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Comparative Stability of vit E isomers Extracted from Unsaponifiable Fractions of Rice Bran Oil under Various Temperature and Oxygen Conditions

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Abstract - Due to the fact that tocopherols and tocotrienols have antioxidant and anticancer properties, the commercial utilization of unsaponifiable fractions in rice bran is increasing. These nutraceutical compounds, however, are fairly unstable and readily break down when exposed to oxygen or lighting conditions. To compare the relative sensitivity of vit E isomers to heat and oxygen, concentrated unsaponifiable fractions extracted from crude rice bran oil were exposed to various temperature, oxygen (nitrogen-balanced), and bathing solvent conditions and resultant concentration changes in α- and γ-tocopherols (T) and tocotrienols (T3) were evaluated. Each isomer exhibited different heat stability. Among them, α-T3 degraded more rapidly compared to other vit E isomers while α-T was the most stable isomer. Oxygen level also showed significant impact on each isomer's stability where severe reductions of γ-T (by 20%) and γ-T3 (by 29%) were observed under 2% oxygen conditions, while under 0% oxygen conditions no degradation could be observed even after exposure to 95℃ for 4 hours. When various blending solvents were mixed with concentrated unsaponifiable fractions, organic solvents such as isooctane and hexane were more effective in maintaining the stability of γ-T3 compared to edible oils, among which corn oil was more efficient than soybean and rice bran oils.

Key words - heat, oxygen, rice bran, tocopherol, tocotrienol, stability, vitamin E

Introduction

Rice is a major staple food crop in Asian countries, including South Korea. To enhance the edible quality, rices are generally consumed in the form of white rice after milling process, during which the rice bran comprising about 10% of the brown rice weight are removed. The rice bran, however, contains many kinds of nutritive and health-beneficial compounds such as dietary fiber, octacosanol, oryzanol, phytic acid, and vitamin E, etc (Jariwalla, 2001). Vitamin E has been well-known as an antioxidative compound protecting biological membranes against free radicals in human body. Vit E is a generic term comprising tocopherols (T) and tocotrienols (T3) commonly containing a chromanol ring and a hydrophobic phytyl side chain (Shin and Godber, 1993). The difference between tocopherols and tocotrienols lies in chemical structure of hydrophobic side chain; tocotrienols have unsaturated bonds in each of the 3 isoprene units in hydrocarbon tails, while tocopherols have a fully saturated phytyl side chain. Both the tocopherols and tocotrienols are further classified into 4 isomers: α-, β-, γ-, δ-forms according to the number and position of methyl groups on the chromanol ring (Lee and Park, 2004). Among 8 isomers, α-tocopherol is the most widely documented and commercialized form of vit E due to its early discovery and prominent function (Evans et al., 1936). Unlike α-tocopherol which has been treated as the most active form of vit E and generally used as a representative of antioxidant, tocotrienol has been regarded as a compound bearing no health-beneficial effects. Recently, however, various pharmaceutical effects of tocotrienol have been noticed; e.g., antioxidative (Lehmann and Slover, 1976; Kamart et al., 1997), anticarcinogenic (Lee and Kim, 2006; Nesaretnam, et al., 1998), cardiovascular system-protecting (Tomeo et al., 1995), blood cholesterol-lowering (Sugano and Tsuji, 1997; Qureshi et al., 1991), diabet risk-reducing (Montonen et al., 2004), although slight differences in

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biological activities among these 8 vit E isomers were observed (Burton and Ingold, 1981; Makpol et al., 1997; Yu et al., 1999).

Based upon such distinctive pharmaceutical effectiveness, tocopherols and tocotrienols extracted from plant resources such as rice bran oil and palm oil are recently commercialized as sources for functional foodstuff, drink and cosmetics, especially in developed countries such as USA and Japan. Rice is the staple food of Korea, and the estimated annual production of rice bran reaches approximately 450,000 tons. Industrialization of rice bran in Korea, however, especially as food resources are very limited; i.e., only 5% of rice bran are used for rice bran oil production and the remaining rice bran are used for animal feeding (55%), farming-site utilization (15%), mushroom media (11%), fertilizer (10%), etc.

