산·학·연 논단

Functional Beef Product Containing Rice Bran Extracts Influence Cholesterol Oxidation and Nutritional Profile

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INTRODUCTION

The beef industry is continually developing new products to improve health and nutrition. The products should meet the demand of today's consumer relative to nutritive value and taste. It is known that rancidity is a major deterrent to acceptable taste in precooked beef products (1-3). Thus, the use of antioxidants would be an approach to reduce rancidity in meat products. The values of certain red meat cuts might be enhanced through restructuring techniques from nutritional and functional points of view. Several chemical compounds in rice bran have functional potential as natural antioxidants, including tocopherols, tocotrienols, γ-oryzanol and inositol phosphates.

Beef is an excellent source of high quality nutrients including most minerals, many water-soluble vitamins and all eight essential amino acids (3). However, beef is high in fat, particularly saturated fat and cholesterol, and is practically devoid of complex carbohydrate which is nutritionally important. Rice bran is high in fibers like cellulose and hemicellulose (3) and fat-soluble vitamin E (4) and possesses several plant sterol compounds that are thought to have a positive effect on cardiovascular diseases (5). However, rice bran has been found to impair mineral bioavailability. Beef enhances mineral bioavailability when rice bran is consumed in the diet concurrently (6-8). Thus, combining these nutritional properties into a single product might constitute a nearly ideal product.

This combination could also provide complimentary functional attributes. This is especially true with regard to lipid and cholesterol oxidation in that rice bran possesses several compounds that can act as antioxidants. The antioxidant compounds, which can influence the nutritional properties and oxidative stability in the meat product, mainly come from the nonsaponifiable fraction of rice bran oil. Furthermore, the nonsaponifiable fraction present in rice bran oil might be responsible for lowering cholesterol. The hypercholesterolemic property is believed to be due to its high content of tocotrienols, γ-oryzanol, β-sitosterols and other nonsaponifiables (9). Those specific compounds also have other important beneficial effects on human health, which is that they act as an antioxidant to protect the oxidative stability of lipids *in vivo* and *in vitro*. By including rice bran in beef products, it may be possible to achieve a high degree of lipid stability; lipid instability is the primary reason for deterioration in precooked beef products. However, previous research has discovered that whole rice bran increased an undesirable cereal flavor and caused a decrease in overall acceptability of ground patties with rice bran (10). Thus, access to a semi-purified fiber component from rice bran may be necessary to serve as a water binding agent and source of complex carbohydrates.

Smith (11) indicated that cholesterol autoxidation proceeds by a free radical mechanism, similar to autoxidation of unsaturated fatty acids (12). Factors affecting rate and onset of lipid oxidation might be the same as those of cholesterol oxidation. As a result, several reactive components like cholesterol oxide products can be generated (12). Because there appears to be no practical method to lower cholesterol in certain foods like muscle tissue, research directed to protect against cholesterol oxidation in a meat product might be more valu-

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able. Techniques are being developed to lower cholesterol oxide content of the product.

The purpose of this research was to develop a beef product that included purified components of rice bran, which would maximize sensory and nutritional properties as well as antioxidative properties and result in a functional food from the point of view that it could possess therapeutic value.

EXPERIMENTAL METHODS

Formulations and processing procedures

Standard meat processing approaches was used by using state-of-the-art facilities in the Muscle Foods Processing Laboratory of the LSU Agricultural Center. Low-fat beef (<10 % fat) was used in all experiments. Added ingredients included rice bran components, water, salt, and phosphate. Types (and levels) of rice bran components included rice fiber (3%), rice bran oil (0%, 1%, 2%) and the mixture of rice fiber (3%) and rice bran oil (2%). Storage stability was evaluated during refrigerated storage.

