</sub> a | <sup>1</sup> a 22.46 a <sup>2</sup> (96.2) <sup>3</sup> | <sup>3</sup> a 21.60 a (92.5) | a 21.80 a (97.6)  | a 21.77 a (93.2) |
| a 23 °C, 55% RH ab       | a 23.91 a (102.4)                                       | a 21.35 ab (91.4)             | b 20.88 ab (89.4) | b 20.22 b (86.6) |
| a 23 °C, 80% RH a        | a 22.23 a (95.2)  | a 21.76 a (93.2)              | b 20.21 a (86.6)  | b 19.97 a (85.5) |
| b 35 °C, 55% RH a        | b 19.50 b (83.5)  | b 17.50 b (75.0)              | c 14.37 c (61.5)  | c 11.93 c (51.1) |
| b 35 °C, 80% RH a        | a 21.80 a (93.4)  | b 17.65 b (75.6)              | c 13.72 c (58.8)  | d 11.07 c (47.1) |

<sup>1</sup> Values in the same column that are followed by the same letter are not significantly different (p < 0.05).<sup>2</sup> Values in the same row that are followed by the same letter are not significantly different (p < 0.05).<sup>3</sup> Percent retention of retinyl palmitate.**Table 6. The rate constant and half-life of retinyl palmitate in rice fortified with Ultra Rice under different storage conditions**

| Storage condition    | Rate constant (day <sup>-1</sup> ) × 10 <sup>2</sup> | Half-life (days) |
|----------------------|--|------------------|
| 0 °C, N <sub>2</sub> | 0.91   | 1240             |
| 23 °C, 55% RH        | 2.21   | 548              |
| 23 °C, 80% RH        | 2.09   | 554              |
| 35 °C, 55% RH        | 6.66   | 169              |
| 35 °C, 80% RH        | 7.77   | 159              |

standard obtained at the same wavelengths. The results are shown in Table 3. Very close values were obtained from samples and standard, indicating the purity of the retinyl palmitate peak. Generally, the results of validation parameters were reliable and satisfactory for the overall analytical method.

### Stability of retinyl palmitate during cooking

Cooking significantly (p < 0.05) reduced the RP content in the samples cooked by the rice cooker (method A) and by boiling in excess water (method C). Boiling without excess water (method B) did not significantly (p < 0.05) affect the RP content (Table 4). Comparison of the three cooking methods indicated that they did not differ significantly (p < 0.05). After cooking with excess water (cooking method C), about 20 % of RP was measured in the excess water. Therefore, the loss in RP was accounted for by leaching into the cooking water. According to 21 CFR 137.350 (revised as of April 1, 1999), washed rice should contain not less than 85 percent of the minimum quantities of the specified amounts of thiamin, riboflavin, niacin or niacinamide, folic acid, iron, vitamin D, and calcium. Although RP is not mentioned in the CFR specifications, UR meets the standard for RP, since more than 85% of the RP was retained after conventional cooking (method B). Murphy and others. (1992) reported that cooking reduced the RP about 4% to 39% in UR and synthetic rice premixes depending upon their formulations. They also reported that cooking stability of RP in UR mixed with rice yielded results similar to stability of the premix alone and there was no loss of RP during rinsing. The cooking method used in their study included cooking for 5 min in boiling water and over low heat for 20 min until all cooking water was absorbed by the synthetic rice premix or UR. This method is similar to method B (boiling until water is absorbed). Flores and others (1994) reported that the loss of RP in a mixture of UR with unfortified rice at the ratio of 2 to 100 was 26% during cooking. The cooking condition included 5 min boiling followed by 20 to 25 min under low heat. Results of the previous studies are close to retention values reported here.

