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Inhibition of Warmed–Over Flavor (WOF) and 7-Ketocholesterol in Refrigerated Precooked Pork Patties containing Commercial γ-Oryzanol and α-Tocopherol

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상업적 γ-Oryzanol과 α-Tocopherol 첨가에 따른 냉장 조리 돼지고기의 Warmed-over flavor(WOF)와 7-Ketocholesterol 제어 효과

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ABSTRACT – The oxidative stability of refrigerated precooked pork patties containing commercial γ-oryzanol and α-tocopherol was evaluated. Precooked pork patties containing either γ-oryzanol or α-tocopherol showed higher oxidative stability ($p<0.05$) during storage at 4°C than did the precooked pork patties without the additives (control). The thiobarbituric acid-reactive substances (TBARs) values and warmed-over flavor (WOF) of the precooked pork patties containing γ-oryzanol or α-tocopherol were lower ($p<0.05$) than those of the control during refrigerated storage (0, 1, 4, and 8 days). The correlation between TBARs and WOF values was significant ($p<0.05$). 7-Ketocholesterol content was lower ($p<0.05$) than those of the control during refrigerated storage (0, 6, 12, 18, and 24 days). The correlation between TBARs values and 7-ketocholesterol content was also significant ($p<0.05$).

Key words: pork, warmed-over flavor, γ-oryzanol, 7-ketocholesterol

Introduction

Warmed-over flavor (WOF), which is caused by the lipid oxidation of uncured precooked meat, is unacceptable to the consumers due to the rancid taste[1,2]. In general, the rate of lipid oxidation in the meat product is affected by the contents of lipid, unsaturated fatty acids, and antioxidant compounds as well as the preparation techniques and the storage time[2,3]. In addition to lipids, cholesterol is also susceptible to oxidation even at an ambient temperature; when exposed to air, cholesterol oxide products (COPs), which may be toxic to cells and possibly be involved in the cardiovascular disease (CVD), are formed. 7-Ketocholesterol is one of most common COPs found in the muscle foods containing high levels of cholesterol during the cooking process or prolonged storage[4,5]. Because both polyunsaturated fatty acid (PUFA) and cholesterol oxidation are preceded by a free radical mechanism, the compounds that inhibit the PUFA oxidation may also inhibit the oxidation of the cholesterol. Extrinsic factors such as the addition of antioxidants during processing, cooking or packaging can be manipulated to enhance the oxidative stability of the cooked meat[6,7]. Both synthetic and natural antioxidants have been used in the meat products to control the lipid oxidation[2].

γ-Oryzanol, a rice bran extract, is known to be a powerful inhibitor of the iron-driven hydroxyl radical formation and has also been reported to exert antioxidant activity in the stabilization of lipids[2,8-10]. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxy-toluene (BHT), have been used for many years to increase the oxidative stability in the processed meat products[2]. However, the current trend in the food industry is the use of natural antioxidants, which are preferred by consumers due to their safety[2,8]. The nutritive value of the pork product and the stability against oxidative degradation would be increased

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by the incorporation of natural antioxidants into the pork product. Our objective was to investigate the oxidative stability of the refrigerated precooked pork patties containing γ-oryzanol or α-tocopherol necessary for preventing the development of WOF and the formation of 7-ketocholesterol during storage.

Materials and Methods

Preparation of pork patties

Lean pork from the shoulder butt of the Korean grade carcasses and the subcutaneous fat from the same carcasses were ground separately through a 1.0-cm plate, then reground through a 0.8-cm plate. The fat was standardized to 30% by combining the lean and the fat proportionately and regrinding through a 0.64-cm plate. The meat was fabricated into 60-g (±0.6 g) patties and placed into the glass Petri plates (100 mm × 200 mm) with covers. The concentrations of the active components used in the meat samples were 120 ppm γ-oryzanol, 120 ppm α-tocopherol, and 60 ppm γ-oryzanol+60 ppm α-tocopherol (on the fresh meat basis). The additives were dissolved in 2 mL ethanol and vigorously mixed for 2 min in a blender. This concentration was chosen because it fulfilled the legal requirement of the maximum usage of additives, which should not exceed 0.02%. After a through mixing, the samples were made into four 60-g (±0.6 g) patties. Ethanol was added to the control samples and treated similarly as the experimental samples. Both patty samples were cooked on a grill for 7 min/side until the internal temperature reached 72±1ºC. The cooked samples were placed in their respective Petri plates and stored in a refrigerator (4ºC) for 1, 4, and 8 days to develop WOF. The sensory and the chemical determinations were made at 0, 1, 4, and 8 days.

