PHENOLOXIDASE AND ANTIOXIDANT IN KOREAN GINSENG

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ABSTRACT

Enzymatic browning is considered desirable in tea and tobacco processing but undesirable in many fruits processing at the present time. It is necessary to understand the nature of the enzyme, phenoloxidase, in order to control browning reactions, and extend its effects to formation of browning products as antioxidants in ginseng.

Ginseng exhibits antioxidant activity when incorporated with turkey dark meat patties. The activity in red ginseng showed about two times stronger than white ginseng. One of the phenolic antioxidants from fresh, white and reprocessed white ginseng was identified as phenol 2.6 Bis (1.1 dimethyl ethyl) 4-methyl among several unknown compounds by GC/mass spectrometer.

In red ginseng, no phenol 2.6 Bis (1.1 dimethyl ethyl) 4-methyl was detected, the compound may be polymerized by phenoloxidase and form some higher molecular compounds which may possess high antioxidant activity.

Phenoloxidase isozymes in fresh Korean ginseng (*panax ginseng* C.A. Meyer) were extracted with phosphate buffer at pH 7.3. The isozymes were purified through ammonium sulfate fractionation, dialysis and chromatography on a DEAE-cellulose column. Two groups of phenoloxidase were shown to be present, one in the floating agglomerated group and the other in the precipitate group from the 0.85 saturation ammonium sulfate. The DEAE-cellulose column chromatography, the phenoloxidase isozyme present in the precipitate appears as the first peak (I), and that in the agglomerate in the second peak (II). Isozyme I showed higher activity with catechin and catechal, and isozyme II showed higher activity with p-cresol. The isozyme showed two optimum pH activity one at pH 4.5 and the other at 8.5 with catechin as substrate.

Korean ginseng phenoloxidase has high heat stability. When heated at 75°C for 2 hours, its activity remained 90% and 80% on phenoloxidase I and II respectively.

Phenoloxidase I was most active on (+) catechin followed by p-cresal, catechol and epicatechin. Phenoloxidase II was most active on p-cresal followed by (+) catechin, catechol, p-coumanic acid and epicatechin.

Sodium bisulfite, sodium cyanide, ascorbic acid glutachion in the oxidized form, sodium diethyl dithiocarbomate and ethylendiamine tetra acetate (EDTA) acted as inhibitors.

Red ginseng color development was initiated by phenoloxidase and finished by a followed sun drying process. The antiaging activity of ginseng may be initiated by the antioxidant in the ginseng.

INTRODUCTION

Korean ginseng (*Panax ginseng* C.A. Meyer) belongs to the araliace family. It is a perennial plant grown in Korea (33-46°C), China and the USSR. It takes an average of five years from seedling to harvest. White ginseng (minimum 4-years-old) is prepared from fresh ginseng by cleaning it in cold water and drying it in sunlight, or in hot air and/or in a vacuum. Red ginseng is prepared from good quality fresh ginseng (6-years-old) by cleaning it in cold water and subsequent heat treatment (near boiling temperature) for about three hours and sundrying it for about one week. In addition to these three major products, at present many kinds of ginseng products are developed from them, such as ginseng tea, cake, candy, drinks, tablets capsules, pills, syrup and wine.

In Asia, ginseng is considered one of the most unique herbs and was used by royal families. According to the Pen-tsao Kang Mu (Chinese Medicinal Herbs) which was compiled by Li-Shih-chen, now translated into English by Forter Smith and G.A. Stuart (1973), "*Panax ginseng* is the medicine *par excellance*; – reserved for the emperor and his household, and conferred by Imperial favor upon high officials whenever they have a serious breakdown that does not yield to ordinary treatment and which threatens their lives". From ancient Korea (Shilla) ginseng was presented to China's emperor (Tang) (Cho, Y.B. 1978). Later Yi dynasty ginseng became one of the important trade items to China and Japan. Today, ginseng is largely managed by the Office of Monopoly in Korea. Processing of red ginseng is authorized only to the Office of Monopoly, and it is hard to buy red ginseng in Korea with Korean currency. The reasons that this unique herb was served to the emperor have not yet been unveiled. But recent scientific research explored some parts of the ginseng medicinal and biological effects with major chemical components; however, many parts remain unexplored. Some recent experimental reports of ginseng effects are as follows:

1. Helps to Cure Diabetes and Heart Disease. (due to insuline-like substance in ginseng).

According to Ando, T. et al (1980) and Yoshida (1980) pathological conditions of diabetic patients were improved by administering red ginseng which contained insuline-like peptides and adenosine. They isolated these two active compounds from ginseng. As reported by Sohn, E.S. et al (1978), the Korean ginseng when given alone, was effective in lowering blood pressure in 13 patients out of 15 (80%). Diuretics and other antihypertensive agents were added to obtain a satisfactory hypotensive effect. However, according to their report, small amounts (10 mg/kg) of ginseng induce hypertension on large amounts (100-200 mg/kg) have a hypotensive effect. Kuwasima, K. et al (1980) reported that ginseng medication resulted in marked improvement of symptoms associated with circulatory disturbance by digital phetysmorgraphy which has been shown to accurately represent heart function and peripheral hemodynamics. Chang et al. (1978) conducted a clinical survey on 120 cases of gynecological laparotomies postoperative recovery with ginseng-triol for a 6-month period at Seoul National University and one of their significant findings was a lower serum cholestoral level in the ginseng-treated group, and the serum protein was significantly increased in the treated group. These results may indicate ginseng effects on diabetes and heart disease remedy.

2. Restore Youth (anti-aging and prevent wimkling)

Han, B.H. et al. (1i78) demonstrated the antioxidant activity from the extract of Korean ginseng in order to prove the anti-aging effect. They isolated and characterized one effective components; 2-methyl-3-hydroxy-r-pyrone (maltol) from Korean ginseng. Kim M.W. et al. (1980) also mentioned that antioxidant from Korean red and white ginseng have anti-aging effects and they obtained two fractions and confirmed both have antioxidant activity. And recently Paik, T.H. et al. (1982) also confirmed the antioxidant activity

in ginseng; however, they did not identify the antioxidant compounds. Stengel, F. and Listbarth, H. (1968) report that reduction of skin dryness and reduction of skin wrinkles in elderly people (approximately 68% between 71 and 90 years old) with creams and cosmetics containing ginseng. Forgo. 1. (1980) conducted several experiments on 60 males and 60 females with two GINSANA capsules per day (equivalent to 1,000 mg of *Panax ginseng* root) for 12 weeks and compared them to a group given placebo (gelatin capsules with glycerin) and concluded that ginseng is not a doping drug and the 40-to-60 year-old group showed significantly shortened reaction time and significantly improved in all the respiratory parameters at the end of the 12-week treatment.

3. Natural Tranquilizer (central nervous system depression and stimulation).

Betterman, A.A. (1980) conducted his experiment at Hamburg Hospital, Germany by a double-blind study with ginseng, with subjects ranging in age from 15 to 78 years (average 48 years) and have concluded that it did not cure all psychosomatic disease but provided a remedy for the "psychosomatic health" of all patients. However, Siegel, R.K. (1980) conducted his experiment on four monkeys (Macaca Mulatta) with ginseng and mentioned that gross behavioral changes included increased in vocalization activity, stereotype movements, and weight loss. These results are consistent with previous animal studies demonstrating stimulant effects on psychosomatic performance from ginseng. And Oh, J.S. (1969) report in the results of their experiment on the central nervous system of rats that small amounts of ginseng acted as stimulants and large amounts acted as sedatives. A more specific report from Saito, H. (1973) concluded that the unique ginseng constituents; ginsenoside Rg_1 exhibits central nervous system stimulating action.