### Stability of retinyl palmitate during 6-mo storage

Stability of RP in the rice fortified with UR during 6-mo storage under different environmental conditions are presented in Table 5. On the whole, the stability of RP appeared to be more affected by temperature than RH. A significant (p < 0.05) difference in RP stability between the samples stored at 23 °C and the

samples at 35 °C was observed, but no difference in the stability was found between 55 and 80 % RH throughout 24-wk storage within the same temperatures. The RP content of the samples stored at 0 °C under nitrogen was not significantly different from the RP in the samples stored at 23 °C. No statistical differences in RP stability were found at 0 °C or 23 °C throughout 24-wk storage, except the samples stored at 23 °C, 80 % RH after 24 wk (p < 0.05). RP decreased throughout storage at 35 °C. About one-half the amount of RP in the mixture stored at 35 °C was lost after 24 wk. Therefore, it is critical to maintain the storage temperature as much below 35 °C as possible to improve RP stability.

Rate constants for loss of RP in the mixture were determined by plotting linear regression of residual RP with time (days) using the SAS statistical package. Half-life (Table 6) of RP was calculated as time (days) required for 50% of RP to degrade. The half-life at 0 °C under nitrogen was 1240 d and 548 and 554 d at 23 °C, 55% RH and 23 °C, 80% RH, respectively. Half-lives decreased to 169 and 159 d at 35 °C, 55% RH and 35 °C, 80% RH, respectively. The half-life of RP in the samples stored at 80% RH is slightly longer than the samples at 55% RH within the same storage temperature (23 °C). This trend was reversed at 35 °C. RP in the samples stored at 55% RH has slightly longer half-lives than in the samples stored at 80% RH. Half-lives for RP reported by Murphy and others (1992) in various synthetic rice premixes ranged from 20 to 902 d at 25 °C with 2 water activities (0.11 and 0.75).

### Conclusion

THE RP IN THE RICE FORTIFIED WITH UR WAS QUITE STABLE UNDER the various cooking procedures used in this study. When stored at 23 °C for 6 m, 85% of the RP was retained. Storage at 35 °C showed that extensive losses in RP can occur during extended storage. This observation shows that under tropical conditions, rice fortified with UR might require special handling to avoid significant RP losses. If temperature control is not possible during extended product storage, more rapid product turnover should be planned or fortification overages instituted to compensate for RP loss.

## Materials and Methods

### Sample description

UR, fortified with all-trans retinyl palmitate (RP, Palma-beads®, Type 500; Roche Vitamins and Fine Chemicals, Hoffmann-LaRoche Inc., Nutley, N.J., U.S.A.), was prepared by PATH and shipped to the University of Georgia, Department of Food Science and Technology. The fortification target level was 2500 IU/g UR. The RP used in UR is dispersed in a gelatin matrix with sucrose, peanut oil, and tricalcium phosphate. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are added as antioxidants.

One gram of UR was mixed with 99 g of long grain rice (Blue Ribbon, Comet American Marketing, Houston, Texas, U.S.A.) for the cooking and storage studies. For the storage study, the 100-g mixtures were prepared, packed in 1.5-mm low-density polyethylene bags, and heat-sealed.

### Rice cooking methods

**Rice Cooker (method A).** One hundred grams of the rice mixture containing 1 g UR and 99 g rice were rinsed two times with excess tap water. The rinsed rice was placed in a rice cooker (Matsushita Electronic Industrial Co. Ltd., Japan). Two hundred milliliters of tap water were added, and the rice was cooked for 20 min. No excess water remained after the cooking process.

**Boiling without excess water (method B).** The rinsed rice mixture (100 g prior to rinsing) was cooked with 200 mL tap water (w:v, 1:2). The rice was cooked over low heat for 20 min after being brought to a boil. No excess water remained after the cooking procedure.

**Boiling in excess water (method C).** All conditions were the same as given above (B), except 400 mL tap water was added to 100 g rice. Excess water remaining after cooking was collected by pouring the cooked rice through a food colander. The cooking water was collected and assayed for residual RP.