Procedures of cholesterol model system

A solid phase extraction (SPF) method was used to extract a purified nonsaponifiable fraction from rice bran using C18-SPE tubes. Cholesterol (500 ppm) and the extracted nonsaponifiable fraction (0 ppm, 700 ppm, 1400 ppm, 2100 ppm) were added to the mixture of SDS and buffer. The pH was adjusted to 5.5 (the typical pH of meat products) and copper II sulfate (0.005 M) was added to promote autoxidation. The cholesterol dispersion model system was incubated at 80°C to increase the rate of cholesterol autoxidation over time (0, 4, 8, 12, 16 h). The cholesterol dispersion was extracted with diethyl ether by liquid-liquid extraction and the measured parameters were sterol compounds such as cholesterol and 7-ketocholesterol, vitamin E vitamers, and γ-oryzanol.

Analytical procedures

Moisture, lipid, and free fatty acid were determined by standard AOAC (13) procedures. Vitamin E vitamers and γ-oryzanol were determined by HPLC procedures that have been reported by Shin et al. (14). Cholesterol and cholesterol oxide product (7-ketocholesterol) were determined using HPLC methods newly developed. Fatty acid composition was determined by gas chromatography using transesterification for fatty acid derivatization (15). Oxidative rancidity was determined by the thiobarbituric acid assay as described by Tarladgis et al. (16).

Consumer sensory analysis

Untrained consumers (n=41) without age or sex were randomly recruited in the Louisiana State University. They evaluated beef roast samples for acceptability of appearance, texture, flavor, and color, and overall liking, using a 9-point hedonic scale (1=dislike extremely, 5=neither dislike nor like, and 9=like extremely). Two sessions (before storage and after 4 d of storage) were conducted with the same panels for each session.

Statistical analysis

A randomized complete block (replication, n=3) design with 3×3 (treatment level×storage time level) factorial arrangement for beef product and 4×5 (treatment level×incubation time level) factorial arrangement for model system were used. The General Linear Model (GLM) was applied at α=0.05 for statistical significance (17). Means were separated ($p \leq 0.05$) using Least Significance Difference (LSD). Analysis of variance (ANOVA) was used to analyze consumer sensory data.

RESULTS AND DISCUSSON

The effect of rice bran oil and fiber on nutritive aspect

Table 1 depicts the fatty acid composition of restructured beef roasts supplemented with rice bran oil. The major fatty acids in restructured beef roasts include the saturated fatty acids (SFA); myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids, and the unsaturated fatty acids (UFA); palmitoleic (C16:1), oleic (C18:1) and linoleic (C18:2) acids. Palmitic acid (C16:0) and stearic acid (C18:0) represented a high proportion of the saturated fatty acids in the beef roasts. Although stearic acid is one of the major saturated fatty acids in beef roasts (14.46% of total fatty acids and 27.78% of saturated fatty acids), this fatty acid is not highly associated with cardiovascular disease (18) and is possibly neutral in terms

Table 1. Fatty acid profiles of restructured beef roasts containing different levels of rice bran oil (RBO)

Treatment		Fatty acids (wt. % of total fatty acids)									
		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	SFA[1)]	UFA[2)]	S/U[3)]
	RBO	0.13[4)]	17.53	1.33	-	44.77	34.91	1.32	18.99	81.00	0.23
0%	RBO	2.85	34.75	4.22	14.46	40.99	2.73	-	52.06[a5)]	47.94[b]	1.09[a]
1%	RBO	2.65	34.00	4.09	13.63	40.98	4.64	-	50.32[a]	49.71[b]	1.01[a]
2%	RBO	2.36	33.08	3.34	12.93	41.88	4.41	-	48.37[a]	51.63[b]	0.94[b]

[1)]Saturated fatty acids (wt. % of total fatty acids)
[2)]Unsaturated fatty acids (wt. % of total fatty acids)
[3)]Saturated fatty acids/Unsaturated fatty acids
[4)]Each mean (n=3) is based on % total fatty acid.
[5)]Means with different letters in each column are significantly different ($p<0.05$).

of its effect on plasma cholesterol in humans (19). On the other hand, palmitic acid, which is the most abundant saturated fatty acid in beef roast (34.75% of total fatty acids and 66.75% of saturated fatty acids), raises plasma cholesterol in humans (18,19). Adding 2% rice bran oil to the beef roast formulation changed the saturated fatty acids/unsaturated fatty acids ratio (S/U) (Table 1).

