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산초유 산패방지를 위한 항산화물질과 혼합유의 영향

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Effects of Blending Oil and Antioxidants to Prevent Rancidity of Sancho Oil

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ABSTRACT

Background: Sancho (*Zanthoxylum schinifolium* Siebold and Zucc) oil is used as a traditional medicinal material to treat severs stomach inflammation and as a diuretic. This study was carried out to investigate the effect of addition of antioxidants and blended oil the storage stability and safety of the biomaterial.
Methods and Results: The effects of temperature and light on sancho oil were investigated, and the ability of antioxidants in preventing rancidity of the oil was discovered. Under fluorescent light and in darkness, the acidity of the oil was much lower than that under direct sunlight. The addition of antioxidants decreased the acid value of sancho oil; the antioxidant that showed the best results in this regard was 0.5% propolis. The acid value of canola oil, which had the lowest acid value compared with that of other oils, and blended oil, containing 5% canola oil in sancho oil, decreased by 5.5% and 15%, respectively. About one acid value decrease was observed for every 1% increase in blending with canola oil. As the concentration of canola oil increased, the viscosity and the elightness (L valu) of sancho oil increased slightly, while the blueness (b value) decreased.
Conclusions: The results of this study may contribute to ensuring food safety during preservation and the industrialization of the presevation of sancho oil.

***Key Words*:** *Zanthoxylum schinifolium* Siebold & Zucc, Acid Value, Antioxidant, Blending Oil, Canola Oil, Sancho Oil

INTRODUCTION

Vegetable oil is oxidized according to the oil extraction method and storage period, and such changes can cause harmful substances, which can cause enormous problems in food hygiene (Song *et al*., 2003). Rancidity is a phenomenon in which the quality of fat foods is reduced during oil extraction and storage due to various chemical and microbiological factors such as changes in smell and taste and loss of nutritional value (Sanders, 1983). In our previous studies, it has been reported that rancidity occurs in sancho (*Zanthoxylum schinifolium* Siebold and Zucc) oil (Kim *et al*., 2012).

Sancho has long been used as an antiseptic, anti-inflammatory, diuretic and anti-parasitic agent (Lee, 1996). Sancho oil has traditionally been used for a long time,

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but extraction methods are not scientifically established and can cause problems with distribution and use.

In order to increase the storage stability of sancho oil, it is advisable to prevent rancidity at the initial stage. It is preferable to add an antioxidant or a blending oil to chemically inhibit rancidity.

A number of synthetic or natural antioxidants have been developed to prevent oxidation of oils and fats. However, because of their effectiveness and economy and stability, there are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and ethylenediaminetetraacetic acid (EDTA) actually used in food, and α-tocopherol is a natural antioxidant (Aluyor and Ori-Jesu 2008).

Blending of vegetable fats/oils with different properties is one of the simplest ways to create new specific products with the desired tissue and oxidative stability (Hashempour-Baltork *et al*., 2016).

Sancho oil has long sold at very high prices as traditional edible oil. However, sancho oil has been reported to rupture very rapidly according to extraction and storage methods (Kim *et al*., 2012; Kang *et al*., 2017). And now there is no proper method to prevent the rancidity of sancho oil. Also, there is no research to prevent rancidity using antioxidants, which is an appropriate method to prevent rancidity of sancho oil. We have devised two methods to prevent rancidity of sancho oil. In other words, the optimal antioxidant was added to sancho oil to prevent rancidity, or to search for edible oil which is resistant to rancidity and to improve storage stability by blending oil.

Therefore, this study was carried out to investigate the antioxidant and blending oil in order to increase the storage stability of sancho oil and to ensure safety to bio materials.

MATERIALS AND METHODS

1. Materials

We have searched for optimal antioxidant and some vegetable oil which is strong against rancidity. As an antioxidant, α-tocopherol (Sigma-Aldrich Co., St. Louis, MO, USA), butylated hydroxytoluene (BHT, Sigma-Aldrich Co., St. Louis, MO, USA) and propolis (Sigma-Aldrich Co., St. Louis, MO, USA) were used. Canola oil, olive oil and grape seed oil, which were used to select suitable plant oils for blending (CJ Cheiljedang Co., Seoul, Korea), were purchased and used in the market. Sancho (*Zanthoxylum schinifolium* Siebold and Zucc) oil was purchased from Jirisan sancho oil farm and used in the experiment. Vegetable oils including sancho oil were used in the experiment for less than 3 months.