Sensory analysis

Sensory analysis was conducted after 1, 4 and 8 days storage by a 10-member panel. The panelist training involved five 1-hour sessions. The panelists were familiarized with the test procedures and the characteristic flavor (WOF) before tasting the cooked pork patties stored at 4ºC for 0 to 8 days. They were then asked to evaluate each treatment using a 9-point continuous scale from 1=extremely low intensity to 9=extremely high intensity based on WOF.

Chemical analysis

Proximate analyses for the contents of moisture, protein, fat, and ash were performed in triplicates for all treatments as outlined by AOAC[11]. TBARs values were determined at 0, 1, 4, and 8 days using a distillation method of Tarladgis et al.[12] as modified by Ockerman[13] to measure the level of lipid oxidation. Results were expressed as mg TBARs/kg of meat.

Cholesterol and 7-ketocholesterol analysis

Lipid extraction and cold saponification

Five-gram portion was prepared from a randomly selected 60-g pork pattie and mixed into 50 mL of 2:1 (v/v) chloroform/methanol for lipid extraction [14]. Subsequently, 10 mL of 1.5 N KOH in methanol was added to the sample, which was then shaken until the mixture became free of the dispersed fat particles. Saponification was conducted at room temperature overnight (18-20 h). The extraction method reported by Kim et al.[2] was used to obtain the nonsaponifiable from the saponified mixture.

High-performance liquid chromatography (HPLC)

The HPLC systems employed for the analyses of 7-ketocholesterol and cholesterol were an LC-Si column (25 cm × 4.6 mm i.d. Supelco Co., Bellefonts, PA, USA) and a C18, 5 μm, 100 A column (Rankin LC and Supplies, Waburn, MA, USA), respectively. The mobile phases consisted of hexane/isopropyl alcohol (97.5:2.5) for 7-ketochoelsterol and methanol/acetonitrile (7:3) for cholesterol. The mobile phases were pumped at the isocratic condition and the UV detection wavelength of Tunable Absorbance Detector (Water™ 486, Waters, Milford, MA, USA) was 230 nm for 7-ketocholesterol and 207 nm for cholesterol. Cholesterol and 7-ketocholesterol contents were determined at 0, 6, 12, 18, and 24 days of storage. Because sensory evaluation of the meat samples is generally not carried out for more than 8 days, this protocol was adopted in the present investigation. However, unlike the sensory analysis, the analysis times of cholesterol and 7-ketocholesterol were extended because these compounds were relatively stable during short storage period.

Statistical analysis

A randomized complete block design with either 4×4 or 4×5 factorial arrangement was used. Replications (n=3) were blocked and the additive treatment and the incubation time were the main treatment factors. The General Linear Model (GLM) procedure was applied to the data at a level of $p<0.05$ for statistical analysis[15] and Least Significance Difference (LSD) was used to compare the mean differences among the treatments. Pearson's correlation coefficient was also used to determine the relationships between among various combinations of the variables such as TBARs, 7-ketocholesterol, and sensory WOF values measured[15].

