嘉南藥理科技大學專題研究計畫成果報告

烏梅抗氧化機制之探討

計畫類別：□個別型計畫　　□整合型計畫

計畫編號：CNFH-91-16

執行期間：91 年 1 月 1 日至 91 年 12 月 31 日

計畫主持人：晏文潔

共同主持人：張黎文

計畫參與人員：

執行單位：

中華民國　九十二　　年　二　　月 十九　　日

Abstract

The antioxidant effectiveness of Ume, smoked plum, (*Prunus mume* Sieb. et Zucc.) was investigated. Ethanol extracts of Ume (EEU) and water extracts of Ume (WEU) showed remarkable antioxidant activity in linoleic acid and liposome model systems. A correlation estabilished between the DPPH radical scavenging and antioxidant activity, yielded a coefficient of r=0.79 and 0.82 for EEU and WEU, respectively. The correlation coefficients between the reducing ability and inhibition of linoleic acid peroxidation were r=0.99 and 0.97 for EEU and WEU, respectively. In addition, the correlation coefficients between the content of phenolic compounds and antioxidant activity were r=0.97 and 0.94 for EEU and WEU, respectively. Moreover, the effectiveness of scavenging DPPH radical by EEU and WEU correlated, r=0.91 and 0.90, well with the amount of phenolic compounds. The reducing ability of EEU and WEU also correlated (r=0.99 for EEU and WEU) well with the amount of phenolic compounds. EEU and WEU exhibited scavenging activity for hydroxyl radical. Thus, the overall antioxidant contributor of EEU and WEU might be the phenolic compounds because of their reducing ability, and free radical and active oxygen scavenging capacity.

Key words: Antioxidant effectiveness, *Prunus mume* Sieb. et Zucc., Ume, phenolic compounds, free radical, active oxygen, reducing ability, peroxide formation, linoleic acid, liposome.

Introduction

The free radical oxidation of the lipid components in the foods by the chain reaction of lipid peroxidation is a major problem for food manufacturers. The radical generation occurs normally in the human body is believed to increase in most diseases.

The inhibitory of lipid peroxidation is governed by the use of antioxidants. There is an increasing interest in the use of natural antioxidants as a result of synthetic antioxidants safety concern (Aeschbach, Lolger, Scott, Murcia, Butler, Halliwell & Aruoma, 1994; Aruoma, 1994). From the human aspect, the best source of antioxidants is from diet. This aspect has resulted in increased interest in the investigation of the effectiveness of naturally occurring compounds with antioxidant action. The fact that various antioxidants occur naturally in plants has been recognized. They can be observed in fruit, vegetables, nuts, seeds, leaves, flours, roots, and barks (Pratt & Hudsou, 1990).

The smoked plums is the dried almost ripe fruit of *Prunus mume* Sieb. et Zucc. and it is called Ume in China. It is collected in spring when almost ripe, baked until turns black in color. Ume has been used as a folk medicine for many centuries in China. Recently beverages prepared from herbs are quite popular. Therefore, Ume not only are extracted as medicine but also processed drinks. Ume has been reported to contain many active components, including volatile oils (n-hexanal, α-terpineol, tetradecanoic acid, benzaldehyde), organic acid (citric acid, succinic acid, fumaric acid), triterpenoids, phenolic compounds (rhamncitrin-3-*O*-rhamnopyranoside, kaempferol-3-*O*-rhamnopyranoside, rhamnetin-3-*O*-rhamnopyranoside, quercetin-3-*O*-rhamnopyranoside) (Dang et al., 1999). The fruit of *Prunus mume* Sieb. et Zucc. have been demonstrated to exhibit various types of biological activities such as mutagenesis, activation of the alternative pathway of complement and activation of clot formation in human plasma (Dogasaki, Hurakami, Nishijima,

Ohno, Yadomae & Miyazaki, 1994; Dogasaki, Hurakami, Nishijima, Ohno, Yadomae, & Miyazaki, 1995). Dogasaki et al. (Dogasaki, Murakami, Nishijima, Yamamoto & Miyazaki, 1992) also reported that oleic acid and linoleic acid in Ume showed antimutagenic activities. In addition, the other literatures of the pharmaceutical effect of Ume have been reported (Dogasaki, Nishijima, Ohno, Yadomae,& Miyazaki, 1996; Shen, Cheng, Qiao, Li, 1995). No studies, however, have been conducted to investigate the antioxidant activity of Ume. Hence, the objective of this work was to examine the antioxidant activity of Ume.

