Determination of γ -Oryzanol in Refined Rice Bran Oil by Nuclear Magnetic Resonance Method

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Summary γ -Oryzanol contained as a minor component in rice bran oil (RBO) is a group of phytosterol ferulates. The existence of γ -oryzanol in rice bran oil is very interesting because of the special biological functions. The methods to determine γ -oryzanol in crude RBO include the absorptiometry, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). However, those methods need much solvent and long operating time. Moreover, some of those methods are not able to apply to refined RBO because of the low sensitivity. We have developed the method to determine γ -oryzanol in refined RBO using proton nuclear magnetic resonance (^1H -NMR). Refined RBO had a specific chemical shift due to the methoxy group of ferulate in γ -oryzanol at 3.9 ppm on the ^1H -NMR spectrum. The intensity of the chemical shift due to the methoxy group of ferulate was consistent with the amounts of γ -oryzanol in refined RBO. This NMR method was able to quickly and easily determine γ -oryzanol in refined RBO.

Key Words NMR, γ -oryzanol, rice bran oil

Rice bran oil (RBO) which is popular edible oil in Japan, has some bioactive compounds such as tocopherols, tocotrienols and phytosterols. Especially, γ -oryzanol contained as a unique minor compound in RBO is a group of phytosterol ferulates. The existence of γ -oryzanol in rice bran oil is very interesting, because γ -oryzanol has special biological functions such as antioxidant, antiinflammatory and antitumor activities (1). Generally, crude and refined RBO contain 1.4–2.9% and 0.2–0.4% γ -oryzanol, respectively. Nowadays, several methods (2–4) to determine γ -oryzanol in RBO include the absorptiometry, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). However, these methods have some problems. The absorptiometry cannot be applied to refined RBO because of the low sensitivity. The TLC method is lack in the accuracy. HPLC methods using normal- and reversed-phase columns have much solvent, although they are effective for determining γ -oryzanol in refined RBO, and separating and identifying molecular species of γ -oryzanol. Moreover, both TLC and HPLC methods need long operating time. In some cases, they need pretreatment such as solid phase extraction to concentrate γ -oryzanol. The rapid and easy method is necessary to routinely determine γ -oryzanol in refined RBO.

Nuclear magnetic resonance (NMR) is a technique used to obtain physical, chemical, and structural information about molecules due to the chemical shift of the resonance frequencies of the nuclear spins in the sample. The NMR analysis is one of nondestructive, easy and rapid operating methods. Moreover, the NMR analysis needs few deuterated chloroform (CDCl_3) and deuterated water (D_2O) as solvents.

The NMR analysis is often applied to fats and oils.

Some researchers (5–7) applied ^1H -NMR to measure the fatty acid composition of edible oils. In particular, polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid contained in fish oils are determined by ^1H -NMR. Mono-, di- and tri-acylglycerol isomers can be identified by ^1H - and ^{13}C -NMR. Oxidation products such as alkanal and alkenal are measured in oxidized oil by ^1H -NMR.

We tried to apply ^1H -NMR to determine γ -oryzanol in refined RBO, because the operating time of ^1H -NMR analysis is short (within several min) and few solvent is necessary.

Materials and Methods

Refined rice bran oil (RBO) and canola oil were provided by Tsuno Food Industrial Co. and The Nisshin Oil-lio Group, Ltd., respectively. γ -Oryzanol (purity >98%) and CDCl_3 containing 0.05% tetramethylsilane (TMS) were purchased from Wako Pure Chemical Industries, Ltd.

Two hundreds micrograms of RBO or canola oil which γ -oryzanol was added at the level of 0.08–1.5% (w/w) was mixed with 400 μL CDCl_3 , and the sample solution was put in a dedicated tube for NMR. The ^1H -NMR analysis was performed using Bruker Ascend 400 NMR in the following condition: Magnetic field strength, 400 MHz; spectral width, 8,012 Hz; integration, 16; temperature, 298 K. The ^1H -NMR analysis was accomplished within 5 min.

Results and Discussion

Figure 1 shows the ^1H -NMR spectrum of γ -oryzanol. Many peaks were observed in chemical shift at the range of 0 to 8 ppm. Multiple chemical shifts due to ferulate were observed at more than 3 ppm, and chemical shifts at the range of 0 to 2 ppm were derived from phytoster-

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ols (mainly 24-methyl cycloartenol, 75%). In particular, the strongest peak of chemical shift at 3.9 ppm was due to methoxy group ($-\text{O}-\text{CH}_3$) in ferulate.

Figure 2 shows the ^1H -NMR spectra of canola oil with and without γ -oryzanol. Chemical shift due to the methoxy group of ferulate at 3.9 ppm was observed for canola oil added with γ -oryzanol, while canola oil had no chemical shift at 3.9 ppm.

^1H -NMR spectra were measured for canola oils which γ -oryzanol was added at the concentration of 0.08 to 1.5% (w/w), in order to obtain the calibration curve.

Fig. 1. ^1H -NMR spectrum of γ -oryzanol.

The calibration curve was produced using the ratio of the peak intensity of chemical shift due to the methoxy group at 3.9 ppm against that of TMS at 0 ppm and the amounts of γ -oryzanol added to canola oil, as shown in Fig. 3. The ratio of the peak intensity of chemical shift due to the methoxy group against that of TMS showed the value between 0 and 7. The ratio was higher with increased amounts of γ -oryzanol added in canola oil. There is a good positive correlation ($r=0.998$) between the intensity of chemical shift due to the methoxy group and the level of γ -oryzanol at the range between 0.08% and 1.5% (w/w). The quantitative limit was about 0.1%.

Commercial refined RBO were applied to the NMR method to determine their γ -oryzanol. Figure 4 shows the ^1H -NMR spectra of two refined RBO containing 0.37% and 1.39% (w/w) γ -oryzanol, respectively. Chemical shift due to the methoxy group of ferulates was observed at 3.9 ppm in two refined RBO, although refined RBO and canola oil had oleic acid (18:1) as major fatty acid and considerable amounts of tocopherols and phytosterols. The peak intensity of chemical shift at 3.9 ppm of refined RBO containing 1.39% γ -oryzanol was stronger than that of refined RBO containing 0.37% γ -oryzanol. The ratio of the peak intensity of chemical shift due to the methoxy group against that of TMS obtained from both refined RBO was applied to the calibration curve. As a result, the level of γ -oryzanol in refined RBO contained at 0.37% and 1.39% were estimated as 0.35% and 1.43%, respectively. The amounts of γ -oryzanol in refined RBO estimated by NMR method were consistent with those by HPLC method.

From these results, the NMR method could be effective to determine the total amounts of γ -oryzanol in refined RBO. The absorptiometry is one of rapid and easy methods to determine γ -oryzanol in RBO. However, it is applicable for crude RBO but not refined RBO, because it

Canola oil

+ 1.5% γ -oryzanol

Fig. 2. ^1H -NMR spectra of canola oil with and without γ -oryzanol.

Fig. 3. Calibration curve of γ -oryzanol. Fig. 4. ^1H -NMR spectra of refined rice bran oils.

is lack in the sensitivity. Thus, HPLC methods are generally used to determine γ -oryzanol in refined RBO. However, HPLC methods sometimes need pretreatment such as solid phase extraction to extract and concentrate γ -oryzanol from RBO. Moreover, HPLC methods using normal- and reversed-phase columns use much solvent. The NMR method we have applied has no pretreatment, few solvent and short operating time. The NMR method could be a rapid and easy method to determine γ -oryzanol in refined RBO but also crude RBO.

Disclosure of State of COI

No conflicts of interest to be declared.

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