

Effects of *Rhus verniciflua* Stokes Extract, Gallic acid, and Fisetin on the Lipid, Protein, and Myoglobin Oxidation in Hanwoo (Korean Cattle) Beef Model System

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Abstract— This research was conducted to investigate the effects of RVS water extract (15 ppm), gallic acid (50 µM), and fisetin (50 µM) on the lipid, protein, and myoglobin oxidation in Hanwoo (Korean cattle) beef homogenate system. The experimental homogenates (*M. longissimus dorsi*) were incubated under oxidized condition by Fe (III)/ascorbic acid at 37°C for 7 hr. Trolox equivalent antioxidant capacity (TEAC), using ABTS⁺ radical scavenging activity assay, and ferric reducing antioxidant power (FRAP) were increased significantly ($P < 0.05$) by all additions. Gallic acid and fisetin resulted in the highest ($P < 0.05$) FRAP and the highest ($P < 0.05$) TEAC, respectively. The inhibition of TBARS level was obtained by all antioxidants and was in following the order: fisetin > RVS extract > gallic acid. Carbonyl content was inhibited by RVS extract and fisetin. Fisetin was more effective than RVS extract for the inhibition of protein oxidation. Myoglobin oxidation was not inhibited by all antioxidants. Furthermore, gallic acid and fisetin accumulated the metmyoglobin formation. These results suggest that RVS extract is more advantageous than gallic acid and fisetin for improving the storage stability of beef products.

Keywords— *Rhus verniciflua* Stokes extract, gallic acid, fisetin, model system, Hanwoo beef.

I. INTRODUCTION

From about 4,000 years ago, *Rhus verniciflua* Stokes (RVS) belonging to the ivy (Anacardiaceae) has been used for medicine in South Korea, China, and Japan [1]. The extract of RVS contains a variety of polyphenol compounds (butein, butin, gallic acid, fisetin, fustin, sulfuretin, and quercetin etc.) [2, 3, 4] and has beneficial effects, such as antioxidant, anticancer, antimutagenic, anti-inflammatory, antithrombotic, and anti-obesity [5, 6, 7]. Fisetin (3,7',3',4'-tetrahydroxyflavone; 5-deoxyquercetin) and gallic acid (3,4,5-trihydroxybenzoic acid) are rich in various plants as well as in RVS [8, 9]. These

compounds have a powerful free radical scavenging activity and ferric iron chelating ability [10, 11], also pharmacological effects, such as anticancer and anti-inflammatory [12, 13, 14, 15].

The antioxidant effect of RVS extract in liposome model system and meat has been reported by Liang et al. [16]. However, there is still little information on effect of extract and compounds of RVS on the radical scavenging activity, ferric iron reducing ability, and chemical oxidation in meat system. Therefore, this research was conducted to investigate the effects of RVS extract, gallic acid, and fisetin on the antioxidant status in beef homogenate model system oxidized by Fe (III) and ascorbate.

II. MATERIALS AND METHODS

A. Preparation of RVS extract

Thirty grams of RVS heartwood powder and 500 mL of deionized water were boiled at 100°C for 3 hr. After cooled in the room temperature, RVS extract was filtered with a Whatman filter paper No. 1 and a 0.45 µm syringe filter. The soluble matter content of RVS extract was 0.5% with a refractometer (PAL-03S, Atago Co., Ltd., Japan).

B. Preparation of meat homogenate model system

Fresh *M. longissimus dorsi* from 28 months-old-Hanwoo (Korean cattle) steer and 9 volumes of 0.12 M KCl-5 mM histidine buffer (pH 7.0) were homogenized at 15,000 rpm for 2 min. Meat homogenate was filtered with a cheese cloth, centrifuged at 2°C, 600 g for 10 min, and then incubated at 37°C under oxidized condition by 30 µM FeCl₃ and 100 µM sodium ascorbate (Final concentration). Before oxidation, deionized water (control), 50 µM gallic acid, 50 µM fisetin, and 15 ppm

RVS extract (Final concentration) in model system. At 0, 3, and 7 hr of incubation, meat homogenates were mixed with 0.02% BHT [Final concentration; 17] for stopping the oxidation and stored at -80°C until measurement.