Rice bran is an excellent source for tocopherols and tocotrienols. Due to their antioxidative properties, however, vit E groups are generally accepted as relatively unstable compounds and they are readily degraded by the presence of light, oxygen and high temperature. Consequently, preserving the activity of these pharmaceutical compounds during the process of industrialization as well as during the market circulation is a great concern. Although autoxidative, photolytic, and high temperature sensitivities of vit E isomers have been reported (Lehmann and Slover, 1976; Simonne and Eitenmiller, 1998, Margherita, et al., 2007), significant inconsistency between reports could be observed, which may depend upon experimental conditions and source of vit E extraction. In western countries most of vit E are extracted from soybean, olive and palm oils and thus information on stability of vit E isomers from rice is very limited. The objective of this study was evaluating the comparative stability of 4 major forms of vit E isomers extracted from rice bran under different temperature, oxygen, and bathing solvent conditions.

Methods

Preparation of unsaponifiable fraction of rice bran oil

Rice bran crude oil was provided from a manufacturer producing rice bran oil. The process of obtaining crude oil consists of pelletizing fresh rice bran, hexane extraction, and subsequent hexane removal by distillation. In general operation condition, the manufacturer produces 10 to 15 of crude oils from 100 M/T of rice bran everyday and crude oils are used for rice bran oil production after further refining. To collect unsaponifiable fraction concentrate from crude oil, one kg of crude oil was added into a pilot-scale (60 L) extractor containing 18 L EtOH and 200 g of ascorbic acids. The extractor was heated at 80℃ prior to the addition of crud oil, and after 10 minutes of extraction at 80℃, 600 mL of 44% (w/w) KOH were added for saponification. After 18 minutes of saponification the solution was quickly cooled down to room temperature by passing through an ice-water cooling system. After adding 18 L of distilled water and 18 L of hexane, the mixture solutions were thoroughly homogenized. After that liquid-liquid separation hexane layers containing unsaponifiable fractions were collected. This hexane-extraction process was repeated two more times and colleted hexane layers were pooled and washed twice with distilled water. After removal of hexane in a pilot-scale vacuum evaporator at 60℃, the remaining unsaponifiable fraction of concentrates was used for further stability test experiments.

Heat stability test

One mL of unsaponifiable fractions were transferred into an 1.5 mL amber vial, and after gas-tight capping the vials were covered by aluminium foil to prevent light-induced degradation and then submerged in a boiling hot water. After 4, 8, 12, and 24 hours of heating treatment durations, the vials were cooled down to room temperature, decapped and changes in concentration of tocopherols and tocotrienols were quantified with an HPLC. Relative stabilities of isomers were expressed as the percentage value in comparison with each isomer's concentrations prior to heat treatment.

Oxygen stability test

In order to induce more unfavourable conditions in elucidating oxygen stability, high temperature conditions were given simultaneously. The autoxidation conditions were given according to Lehmann and Slover (1976) after modification. The 0.1 mL of unsaponifiable fractions were mixed with 99.9 mL of isooctane and transferred into a glass test tube. The test tubes were covered by aluminium foil for light protection, and placed in a water bath adjusted to 95℃.

Artificially modified gases containing 0, 2, 8, and 21% oxygen (nitrogen balanced) were blown into the bottom of the test tubes through a bronze tube at a flow rate of 14 mL min^{-1}. After 4 hours of oxygen blowing, final volume (100 mL) were adjusted by adding isooctane and resultant concentration changes in tocopherol and tocotrienol were evaluated and compared to non-treated value.

Blending solvent test

The unsaponifiable fractions extracted from rice bran needs to be dissolved in a certain kind of solvent for final product development, short-time storage, or for market circulation, etc. Consequently the selection of blending solvent that can provide higher stability of target compound is important. To address this question, unsaponifiable fractions were mixed with 100 times volume of organic solvents (isooctane and hexane) or commercially-purchased edible oils (rice bran oil, soybean oil, and corn oil) and transferred into a gas-tight vial. After capping and light protection, vials were submerged in boiling water for 24 hours and resultant concentration changes in γT3 were compared to pre-heating value. In the case of soybean oil which contains γT3, its concentration was measured prior to experiment and considered as a blank.