4. Tonic (anti-fatigue and rapid recovery from abnormal conditions)

Fulder, S. et al. (1980) reports his experimental results on students nurses at Maudsley Hospital, Camberwell, London. They described that ginseng consistently improved the ratings of tests on mood, competence and general performance, even though the subjects did not sleep as much after taking it. There were also improvements in the objective tests of psycho-physical performance. Finally, they concluded that ginseng does have anti-fatigue and tonic effects on man. A report (Yonezawa et al. 1980) on X-ray (700-Rad) radiated mice (40), showed that 82.5% of the mice who received ginseng (6.8 mg ginseng extract/animal) recovered from the radiation, whereas only5% of the control group recovered. Johnson, A. et al. (1980) conducted their double-blind clinical study with Korean, American and Siberian ginseng on 36 male and 2 female student volunteers (average 25-years-old) and concluded that ginseng improved proof reading error detection and improved the mood-fatigue level (Korean and American ginseng only) but not significant affect occurred on mathematical performance and final grade performance. A more specific report made by Saito, H. et al (1974), ginsenoside Rg_1 showed antifatigue effects in every test of male mice (ddy-strain 20-23 g). As mentioned above, ginseng has tonic effects which show anti-fatigue and rapid recovery from abnormal conditions.

5. Gonadotropic Activity

According to the report of Ishigami, J. (1973), he cured 14 out of 24 (58%) men with a weak and low sperm count (2 to 3 years they remained childless after marriage) using medicine containing ginseng for about 4 to 8 weeks of treatment. A report by Yamanoto, M. (1973) demonstrated that ginseng increased testicle cell division and DNA bio-syntheses. Kim, Chul, et al. (1978) reported that the interintromission period, the ejaculatory latency and the post-ejaculatory interval were significantly reduced in the ginseng group while the occurrence of ejaculation was significantly greater and the number of the copulation plugs deposited was

significantly larger in the ginseng group, based on their experiment with male rats.

6. Prolonged Life

Nagasawa, T. et al. (1977) reported that ginseng consists of some active components which induce RNA polymerase in the liver, accelerates biosynthesis of Renal Nuclear RNA, protein synthesis, and blood protein synthesis. Han, B.H. et al. (1972) reported that of 60 species of crude drugs screen tests, ginseng showed anti-inflammatory (anti-rheumatic) activity based on the stabilizing activity of serum albumin. A report (Fulder, S. 1977) indicated an increased dose of ginseng extract increased the mitogenic action of cultured cells similar to hydrocortison. He indicated that these are also capable of delaying cellular necrosis! A recent report was conducted (Chao, C.Y. et al. 1980) about ginsenoside Rg_1 and Rb_1 of Panax ginseng on mitosis in cultured human blood lymphocytes. Rg_1 promotes mitosis in human lymphocytes activated by phytohemagglutinin. Rb_1 inhibits mitosis in activated human lymphocytes and lengthens the period, but both did not arrest the mitotic cells at any particular stage. According to these results, ginseng could extend our life if one consumed it appropriately (1,000-3,000 mg/day) and cure or prevent many kinds of diseases.

Antioxidants need not only fatty foods but also food that can be stored for long periods of time. For this, several antioxidants such as Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallat, (PG) and tertbutyl hydroquinene (TBHQ) are allowed to use food-system levels up to 0.01% based on the fat content of the food. However, consumers do not understand the benefits of preservatives and avoid eating them. Thus, there is a recent trend toward natural food products. Hence, artificial preservatives may need to be replaced by natural antioxidants to preserve our food. At present, the well-known natural antioxidants are tocopherol and ascorbic acid (Renton and Rogstand 1981). In addition of these compounds, there are some other phenolic compounds such as flavonoids (Yamaguchi, N. 1975) and amino acid, peptide and related compounds (Yamaguchi, 1977, Hayes, R.E., et al. 1977 and Lingnert and Erickson 1980). In the food system, natural spices used mainly for flavor, but it contributes antioxidant activity (Chipault, J.R. et al. 1952) to the food. And some other compounds such as hydroxy N-heterocyclic compounds were also reported as antioxidant (Kawashima, et al. 1979). Many kinds of natural antioxidants are explored for preserving our food system. Among them, we will focus only on antioxidants, and browning products (enzymatic and non-enzymatic) which are related to red ginseng.

Oura, et al. (1975) reported that oxidation of linoleic acid was strongly retarded by several phenolic compounds such as catechol, 3-4 dihydoxytoluene and resorcinol. Some moderate antioxidants were DOPAMINE, DOPA, noradrenaline, adrenaline, and gallic acid. However, no activity was seen on chlorogenic acid, pyrogallol, phloroglucinol or phenylalanine. On the other hand, when browning product of the phenolic compounds was induced by polyphenoloxidase, the antioxidative activity was much enhanced: such as 3.4-dihydroxytoluene, catechol, DOPAMINE, pyrogallol, noradrenaline, adrenaline DOPA, and gallic acid based browning product, in that order of strength. However, some substances such as chlorogenic acid, and resorcinol based products did not increase antioxidant activity. The most dramatically increased antioxidant activity item was pyrogallol (POV% from 80.0 to 7.2) and the next was adrenaline (POV% from 63.3% to 10.0) based enzymatic browning compounds.

Similarly, non-enzymatic browning compounds also exhibit antioxidant activity. Beckel and Waller (1983) report that antioxidant from the maillard reaction product of xylose and arginine. Kawashima, et al. (1977) report that carbonyl compounds (methylglyoxal, glyoxal, glyoxylic acid and dihydroxyacetone) when heated (100-175°C) with common amino acid in corn oil, the browning compounds obtained from the reaction exhibit antioxidant activity. Yamaguchi, N. (1980) report that browning

products were generated by the reaction of reducing sugar and amino acids. He fractioned it and found such a high antioxidant activity substance as melanoidin. Its molecular weight was estimated at 4,500 dalton. They reported that measured antioxidant activity from these products was higher than BHA but lower than BHT. Then certain browning products generated by enxymatic or non-enzymatic reaction, exhibit antioxidant activity.

Several natural antioxidants are explored at present. A report (Williams and Harris 1983) described that dried oranges exhibit antioxidant activity when incorporated with frozen and unfrozen cooked meats but not in raw meat. They did not identity the responsible compounds. And Pratt, D.E. et al. (1981) report phenolic antioxidant of soy protein hydrolyzates. They indicate that responsible antioxidants are three isoflavones: genistein, daidzein and glycitein and eight phonolic acids are caffeic, ferulic, p-coumaric, syringic, vanillic, gentisic and p-hydroxy-benzoic acid. Catiganani and Carter (1982) report that Lignin exhibits antioxidant properties in the presence of oil and probably in the presence of other fat soluable substances (e.g. vitamin A and E). Antioxidant activity in *glycyrrhizae radix* was reported by Hirosue, T. et al. (1982) the activity was greater than BHA in methyl linolate system. The responsible compounds were not identified. They assumed it as a melanoidin like compound and its molecular weight was estimated at greater than 10,000 dalton.

As already discussed, ginseng exhibits antioxidant activity; however, white and red ginseng may have different antioxidant activity because red ginseng is obtained from fresh ginseng through the heating and sundry process, thus enzymatic non-enzymatic browning products are generated by the processing. Then this study was conducted to detect the presence of polyphenoloxidase (PPO) enzyme in fresh Korean ginseng and initiation of color development in red ginseng processing. And to investigate the antioxidant properties of fresh white and red.

