Study on the Antioxidant Effect of Pycnogenol Extracts on Slice Dried Meat

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Abstract: Pycnogenol is one of the strongest natural antioxidants found in pine bark. Pycnogenol has been used in Europe for a long time, but there are not many domestic applications. To study the effect of Pycnogenol on antioxidative preservation of preserved meat under -18 ℃ storage condition. The results showed that Pycnogenol had significant effects on the growth and development of Pichia pastoris under the conditions of -18 ℃ storage condition, MDA content, POV value and AV value.The MDA content, the peroxide value and the acid value of the blank control group increased significantly during the five-month storage period, and the addition of Pycnogenol could significantly inhibit the rancidity of the fat. In the course of induction, that is, in 200 min, the MDA of Pycnogenol group was lower than that of the control group, and the change was stable and decreased slightly, which indicated that Pycnogenol could not only inhibit the production of oxidation products, The resulting oxidized product, MDA, may be degraded. The POV of Pycnogenol group was not changed within 1 month in the experiment of peroxide value determination, and the POV of Pycnogenol was lower than that of the control group in 2 to 4 months, and decreased obviously in 5 months. Radish can inhibit the peroxidation of meat increased. Conclusion: Pycnogenol antioxidant was added to the preserved meat of common packaging. During the five - month frozen storage period(-18 ℃) can significantly inhibit the MDA value, peroxide value and acid value of the increase.

Key words: Pycnogenol; miced meat; antioxidative effect; malondialdehyde

1. Introduction

As a kind of processing meat product with a long history, slice dried meat is much loved by people. It is made of the pork cured by salt, starter liquor, soy sauce, sugar, etc. through baking. It is characterized by beautiful color, fragrant flavor and delicate and soft meat. However, it is very apt to become rancid in the storage process, resulting in the rancid flavor and dark color of the meat. The rancid meat not only carries the bad flavor and odor but also contains the substances harmful to people's health, so it loses the edible value.

At present, antioxidants are added to the meat to prevent fat rancidity and extend the shelf life. The applied antioxidants include BHA, BHT, TBHQ, PG, etc. With the development of the extraction technology, natural antioxidants have also been widely used in the meat product industry, such as tea polyphenol[1], clove,[2] star anise[3-4], etc.

Pycnogenol is the effective component extracted from the pine bark. As a new natural antioxidant, it has not only the strong antioxidant effect but also the hypolipidemic, antihypertensive, anti-aging and anti-bacterial effects and has received the attention of people in recent years[5-6]. Now it has been applied in pharmaceutical and other industries. However, its application in the meat products has not yet been reported. The paper explores the antioxidant and preservation effects of pycnogenol on the slice dried meat packed ordinarily and stored at -18 ℃.

2. Test Materials, Instruments and Methods

2.1 Materials and Reagents

2.1.1 Materials

Slice dried meat and antioxidant extracts (antioxidant components in pycnogenol, citrus aurantium, ferulic acid, emblic leaf flower fruit, rosemary) are shown in Table 1.

Table1 Antioxidant extracts

Name	Components	Solubility	Color
Pycnogenol	Proanthocyanidins, organic acid and other bioactive components	Soluble in hot water and alcohol Insoluble in cold water	Brown
Rosemary extract	Rosemanol, carnosol, rosemary dialdehyd, ursolic acid	Insoluble in water, Slightly soluble in 50% alcohol, Soluble in high concentration alcohol	Light Yellow
Citrus aurantium extrat	Naringin, hesperidin	Soluble in water and slightly soluble in alcohol	Light Yellow
Ferulic acid	Phenolic acids	Insoluble in water and soluble in alcohol	White
Emblic leaf flower Fruit extract	Gallic acid	Soluble in water and50% alcohol and insoluble in alcohol	White

2.1.2 Reagents

The reagents used in the present study is shown in Table 2.