C. Trolox equivalent antioxidant capacity and ferric reducing antioxidant power

Trolox equivalent antioxidant capacity (TEAC) was determined by ABTS⁺ radical scavenging activity assay of Re et al. [18] and calculated as μM trolox using inhibition rate (%) of ABTS⁺ radical and trolox (0–20 μM) standard curve. Ferric reducing antioxidant power (FRAP) was performed by Benzie and Strain [19] and calculated as μM Fe (II) using standard (1000 μM ascorbate) and curve of 100–1000 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$.

D. TBARS and DNPH-carbonyl

TBARS (2-thiobarbituric acid reactive substances) was performed by described as Siu and Draper [20] and expressed as ng malondialdehyde (MDA) per mg protein. Protein concentration was performed by biuret method [21]. DNPH-carbonyl was determined by Oliver et al. [22] and calculated as nmol DNPH per mg protein using millimolar extinction coefficient of protein hydrazones [$21.0 \text{ mM}^{-1}\text{cm}^{-1}$; 23] and BSA standard curve.

E. Oxymyoglobin and metmyoglobin concentrations

Oxymyoglobin (OxyMb) and metmyoglobin (MetMb) concentrations were performed by described as Krzywicki [24] and calculated as relative percentage (%) of myoglobin derivatives (OxyMb + MetMb + DeoxyMb).

F. Statistical analysis

Data was analyzed by ANOVA (Analysis of variance) of SPSS [25]. Significant differences among means were determined by the Duncan's multiple range tests at $P < 0.05$.

III. RESULTS AND DISCUSSION

Fig. 1 shows the effect of RVS extract, gallic acid, and fisetin on the TEAC and FRAP in Hanwoo beef homogenate model system. Both TEAC and FRAP were significantly ($P < 0.05$) increased by all additives. Particularly, gallic acid- and fisetin-added beef homogenates had the highest ($P < 0.05$) TEAC and highest ($P < 0.05$) FRAP, respectively. But RVS extract-added beef homogenate showed the lowest ($P < 0.05$) effects in both TEAC and FRAP. Fisetin and gallic acid have antioxidant effects on free radicals and ferric iron [10, 11]. As well, fisetin is one of quercetin derivatives as 5-deoxyquercetin. According to Rice-Evans et al. [10], quercetins have higher radical scavenging activity compared with gallic acid.

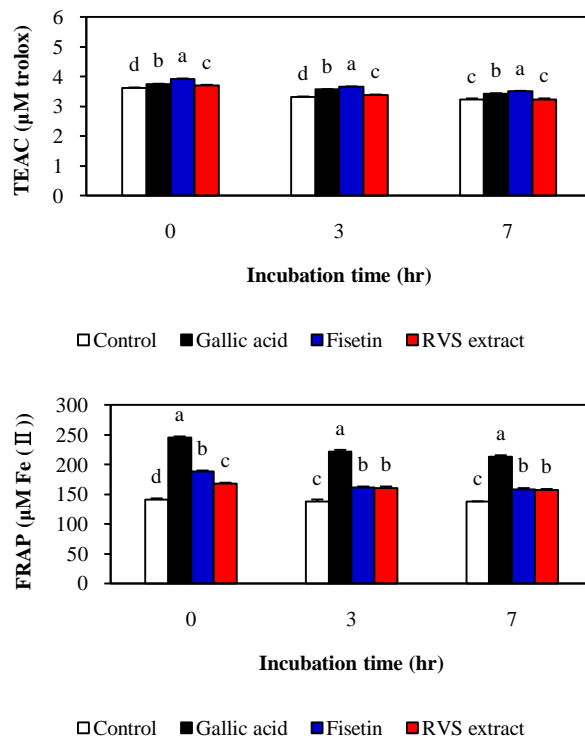


Fig. 1 Effect of RVS extract, gallic acid, and fisetin on the TEAC and FRAP in Hanwoo (Korean cattle) beef homogenate model system oxidized by Fe (III) and ascorbate.

^{a-d} Means \pm S.E. with different letters indicate significant differences between treatments at $P < 0.05$.

As shown in Fig. 2, TBARS level in beef homogenate was retarded by addition of all additives. Fisetin-added beef homogenate had the strongest antioxidant effect on TBARS development but gallic acid-added beef homogenate showed the lowest effect. DNPH-carbonyl content (Fig. 2) was inhibited by RVS extract and fisetin. Fisetin-added beef homogenate had the highest antioxidant effect on carbonyl accumulation. In experiments of liposome and beef homogenate model systems of Liang et al. [16], similar results, RVS extract strongly inhibited lipid oxidation, have been reported.