METHODS AND MATERIALS

Materials

Red ginseng 6-year-old earth brand was obtained from the Office of Monopoly in the Republic of Korea. White ginseng 6-year-old first grade was purchased at an herb store in Seoul, Korea. Both were ground separately into powder using a standard wiley cutting mill with ½ mm sieve (Fisher cat no 8-326-10) and stored in air-tight plastic or glass sample containers. Fresh ginseng was brought from Korea (Kimpo fresh ginseng market) by air. And all samples were stored in a cold room 4°C for the experiment. Three turkeys (young tom) were purchased from a local grocery store and thawed overnight in room temperature before the experiment. U.S.P. or ACS reagent grade chemicals were used for the experiment unless otherwise stated.

Methods

Extraction of Ginseng Constitutents

200 g of red and white ginseng powder was placed separately in 2,000 ml flasks and 95% ethyl alcohol (Ca 1000 ml) was added and allihn condensor with heating mantle except soxhlet extraction tube were used. Extraction was conducted under a hood for 4 hours continuously and repeated 3 times, and the extracts were combined and condensed using the rotary vacuum evaporator. The temprature of the water bath was 45°C and vacuum was 140 mm Hg generated by an aspirator.

Turkey Meat Pattie

The turkey dark meat was deboned with skin attached so that the meat and skin were in natural propertions. The meat was cut into cubes. (2.5 cm one side) and then ground through the 10 mm plate of Hobart grinder (model 14532) and a portion of meat (227 g) was removed for fat analysis. The meat was divided into designed treatment (Table 1) and mixed with appropriate

antioxidants, ginseng powder or ginseng extract in a Leland food mixer (Model 100 DA) on low speed for 30 seconds then reground through the fine plate (4.5 mm) of the Hobart grinder. Meat patties were prepared with Hollymatic hand operated hamburger molds (Hollymatic corp.) in 100 g and placed one layer deep in 3 mili polyethylene bags, and stored at 4°C on a single layer on trays in the refrigerator. Tween 20 (Polythylene sorbitan monolaurate) was employed as the antioxidant carrier. TBHQ was chosen as the food grade antioxidant with which to compare the activity of ginseng. TBHQ was dissolved in Tween 20, with heating and stirring one day prior to the turkey processing to ensure complete dissolution. Ginseng extract was precipitated 3 to 4 hours later when dissolved in Tween 20; therefore, it was applied immediately after mixed in Tween 20.

Table 1. Experimental design for antioxidant activity confirmation in ginseng using turkey dark meat patties.

Treatment number	Antioxidant %, based on fat content	Fat content turkey meat (%)
1.	0	11.3
2.	0.01% TBHQ	11.3
3.	0.1% Red ginseng powder	11.3
4.	0.1% White ginseng powder	11.3
5.	0.005% Red ginseng powder	11.3
6.	0.05% White ginseng powder	11.3
7.	0.1% Red ginseng extract	11.3
8.	0.1% White ginseng extract	11.3
9.	0.2% Red ginseng extract	11.3
10.	0.2% White ginseng extract	11.3

Tween 20, on antioxidant carrier solution, was added to 0.27 g/kg meat for all treatments.

Thibarbituric Acid (TBA) Test

The stored ground turkey patties were evaluated in duplicate for rancidity using the 2-thiobarbituric acid (TBA) distillation method as described by Tarladgis et al. (1960) with minor modifications where the TBA dissolved in distilled water instead of acetic acid and a sorvall omni mixer (E.I. Dupont de Nenueurs and Co., Inc.) used for blending, and after adding the TBA reagent to the distillates the tubes wer stoppered and set aside for 15 hours in the dark room temperature to avoid heating in a hot water bath. And then absorbancy was measured using Bechman spectrophotometer model 35 at 530 nm.

Preparation of Red Ginseng from White and Fresh Ginseng

Fresh ginseng (water contents 7.25%) were placed on a perforated plate just above the water level in a covered water bath 75°C and held for 1, 2 and 3 hours. The heated product was sun dried for one wek. Reprocessing of white ginseng to red ginseng was done by rehydrating the white ginseng in cold water (4°C) for 24 hours (water content 72.5%) and then followed the same methods as stated above in making red ginseng from fresh ginseng. For experiment analysis, it was ground into powder as the others.

Extraction and Purification of PPO

For extraction of the enzyme, 250 g of fresh ginseng was cleaned with cold water and deionized water, dried using paper towel, and then cut into small pieces (4 cm cubes). It was divided into two parts, each part was blended with 500 ml of 0.5 M potassium phosphate buffer pH 7.3 which contained 0.5% polyethylene glycol. The resulting slurry was centrifuged at 8000 x g for 10 minutes at 2°C. The combined supernatant was brought to 0.4 saturation of Ammonium sulfate and centrifuged as the previous one, and the precipitated protein was removed. The supernatant was brought to 0.85 saturation of Ammonium sulfate and centrifuged at 20,0000 x g for 20 minutes. There were two mass groups; a precipitated one and a floating one, in the centrifuged tube. These were separately dissolved in small portions of 0.005 M potassium phosphate buffer pH 6.3 and dialyzed at 2°C in the same buffer, using a spectrapo for standard cellulose dialysis tubing (Scientific product, McGraw Park III 60085. Mol. Wt. cut off 12,000-14,000. D-1612-2) for 6 hours, with three changes of the buffer during

dialysis. Aliquot of the dialyzed enzyme extract was fractionated through a DEAE-cellulose column (Sigma corp. 0.88 meq/g), 26 x 700 mm with 500 ml of linear gradient of 0.005 M-0.05 phsophate buffer pH 6.3 (250 ml each) and then 500 ml of 0.05-0.5 M phosphate buffer pH 6.3 (250 ml each). The fractions were collected 7 ml per tube with 1 ml per minute flow rate. The fractions were monitored and recorded with a Gilson LC detector model III at 280 nm. The PPO containing protein fractions were pooled, dialyzed against 0.005 M phosphate buffer pH 6.3 at 2°C for 6 hours and then concentrated by Osmosis in a standard dialysis tubing which was surrounded by Polyethylene glycol (approximate mol. wt. 20,000 #2263, Sigma Chemical Co.) in flake form. This operation lasted for 3 hours at 2°C room.

Electrophoresis

Polyacrylamide gel electrophoresis was conducted to identify PPO enzyme and its mol. wt. according to a method described by Hedrick and Smith (1968) Gabiel (1971) and Jolly and Mason (1965) with some modification. The gel tubes (bio-rad 5.5 mm id 125 mm L) were soaked in cleaning solution (Bio-rad cleaning concentrate diluted 50 times) overnight and soaked in water and deionized water subsequently. Then the tubes were dried in an oven and cooled before use. The separating gel (small pore gel) was prepared by mixing 15 g of acrylamide (Bio-rad 99.9%) wit 0.5 g bis-acrylamide dissolved in deionized water to make 100 ml. The buffer was prepared by adding 48 ml of 1N HCI with 6.85 g TRIS per 100 ml, and 0.46 ml of TEMED was added just before use. The pH of the buffer solution was 7.6. The stacking gel was prepared mixing 10 g of acrylamide with 2.5 g of bisacrylamide, 4.0 mg of riboflavin and 40 g of sucrose dissolved in deionized water and adjusted the volume to 100 ml. 5, 6, 7 and 8% gel were prepared using abovementioned stock solution. The separating gel was filled (8 cm) in glass tubes and stacking gel was layered (2 cm) on it after the separating gel was polymerized. A fluorescent light was placed 5 cm above the upper end of the column to polymerize the gel.