Table 2 Reagents

Reagent	Specification	Production Factory
Chloroform	AR	Tianjin Fuyu Fine Chemicals Co., Ltd.
Acetic acid	AR	Tianjin Damao Chemical Reagent Factory
Potassium iodide	AR	Tianjin Fuchen Chemical Reagent Factory
Sodium thiosulfate	AR	Guangzhou Photography Chemicals Factory
Potassium hydroxide	AR	Tianjin Jingu Industrial and Commercial Company
Ether	AR	Tianjin Damao Chemical Reagent Factory
Alcohol	AR	Tianjin Damao Chemical Reagent Factory
Potassium chromate	AR	Tianjin Fuchen Chemical Reagent Factory
Sulfanilic acid	AR	Tianjin Tianxin Fine Chemical Development Center
N-1-naphthylethylenediamine	AR	Tianjin Chemical Reagent Research Center
Folin- phenol reagent	AR	Beijing Dingguo Biotechnology Development Center
Acetylacetone	AR	Tianjin Fuchen Chemical Reagent Factory
Formaldehyde	AR	Guangzhou Chemical Reagent Factory
Thiobarbituric acid	AR	Sigma Company
Tris buffer	AR	Shanghai Yuanju Biotechnology Co., Ltd.
Bovine serum albumin	AR	Shanghai Boao Biotechnology Co., Ltd.
Silver nitrate	AR	Guangzhou Lixin Chemical Factory

2.2 Instruments and Equipments

The instruments and equipments used in the present study is shown in Table 3.

Table 3 Instruments and Equipments

Instrument Name	Type	Manufacturer
Meat grinder	Type TC12I	Panyu Henglian Food Machinery Factory
Far-infrared oven	Type FO-24B	Guangzhou Baiyun Zhujiang Kitchen Equipment Factory
Freezing centrifuge	Type TGL-16G-A	Shanghai Anting Science Apparatus Factory
Water bath kettle	Type LSY	Beijing Medical Equipment Factory
Electronic balance	Type FA2104	Shanghai Jingke Instrument Factory
Air dry oven	Type 510A	Shanghai Medical Electronics Factory
Magnetic heating stirrer	Type 78-1	Shenzhen Guohua Instrument Factory
Tissue blender		Produced in Japan
722 Type spectrophotometer		Shanghai Analysis Instruments Plant

2.3 Methods

Preparation of Slice Dried Meat:

With reference to Hu Yue's method[7-8]. Ingredients (unit: g): raw meat (pork hind leg) 500; sugar 85; Salt 1.5; soy sauce 40; five-spice powder 0.8; monosodium glutamate 2.5; liquor (50°) 7.5; egg 20; D-isoascorbic acid 0.5; sodium nitrite, 0.08; adding the antioxidant (500 mg/kg).

Preparation technology: removing all bone and fascia from the raw pork; grinding the pork and mixing it with the ingredients; curing the mixture at less than 10 ℃ for 1 to 2h; after that, spreading the ground pork on the stainless steel plate and baking it at 65 ℃ for 5 to 6 h; turning it once at half time; then baking it at 220 ℃ for 1 min to make the oil out of the meat and show the color of reddish brown; cutting the meat in slice and packing it (ordinary package); storing the prepared slice dried meat (12each treatment) at -18 ℃ and measuring the MDA, POV and AV once a month.

2.4 Measuring Method

2.4.1 Induced Oxidation Test

1.000 g of samples and 0.15 N of KCL were weighed and then mixed evenly in the tissue grinder. 0.1 ml of supernatant was taken. 0.5ml of Tris buffer, 0.2 ml of 2nM ascorbic acid and 2ml of 5 mM ferrous sulfate were added in the supernatant successively. The mixture was preserved in the water bath at 41 ℃ for 0 min, 50 min, 100 min and 200 min. At the same time, 2 ml of TBA-TCA-HCL mixture was added at each time point. The mixture boiled at 100℃ for 15 min and then centrifuged at 3000 rpm and 8℃ for 15 min. The supernatant was moved to the colorimetric tube for color comparison and absorbance measurement (O.D). 0.1 ml of 0.15 N KCL was used to replace the tissue mash liquid to do the blank control test. Other steps were the same. The protein content in the sample was measured finally.

Compute:

$$\text{MDA(nmol/mg Protein)} = [6.1402 \times 1000 \times 3 \times \text{O.D}]/[100 \times \text{Protein content(mg/ml)}] \quad (1)$$

2.4.2 Determination of MDA

1) TBA Experiment.

It referred to Xu Yongcai's method[9]. 10.0g of sample was weighed accurately and put in the stirrer. 60 ml of distilled water, 1 ml of EDTA solution and a small amount of PG were added in it to mix well. 30 ml of water was used to wash the stirrer. Then, all the solutions were moved to the distillation flask for heating until 50 ml of distilled liquid was collected. 5ml of collected liquid and 5ml of TBA reagent were transferred by the pipette to a

glass tube for heating for 30 min (5ml of water was used for the blank test). Then, the glass tube was cooled in the cold water for 10 min to measure the OD value at 538 nm.