HPLC analysis

Quantitative determination of tocopherol and tocotrienol in samples were conducted according to Lee and Park (2004) by using a normal phase HPLC (S1201, Sykam, Germany). For HPLC column, Zorbax SiL (4.6 × 250 mm, 5 μm) was used and for isocratic mobile phase, isooctane : acetic acid : ethyl acetate : DMP (2,2-dimethoxypropane) = 98.5 : 0.7 : 0.7 : 0.1 was used. Injection volume was 20 μL, and the measurement wavelength was 290 nm. Authentic standards for tocopherols and tocotrienols purchased from CalBiochem (U.S.A.) were used for identification of corresponding isomers and subsequent quantification.

Results

Heat stability

All vit E isomers in unsaponifiable fraction of rice bran decreased as the heating duration increased. Each isomer, however, showed somewhat different heat sensitivity during the 24 hours of heat treatment. In the case of initial 4 hours of heating at 100℃, all tested isomers showed similar decrease less than 9% compared to pre-heating. These relatively low decrease at initial stage of heating suggested that most of vit E existing in rice, especially in brown rice which contains vit E-rich bran, could be stable under a general rice cooking conditions: i.e., less than 1 hour at temperature lower than 100℃. Extended heat treatment up to 8 hours, however, resulted in more severe and isomer-dependent degradation; i.e., more rapid decrease could be observed in α-T3 (by 34.9%) compared to αT (by 7.4%), γT (25.9%), and γT3 (by 13.5%). When heating duration was extended up to 24 hours, αT, γT, αT3, and γT3 exhibited concentration decrement by 27.3, 47.4, 46.4, and 32%, respectively compared to their concentrations prior to heat treatment. These results suggest the order of heat stability of vit E isomers from rice bran in descending order of αT > γT3 > αT3 ≈ γT. The fact that αT was the most stable isomer was in agreement with Simonne and Eitenmiller (1998) who reported stability of αT during deep-fat frying was higher than other form of vit E isomers in soybean oil, corn oil, and palm olein.

Oxygen stability

Vit E isomers carrying antioxidative properties may suffer

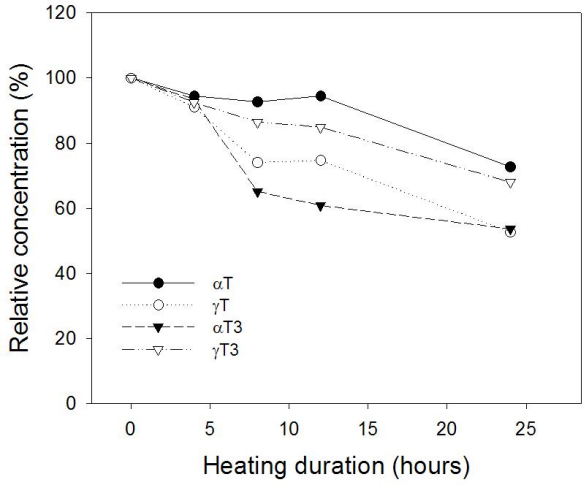

Fig. 1. Time-series changes in tocopherol and tocotrienol concentrations in unsaponifiable fractions extracted from rice bran under high temperature conditions.

easy degradation when exposed to oxygen. Relative sensitivities of vit E isomers against oxygen were evaluated by exposing to different levels of oxygen under high temperature conditions. When exposed to 0% oxygen, all tested vit E isomers exhibited no degradation even after 4 hours' heating at 95℃. Increased oxygen level, however, induced significant reduction in vit E concentrations. The most affected one was γT, which decreased by 45.8% after 4 hours of heating under 21% oxygen. The stability of vit E isomers against oxygen in descending order was αT3 > αT > γT3 ≈ γT which decreased by 19.3%, 24.2%, 42.5%, and 44.8%, respectively. In both tocopherols and tocotrienols, gamma form isomers exhibited more severe degradation than alpha form isomers in that even at 2% low oxygen level γT and γT3 decreased by 19.9% and 29.0%, while αT and αT3 decreased by 13.3% and 12.2%, respectively. In both alpha and gamma forms of vit E isomers, oxygen sensitivity differences between tocopherols and tocotrienols were not prominent, indicating that oxygen sensitivity of vit E isomers extracted from rice may depend more upon the presence of methyl groups in chromanol ring rather than the presence of double bonds in phytyl side chain. Our results concluded that the presence of oxygen even as low as 2% may easily induce significant degradation of vit E isomers in comparison with no degradation at 0% oxygen (Fig. 2) and again suggested the importance of keeping oxygen-free conditions in storage or during commercialization of tocopherols and tocotrienols by using rice bran. However, as reported by Lehmann and Slover (1976) the stability of tocopherol and tocotrienol isomers may differ depending upon the blending solvent, and consequently more precise experiments need to be done corresponding to bathing solutions to be used in commercialization.