The enzyme in 50% glycerol with 0.001% bromphenol blue was applied per tube, the enzyme protein applied per tube was 500-100μg. Electrophoresis was conducted in a cold room at 4°C with 2mA of current per tube on stacking gel and 4 mA of current on the separating gel. The reservoir buffer was prepared with diethylbarbituric acid 5.52 g/l with TRIS 1.0 g/l at pH 7.3. Enzyme staining solution was prepared with 0.03M L-proline with 0.03M catechol in 0.1M citrate-0.2M phosphate buffer pH at 7.3 just before use, and finished gel electrophoresis gels were soaked in the above mentioned enzyme staining solution and pinkish color bands developed in an hour. After relative mobility (Rm) value was obtained, the gels were destained in 0.1M Ascorbic solution. And the gels were soaked in the protein staining solution overnight which was prepared with 0.1% Coomassie blue R-250 (Bio-rad) in 45% methanol with 10% acetic acid. Coomassie blue R-250 (Bio-rad) in 45% methanol with 10% acetic acid. The stained gels were dipped in the destaining solution until clear protein bands were seen, using gel electrophoresis diffusion destainer (Bio-rad Model 172). The destaining solution was prepared the same as staining solution except the dye was eliminated.

The molecular weight of the enzyme protein was compared with standard protein (SDS-PAGE molecular weight standards low molecular weight range 10,000-100,000 Bio-rad). And SDS-discontinue gel electrophoresis described by Laemmli, U.K. (1970) was also applied.

Protein Determination

Protein was determined by the protein dye-binding method using Commassie brilliant blue G-250 as described by Bradfort (1976).

Assay for Polyphenoloxidase (PPO)

The PPO activity was determined by measuring the initial rate of increase of absorbance at 420 nm. unless otherwise stated, activity was assayed in a 3 ml reaction mixture, consisting of

2.8 ml of 0.01M catechol or catechin in 0.1M citrate-0.2M phosphate buffer pH 7.3 with prepared enzyme aliquot 0.2 ml at 30 ± 0.5°C using a Perkin Elmer Model 575 Spectrometer with thermostatical cell compartment, and a recorder was used to monitor absorbance change with time. Enzyme PPO activity was calculated from the initial linear portion of the curve. (1 unit equal to ΔOD of 0.001 per minute at 420 nm).

Substrate Specificity

Some less soluble compounds in cold buffer were heated in a flask in a water bath 70°C for 5 minutes. Then 2.8 ml of 0.01M each of the substrate (Table 3) in 0.1M citrate-0.2M phosphate buffer pH 7.3 reacted with prepared enzyme aliquot 0.2 ml at 30°C as stated above.

Heat Stability

A bath of water was heated to 50 ± 0.2°C with two test tubes in it. An aliquot (8 ml) of enzyme PPO was pippetted into a heated test tube, swirled quickly and at the same time timing was started. An aliquot (0.5 ml) of heated enzyme aliquot was withdrawn at predetermined time intervals and put into prelabelled test tubes which were placed in an ice bucket. This aliquot of anzyme PPO was assayed for residual activity at 30°C. The same procedure was followed at 75°C. Catechol and (+) catechin were employed as substrate.

Effect of pH

A study was made of the effect of pH on the catechol and (+) catechin oxidation by ginseng PPO. Enzyme activity was determined in 0.1M citrate-0.2M phosphate buffer at different pH values, ranging from 3.0 to 9.0.

Effect of Enzyme Concentration

The effect of enzyme concentration was investigated by holding the substrate concentration constant (2.0 ml of 0.01M catechin in 0.1M citrate-0.2M phosphate buffer pH 7.3) but varying concentration of enzyme added to the reaction mixture. The total values of the reaction mixture was also held constant (3.0 ml) by adding an appropriate amount of deionized water.

Effect of Substrate Concentration

Substrate; (+) catechin varying from 2×10^{-3} to 40×10^{-3} M were employed to study the effect of substrate concentration. In a cuvette, 0.2 ml of the enzyme solution was mixed rapidly with 2.8 ml of (+) catechin at different concentrations in 0.1M citrate-0.2M phosphate buffer at pH 7.3. Michaelis constant and maximum velocity from two isozymes were calculated from a plot of 1 over activity versus 1 over substrate by the method of Linweaver and Burk (1934).

Effect of Inhibitor

Inhibitors; sodium bisulfite, sodium cyanide, glutathione, EDTA, ascorbic acid and sodium diethyl-dithiocarbamate at 0.01M were preapred as stock solution in 0.1M citrate-0.2M phosphate buffer at pH 7.3. And added 0.1 ml of inhibitor to the 2.7 ml of (+) catechin in the same buffer solution in cuvette just before the reaction and then added 0.2 ml of PPO aliquot to see the activity of the PPO with inhibitor.

Determination of Activation Energy of Reaction

In the study of activation energy, the enzymatic reactions were run at 10, 20, 30, 40, 50, 60 and 70 degrees celsius. The non-enzymatic reactions were run at the same temperature. When the log of the slope obtained from above were plotted log K versus I over absolute temperature, the activation energy of a particular reaction could be obtained from the slope. The Arrhennius equation was employed for the more reliable determination of activation energy.

Detection of Phenolic Compounds

Detection of phenolic compounds in Korean ginseng was carried out by methods described by Walter, Jr. and Purcell (1979) with some modification. 20 g of sample (red, white and reproces-

sed) ginseng was separately poured into 500 ml of round-bottomed flasks and decocted with 100 ml in the water bath (80°C) for 30 minutes. But the fresh ginseng 30 g was sliced into Virtis mixer (The Virtis Co. Gardiner, New York, 12525) with 100 ml of boiling ethanol (95%) and homogenized (speed at 80 on the scale) for 2 minutes and cooled to room temperature and filtered using whatman filter paper #1 and the residue was extracted twice with the same method. The filterate was combined and evaporated in vacuo at 40°C. The syrup remaining was taken up in double deionized water and adjusted to 25 ml in a volumetric flask. The sample aliquot was extracted three times with equal volume of hexane to remove lipid, and centrifuged at 4000 x g and filtered through a 0.45μm membrane filter (millipore type HA). The filterate was mixed with an equal volume of n-propanol and sodium chloride was added with shaking in a separatory funnel until two phases formed. The lower layer was discarded and the upper portion removed and evaporated in rotary vacuum evaporator. The residue was dissolved with methanol and made up to 25 ml. A portion (50μl) of methanol extract was applied on thin layers of chromatography glass plate coated with Silica gel G 100μm thick (Silica gel G for ILC ACC. To sthal. EM. Laboratory Inc.) and developed by Butanol, Acetic acid and Water (4:1:2) ascending method. Then the plate was air dried and illuminated by uv-lamp in a dark place. The fluroscent spots were separately removed and placed in 80% ethanol in a beaker and eluted overnight. Then it was filtered through a 0.45μm millipore filter and dried in a rotary vacuum evaporator (Brinkman Rotovapor-r) and then it was hydrolized by 5 ml of 2N HCl at 90°C for 2 hours. The hydrolysate was extracted three times with ethyl ether, and combined extracts were dried under a stream of N2. Then it was dissolved in 1 ml of methanol for antioxidant, HPLC and GC/mass spectral analysis. The aqueous phases (ethyl ether extract) were dried in rotary vacuum evaporator. Then it was dissolved in 1 ml of double deionized water. The prepared sample aliquot was tested antioxidant activity and subjected HPLC and GC/mass spectrometry.

Antioxidant Activity Test

1.1 diphenyl-2-picryhydrazyl (DPPH) (90%) 0.0243 g was dissolved in 100 ml of ethanol (95%) and 7 ml of the above solution was diluted and made up to 50 ml using ethanol (95%). Then an adequate absorbance reading was obtained by spectronic 20 at 517 nm. 6 ml of prepared reagent (DPPH) and 0.2 ml of antioxidant aliquot was mixed in the tube and the absorbance was read, the DPPH violet color was decolorized by antioxidant, such as ascorbic acid and tocoperol, etc. The principle of the test method was referred from Blois, N. (1958).