2) Compute

$$MDA=(mg/kg)=7.8\times O.D \quad (2)$$

2.4.3 Determination of POV

20g of the sample was weighed and put in a 500 ml conical flask with joint. 150ml of petroleum ether was added in it. The mixture was placed for 12 to 24 hours and then filtered by the filter rapidly. The solvent was recovered to get the grease. 2 to 3g of the grease was weighed and added in 250 ml of iodine flask. Then, 30ml of the chloroform-glacial acetic acid mixture was added in the flask to vibrate immediately, so as to make the sample dissolved quickly. 1ml of saturated solution of potassium iodide was added in the flask and then shaken well with the flask covered. After that, the mixture was placed in the dark place for 3 min. Then, 50 ml of distilled water was added and then shaken well. 0.002 N sodium thiosulfate standard solution was immediately used for titration until the mixture became pale yellow. 1 ml of starch indicator was added to continue titration until the blue color disappeared. The blank test was done at the same time.

Compute:

$$POV=(V_1-V_2)\times N\times 0.126/W \quad (3)$$

where V_1 refers to the volume of sodium thiosulfate solution used by the sample, ml;

V_2 refers to the volume of sodium thiosulfate solution used by the blank test, ml;

N refers to the equivalent concentration of sodium thiosulfate solution;

W refers to the weight of the sample, g;

0.1269: 1 mg equivalent sodium thiosulfate is equivalent to 0.1269 g of iodine

2.4.4 Determination of AV

20g of the sample was weighed and put in a 500 ml conical flask with joint. 150ml of petroleum ether was added in it. The mixture was placed for 12 to 24 hours and then filtered by the filter rapidly. The solvent was recovered to get the grease. 2 to 3g of the grease was weighed and added in an iodine flask. Then, 50ml of the mixed solvent was added and shaken to make the sample dissolved quickly. After that, three drops of phenolphthalein indicator were added. 0.05 N alkaline solution was used for titration until the light red color did not disappear within 30s. The volume of alkaline solution consumed was written down (V).

Compute:

$$AV\ (mg\ KOH/g\ oil) = V\times N\times 56.1/W \quad (4)$$

where V refers to the volume of hydroxide potassium consumed in titration, ml;

N refers to the equivalent concentration of hydroxide potassium solution;

W refers to the weight of the sample, g;

56.1 refers to the mg equivalent of hydroxide potassium.

2.5 Data Statistical Analysis

The samples of each variety were determined for 4 times (n=4). The data received the variance analysis and the processing by SPSS statistical software.

3. Results and Discussion

3.1 Induced Oxidation Result Analysis

Fig.1 showed the oxidation stability comparison between 5 kinds of natural antioxidants including pycnogenol and rosemary and the blank control under the induced oxidation condition. All antioxidants had the inhibition effect on the oxidation of slice dried meat. Before induction (0 min), the MDA of the oxidation product from the group of slice dried meat with pycnogenol added in it was lower than the blank control, indicating that pycnogenol had the antioxidant effect in slice dried meat. In the process of induced oxidation, it showed the same result. Within 200 min, the MDA value of the group with pycnogenol was always lower than the control group. The MDA value of the control group was on the rise within 100 min, indicating that the slice dried meat with lean meat as the raw material was still prone to oxidation. It was mainly because the fatty membrane of the muscle tissue contained many unsaturated fatty acid. The MAD value of the group with pycnogenol was stable and slightly down within 200 min, indicating that pycnogenol could not only inhibit the production of oxidation products, but also had the degradation effect on the MDA of existing oxidation products.