Results and Discussion

Proximate analysis indicated the contents of moisture (39.97% ± 2.58), fat (28.25% ± 2.17), protein (24.37% ± 2.18), and ash (4.57% ± 0.58) of each treatment and replication were not affected by the additive treatments. The TBARs values and WOF scores of the precooked pork patties are presented in the Table 1. TBARs value for the control was higher ($p<0.05$) than those of the samples with additives after 4 days storage. After 1 day of storage at 4°C, the TBARs values increased drastically ($p<0.05$) in the control samples, but not in the treated samples. Moreover, the TBARs values of the treated samples were not significantly changed at day 4 compared to day 1. The higher TBARs of the control may be due to an increased interaction between the substrate and the catalyst during storage and cooking. Such process may result in an increased degradation of the heme compounds that are hypothesized to be responsible for the lipid peroxidation[1-3]. Sensory WOF scores (Table 1) also increased as the storage time increased. The WOF scores of the treated samples were not significantly changed at day 4 compared to day 1, in particular, the WOF scores of the samples treated with γ-oryzanol (120 ppm) did not change significantly up to 8 days of storage. This could be due to the interruption of the radical chain mechanisms by the phenolic compounds such as γ-oryzanol and α-tocopherol. The TBARs values and the sensory WOF scores showed similar trends during storage. The correlation between TBARs values and WOF scores determined through the sensory analysis was significant ($p<0.05$) at 0.9008 (r^2) (Fig. 1) (Table 3), an indication that

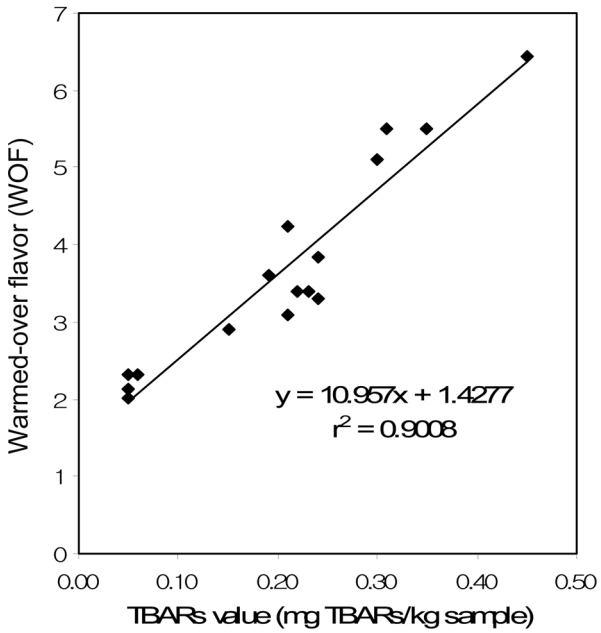

Fig. 1. Correlation of thiobarbituric acid reactive substances (TBARs) with sensory warmed-over flavor (WOF) intensity of precooked pork patties stored at 4°C.

Table 1. TBARs values and sensory warmed-over flavor (WOF) in precooked pork patties containing additives stored at 4°C for 0, 1, 4, and 8 days

	Variables							
	TBARs (mg/Kg) [a]				Sensory WOF [a]			
Treatment	0	1	4	8	0	1	4	8
A	0.06 a	0.24 b	0.35 c	0.45 d	2.32 a	3.31 b	5.51 c	6.43 d
B	0.05 a	0.22 b	0.24 b	0.31 f	2.31 a	3.40 b	3.84 b	5.50 c
C	0.05 a	0.21 b	0.23 b	0.30 f	2.01 a	3.10 b	3.40 b	5.11 c
D	0.05 a	0.15 e	0.19 be	0.21 b	2.13 a	2.90 ab	3.61 b	4.23 bc

[a]For each variable, means (n=3) within each row and each column with unlike letters are significantly different ($p<0.05$). A=control, B=60 ppm α-tocopherol+60 ppm γ-oryzanol, C=120 ppm α-tocopherol, D=120 ppm γ-oryzanol.

Table 2. TBARs values and 7-ketocholesterol concentrations of precooked pork patties containing additives

	Variables								
	TBARs (mg/Kg) [a]				7-Ketocholesterol (ìg/g) [a]				
Treatment	0	1	4	8	0	6	12	18	24
A	0.06 a	0.24 b	0.35 c	0.45 d	0.27 a	1.88 b	5.32 d	5.98 d	8.60 f
B	0.05 a	0.22 b	0.24 b	0.31 f	0.29 a	0.80 c	2.92 e	3.05 e	5.03 d
C	0.05 a	0.21 b	0.23 b	0.30 f	0.28 a	0.78 c	2.99 e	3.11 e	5.11 d
D	0.05 a	0.15 e	0.19 be	0.21 b	0.30 a	0.73 c	1.92 b	2.99 e	3.04 e

[a]For each variable, means (n=3) within each row and each column with unlike letters are significantly different ($p<0.05$). A=control, B=60 ppm α-tocopherol+60 ppm γ-oryzanol, C=120 ppm α-tocopherol, D=120 ppm γ-oryzanol. Patties were stored at 4°C.