The oil was cooled at room temperature and centrifuged at 2,500 rpm for 5 minutes using a centrifuge (HA-1003-3, Hanil Science Industrial Co., Ltd., Seoul, Korea). The supernatant was collected and stored at −20℃ and dark and used as a sample.

2. Acid value for light sources and temperature of sancho oil

The acid value of the light source of sancho oil was investigated.

The acid value of the sancho oil was measured at 30 h intervals in an incubator (SH-75B, Seyoung Scientific Co., Ltd., Bucheon, Korea) at 3℃, 6℃, 9℃, 12℃, 15℃, 18℃, 21℃, 24℃ and 27℃ after direct sunlight and fluorescent lamp irradiation. In addition, the acid value of the sancho oil was measured in the dark at an incubator (SH-75B, Seyoung Scientific Co., Ltd., Bucheon, Korea) adjusted to 25℃.

The acid value of the temperature change of the sancho oil was investigated. The acidity of the sancho oil was measured at −20℃, 5℃, 25℃, 35℃, 50℃, and 70℃. The acid value of sancho oil was measured after 3 h, 6 h, 9 h, 12 h, 15 h, 18 h, 21 h, 24 h, 27 h and 30 h intervals for measuring the acid value of sancho oil with time.

3. Determination of acid value

Acid value of sancho oil and blending oil was measured according to AOCS (1990). 1.0 g of the sample was taken in an erlenmeyer flask and mixed with 1 : 2 (v/v) ethanol (Sigma-Aldrich Co., St. Louis, MO, USA, 20 ㎖) was added to dissolve the oil.

The phenolphthalein solution (Sigma-Aldrich Co., St. Louis, MO, USA) was used as an indicator and titrated with 0.1 N potassium hydroxide (KOH) (Sigma-Aldrich Co., St. Louis, MO, USA) until the ethanol solution became pale red.

The acid value was determined by the following equation, and the experiment was repeated three times. The acidity was measured in an incubator (SH-75B,

Seyoung Scientific Co., Ltd., Bucheon, Korea) adjusted to 25℃.

$$\text{Acid value} = 5.611 \times A \times F/\text{sample weight (g)}$$

A; Volume of 0.1 N KOH (㎖), F; Titer of 0.1 N KOH (㎖)

4. Determination of vegetable oils suitable for blending oil production

Three vegetable oils were mixed with sancho oil in order to search for edible oil resistant to rancidity. Three vegetable oils were mixed with sancho oil at 5% (w/v) and 15% (w/v) concentration.

Each concentration of vegetable oils were slowly added to the sancho oil at 25℃ and stirred for 10 min. The vegetable oil was incubated at 25℃ for 24 h and the acid value was measured.

5. Measurement of acid value by antioxidant treatment

After addition of α-tocopherol, BHT and propolis to the sancho oil, the acid value was measured at 35℃ for 30 hours every 3 hours. Acid value of sancho oil measured by AOAC (1984).

6. Determination of proper concentration of canola oil

The acid value of each concentration of canola oil, which was strongest in rancidity, was measured. Canola oil was divided into 1 to 15% and mixed with sancho oil.

Each concentration of canola oil was slowly added to sancho oil at 25℃ and stirred for 10 minutes. The canola oil was incubated at 25℃ for 24 hours and the acid value was measured.

7. Chromaticity measurement of blending oil

The blending oil color value was measured with a colorimeter (Chromateter, CR-300, Minolta Co., Tokyo, Japan) using a Hunter system.

After calibrating with a standard color plate (L = 96.96, a = 0.17, b = 1.96), the color of each sample was measured and the lightness, redness, blueness and color difference indicated by L, a, and b values were identified. The brands were prepared by adding 5%, 10%, 15%, 20%, and 25% canola oil to the sancho oil, and the color value was measured after mixing for 10 minutes.

8. Viscosity measurement of blending oil

The viscosity of the brand oil was measured by using a viscometer (Brookfield engineering laboratories, Lorch, Germany) at 25℃ in a beaker with 100 g of the sample at 6 at a rate of 60 rpm for 2 min.

The blending oil was prepared by adding 5%, 10%, 15%, 20% and 25% canola oil to sancho oil and mixing for 10 minutes. The blending oil immediately measured the viscosity at 25℃.

9. Statistical analysis

The changes in acid value of commercially available vegetable oils and sancho oil and the type and concentration of vegetable oil were analyzed by one-way ANOVA using the IBM SPSS statistical package (Ver. 24, IBM Co., Armonk, NY, USA).