The total vitamin E content was significantly higher ($p<0.05$) in roasts containing 2% RBO and 3% RF. There was no difference ($p>0.05$) between the controls and the samples with 3% RF (Table 3). Rice-bran oil contains 0.1%~0.14% vitamin E vitamers and 0.9%~2.9% γ-oryzanol (20), which are known as potent antioxidants. Rice-bran oil also contains a relatively high level of tocotrienols, which inhibit cholesterol synthesis and lower serum cholesterol (21). The hypocholesterolemic properties of rice bran oil might be due to its high content of tocotrienols, γ-oryzanol, β-sitostenols and other nonsaponifiables (9). Those specific chemical compounds also have another important beneficial effect on human health in that they act as antioxidants to protect oxidative stability of lipids *in vivo* and *in vitro*.

Rice bran also contains both insoluble fiber such as cellulose and soluble fiber such as hemicellulose. Soluble fiber in bran binds to cholesterol and bile acids in the gastrointestinal tract to reduce the absorption of cholesterol (12). Therefore, soluble dietary fiber from rice bran and the nonsaponifiable fraction from rice bran oil have a positive effect on blood cholesterol levels.

The effect of rice bran oil and fiber in oxidative stability

Data in Table 2 shows that at 0 day, the TBARs for the control was higher ($p<0.05$) than for samples containing RF and RF/RBO. The higher TBARs of the controls (Table 2) may be due to an increase of reaction between substrate like linolenic acid and catalyst like heme iron during storage and the cooking process. TBARs of samples with either 3% RF or the 2% RBO/3% RF mixture were not significantly different during 4 and 8 days refrigerated storage and those values were lower than those of the controls (Table 2). This may result from phytic acid in rice fiber, which acts as an antioxidant. Phytic acid is a powerful inhibitor of iron-driven hydroxyl radical formation because it can form a unique iron chelate, which becomes catalytically inactive (23). In addition, inositol 1,2,3-trisphosphate and inositol 1,2,3,6-tetrakisphosphate, which can be pro-

Table 2. Main effects of storage and treatments for measured properties of restructured beef roast

Variables	Storage days	Treatment		
		Control	3% RF	2% RBO & 3% RF
Moisture (w/w %)	0	67.02[a]	66.56[a]	63.65[b]
	8	66.11[c]	65.82[c]	63.28[b]
Total lipid (w/w %)	0	5.09[a]	5.02[a]	7.59[b]
	8	5.55[a]	5.19[a]	7.61[b]
Cholesterol (mg/100 g)	0	45.90[a]	45.46[a]	45.39[a]
	8	45.01[a]	44.34[a]	44.19[a]
7-Keto cholesterol (μg/g)	0	1.29[a]	1.34[a]	1.63[a]
	4	2.10[b]	1.69[a]	1.88[a]
	8	8.02[c]	2.24[ab]	1.91[a]
TBARs (mg TBARs /kg)	0	0.15[a]	0.06[d]	0.08[c]
	4	0.19[d]	0.10[e]	0.09[ce]
	8	0.39[f]	0.12[e]	0.11[e]

For each variable, values within each row and each column with unlike letters are different ($p<0.05$). Each value represents a mean of experimental replication (n=3) with duplicates of each sample.

duced by dephosphorylation of phytic acid by either chemical or enzymatic hydrolysis, have been reported to be responsible for the inhibition of iron-catalyzed hydroxyl radical formation (23,24).