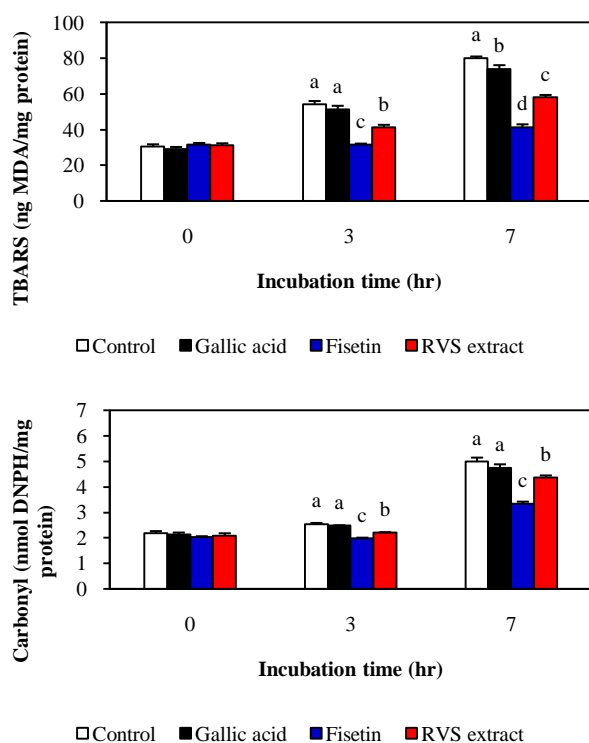


Fig. 2 Effect of RVS extract, gallic acid, and fisetin on the TBARS level and DNPH-carbonyl content in Hanwoo (Korean cattle) beef homogenate model system oxidized by Fe (III) and ascorbate.

^{a-d}Means \pm S.E. with different letters indicate significant differences between treatments at $P < 0.05$.

Fig. 3 indicates the effect of RVS extract, fisetin, and gallic acid on the myoglobin oxidation in beef homogenate. The generation rate of MetMb was faster in RVS extract-, fisetin-, and gallic acid-added beef

homogenates compared with the control. Thus, all additives decreased myoglobin oxidation stability in beef homogenate. Hayes et al. [26] also have suggested that sesamol, one of polyphenol compounds, promoted myoglobin oxidation in meat homogenate model system.

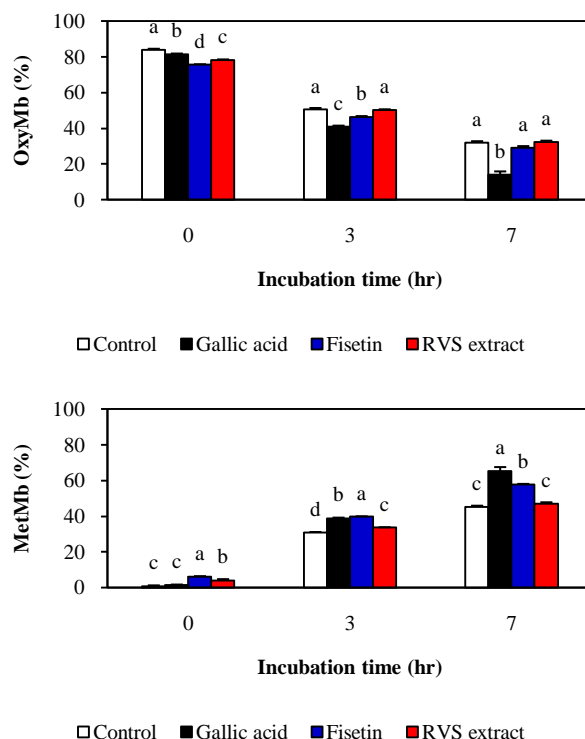


Fig. 3 Effect of RVS extract, gallic acid, and fisetin on the myoglobin oxidation in Hanwoo (Korean cattle) beef homogenate model system oxidized by Fe (III) and ascorbate.

^{a-d}Means \pm S.E. with different letters indicate significant differences between treatments at $P < 0.05$.

IV. CONCLUSIONS

Although addition of RVS extract, gallic acid, and fisetin increased total antioxidant activity in beef homogenate, gallic acid had small effect on lipid and protein oxidation. Moreover, all additives did not have antioxidant effect on myoglobin oxidation.

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