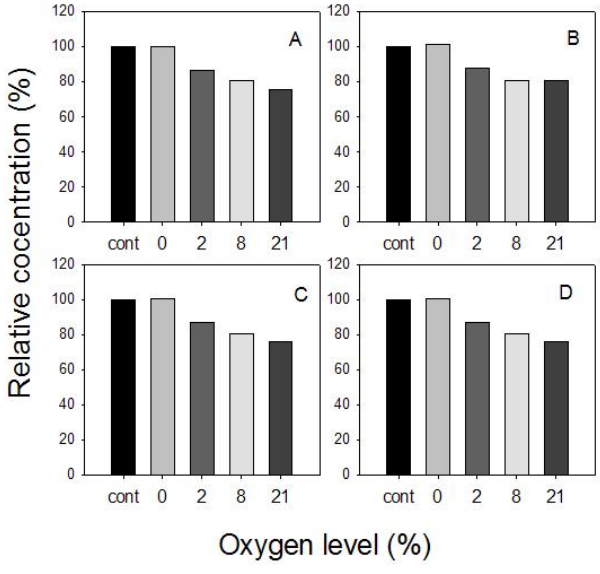

Fig. 2. Oxygen stability of vit E isomers (A: αT, B: γT, C: αT3, D: γT3) extracted from rice bran.

Blending solvent effects

Isolated vit E isomers are to be dissolved in bathing or blending solvents. Organic solvents such as isooctane and hexane are generally used as a bathing solvent in the middle of extraction and short term storage prior to the production of final products. In contrast plant-based edible oils could be used as a blending solvent in final process for products, especially in such cases as functional food. Different blending solvents, however may result in significantly different order of stability among vit E isomers (Lehmann and Slover, 1976, Yoon et al., 1986). In this report, relative stabilities of γT3 in various solvents at high temperature conditions were evaluated. As shown in Fig. 3 organic solvents prevented γT3 from degradation more effectively than edible oils in that isooctane and hexane resulted in degradation of γT3 by 27.5% and 30.2%, while corn oil, rice bran oil, and soybean oil resulted in γT3 decrease by 52.4%, 68.7, and 72.2%, respectively. These results suggest that organic solvents are preferable in temporary storage of vit E

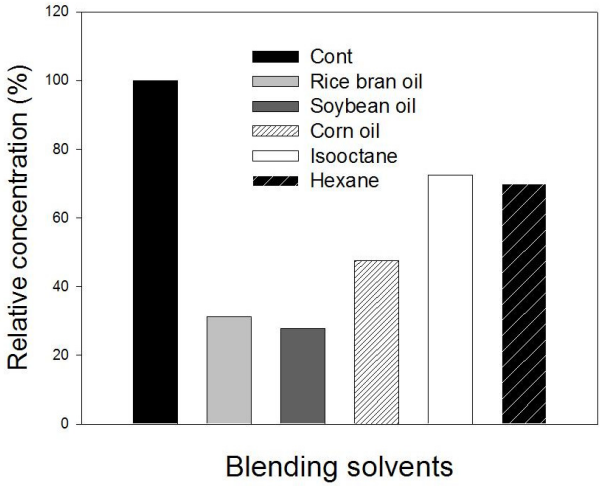

Fig. 3. Relative stability of γ-tocotrienol extracted from rice bran and mixed with various blending solvent.

isomers extracted from rice bran prior to the preparation of final products. Besides, corn oil seems to be preferable as a blending solvent.

In conclusion, each tocopherol and tocotrienol isomers showed different stability under high oxygen and temperature conditions, in that α-tocotrienol was the most stable isomer to heat and α- form of tocopherols and tocotrienols exhibited higher autoxidation stability compared to γ- forms. As a blending solvent, organic solvents such as isooctane and hexane were more effective for maintaining stability of γT3 compared with edible oils, among which corn oil was more efficient than soybean and rice bran oils.

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