Cholesterol oxidation may decrease the concentration of cholesterol in food during storage. There was no significant change in the content of cholesterol during 8 days of storage even though cholesterol oxidation may have occurred (Table 2). 7-Ketocholesterol is one of the first COPs to form and one of the oxidized compounds found in highest abundance in food. Without exception, 7-ketocholesterol significantly increased ($p<0.05$) during storage in control samples (Table 2). The increase during storage was similar to that of TBARs. These trends suggest that lipid oxidation and cholesterol oxidation possess similar mechanisms. The correlation between 7-ketocholesterol and TBARs values was 0.96 (r^2) as shown in Fig. 1. The inclusion of RF and RBO showed a significant and beneficial effect in suppressing cholesterol oxidation, the formation of 7-ketocholesterol and lipid oxidation in restructured beef roasts (Table 2).

The loss of total vitamin E during storage (Table 3) may be due to the interaction between vitamin E and the free radicals generated during lipid oxidation. Tocopherol has been identified as a sacrificial inhibitor of the propagation stage of lipid oxidation (25). The significant decrease in α-tocopherol in all samples during storage (Table 3) was also seen by Aoyama et al. (26). α-Tocopherol is unstable in many food products during storage. In this experiment, α-tocopherol was depleted first during storage and γ-tocopherol was destroyed more slowly (Table 3). Yet, α-tocotrienol was not different until 4 days of storage, similar to γ-tocopherol (Table 3).

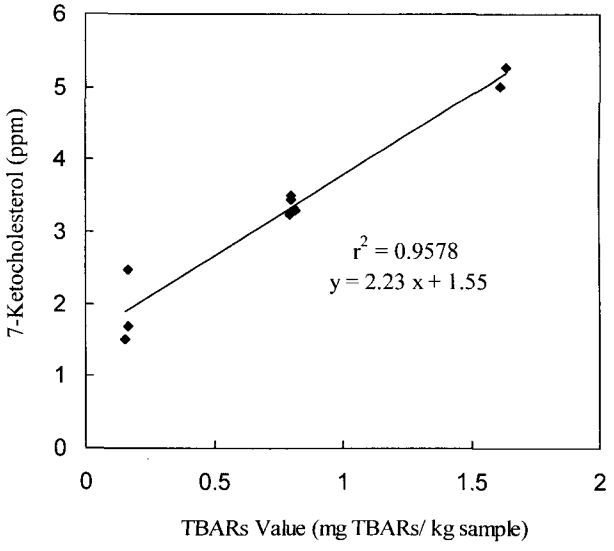

Fig. 1. Correlation of thiobarbituric acid reactive substances (TBARs) value with 7-ketocholesterol content of restructured beef roasts during refrigerated storage.

Table 3. Changes in vitamin E vitamer content (μg/g) of restructured beef roasts during storage at 4°C

Storage day	Treatment	α-T	α-T3	γ-T	γ-T3	Total
0	Control	2.61^a	0.29^a	0.04^a	0.27^a	3.21^a
	3% RF	2.58^a	0.30^a	0.07^b	0.29^a	3.24^a
	2% RBO & 3% RF	2.77^b	0.30^a	0.17^c	2.28^b	5.52^b
4	Control	1.17^c	0.31^a	0.03^a	0.08^b	2.13^c
	3% RF	1.95^c	0.29^a	0.06^b	0.11^b	2.43^c
	2% RBO & 3% RF	2.25^d	0.28^a	0.16^c	0.73^c	3.42^e
8	Control	1.09^e	0.14^b	0.03^d	0.01^c	1.28^d
	3% RF	1.25^e	0.06^b	0.04^d	0.05^c	1.40^d
	2% RBO & 3% RF	1.64^f	0.21^b	0.13^f	0.50^d	2.48^f

For each storage period and each column, values with unlike letters are different ($p<0.05$). RBO is rice bran oil; RF is rice fiber; T=tocopherol; T3=tocotrienol.