Means were compared at 5% significance level using Duncan's Multiple Range Test (DMRT) comparison ($p < 0.05$).

RESULTS

1. Acid value change for temperature of sancho oil.

The acid value with temperatures change of blended oil with canola oil was investigated (Fig. 1).

Blending oil increased rapidly until 3 - 6 h, however, there was no significant change after six hours. As a result, about 1 of acid value was decreased for each 1% increase in blending oil. Also, the acidity of blending oil was very low at less than 2 at 25℃ and there was little

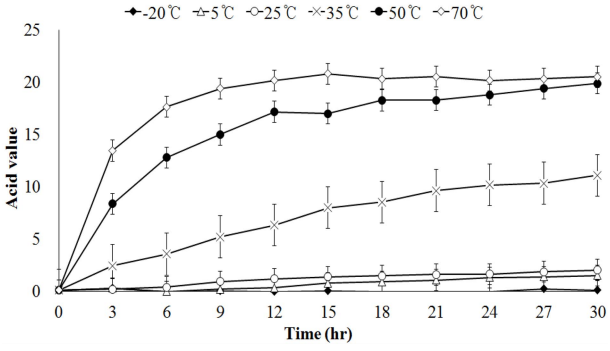

Fig. 1. Change in acid value according to different temperature of sancho oil. The acidity of sancho oil was measured at –20℃, 5℃, 25℃, 35℃, 50℃ and 70℃ for 3 - 30 h.

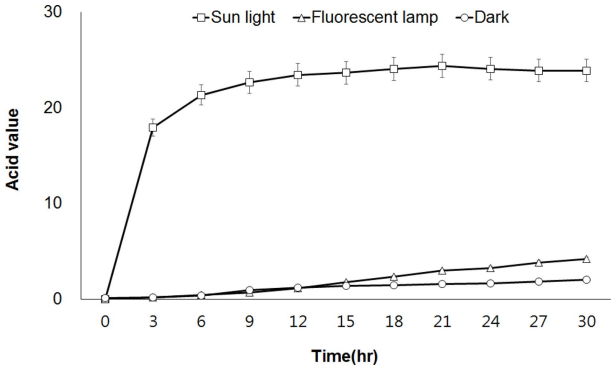

Fig. 2. Change in acid value and light source of sancho oil. The acidity of the sancho oil against sunlight, fluorescence and darkness was measured at 25℃ for about 0 h - 30 h at 3 h intervals.

change after storage days.

2. Acid value change for light of sancho oil.

The change of acid value with temperature of blended oil with canola oil was investigated (Fig. 2).

Blending oil with 5% canola oil showed similar acid value to that of controls not placed under direct sunlight. Acid value of sancho oil under fluorescent lamps and dark are dramatically lower than direct sunlight. The acid value of sancho oil for fluorescent light irradiation and dark condition treatment was increased from about 12 h to 15 h.

3. Change of acid value of sancho oil by antioxidant treatment.

Antioxidants were added to sancho (*Zanthoxylum schinifolium* Siebold and Zucc) oil blended with three edible oils and acid value changes were measured (Fig. 3).

As a result, the acid value varied depending on the type and concentration of antioxidants. Among the four antioxidants, α-tocopherol has the highest acid value in antioxidants whereas the lowest acid value among the antioxidants was 0.5% propolis. The acid value of control was 8.15 at 30 hours, 6.97 for 0.02% a-tocopherol, 5.99 for 0.01% propolis, 5.31 for 0.2% BHT and 4.89 for 0.5% propolis, respectively.

The longer the antioxidant treatment time, the higher the acid value. Acid value increased rapidly after 18 hours of antioxidant treatment. The acid value of propolis, which had the lowest acid value, was different according to treatment concentration (Fig. 3).

The acid value of 0.5% propolis treatment was lower than that of 0.1% treatment. Propolis treatment at two concentrations showed the same tendency even after incubation time. Antioxidant added sancho oil was shown to retard oxidation for about 15 hours compared to sancho oil without antioxidants even in the dark and 35℃ (data not shown).