### Storage conditions and sampling plan

The rice mixtures in the polyethylene bags were stored in 5 air-tight glass storage chambers with two temperatures and two humidity conditions listed in Table 7. The storage chambers were protected from light by wrapping with aluminum foil. Relative humidity (RH) was adjusted by saturated salt solutions. Saturated magnesium nitrate solution was used for 55 ± 5% and saturated potassium chloride solution for 80 ± 5% RH. At 6-wk intervals, 3 samples were removed from the environmental chambers (2 samples for condition 1, Table 1) and finely ground with an Ultra Centrifugal Mill ZM 100® (Glen Mills Inc., Clifton, N.J., U.S.A.) and analyzed for RP and moisture content. All samples were independently assayed for RP (3 replicates).

### Retinyl palmitate and moisture determination

**Direct Solvent Extraction for Stored Rice.** RP was extracted by a direct solvent extraction procedure modified from a method developed by Landen (1982) which used isopropanol and methylene chloride to extract lipid-soluble components, followed by dehydration of the extract with anhydrous magnesium sulfate. In Landen's original procedure, high-pressure gel permeation chromatography (HP-GPC) was used to fractionate vitamins from the lipid material, and reversed-phase HPLC was used for the quantitative determination of RP and  $\alpha$ -tocopheryl acetate. In this study, the procedural modifica-

**Table 7—Storage condition**

| Storage conditions | Temperature (°C) | Relative humidity (%) <sup>a</sup> |
|--------------------|------------------|------------------------------------|
| 1                  | 0 ± 5            | under nitrogen                     |
| 2                  | 23 ± 5           | 55 ± 5                             |
| 3                  | 23 ± 5           | 80 ± 5                             |
| 4                  | 35 ± 5           | 55 ± 5                             |
| 5                  | 35 ± 5           | 80 ± 5                             |

<sup>a</sup> Relative humidities were adjusted by saturated salt solution. Potassium chloride and magnesium nitrate were used for 80 and 55% relative humidity, respectively.

tion eliminated HP-GPC and included addition of 80 °C water to the dry sample and substitution of hexane for methylene chloride (Lee and others 1999). The samples of 1.0 g finely ground rice mixture were accurately weighed into a 125-mL round-bottom glass bottle. Hot deionized water (80 °C, 4 mL) was added and mixed with the sample. Ten mL isopropanol was added to the mixture. Approximately 5 g anhydrous magnesium sulfate was added followed by 25 mL of extraction solvent (hexane containing 0.01% BHT, w/v). The mixture was homogenized with a Polytron® homogenizer (SOURCE/) for 1 min at medium speed. The Polytron was rinsed with 5 mL extraction solvent. The mixture was filtered through a medium-porosity glass filter with a vacuum bell jar filtration apparatus (Kontes, Vineland, N.J., U.S.A.). The vacuum was released and the filter cake was broken with a spatula and washed with 5 mL extraction solvent. The filter cake was transferred to the same 125-mL round-bottom glass bottle for the repeat extraction. Five mL isopropanol and 30 mL extracting solvent were added to the mixture followed by homogenization and filtration. The combined filtrate was transferred to a 100-mL volumetric flask and diluted to volume with extracting solvent followed by filtration using a 0.45- $\mu$ m nylon membrane filter (MSI Inc., Westboro, Mass., U.S.A.). All steps were carried out under yellow light and all solvents were HPLC grade.

**Direct solvent extraction for cooked rice.** Initial studies showed that in order to obtain acceptable measurements of residual RP, small weighings of the final cooked products could not be used. Therefore, the entire batch of cooked rice was transferred to a 500-mL beaker, homogenized with a Polytron homogenizer for 3 min after adding 500 mL water, and quantitatively transferred to a 1000-mL volumetric flask and diluted to volume with water. Ten mL of the homogenized cooked rice slurry was accurately weighed into a 125-mL round glass bottle. Twenty mL isopropanol and 10 g anhydrous magnesium sulfate were added to the sample aliquot followed by 50 mL of the extracting solvent. The mixture was homogenized with the Polytron homogenizer for 3 min at medium speed. The Polytron was rinsed with 5 mL extracting solvent. After filtering through a medium-porosity glass filter using a vacuum bell jar filtration apparatus, the filtrate was transferred to a 100-mL volumetric flask and diluted to volume with the extracting solvent followed by filtration using a 0.45- $\mu$ m nylon membrane filter.