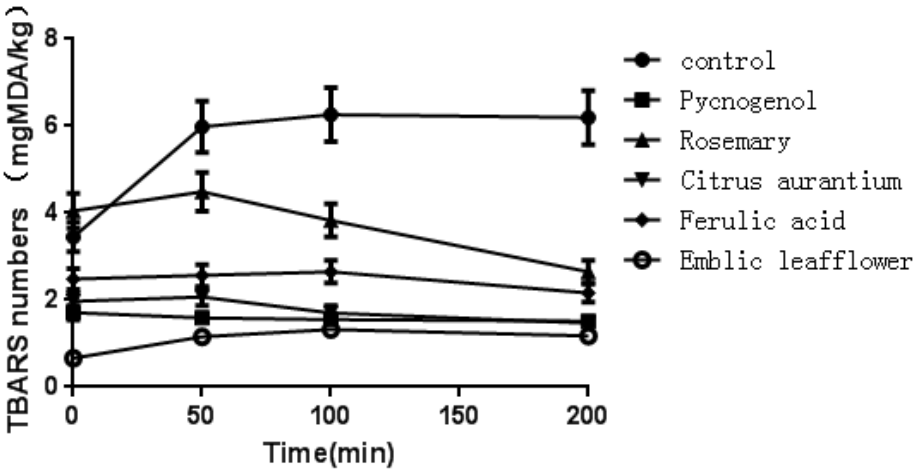

Fig.1 Comparison of the Induced Oxidation Results of Slice Dried Meats under Different Processing Methods (n=4)

Pycnogenol was compared with other antioxidants. From Fig.1, in the whole induction phase (0 to 200 min), the MDA value of the group with emblic leaf flower fruit increased slowly within 100 min, but it was the lowest, indicating the good antioxidant effect at the early stage of oxidation. The MDA value of the group with rosemary was the highest, but it showed a trend of sharp reduction after 50 min, indicating that rosemary had the strongest degradation effect on MDA. Pycnogenol had the similar antioxidant effect as citrus aurantium within 200 min and as ferulic acid within 50 min. However, over time, the antioxidant activities of different groups were significantly different ($P < 0.05$). Pycnogenol showed the stronger antioxidant property, indicating its long-term effect.

3.2 Analysis on the MDA Values Determined by the TBARS Method

According to Fig.2, OVs of all samples showed a rise trend. That of the group with rosemary, increased fastest and with emblic leaf flower fruit increased slowest. Compared with the blank control group, pycnogenol had the obvious inhibition effect on the oxidation of slice dried meat. In one to two months, the MDA values of the control group increased significantly, but that of the group with pycnogenol had no change (see Fig.2), indicating that pycnogenol had the strong antioxidant capacity at the early stage of oxidation. In two to three months, the MDA value of the group with pycnogenol increased rapidly. After comparison with the control group, slice dried meat was oxidized the fastest during this period of time with a large number of oxidation products. In addition, the effective ingredients in pycnogenol were consumed in the early stage of oxidation, so the MDA value of the group with pycnogenol increased. However, compared with other control groups (except the group with emblic leaf flower fruit), the antioxidant capacity of pycnogenol was still the strongest. Within three to five months, the MDA value of the group with pycnogenol changed steadily, indicating the similar antioxidant effect as other control groups (except the group with rosemary). The result was the same as that of the induced oxidation (see Fig.1).

It is worth noting that rosemary has certain antioxidant effect in the experiment, but the slice dried meat with rosemary has been oxidized very fast in the whole storage period and its MDA value has been close to the blank control group after 3 months, indicating its poorest antioxidant activity among 5 kinds of antioxidants. Rosemary's good antioxidant effect in lard and vegetable oil has been reported [10-11]. The result above may be caused by the negative effect of the high temperature processing (250℃) in the production of slice dried meat on the composition of rosemary or by the adding amount. Adding too small amounts of the antioxidants can promote the oxidation effect in turn[12]. Thus, the antioxidant activity of pycnogenol may also be affected by the high temperature processing in the production of slice dried meat or the adding dose. It needs further research.