High Pressure Liquid Chromatography (HPLC)

Isolated and purified phenolic compounds from the above method were analyzed using the HPLC system, according to the method described by Walter et al. (1979) with some modification. The instrument was a Water Associates Chromatography equipped with a model-6000 A solvent delivery system, a model 440 absorbance detector, and R401 differential refractometer and a μbk universal injector and a variable speed omniscribe recorder. The column was 30 x 3.9 mm ID stainless steel μ-Bondpak C18 (Water Associates) with precolumn packed with CO : PELL ODS (octadecyl C18) groups chemically bonded to 30-38 μ, glass bead by whatman. Several eluents (30-35% methanol was prepared under vacuum using deionized water through milli-Q system) were delivered at 1 ml/min. The column eluent was monitored at 254 nm (0.5 aufs) and the response recorded on a strip chart recorder. Several concentrations of some phenolic compounds (SIGMA Chemical Co.) were prepared in deionized water, and filtered through Mili-Q-system as standard solution and injected with some 10 to 25μl into HPLC system. The retention time and peak height were measured in order to detect unknown phenolic compounds in the samples. The sample aliquot prepared as mentioned above was also injected with 10-25μl into HPLC system and the retention time was mea-

sured with peak height.

Gas Chromatographic and Mass Spectral Analysis

A sample of aliquot (dissolved in methanol which was obtained from ethyl ether soluble portion after HCI hydrolysis) 1.5μl was injected into a Finnegan 3200 gas chromatographic-Mass spectrometer, equipped with a 30m DB-1 fused silica capillary column (J & W scientific) with helium as carrier gas (28 cm/sec) and temperature programmed 8°C/min. from 80°C to 300°C. Eluting compounds were ionized at 70 ev and the Mass spectra of the individual peak was obtained and for identification the peaks were matched against computerized systems of library mass spectra data.

RESULTS AND DISCUSSION

Antioxidant Activity Confirmation of Ginseng Using Turkey Meat

Ethanol extract from red and white ginseng were condensed by a vacuum evaporator (120 mm Hg pressure) until no ethanol smell remained, and measured the weight of each extract. The resulting products were 31% and 17% on red and white ginseng, respectively. The Higher extract obtained from red ginseng may be caused by the good quality of the ginseng selected for the red ginseng, the heat treatment, and the sun drying process may enhance and increase the extractable material. To compare and confirm the antioxidant in the ginseng powder and the extract of red and white ginseng, the substances were incorporated with turkey dark meat as mentioned in the materials and methods.

The absorbance was measured at 530 nm using spectrophotometer model 35 to obtain TBA values from the distilates. The TBA values indicate that as shown on Fig. 1, oxidative rancidity development in the meat during storage was somewhat prevented by adding ginseng powder or extracts. Turkey meat patties stored for 4 days obtained TBA values of 23.3, 11.1, 11.6 and 22.5 on control, 0.01% TBHQ, 0.1% red, 0.1% white ginseng powder incorporated patties respectively. The powder form exhibits 3 times higher antioxidant activity than the extracted form, based on the original weight of ginseng. TBA value from 0.005% red ginseng powder incorporated one similar to 0.05% white ginseng powder incorporated one. However, at the other level, the value was similar to the white one. Compensating for the value, the resulting value estimate of the antioxidant activity of red ginseng was twice higher than the white ginseng.

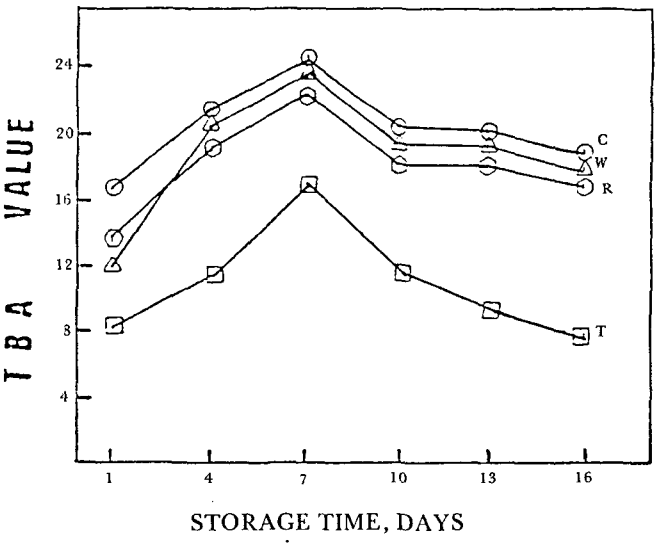

Fig. 1. TBA values of ground turkey dark meat patties treated with 0.01% TBHQ (T), 0.005% red ginseng powder (R), and 0.05% white ginseng powder (W) stored at 4°C the control sample is labeled C.

Red Ginseng Made from Fresh and White Ginseng

While preparing red ginseng from fresh ginseng, it developed it red-brown color after 3 days exposure under the sunlight, and for one week, its color was similar to that of the normal red ginseng; however, increasing the length of heating time, increased the intensity of the red-brown color as shown on Plate 1. For 3 hours heating at 75°C, turn out the most intense red-brown color which is almost similar to commercial red ginseng products. Reprocessing white ginseng to red ginseng, as expected, produced almost no color. As the result, red ginseng color

development originated by polyphenol oxidase and phenolic compounds, and subsequently proceeded its chemical reaction by sun drying, and developed its unique color, bright red-brown, which contains higher antioxidant activity.

Plate 1. Color development of fresh ginseng and reprocessed white ginseng to red ginseng.

Fresh ginseng: #7, 9, and II with 1, 2 and 3 hours heated at 75°C respectively.

Reprocessed white ginseng to red ginseng: #1, 3, and 5 heated at 75°C for 1, 2 and 3 hours respectively.

Isolation and Purification of PPO

The polyphenoloxidase extract from fresh Korean ginseng by 0.5M potassium phosphate buffer pH 7.3 showed rather weak activity where the crude extract basis, PPO from ginseng was 2×10^3 unit/g ginseng compared to PPO from kiwifruit which is 20×20^3 to 23×10^3 units/g of kiwifruit observed before the DEAE-cellulose column fractionation step in this laboratory. This may be caused by a rather long time between harvest and enzyme extraction and by exposure to higher temperatures during air shipping periods. On the purification step, there were two distincitive solid masses from 0.85 saturation of ammonium sulfate. The floating agglomerated mass was somewhat difficult to collect where substantial amounts of enzyme was present. However, the PPO enzyme was present more in the precipitated mass. (Figure 2) During dialysis, the PPO activity was decreased very much, and also lost, then recovery was about half of the original enzyme as shown in Table 2. Through fractionation of DEAE-Cellulose column chromatography, the precipitated portion of the PPO appeared at the first peak which showed up near the middle part of the void volume and ended with the void volume. This PPO protein may be negatively charged at the pH 6.3 of elution buffer. The agglomerated portion of the PPO appeared at the second peak which showed up near the end of fractionation (720-840 ml of elution) which PPO protein may be positively charged at pH 6.3 of elution buffer. It was eluted out pretty high ionic strength, about 0.5M phosphate elution buffer. By DEAE-Cellulose column chromatography the PPO enzyme lost its activity very much, similar to dialysis process, these may be caused by loss of cuppric ion, an essential element of activity site by elution process. Both the first and second fraction peak, the PPO activity was observed at the front slope of the protein peak by column fractionation.