Results and Discussion

Figure 1 shows that the antioxidant activity of EEU and WEU during linoleic acid oxidation, determined by the thiocyanate method. The antioxidant activity of the extracts are compared with that of two commercial antioxidants, Toc and BHA. For all treatments there was a rise in absorbance for extracts stored at 40℃ indicating linoleic acid oxidation. Control(E) and control(W), with antioxidants substituted by ethanol and water, respectively, had the highest PV of all treatments during 36 hr storage at 40℃, showing the highest intensity of oxidation. The significantly (p<0.05) lower absorbance for different extracts and commercial antioxidants (compared with that of the control) indicate greater antioxidant activity of the extracts. Control (E) and control (W) reached a maximum absorbance of 0.803 and 0.815, respectively, indicating that the highest PV, at 32 hr of testing, was oxidized to the highest extent. At 200 ppm, EEU, WEU, Toc and BHA showed a gradual increase in PV, which reached an absorbance of 0.256, 0.396, 0.529 and 0.337 respectively, indicating that they exhibited 68.1, 51.4, 34.1 and 58.0% inhibition of oxidation after 32 hr of storage, compared with that the control. Significant differences (p<0.05) were found between these values. Furthermore, EEU was significantly more effective (p<0.05) than WEU, Toc and BHA. However, WEU was more effective (p<0.05) than Toc, but less effective (p<0.05) than BHA. These results clearly indicate that EEU and WEU possessed marked antioxidant activity. In addition, the yields of EEU and WEU were 16.6 and 10.5%, respectively, indicating that the yield and antioxidant activity of EEU were greater than those of WEU.

Linoleic acid was assumed not to reflect completely the lipid peroxidation, due to its unique physical properties in aqueous micelles (Yi, Meyer & Frankel, 1997). Phospholipid is generally thought to be the mainly responsible for the oxidative deterioration and off-flavor development of foods, due to its greater degree of unsaturation (Wu & Sheldon, 1988). Hence to study in detail the EEU and WEU in biological systems, the liposome model system, prepared from lecithin, was used for evaluation of antioxidant activity. Antioxidative action of EEU and WEU in a liposome model system, as measured by the thiobarbituric acid method, was plotted in Figure 2. EEU and WEU in the range of 0.01~10 mg show 0-35.6 % and 5.5-15.7 % inhibition of thiobarbituric acid reaction substances (TBARs), respectively, compared with the control, indicating that the observed inhibition varied with the amount of extracts. The inhibitory effect of 10 mg of EEU (35.6%) and 10 mg of WEU (15.7%) on TBARs was significantly (p<0.05) weaker than that of 10 mg of BHA (55.5 %) and 10 mg of Toc (60.6 %). Moreover, the inhibitory effect of 10 mg of EEU

on TBARs formation was greater than that of 10 mg of WEU.

The results obtained from Figure 1 and Figure 2 show that the antioxidative action of EEU on linoleic acid model system and liposome model system were greater than that of WEU. This conclusion is particularly meaningful to consumers because wine, prepared from ume, and ume drinks, prepared from water extracts, still retain a strong antioxidant effect, and the effect of the former is greater than that of the latter. These properties make EEU and WEU excellent candidates for use as a natural antioxidant in processed food.