Consumer acceptability

The sensory attributes of a food are very important to overall acceptance (27). An effect of the treatment on sensory attributes was not detected ($p>0.05$) among samples (Table 4). This indicates that the samples containing additives may be as acceptable as the control. Nevertheless, samples containing rice fiber, either 3% RF or the 2% RBO/3% RF mixture, had slightly lower acceptability compared with the control and samples with only added 2% RBO. This is an effect of the cereal flavor note from fiber that could be imparted to the beef products. Sharp and Kitchens (28) indicated that the addition of rice bran into bakery products imparted an unpleasant cereal flavor.

There were no effects of treatment on sensory attributes after 4 days of storage (Table 4). This indicates that oxidized flavor was not evident in cooked meat during 4 days of refrigerated storage. The off-flavor that occurs after the cooking of meats is generally called warmed-over-flavor (WOF) and is due to lipid oxidation. However, this data indicate that TBARs values in the range of 0.08~0.19 do not affect the sensory attributes. The use of either 3% RF or the mixture of 2% RBO

Table 4. Sensory attribute data for restructured beef roasts

Treatment	Storage days	Sensory attributes mean score					Acceptability (%)
		Appearance	Flavor	Color	Texture	Overall liking	
Control	0	4.88[a]	5.66[a]	5.07[a]	5.66[a]	5.46[a]	73.2
	4	4.83[a]	5.39[a]	4.83[a]	5.54[a]	5.32[a]	61.0
3% Fiber	0	5.10[a]	5.41[a]	4.98[a]	5.44[a]	5.47[a]	58.5
	4	5.34[a]	5.12[a]	5.29[a]	5.41[a]	5.32[a]	61.0
2% Oil	0	5.12[a]	6.05[a]	5.34[a]	5.44[a]	5.66[a]	73.2
	4	5.24[a]	5.76[a]	5.34[a]	5.71[a]	5.66[a]	80.5
2% Oil & 3% Fiber	0	5.10[a]	5.39[a]	4.85[a]	5.51[a]	5.37[a]	61.0
	4	5.37[a]	5.29[a]	5.29[a]	5.46[a]	5.29[a]	68.3

Numbers in parentheses refer to standard deviation of 41 consumer responses. A 9-point hedonic scale was used (1=dislike extremely, 5=neither dislike nor like, 9=like extremely). Mean values with common letters in the same column are not different ($p<0.05$).

and 3% RF in beef roast formulations resulted in similar TBARs values after 4 days of refrigerated storage. TBARs data indicate that the addition of RBO and RF to beef roasts may effectively inhibit oxidation. Nevertheless, this sensory data showed that there were no significant differences in acceptability of the beef roasts containing RF/RBO compared to the control.

Antioxidative properties of nonsaponifiable fraction from rice bran

Table 5 shows the retention of total vitamin E vitamers and γ-oryzanol during cholesterol autoxidation in a model system. The trends of retention of total vitamin E vitamers and γ-oryzanol in different concentrations of the nonsaponifiable fraction during the cholesterol autoxidation at 80°C were similar. However, in the case of the higher concentration (2100 ppm) of the nonsaponifiable fraction, the retention remained more stable after 4 h cholesterol autoxidation compared with the other two concentrations in which there was a significant decrease in total vitamin E during oxidation. This may be due to the relatively high concentration of γ-oryzanol, which is more heat-stable, and a sacrificed consumption of vitamin E vitamers during the initial stage of oxidation. The molar concentration of γ-oryzanol in rice bran is about five times higher than total vitamin E in rice bran (14), and the nonsaponifiable fraction (4.2%) present in rice bran shows total vitamin E vitamers at 0.08 % and γ-oryzanol at 1.6% (5). Vitamin E vitamers are stable at elevated temperature in the absence of oxygen. The rate of oxidation of vitamin E vitamers, however, is accelerated by heat under aerobic conditions. Vitamin E vitamers are lost if peroxidizing lipids are present (29). Therefore, vitamin E vitamers may be oxidized by the co-oxidation reaction of cholesterol in an aqueous dispersion system. Fig. 2 shows the time course for the loss of individual vitamin E isomers. The isomers had similar trends with time. The decomposition of vitamin E isomers increased with increasing