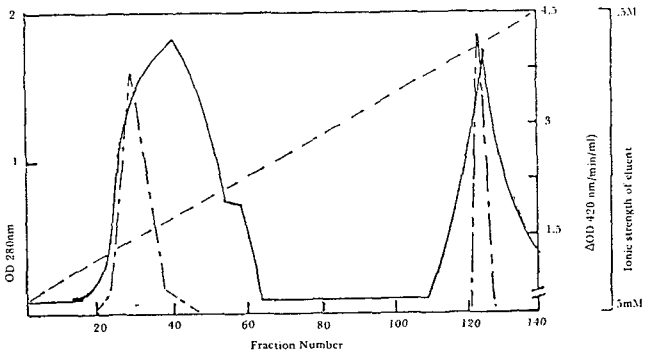

Fig. 2. Chromatogram of fresh Korean ginseng polyphenol oxidase on a DEAE-cellulose column (26 x 700 mm). The column was eluted with 500 ml of 0.005M-0.05M phosphate buffer, Ph 6,3. and 500 ml of 0.05-0.5M phosphate buffer, (———), PPO activity (—·—·—), and lonic strength of buffer (– – – –).

Electrophoresis

5, 6, 7 and 8% of gel electophoresis results could estimate the isozyme and its molecular weight by comparing the known standard protein

Table 2. Purification of polyphenoloxidase from fresh Korean ginseng .

Purification step	Volume (ml)	Activity (unit/ml)	Activity (unit)	Protein conc. (mg/ml)	Total protein (mg)	Specific activity (unit/ mg pro)	Recovery (5)	Purification (fold)
Crude extract	980	540	529×10^3	3.67	3,597	147	100	1
Ammonium sulfate cut (40%)	748	660	494×10^3	3.34	2,498	198	93	1.3
Ammonium sulfate fractionation (85%)	60	7,800	468×10^3	2.48	149	3,145	88.5	21
Dialysis	55	5,400	297×10^3	1.95	107	2,769	56	19
DEAE-cellulose column chrom., dialysis & conc.	(PPO I) 50	2,840	142×10^3	1.46	73	1,945		
	(PPO II) 20	950	19×10^3	0.925	18.5	1,027		
Total PPO (I) + PPO (II)			161×10^3		91.5	1,760	30.4	12

1 unit = ΔOD of 0.001 at 420 nm per minute.

molecular weight. Estimated molecular weight of PPO (I) isozyme was 12K, 18K, 54K and 64K dalton and PPO (II) was 28K, 40K, 44K and 48K dalton. But these molecular weights were somewhat deviated from the molecular weight estimated by the SDS-discontinuous method where these methods only estimated the protein molecules. PPO (I) showed six bands, estimated molecular weight was 13.5K, 14K, 18K, 54K, 64.5K and 84K, and PPO (II) was five bands, 22K, 29K, 33K, 54K and 63K dalton. A higher number of bands may indicate that there were some other proteins besides the enzyme protein. The enzyme staining was not stable and the catechol was not a good substrate to this PPO, thus, detecting the enzyme bands staining was a somewhat difficult task. In a better substrate, (+) catechin in a reaction mixture, substrate to catching for gel enzyme staining, no bands were detected. The (+) catechin may not penetrate to the gel system because of a larger molecular structure than the catechol or it may have some other reasons.

CHARACTERIZATION OF PPO

Substrate Specificity

A number of ortho-diphenol, some monophenol and some other phenolic compounds were tested in a study of substrate specificity of the isozyme (Table 3). PPO (I) showed higher activity on the O-diphenol except in the case of epicatechin. On the other hand, PPO (II) showed higher activity on monohydroxyphenol. However, both isozymes did not act on phloroglucinol, resorcinol and orcinal as substrate. Unlike kiwifruit PPO, the first fraction (PPO (I)) showed high catecholase activity and PPO (II) showed high cresolase activity. In PPO from kiwifruit, the first fraction showed cresolase activity and the 4th peak showed catecholase activity. But in the case of the epicatechin, similar to PPO from kiwifruit, the specificity of PPO (I) was lower than PPO (II).

Heat Stability

Table 3. Substrate specificity of ginseng PPO (relative activity).

Substrate	PPO (I)	PPO (II)
Dihydroxyphenol		
Catechol	189	100
Epicatechin	7	32
(+) Catechin	1,438	695
Chlorogenic acid	10	1
DOP Amine	6	4
DOPA	1	1
Caffeic acid	1	nil
Gallic acid	2	1
Protocatechuic acid	nil	nil
4-methyl catechol	3	1
Monohydroxyphenol		
P-cresol	472	3,150
P-coumaric acid	0	77
m-coumaric acid	2	5
Phenol	0	0
Feulic acid	0	0
Tyrosine	0	0
Other phenolic compounds		
Phyloroglucinol	0	0
Resorcinol	0	0
Orcinol	0	0

nil = less than 1%.

Observed heat stability of PPO from ginseng was very high. The PPO started to inactivate around 50°C but with (+) catechin as substrate, its activity remained 90% and 80% when heated at 75°C for 2 hours on PPO (I) and PPO (II), respectively. A larger quantity, and purer enzymes were needed to find out the exact character of the enzyme against heat.

Effect of pH

With catechol as substrate, two peak from 3.0-9.0 pH range, PPO (I) showed at 4.5 (40%) and 7.0 (100%) and PPO (II) showed at 3.5 (60%) and 7.0 (100%). When (+) catechin as substrate, the optimum pH was increased somewhat, where both enzyme low pH optimum was 4.5 (30-35%) and high pH optimum was 8.5 (80-100%) as shown on the Fig. 3, moderately higher activity range (70% activity or above) was from pH 6.5 to 8.5; however, when catechol as substrate the range was pH 6.5 to 7.5, one pH unit narrowed.

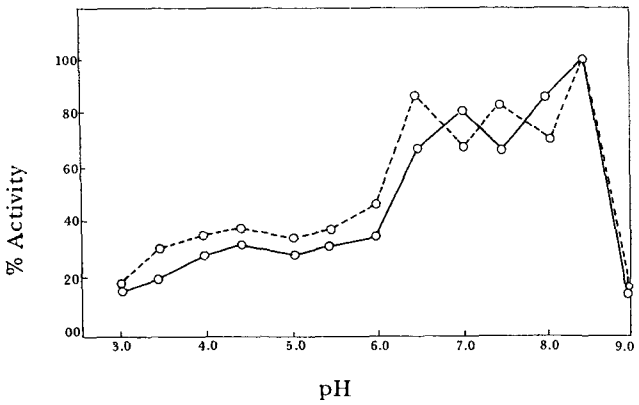

Fig. 3. pH optima of fresh Korean ginseng polyphenol oxidase with 0.01M catechin as substrate in 0.1M citrate-0.2M phosphate buffer at different pH value. Enzyme activities were assayed at 30°C as follows:

Sample cell: 2.8 ml of 0.01M substrate + 0.2 ml of enzyme solution reacted.

Solid line: PPO (I) and dotted line: PPO (II).

Effect of Enzyme Concentration

Both, PPO (I) and PPO (II) enzyme deviated from zero point which may be caused by some kind of inhibitor (irreversible) present in the enzyme aliquot. In addition to that PPO (I) did not show straight-line relationship with increasing enzyme concentration as shown on the Figure 4, this may be caused by competition of two enzymes for common cofactors, say Cu^{++} ion. As described by Whitaker, J.R.(1972), at heat stability test, enzyme activity was increased somewhat at the beginning up to 30 min. at 50°C and 75°C but later on it declined, these phenomena indicated that there are some other enzyme proteins or inhibitor proteins in the prepared enzyme aliquot. At lower concentrations of enzyme, PPO (I) showed a steeper curve than the PPO (II) curve, but at increased concentration, both show similar slope (0.01).