DPPH is a stable free radical which can accept an electron or hydrogen radical converting it into a stable, diamagnetic molecule. Due to its odd electron, DPPH has a strong absorption at 517nm. As this electron becomes paired off, the absorption decreases stoichiometrically with respect to the number of electrons taken up. This reaction change in absorption has been widely used to test the ability of antioxidants as free radical scavengers (Dinis, Madeira & Almeida, 1994; Pekkarinen, Stockmann, Schwarz, Heinonen & Hopia, 1999; Duh, Tu & Yen, 1999). The scavenging effect of EEU and WEU on DPPH radical is shown in Figure 3. The activity of EEU and WEU in scavenging DPPH radical varied with the quantity of extracts (Figure 3A and 3B). The correlation coefficients between the results of DPPH radical scavenging activity and inhibition of linoleic acid peroxidation were r=0.79 (p<0.05) and r=0.82 (p<0.05) for EEU and WEU, respectively. Hence, the antioxidant activity of EEU and WEU may be attributed to their hydrogen-donating ability. This indicates that EEU and WEU are free radical inhibitors, possibly as primary antioxidants that react with free radicals.

The reducing ability of EEU and WEU is shown in Figure 4. The reducing ability (absorbance at 700 nm) of EEU and WEU increased with increasing quantity of extracts. Thus, the reducing ability of EEU and WEU was amount dependent. The correlation coefficients between the results of reducing ability and inhibition of linoleic acid peroxidation were r=0.99 and r=0.97 for EEU and WEU, respectively. The highly positive correlation between antioxidant activity and reducing ability reveals that reducing power in EEU and WEU contributes to their antioxidant activity. The reducing ability is considered to associate with the presence of reductone, which helps in the breaking of the radical chain by donation of a hydrogen atom (Duh, Tu & Yen, 1999; Gordon, 1990). Therefore, the reductones in EEU and WEU are suggested to contribute to the inhibitory effect on lipid peroxidation.

Yen et al. (Yen, Duh& Tsai, 1993) reported that high content of total phenolic compounds in peanut hulls is responsible for the antioxidant activity. Ramarathnam et al. (Kalt, Forney, Martin & Prior, 1999) also noted that the amount of total phenolic compounds in rice hulls has a remarkable influence on the storage ability of rice seeds. In other words, the high content of phenolic compounds may retard fat rancidity and improve the stability of lipid peroxidation. Ume has been reported to contain phenolic compounds (Dang et al., 1999), no studies, however, have been demonstrated the effect of phenolic compounds on antioxidant activity of Ume. As shown in Figure 5, the content of phenolic compounds in EEU and WEU increased with an increasing amount of extract. The correlation coefficients between the content of phenolic compounds and inhibition of linoleic

acid peroxidation were r=0.97 (p<0.05, Figure 5A) and r=0.94, (p<0.05, Figure 5B) for EEU and WEU, respectively. These results show that the antioxidant efficiency of EEU and WEU may be attributed, to a great extent, to their content of phenoleic compounds. This is in agreement with previous reports (Kalt, Forney, Martin & Prio, 1999, Kahkonen, Hopia, Vuorela, Rauha, Pihlaja, Kujala & Heinonen, 1999) which state that the antioxidative capacity of some fruits strongly correlates with the content of total phenolic compounds.

Lipid peroxidation is induced and developed by free radicals. As mentioned above, EEU and WEU as free radical scavengers inhibit lipid peroxidation. Phenolic compounds that exhibit scavenging efficiency on free radical are numerous and widely distributed within the plant kingdom (Kahkonen, Hopia, Vuorela, Rauha, Pihlaja, Kujala & Heinonen, 1999; Namiki, 1990). In view of the high content of phenolic compounds in EEUand WEU, an investigation of the relationship between the reactivity of free radical and phenolic compounds in EEU and WEU may shed light on the mechanism of antioxidant activity of EEU and WEU. Figure 6 shows the relationship between the content of phenolic compounds and DPPH radical scavenging. The scavenging activity of DPPH radical of EEU correlated (r=0.91, p<0.05) well with the amount of phenolic compounds (Figure 6A). Similarly, the scavenging activity of DPPH radical of WEU also correlated (r=0.90, p<0.05) well with the amount of phenolic compounds (Figure 6B). These strong positive correlations indicate that the phenolic compounds in EEU and WEU are significantly responsible for anti-radical ability.