4. Selection of proper vegetable oils for blending oil preparation.

The acid value of vegetable oil was different for each oil (Fig. 4). The acid value of olive oil and grape seed

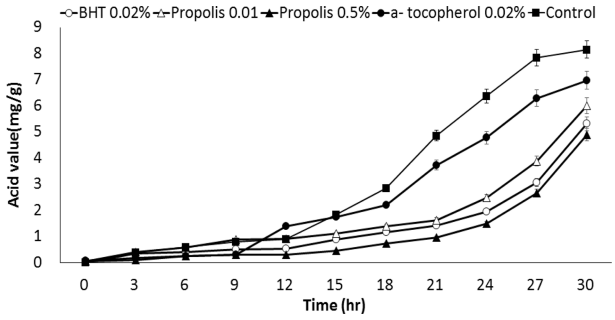

Fig. 3. Change of acid value of sancho oil by addition of antioxidants. α-tocopherol, BHT and propolis were added to sancho oil, respectively. After 30 h at 35℃, the acid value was measured at intervals of 3 h.

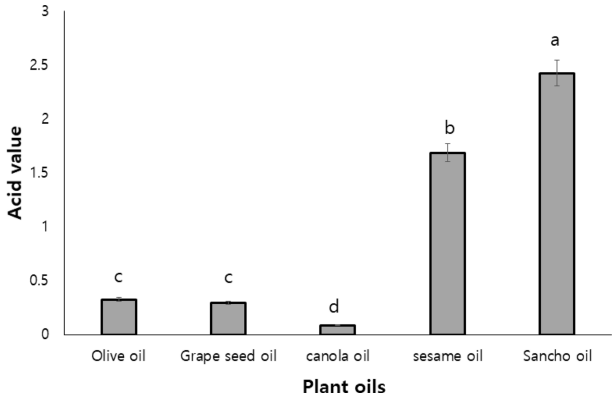

Fig. 4. Acid value of commercially available vegetable oils and sancho oil. Three vegetable oils were mixed with sancho oil in concentrations of 5% (w/v) and 15% (w/v). The oil was stirred at 25 degrees for 10 min, and the acid value was measured after 24 h. Data represent the means ± S.E. of three replicates. *Different letter for each treatment shows significant difference ($p < 0.05$) using Duncan's Multiple Range Test (DMRT) comparison.

oil was 0.326 and 0.294, respectively. The acid value of canola oil was 0.08, which was the lowest in comparison with other vegetable oils. The acid value was less than 1 in the case of commercial edible oil after degumming process, and it was relatively high for sesame oil sold in the absence of the degumming process and green tea oil classified as other edible oils.

5. The acid value of blending oil.

The acid value was investigated by blending sancho oils with other various vegetable oils (Fig. 5).

As a result, it was confirmed that canola oil inhibited rancidity compared with other vegetable oils. In case of blended oil containing 15% of canola oil in sancho oil, acid value decreased compared to other blended oils but the 15% canola oil treatment had the lowest acid value. When the olive oil, grape seed oil, and green tea seed oil were blended by 15%, the acid value was about 1.8, 2.2 and 1.3, respectively.

6. Acid value of sancho oil blended with canola oil.

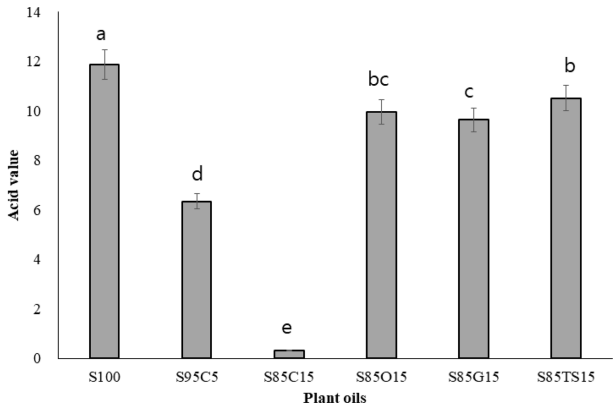

Fig. 5. Acid value change of Sancho oil depending on the type and concentration of vegetable oil. Sancho oil and each plant oil were mixed at 25℃ at different concentrations and acid value was measured after 3 hours. S; sancho oil, C; canola oil, O; olive oil, G; grape seed oil, TS; tea seed oil. S100; 100% sancho oil without canola oil, S95C5; blending oil with 95% sancho oil and 5% canola oil, S85C15; blending oil with 85% sancho oil and 15% canola oil, S85O15; blending oil with 85% sancho oil and 15% olive oil, S85G15; blending oil with 85% sancho oil and 15% grape oil, and S85T15; blending oil with 85% sancho oil and 15% tea seed oil. Data represent the means ± S.E. of three replicates. *Different letter for each treatment shows significant difference ($p < 0.05$) using Duncan's Multiple Range Test (DMRT) comparison.

The concentration suitable for blending of canola oil was determined (Fig 6). As the concentration of canola oil increased, the acid value decreased.