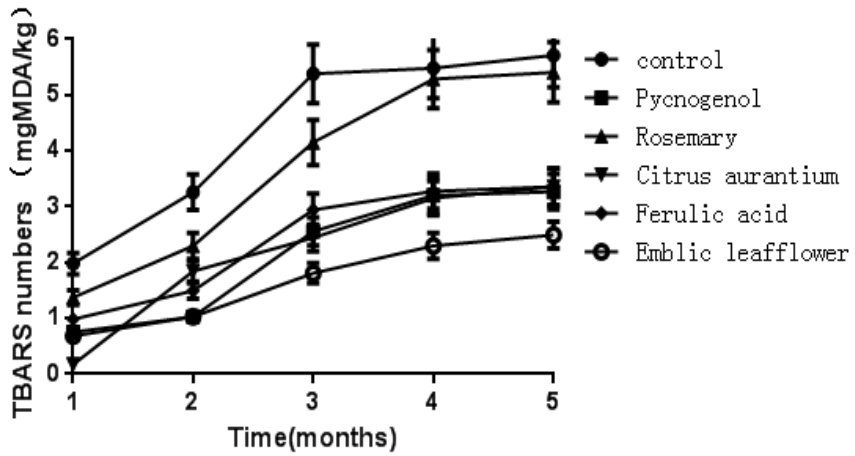

Fig.2 Comparison of the MDA Values of Slice Dried Meats under Different Processing Methods (n=4)

3.3 Peroxidation value (POV) results analysis

In January, the group with pycnogenol had little difference with the blank control group, but over time (1 to 4 months), the POV value of the group with pycnogenol increased slower than that of the blank control group. POV values of two groups had significant difference. During 4 and 5 months, the POV value of the blank control group declined slightly, but that of the group with pycnogenol declined sharply, indicating that adding pycnogenol could restrain the rise of the POV value of slice dried meat. The results were shown in Fig.3. Compared with the other control groups, the antioxidant capacity of pycnogenol was better than that of rosemary and ferulic acid, but worse than that of emblic leaf flower fruit. The antioxidant effect was similar as that of other groups without significant difference, which was the same as the results determined by the induction method and the TBARS method.

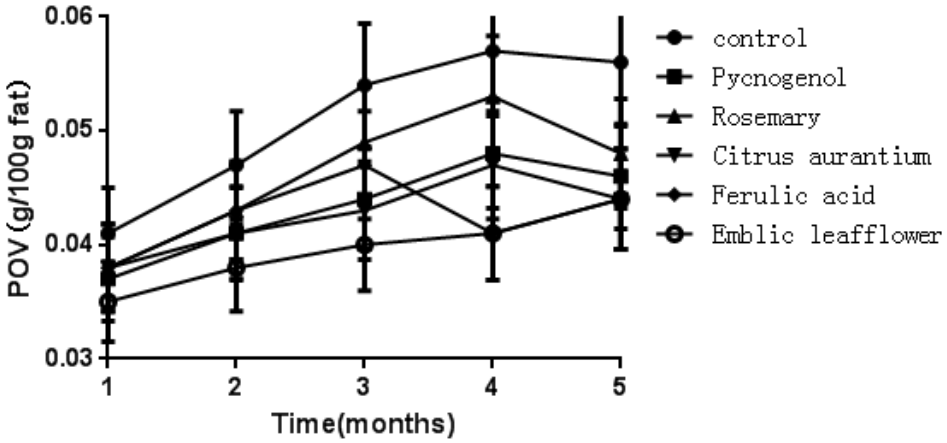

Fig.3 Comparison of the POV Values of Slice Dried Meats under Different Processing Methods (n=4)

It could be found after comparing Fig.2 and 3 that the MDA values of all groups showed a rise trend within 1 to 5 months and the POV values of all groups showed a rise trend and reached the peak within January and March. Then they declined to different degrees in next two months. It indicated that POV and MDA values had good correlation in the early stage of oxidation and no correlation at the late stage of oxidation. This was because in the storage process, due to the influence of temperature, humidity, oxygen, light and metal, the double bonds in the unsaturated fatty acid in the grease structure and oxygen in the air formed hydroperoxide and gradually reached the peak. Under the continuous effect of oxygen, hydroperoxide was further decomposed into aldehyde, ketone and other low molecules, which made the POV value low. At this time, although the POV value was low, the fat oxidative rancidity still went on, which could be seen from Fig.2. Therefore, it is suitable to use the TBARS method to evaluate the fat oxidation degree of the foods containing fat with long-term storage.

3.4 AV Result Analysis

The rancidity and oxidation of the fat are two different chemical reactions, but there is a certain relationship between them[13-14]. The experiment studied the effect of pycnogenol on the rancidity of slice dried meat. The results were shown in Fig.4. With the extension of time, the AV of the blank control group increased gradually. That of the group with pycnogenol showed a downward trend during January and February and then increased slowly, but the change was not big, showing the strong stability. It indicated that pycnogenol not only had the obvious inhibitory effect on the rancidity in the early stage of fat hydrolysis, but also had the effect of reducing the fat hydrolysis degree in a long period of time (5 months).