As expected, the reducing ability of EEU and WEU correlated (r=0.99, p<0.05 for EEU and WEU) well with the amount of phenolic compounds (Figure 7). This correlation analysis between reducing ability and phenolic compounds in EEU and WEU showed a highly positive correlation, indicating that phenolic compounds in EEU and WEU have a marked reducing ability which may contribute to their antioxidant capacity (Duh, Yen, Du & Yen, 1997).

From on the data above, phenolic compounds in EEU and WEU exhibited marked antiradical properties and possessed significant reducing ability, indicating that phenolic compounds in EEU and WEU can donate hydrogen atoms to lipid peroxy radicals to act as primary antioxidants (Gordon, 1990). This implies that phenolic compounds are the main contributor to the antioxidant activity.

To investigate the reaction of hydroxyl radical with EEU and WEU, hydroxyl radical was generated by photolysis of H_2O_2 with ultraviolet light and was trapped with salicylic acid. It is then hydroxylated to produce hydroxyl radical adduct products 2,3-and 2,5-dihydroxybenzoic acid (2,3-DHBA and 2,5-DHBA). The decrease in hydroxyl radical adduct products may be used to test the ability of antioxidants as hydroxyl radical scavengers. The effect of different amounts of EEU and WEU (0.01-10 mg) on the formation of 2,3-DHBA and 2,5-DHBA generated by H_2O_2 was shown in Figures 8 and 9, respectively. As seen in Figure 8, EEU in the range of 0.01-1.0mg showed 54.4-60.5% inhibition of 2,3-DHBA formation, and there was no significant difference (p>0.05) between the various amounts of EEU. Similarly, WEU in the range of 0.01-1.0 mg showed 31.3-31.8% inhibition of 2,3-DHBA formation, and there was no significant difference (p>0.05) between the various amounts of WEU. At 10 mg,

EEU and WEU showed 100±0.6 % and 83.6±0.4 % inhibition of 2,3-DHBA formation, respectively. As seen in Figure 9, EEU in the range of 0.01-10 mg exhibited 15.6-100% inhibition of 2,5-DHBA formation. WEU at the same quantity also showed 8.2~99.4% inhibition of 2,5-DHBA formation. Apparently, the inhibition of the formation of 2,3-and 2,5-DHBA with 10 mg of EEU and WEU was almost complete, indicating that EEU and WEU are powerful scavengers of hydroxyl radical, which is known to abstract hydrogen atoms from membrane lipids and bring about lipid peroxidation. Obviously, the ability to scavenge the hydroxyl radical by EEU and WEU seem to relate directly to the prevention of propagation of the process of lipid peroxidation. Hydroxyl radical is so reactive with all biological molecules that it causes damage to DNA, proteins, carbohydrates and lipids (Aruoma, 1994). Many previous studies (Prasad & Kalra, 1993; Steinberg, 1992; Prasad, Kalra & Bharadwaj, 1989) reported that oxygen free radicals have been suggested to be associated with hypercholesterolemic atherosclerosis, ischemiareperfusion cardiac injury and heart failure. According to the above data, EEU and WEU show remarkable scavenging effect on hydroxyl radical, suggesting that ume wine (EEU) and ume drink (WEU) may be beneficial to health.

According to the results, EEU and WEU scavenge free radical and hydroxyl radical and possess reducing ability. These properties contribute to the antioxidant activity. In particular, phenolic compounds in EEU and WEU strongly correlate with the free radical scavenging as well as reducing ability which contribute significantly to antioxidant activity. The supply of natural antioxidative phenolic compounds through consumption of EEU or WEU may provide additional protection against oxidative damage of cellular biomolecules. However, further studies are needed to determine whether EEU and WEU can act as antioxidant in vivo.