As a result, about 1 of acid value was decreased for each 1% increase in blending of canola oil. As shown in Fig. 6, when the canola oil was blended by 9% or more, the reduction effect of acid value was reduced. When 13% canola oil was mixed, the acid value reached zero.

7. Viscosity of sancho oil blended with canola oil.

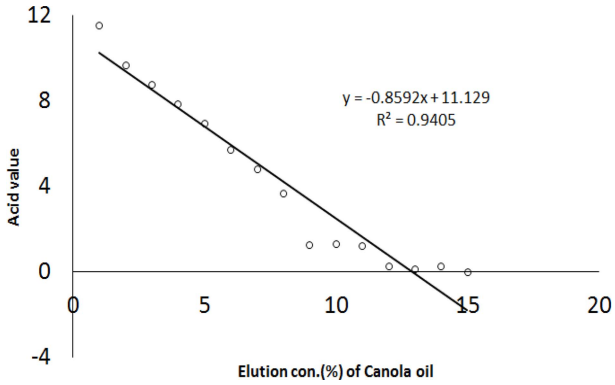

Fig. 6. Acid value change of mixed sancho oil according to canola oil blending concentration. Canola oil was mixed with sancho oil at different concentrations ranging from 1% to 15% and the acid value was measured.

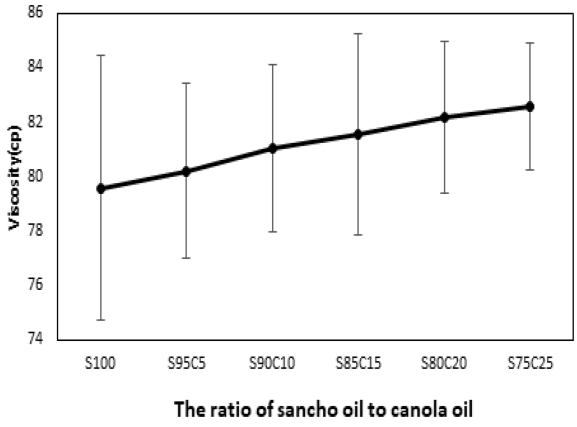

Fig. 7. Viscosity change with concentration of sancho oil and canola oil. The viscosity of sancho oil and canola oil were measured after mixing with different concentrations. S; sancho oil, C; canola oil, S100; 100% sancho oil without canola oil, S95C5; blending oil with 95% sancho oil and 5% canola oil, S90C10; blending oil with 90% sancho oil and 10% canola oil, S85C15; blending oil with 85% sancho oil and 15% canola oil, S80C20; blending oil with 80% sancho oil and 20% canola oil, and S75C25; blending oil with 75% sancho oil and 25% canola oil.

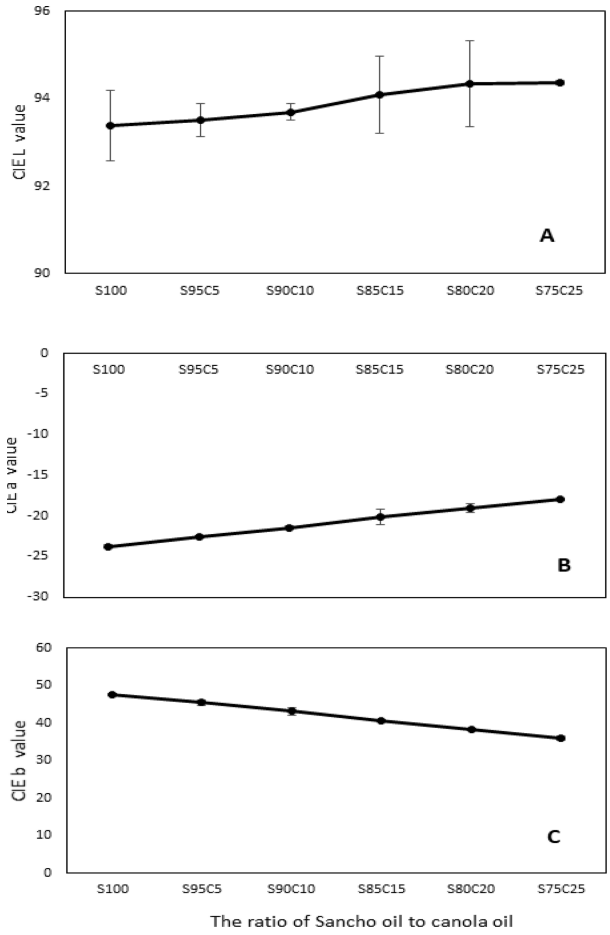

Fig. 8. Changes in chromaticity according to concentration of sancho oil and canola oil. Blended oil of the sancho oil and the canola oil were mixed at different concentrations and measured using a colorimeter. A; CIE L value, B; CIE a value, and C; CIE b value.

