Stability of tocopherols and tocotrienols extracted from unsaponifiable fraction of rice bran under various temperature and oxygen condition

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Abstract

The rice bran has been known to contain both tocopherols and tocotrienols (vit E) showing high antioxidant and anticancer activity. These nutraceutical compounds, however, are very sensitive to high temperature and oxygen. This study examined comparative stability of vit E isomers: a-, b-, g-, and d-tocopherols (T) and tocotrienols (T3) extracted from unsaponifiable fractions of rice bran oil under different temperature and oxygen conditions. Each isomer exhibited different sensitivity to high temperature and among tested isomers, aT3 degraded more rapidly and severely than others. Under 0% oxygen conditions, no degradation could be observed even when isomers were exposed to 95^{o}C for 4 hours, while as low as 2% oxygen resulted in reduction of gT and gT3 by 20% and 29%, respectively. When blending solutions were mixed with unsaponifiable fractions, organic solvents such as isooctane and hexane were more effective for maintaining stability of gT3 compared with edible oils, among which corn oil was more efficient than soybean and rice bran oils.

Media summary

Each vit E isomer extracted from rice bran has different stability and aT3 and gT3 were isomers which degraded more easily under high temperature and oxygen conditions.

Key Words

tocopherol, tocotrienol, vitamin E, stability, rice bran oil.

Introduction

Rice is a major crop in Asian countries, including South Korea. Rice is consumed after milling, during which process bran containing various kinds of functional compounds such as ferulic acid, octacosanol, oryzanol, and vitamin E, etc. are removed. Vitamin E is generally known as an antioxidant protecting biological membranes from free radicals in human body and is a generic term including 8 isomers: alpha (a)-, beta (b)-, gamma (g)-, and delta (d)- tocopherols (T) and tocotrienols (T3). In the Vitamin E groups, aT has been considered to be the most active form. However, recent research has suggested tocotrienol to be a better antioxidant with anticancer activity. Vit E groups are very sensitive to light, temperature and oxygen. The objectives of this study were to investigate the comparative stability of vit E isomers extracted from unsaponifiable fractions of rice bran crude oil.

Methods

Preparation of unsaponifiable fraction of rice bran oil

Rice bran crude oils were collected after extracting fresh bran in hexane followed by hexane removal by evaporation. One kg of crude oil was added into a pilot-scale extractor (60 L) containing 18L EtOH and 200 g of ascorbic acids were added. The temperature of extractor was maintained at 80^{o}C, and after 10 minutes 600 mL of 80 % KOH were added for saponification. After saponification the solution was quickly cooled and 18L of distilled water and hexane were mixed. Hexane layers containing unsaponifiable

fractions were washed twice with distilled water. After removal of hexane in a pilot-scale vacuum evaporator the remaining unsaponifiable fractions were used for further stability test experiments.

Heat stability test

One mL of unsaponifiable fractions were transferred into a 1.5 mL vial and after gas tight capping, the vial was submerged in boiling water. After 4, 8, 12, and 24 hours the vial was decapped and unsaponifiable fractions were diluted with isooctane and tocopherol and tocotrienol concentration was analysed with an HPLC.

Oxygen stability test

Unsaponifiable fractions were mixed with 10 times volume of isooctane and transferred into a test tube placed in a bath with boiling water. Gases containing 0, 2, 8, and 21 % oxygen (nitrogen balance) were blown into the test tube at a flow rate of 14 mL/min for 4 hours, and resultant changes in tocopherol and tocotrienol concentration were analysed.

Blending solution test

Unsaponifiable fractions were mixed with 100 times volume of organic solvents (isooctane and hexane) or edible oils (rice bran oil, soybean oil, and corn oil) and transferred into a gas-tight vial. After capping vials were submerged in boiling water for 24 hours and changes in gT3 concentration was analysed. The amount of gT3 in edible oil was subtracted as a blank.

HPLC analysis

Tocopherol and tocotrienol contents were analysed by using an HPLC; column: Zorbax SiL 4.6 x 250, mobile phase were isooctane : acetic acid : ethyl acetate : DMP (2,2-dimethoxypropane) = 98.5 : 0.7 : 0.7 : 0.1, detector UV at 290 nm.

Results

Heat stability

All vit E isomers in unsaponifiable fraction of rice bran decreased in proportion to heating duration at 95 ^{o}C. However, it was interesting to note that each isomer showed different heat sensitivity in that after 24 hours of heating aT, aT3, gT and gT3 decreased by 27.3, 46.4, 47.4, and 32 %, respectively. Alpha T3 also exhibited the most rapid degradation in that its concentration decreased by 14.9 % (from 11.3 mg mL^{-1} to 7.4 mg mL^{-1}) within initial 8 hours of heating, compared to gT3 which decreased by 13.5 % for the same duration.