Table 3. Correlation coefficients for important relationships

	Correlation coefficients	
	TBARs [a]	Sensory WOF [a]
7-Ketocholesterol	0.9529*	
TBARs		0.9491*

[a] Rhiobarbituric acid reactive substances (TBARs), warmed-over flavor (WOF).

*$p<0.05$ for H_0: $\rho=0$ (H_0: $\rho=0$, null hypothesis: the relationship between 2 variables is not correlated).

they may be useful for studying the WOF development. The low scores of the sensory WOF among the samples with the added antioxidants (Table 1) could be related to the presence of the antioxidants, which inhibited the reactive peroxy radicals to yield hydroperoxides responsible for the increase of the TBARs values. WOF is generally accepted as a byproduct of the oxidation of fatty acids in the intramuscular phospholipid of meat, and thus can be inhibited by the same means of the lipid oxidation inhibition[16]. The significant correlation ($p<0.05$) between TBARs values and sensory WOF scores suggests that the TBARs values or the sensory WOF scores with their corresponding times could be used for the kinetic study on the WOF development in the meat products. No significant differences in the cholesterol contents were found among the additive treatment samples during storage; whereas the cholesterol contents of the control samples changed significantly after 12 days (Fig. 3). The decrease in the cholesterol content during storage of the samples may be due to the cholesterol autoxidation. 7-Ketocholesterol significantly increased ($p<0.05$) during storage in all treatment samples; however, no significant difference was observed between 12 and 18 days (Table 2).

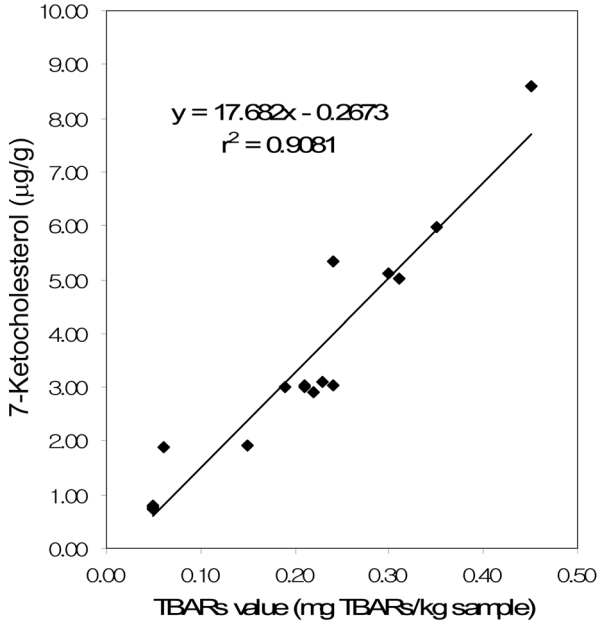

Fig. 2. Correlation of thiobarbituric acid reactive substances (TBARs) with 7-ketocholesterol concentration of precooked pork patties stored at 4°C.