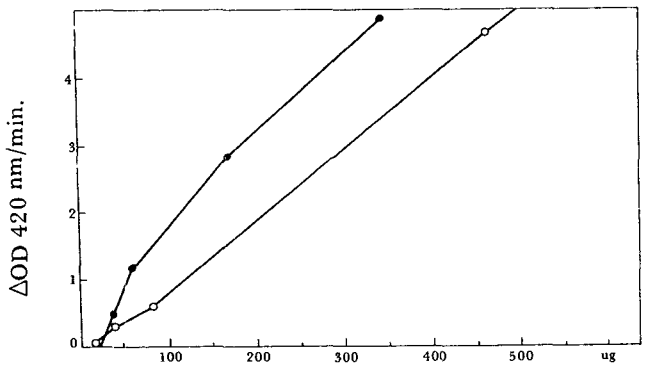

Fig. 4. Effect of enzyme concentration.

Enzyme activity was assayed at 30°C as follows: Sample cell: 2.0 ml of 0.01M catechin in 0.1M citrate-0.2M phosphate buffer, pH 7.3 + X ml of enzyme + Y ml of Deinoized water = total 3.0 ml. X + Y = 1.0 ml.

Black dot = PPO (I) : Circle = PPO (II).

Effect of Substrate Concentration

Concentration of (+) catechin ranging from 2.0×10^{-3} to 40×10^{-3} M were employed for this study. The PPO (I) and (II) both showed sigmoidal behavior. Then 2/3 upper higher concentration range were applied to deliver Km and Vmax value suing Lineweaver and Burk Plot; therefore, this method may not be appropriate. Based on (+) catechin as substrate, the values obtained for Km were 23 m mole and 26 m mole on PPO (I) and PPO (II) respectively. And Vmax values were 3.23 ΔOD/min/ml and 2.63 ΔOD/min/ml on PPO (I) and PPO (II) respectively.

Effect of Inhibitor

A bright red ginseng color may be hindered by some inhibitor, PPO (I) and PPO (II) siozyme showed similar effect on applied inhibitor; however, sodium diethyl dithio carbamat acted rather as activator on PPO (I) and similarly EDTA acted to PPO (II) as shown in Table 4. Applied concentration 0.1 ml of 0.01M inhibitor to 3.0 ml reaction mixture showed around 20% inhibition. Considering these results one should care about contamination of chemicals from beginning on red ginseng processing.

Table 4. Effect of inhibitor: % activity remained after addition of inhibitor.

Inhibitor	PPO (I)	PPO (II)
Buffer solution	100	100
$NaHSO_3$	83	85
NaCN	82	83
Glutathion (oxid)	87	88
EDTA	81	109
Ascorbic acid	90	91
Sodium diethyl dithio carbamate	107	91

Reaction was conducted at 30°C and absorbance read at 420 nm by spectrophotometer (Perkin-Elmer #575).

Reaction mixture: 2.7 ml of 0.01M (+) Catechin in 0.1M Citrate-0.2M Phosphate buffer pH 7.3 and 0.1 ml of inhibitor (0.01M) in same buffer was added and equivalent temperature, and then 0.2 ml of enzyme aliquot was added.

Determination of Activation Energy of Reaction

The formation of brownish color development with PPO with substrate especially (+) catechin was very rapid. According to arrhenius equation, observed and calculated activation energy were 3.04×10^3 cal/mole and 3.59×10^3 cal/mole on PPO (I) and PPO (II), respectively. And substrate alone 14×10^3 cal/mole was obtained.

Phenolic Compounds in the Ginseng

There were two spots which exhibited flourescent light from the TLC plate when placed under a UV lamp in a dark place (RF value obtained was 0.90, 0.15). The lower spot had stronger fluorescence when the plate was illuminated under the UV lamp in a dark room. The middle part of the TLC, faint line of fluorescence were observed. The upper and lower spots were scratched into a breaker separately and eluted by 80% ethanol for overnight. The sample containing 80% ethanol was filtered through a 0.45μm millipore filter and dried in a rotary vacuum evaporator.

And then hydrolized using 5 ml of 2N HCL

at 90°C for 2 hours. The hydrolysate was extracted three times with ethyl ether. The combined ether extract was dried and concentrated under a stream of nitrogen gas. The aqueous phase was condensed by vacuum evaporator. Then it was dissolved in 1 ml of methanol for analysis. For screen test, the samples were scanned under a UV spectrophotometer at 200 nm to 300 nm. There were two peaks, one at 225 nm and the other was at 276 nm. These compounds showed antioxidant activity by DPPH test. All samples from upper spot ether soluble portion, except red ginseng, which was from the lower spot ether soluble portion, showed good peak at 225 nm and 276 nm as shown in Figure 5. Then the samples were assayed under a GC mass spectrometer. The chromatograms were as shown on Figures 6, 7, 8 and 9. Through computer library search, one compound, 2, 6 Bis (1, 1-dimethylethyl) 4 methyl phenol was identified from fresh, white, and reprocessed white ginseng (Figure 10) which is a very good antioxidant, but not detected from red ginseng. The compound disappeared from red ginseng, which may have been caused by polyphenoloxidase which polymerized the 2.6 Bis (1. 1 dimethylethyl) 4 methyl phenol while

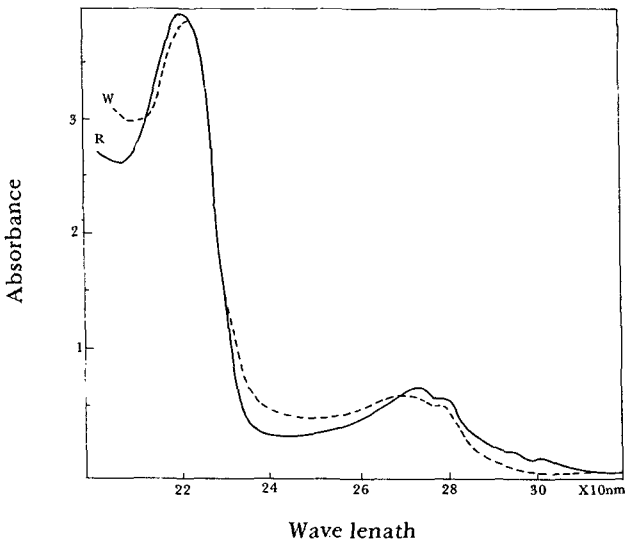

Fig. 5. UV spectrum of phenolic compounds from Korean ginseng.

R = red ginseng extract, TLC lower spot,
W = White ginseng extract, TLC upper spot, Fresh and reprocessed white ginseng were similar to white ginseng.

heating the fresh ginseng to process red ginseng. There were some other peaks from each sample but library computer search could not match these well with known compounds.

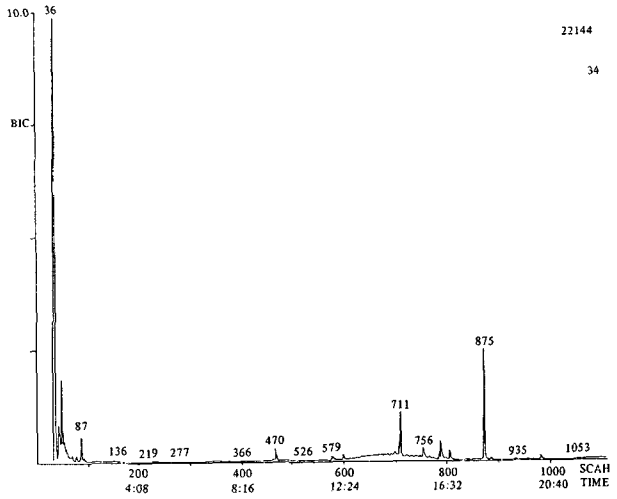

Fig. 6. Chromatogram of phenolic compounds by GC, extracted from fresh Korean ginseng. Ether soluble compounds (TLC upper spot). Finnegan 3200 gas chromatographic-mass spectrometer, equipped with a 30 m DB-1 fused silica capillary column (J&W Scientific) with helium as carrier gas (28 cm/sec). Temperature programmed from 80°C to 300°C at 8°C/min.