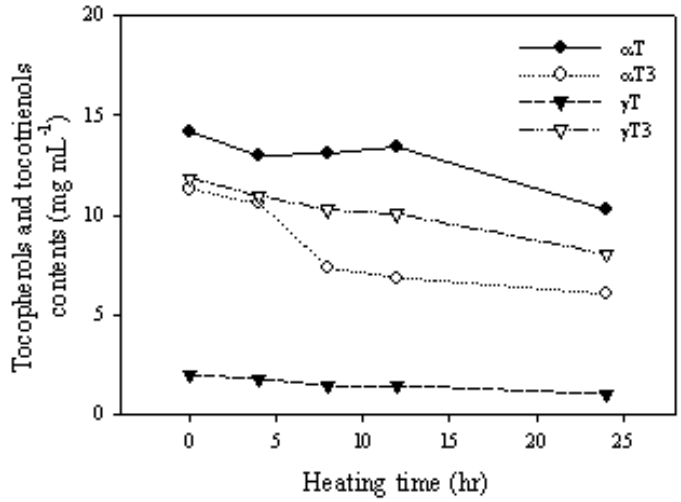

Fig. 1. Time-series changes of tocopherols and tocotrienols concentration in unsaponifiable fractions from rice bran under high temperature conditions.

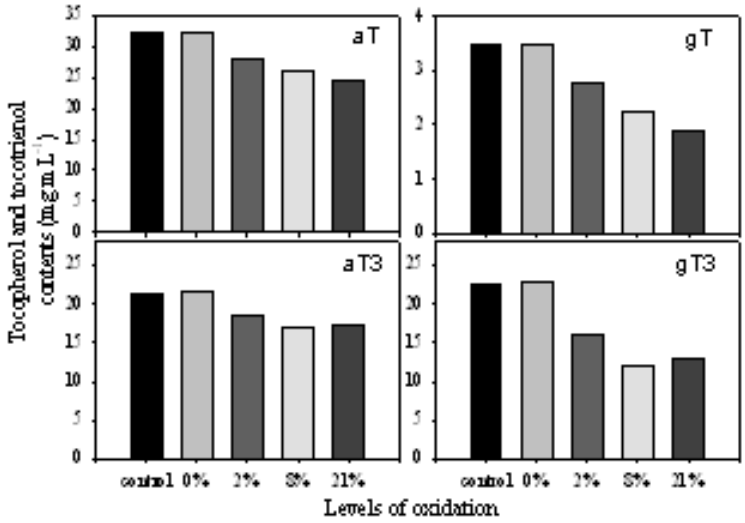

Fig. 2. Stability of alpha (A) and gamma (B) tocopherols and alpha (C) and gamma (D) tocotrienols under different oxygen conditions.

Oxygen stability

All tested isomers exhibited no degradation even after heating for 4 hours at 95 ^{o}C when they were exposed to 0% oxygen condition, while over 20% degradation could be observed under 21% oxygen conditions. Alpha isomers (aT and aT3) were more stable than gamma isomers (gT and gT3) in that gT and gT3 decreased by 45 %, and 43 %, respectively at ambient oxygen level (21 %), while aT and aT3 decreased by 24 % and 19 %, respectively. Those gamma form isomers even showed significant degradation (over 20 %) at oxygen concentrations as low as 2%.

Blending solution stability

Different blending solutions significantly affected the stability of gT3 in unsaponifiable fraction of rice bran. Organic solvents such as isooctane and hexane were more effective in preventing gT3 degradation than edible oil. Among tested edible oils, gT3 were more stable in corn oil compared with soybean and rice bran oils.

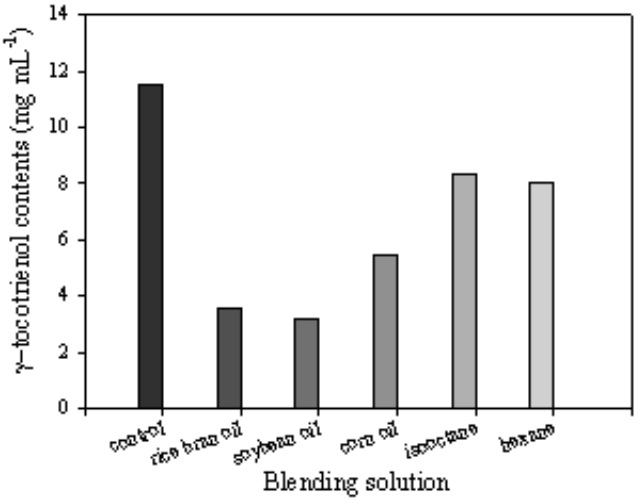

Fig. 3. Stability of gamma-tocotrienol extracted from rice bran under various blending solutions.

Conclusion

Each tocopherol and tocotrienol vitamers showed different stability under high oxygen and temperature conditions. Alpha tocotrienol was most sensitive to heat and alpha form of tocopherols and tocotrienols exhibited less degradation at high oxygen compared to gamma ones. As a blending solution, organic solvents such as isooctane and hexane were more effective for maintaining stability of gT3 compared with edible oils, among which corn oil was more efficient than soybean and rice bran oils.

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