Table 5. Retention of vitamin E vitamers and γ-oryzanol in a model system during a 16 h incubation at 80°C

(Unit = ppm)

Time(h)	Vitamin E vitamers			γ-Oryzanol		
	700 ppm	1400 ppm	2100 ppm	700 ppm	1400 ppm	2100 ppm
	(Nonsaponifiable fraction)			(Nonsaponifiable fraction)		
0	8.30[a]	17.06[a]	24.60[a]	255.00[a]	523.47[a]	739.88[a]
4	5.36[b]	10.76[b]	18.53[b]	168.17[b]	401.73[b]	618.61[b]
8	1.61[c]	4.16[c]	10.10[c]	118.12[c]	311.35[c]	613.18[b]
12	0.47[d]	1.34[d]	6.30[d]	92.46[d]	234.63[d]	599.92[b]
16	0.20[d]	0.74[d]	3.09[d]	73.70[d]	224.65[d]	528.23[b]

For each column, means with different letters are significantly different ($p<0.05$).
Each value represents mean of replication (n=3) with duplications of each sample.

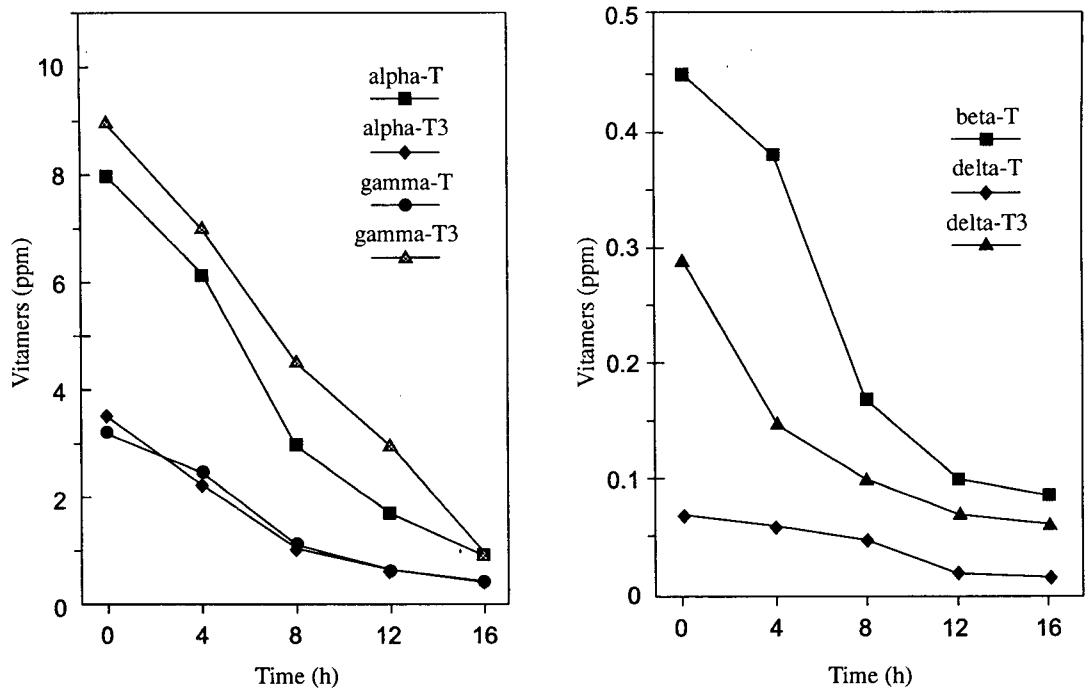

Fig. 2. Time course of changes of tocopherols (T) and tocotrienols (T3) in a cholesterol aqueous dispersion at 80°C.

oxidation time.