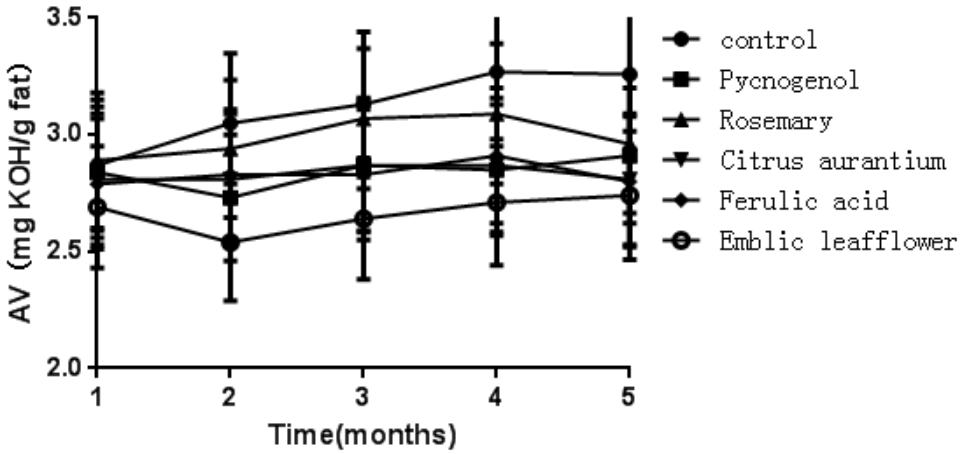

Fig.4 Comparison of AVs of Slice Dried Meats under Different Processing Methods (n=4)

The reason that pycnogenol can inhibit the AV was the fat rancidity reaction is produced by fat oxidation and hydrolysis. In a series of oxidation processes, the main decomposing products are hydroperoxide, carbonyl compound, low molecular fatty acids, alcohols, esters, fatty acid polymer and condensation product. The hydrolysis reaction can produce free fatty acids and glycerin which make the AV rise[15]. Pycnogenol inhibits the rise of the AV through controlling the fat oxidation speed, so as to achieve the effect of keeping fresh.

According to Fig.4, the AVs of the slice dried meats with antioxidants under -18℃ storage condition had little change, basically in a straight line. It indicated that low temperature could effectively inhibit the activity of lipase, reduce the production of free fatty acids and control the AV in a low range. Relatively speaking, the AV of the group with emblic leaf flower fruit was the lowest and that of the group with rosemary was the highest. It could be seen clearly from Fig.2 and 3 that the change trend of AV was different from that of POV. POV showed an obvious rise trend in the whole storage stage. It indicated that despite the little change in AV, the fat oxidation speed was quite fast. Therefore, there is no obvious corresponding relation between AV and POV.

3.5 Antioxidant Effect Mechanism of Pycnogenol

Pycnogenol is the natural polyphenol compound extracted from pine bark. It is mainly composed of (+)-catechin and (-)- epicatechin through C-C key polymerization, belonging to Oligomeric Procyanidins (OPC). Pycnogenol structure contains multiple phenolic hydroxyls which can provide active protons and have very strong reducing ability. When the slice dried meat produces peroxy radicals in the autoxidation process, the hydrogen donor on the hydroxyl can capture them and interrupt or delay the chain reaction, so as to prevent the fat rancidity and deterioration in the slice dried meat and achieve the antioxidant effect. In addition, the catechol structure in pycnogenol can chelate iron ions to form inactive iron compounds, thus affecting the oxidation process[16].

4. Conclusion

Adding pycnogenol antioxidant in the slice dried meat packed ordinarly can obviously inhibit the rise of MDA, POV and AV within 5 months of frozen storage (- 18 ℃). Within 5 months, the MDA value has been on the rise; POV first rises and then drops; AV has little change. It indicates that there is no correlation among those three indicators. TBARS method can well reflect the oxidation tendency of the meat products with long-term storage. POV is suitable to reflect the oxidation effect at the early stage of storage. Rosemary has the lowest

antioxidant capacity in the experiment. It can be induced that the number of effective phenol hydroxyls in pycnogenol, the stability of free radicals in the newly generated antioxidant, its dispersion degree and ability of complexing metal ions can all affect its antioxidant activity. It needs further research.

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