At 18 days, 7-ketocholesterol content of the control increased two-fold compared to those treated with the additives (Table 2). Similar trends were found in TBARs values and 7-keto-cholesterol values during storage. The correlation between 7-ketocholesterol and TBARs values was 0.9081 (r^2) (Fig. 2), an indication that the null hypothesis (H_0: $\rho=0$) was rejected at the significance level of $p<0.05$ (Fig. 3). Because PUFA and cholesterol are integral components of the membrane structure and are susceptible to autoxidation[2,3], the free radicals formed by phospholipid oxidation may

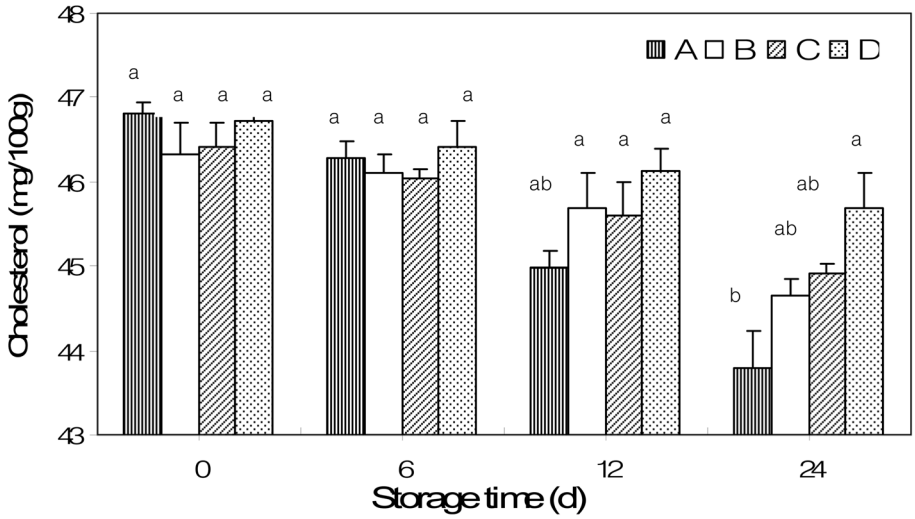

Fig. 3. Cholesterol concentration in precooked pork patties containing additives. Patties were stored at 4°C for 0, 6, 12, and 24 days. A=control, B=60 ppm α-tocopherol+60 ppm γ-oryzanol, C=120 ppm α-tocopherol, D=120 ppm γ-oryzanol.

initiate the cholesterol oxidation in the tissue membranes of the precooked pork patties. This study demonstrated that the treatment of the precooked pork patties with a commercial natural γ-oryzanol extracted from the rice bran could effectively improved the flavor and the oxidative stability of the patties during refrigerated storage compared to the treatments with a commercial α-tocopherol alone or with a combination of γ-oryzanol and α-tocopherol. Thus, inclusion of a natural-based commercial γ-oryzanol into the precooked meat products provides a safe alternative way to prevent the meat product from undergoing damages caused by lipid and cholesterol oxidation during the refrigerated storage.

요 약

가열 조리된 냉장 돼지고기 패티에 사용된 상업용 유통 γ-oryzanol과 α-tocopherol 의 항 산화 효과가 돼지고기 육질 내의 지질 산화로부터 기인되는 warmed-over flavor (WOF) 값과 콜레스테롤 산화로부터 기인되는 이차 산화물인 7-ketocholesterol의 생성 추이 변화로 비교 분석되었다. 지질산화로부터 측정된 파라미터는 이화학 데이터인 TBARs 값과 관능 데이터인 WOF 값에 의해 비교 분석되었으며, 콜레스테롤 산화로부터 유래된 측정 파라미터는 산화 유도기간에 따른 가열 조리된 돼지고기의 콜레스테롤 함량 변화 정도와 주요 콜레스테롤 산화물인 7-ketocholesterol 생성 정도에 의해 비교 분석 되었다. 콜레스테롤과 콜레스테롤 산화물은 cold saponification 추출 기법과 고속액체크로마토그래프 (HPLC) 테크닉을 이용하여 정량적으로 분석되었다. 아울러, 측정된 각 파라미터 변수들 간의 통계적 상관관계가 검토되었으며, 가열 조리된 돼지고기 패티의 산화로부터 기인되는 이차 산화물에 대한 γ-oryzanol과 α-tocopherol의 항 산화력이 비교 분석되었다. 분석 결과 모든 파라미터 변수들은 서로 간에 유의적인 상관관계를 나타내었으며, 특히, γ-oryzanol 처리 군에서 유의적인 지질 및 콜레스테롤 항 산화 효과를 보였다.

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