Fig. 3 illustrates the decrease of ratio of 7-keto-cholesterol/cholesterol due to the increasing concentration of nonsaponifiable fraction. In contrast to the nonsaponifiable fraction treatment, the ratio of 7-keto-cholesterol/cholesterol in the control sample increased greatly during cholesterol oxidation. Okada and Yamaguchi (30) reported that in a 0.01% α-tocopherol and oryzanols solution, α-tocopherol had higher antioxidant activity than oryzanol. Increasing the concentration of α-tocopherol up to 0.5% did not increase the antioxidant activity, but with a smilar increase in oryzanols the activity increased proportionally. An antioxidative function of γ-oryzanol has been reported (30). γ-Oryzanol is a mixture of ferulate esters with sterols and triterpene alcohols, predominantly β-sitosterol, campasterol, cycloartenol, and 24-methylene cycloartenol (20). It has been suggested that the phenolic hydroxyl group in the ferulate esters of oryzanol might be responsible for its antioxidative function. Generally, phenolic antioxidants inhibit lipid oxidation by trapping the peroxy radical to yield the hydroperoxide. They prevent the reaction of the peroxy radical to produce a lipid radical and propagate a free radical chain reaction. Chimi et al. (31) reported that the rates of reaction of aroxyl radical, which is produced after penolic acid donates a hydrogen proton to a peroxy radical, exceed the rates of reaction of another aroxyl radical, which produces free radicals. This mechanism may be related to oxidation inhibition by phenolic compounds.

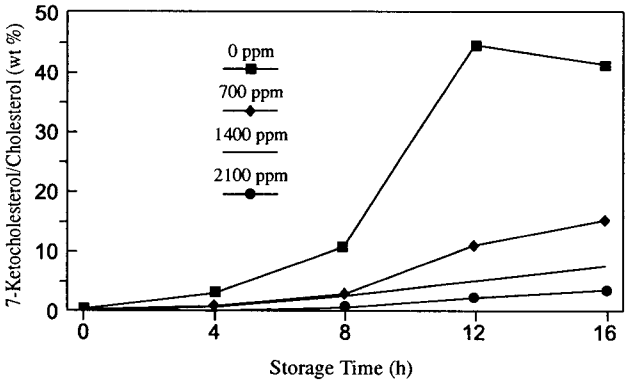

Fig. 3. Time course of cholesterol autoxidation in aqueous dispersions with different concentrations of nonsaponifiable fraction of rice bran.

As seen in Fig. 3, this indicates that additions of 700 to 2100 ppm of nonsaponifiable fraction from rice bran had an antioxidant effect in an aqueous model system during the 16 h incubation at 80°C. Vitamin E vitamers and γ-oryzanol in the nonsaponifiable fraction from rice bran may inhibit the formation of 7-hydroperoxy cholesterols formed at the initial stage of cholesterol oxidation

and reduce the formation of 7-ketocholesterol. There is some evidence that the hydrophilic-lipophilic properties of antioxidants may influence their effectiveness (12). Based on the greater antioxidative effect at higher concentration of nonsaponifiable fraction, cholesterol autoxidation in aqueous model system may require a more lipophillic antioxidant.

CONCLUSIONS

The addition of natural antioxidants in rice bran into food containing a high level of lipid and cholesterol might bring several benefits in that it increases nutritional value, improves oxidative stability, and provides many phyto-chemicals related to nutraceutical or health-promoting ingredients. This study shows the antioxidative effects of semi-purified compounds such as RBO and RF and nonsaponifable fraction from rice bran, but it does not indicate the antioxidant effects of each compound from vitamin E isomers and γ-oryzanol in inhibiting the lipid and cholesterol oxidation. The effects of each compound need to be further researched to understand its mechanism more completely in preventing the oxidation of food with high levels of lipid and cholesterol during processing and prolonged storage.

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