By HPLC, using RI and UV absorbance detector could not detect any compounds; however, from red ginseng TLC upper and lower spot aqueous phase, by absorbance detector at 254 nm, showed same retention time as quinic acid; however, the quinic acid showed no RI detector peak, same as upper portion but the lower portion aliquot showed one peak by RI detector. Then upper spot aqueous phase may contain the quinic acid-like compound, presumably such as 2-methyl 3-hydroxy-r-pyrone (maltol) which was detected by Han et al. (1978) as an antioxidant from red ginseng.

CONCLUSION

Antioxidant activity was detected from Korean red and white ginseng using turkey dark

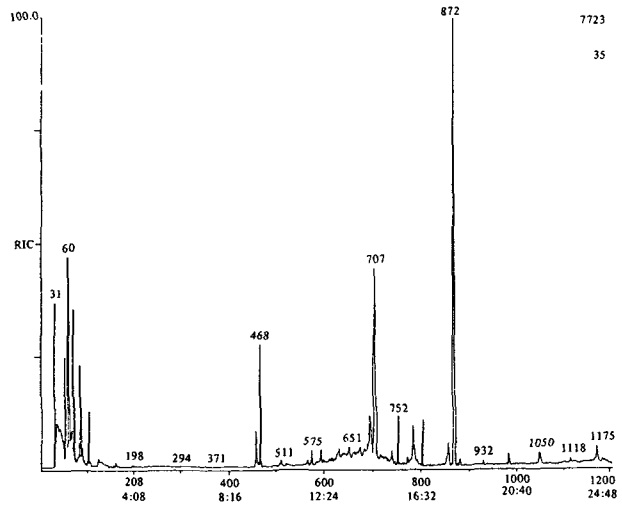

Fig. 7. Chromatogram of phenolic compounds by GC, extracted from Korean white ginseng. Ether soluble compounds (TLC upper spot). Finnegan 3200 gas chromatographic-mass spectrometer equipped with a 30 m DB-1 fused silica capillary column (J&W Scientific) with helium as carrier gas (28 cm/sec). Temperature programmed from 80°C to 300°C at 8°C/min.

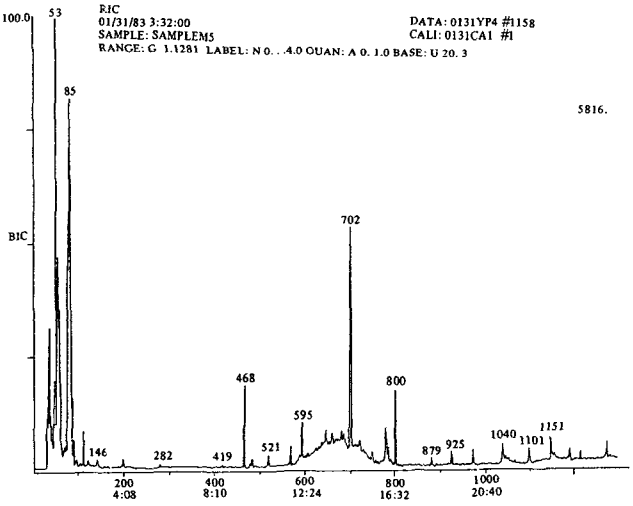

Fig. 8. Chromatogram of phenolic compounds by GC, extracted from reprocessed white ginseng to red ginseng. Ether soluble compounds (TLC upper spot). Finnegan 3200 gas chromatographic-mass spectrometer equipped with a 30 m DB-1 fused silica capillary column (J&W Scientific) with helium as carrier gas (28 cm/sec). Temperature programmed from 80° to 300°C at 8°C/ min.

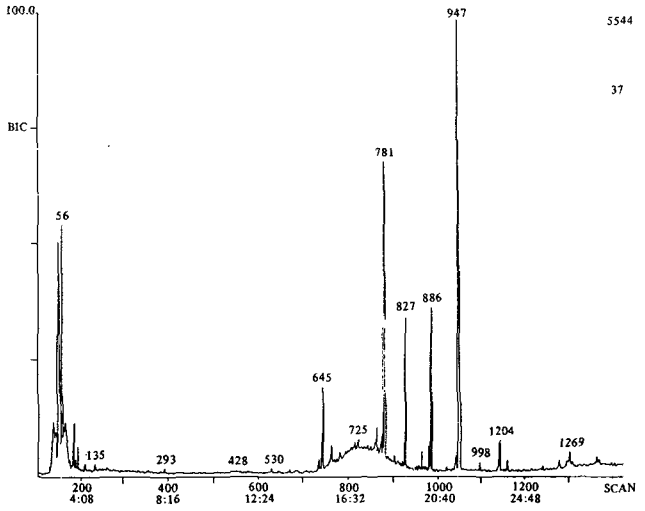

Fig. 9. Chromatogram of phenolic compounds by GC, extracted from red ginseng. Ether soluble compounds (TLC lower spot). Finnegan 3200 gas chromatographic-mass spectrometer equipped with a 30 m DB-1 fused silica capillary column (J&W Scientific) with helium as carrier gas (28 cm/sec). Temperature programmed from 80°C to 300°C at 8°C/min.

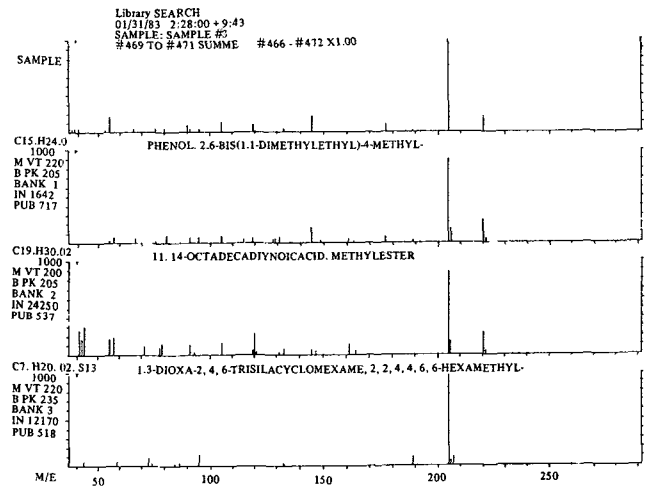

Fig. 10. Mass spectrum of GC, Peak #470 from fresh ginseng. Eluting compound was ionized at 70 ev, using Finnegan 3200 gas chromatographic-mass spectrometer. Peak #468 from white ginseng and reprocessed white ginseng to red ginseng were just the same as this spectrum.

meat patties. The activity in red ginseng showed about two times stronger than the white ginseng. One of the phenolic antioxidant from fresh, white, and reprocessed white ginseng was identified as 2.6 Bis (1.1 dimethylethyl) 4-methyl phenol among several unknown phenolic compounds. In red ginseng, no 2.6 Bis (1.1 dimethylethyl) 4-methyl phenol was detected at this experiment; however, some other unknown compounds showed high antioxidant activity. In fresh ginseng, there are two groups of polyphenoloxidase: high cresolase and high catecholsase activity. These enzymes react with phenolic compounds in ginseng while heating to process red ginseng. Then the intermediate products proceed through its reaction while sun drying by UV light and heat, and end up browning product, so-called red ginseng, which showed higher antioxidant activity. The browning products showed four major peaks which may consist of enzymatic browning products and non-enzymatic browning products, both of which could act as an antioxidant. The antioxidant may act as an anti-aging substance, as mentioned previously in the introduction. Ginseng is one of the most unique herbs because of this antiaging activity; along with this, it possesses tonic, stimulating, insulin-like activity, and antifatigue activities. It may be necessary to increase the cultivation of ginseng, not only in Korea but also in the United States, Japan, and all over the world. Then common people could benefit from its uses with meat to enhance good health.

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