**Moisture determination.** Moisture content was determined according to procedure 925.09 of the AOAC International (1995).

### Saponification of retinyl palmitate beadlet and UR

Ten mL of ethanol containing pyrogallol (6% w/v) was added to each sample (0.05 g for RP beadlet and 0.2 g for UR) and agitated to avoid agglomeration. After sonicating for 10 min, 2 mL of 60% potassium hydroxide in deionized water (freshly prepared) was added. The container was flushed with nitro-

gen for 1 min and connected to an air condenser. The contents were then digested at 70 °C for 30 min in a shaker water bath. Following cooling in an ice bath, 20 mL of 2% sodium chloride in deionized water was added and the digest was extracted three times with 10 mL of extracting solvent (hexane:ethyl acetate, 90:10, v/v) containing 0.01% BHT. The extracting solvent fractions were collected into a 50-mL volumetric flask. The volume was adjusted to 50 mL with extracting solvent followed by filtration using a 0.45- $\mu$ m nylon membrane filter. A 1.0 mL aliquot of the combined filtrates was evaporated with nitrogen and then diluted with mobile phase (Lee and others 1999).

### Standard preparation

The RP and retinol standards were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Purity and stability were monitored by  $E^{1\%}_{1\text{cm}}$  values (960 at 326 nm for RP and 1845 at 325 nm for retinol (Olson 1990) measured by using a DU-64 Spectrophotometer (Beckman Instruments Inc., Fullerton, Calif., U.S.A.). Accurately, 25 mg of each RP and retinol was weighed and dissolved in 25 mL of hexane and isopropanol mixture (90:10, v/v) containing 0.01% BHT (w/v), separately. Appropriate dilution was made with the mobile phase to give a stock standard concentration of 29.15 and 64.84  $\mu$ g/mL for RP and retinol, respectively. For a daily working standard, 1 mL of the stock standard solution was diluted in a 100-mL volumetric flask with mobile phase. The working standard concentrations of RP and retinol were 0.29 and 0.65  $\mu$ g/mL, respectively. Concentrations of the RP and retinol were calculated from peak area determined by the Waters 764 integrator (Millipore Corp., Cary, N.C., U.S.A.).

### HPLC quantification

The normal-phase HPLC system consisted of a Shimadzu LC-6A pump equipped with a Shimadzu RF-10A spectrofluorometric detector (Shimadzu Corp., Columbia, Md., U.S.A.), a SpectraSeries AS100 autosampler (Thermo Separation Products Inc., San Jose, Calif., U.S.A.), and a 25 cm  $\times$  4 mm, 5  $\mu$ m Li-chrosorb Si60 column (Hibar Fertigsäule RT., Darmstadt, Germany) equipped with a precolumn packed with Perisorb A 30-40 $\mu$ m (Hibar, Darmstadt, Germany). The isocratic mobile phase contained 0.9 % isopropanol in *n*-hexane (J.T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.). The flow rate was 0.5 mL/min. The mobile phase was filtered using a 0.22- $\mu$ m nylon membrane filter (MSI Inc., Westboro, Mass., U.S.A.) and degassed by stirring under vacuum. The wavelengths were set at 325 nm for excitation and 470 nm for emission for the determination of RP. The concentration of RP in the samples was calculated using the average peak area compared between standard and sample after duplicate injections. After saponification, retinol was determined using the same LC system with 5% isopropanol in *n*-hexane as a mobile phase. For recovery studies, 1 mL of the stock standard solution of RP was added to the sample with the extracting solvent. The spiked level of RP was 29.15  $\mu$ g/g sample. Recoveries for raw rice mixtures and cooked rice mixtures were 98% (n = 16) and 104% (n = 4), respectively.

### Statistical analysis

Statistical analysis (1-way and 2-way analysis of variance) were performed by using the Statistical Analysis System (SAS, 1990). Means were compared by the Duncan test at = 0.05.

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