The viscosity of the blending oil varied according to the concentration of canola oil and sancho oil (Fig. 7).

The viscosity of canola oil was higher than that of sancho oil. The viscosity of canola oil was 90 and the viscosity of sancho oil was 80. Therefore, the higher the concentration of canola oil, the higher the viscosity.

8. Chromaticity of sancho oil blended with canola oil.

The chromaticity of blending oil was also dependent on the dilution of canola oil (Fig. 8).

The L value (lightness) was slightly increased with the dilution of canola oil, and the degree of a value (redness) gradually increased. The value of b value (blueness) decreased as the concentration of canola oil increased.

DISCUSSION

Blending oil also affected acidity by temperature. The vegetable oil is changed in color by high temperature, increase of free fatty acid, increase of peroxide, decrease of unsaturated fatty acid, change of taste aroma, and the like, and the quality of the oil is deteriorated (Lee and Park, 2010).

The oxidation of the oil can be divided into automatic oxidation, photo oxidation and thermal oxidation, and their oxidation mechanisms are somewhat different (Lee, 1990). Among them, the cause of the oxidation of the sancho oil is reported to occur mainly in photo oxidation and heating oxidation (Kim *et al*., 2012).

In addition to the automatic oxidation, the thermal oxidation occurs when the oil is heated at a high temperature. The formation of carbonyl compounds by pyrolysis of the carbon-carbon bonds of fatty acids, the formation of free fatty acids due to the decomposition of ester bonds, the neutralization reaction, and foam phenomenon occurs (Min and Smouse, 1985).

The light source also affected the acidity of the branding oil (Fig. 2). Light is also part of the external factors that adversely affect the composition and quality characteristics of fats and oils. Light is the initiator and the cause of the reaction leading to the decay of fat and oil. Fats do not absorb visible light spectra, but oxidation can be induced by light absorbed by oil impurities (e.g. pigment, chlorophyll). Oxidation generally involves the production of free radicals (Slavica *et al*., 2011).

Exposure of corn, coconut, rapeseed, and soybean oil to 500 ft-c fluorescent lamps significantly increased the rate of oxidation of the retention of light (Sattar *et al*., 1976). Oxidation rates increased under higher wavelength light, and this effect was more pronounced in vegetable oils than in butter fats.

Vegetable oils are easily oxidized and have been tried to treat antioxidants to prevent rancidity. The most commonly used antioxidants are synthetic antioxidants such as BHA and BHT, and natural antioxidants such as tocopherol and propolis (Yoon *et al*., 1988).

In this study, the differences in the brand equity were also different according to the kinds of antioxidants.

Especially, the acid value of propolis treatment was kept lowest.

Tocopherol belongs to the family of phenolic antioxidants and removes free radicals and reacts with singlet oxygen to inhibit lipid auto oxidation. In vegetable oils, alpha tocopherols inhibit the effects of singlet oxygen during sensitive photo-oxidation.

Tocopherol belongs to the family of phenolic antioxidants and removes free radicals and reacts with singlet oxygen to inhibit lipid auto oxidation. In vegetable oils, alpha tocopherols inhibit the effects of singlet oxygen during sensitive photo-oxidation. Frankel (1989) also reported that tocopherol showed antioxidant activity in plant oils, which is consistent with our study.

It was found that vegetable oil added to sancho oil could delay rancidity. First, we searched for plant maintenance which is resistant to rancidity. The acid value of vegetable oil varies depending on the type of vegetable oil, and the acid value of canola oil is the lowest (Fig. 4). The acidity of vegetable oil is known to increase significantly during the induction period (Lee *et al*., 2007). Grape fruit seed oil were increased from 5.05 to 15.24 to 3.02, olive oil to 1.11, and sesame oil from 1.73 to 4.79 to 2.77, respectively at higher temperatures (Kim, 2017). Also, the acid value of vegetable oil was highest in rice bran oil, followed by corn, rape oil and soybean oil. The acid value for canola oil was 0.071, which is very low compared to corn and virgin olive oil (Kardash and Tur'yan, 2005).

Canola (*Brassica napus* L) may refer to an edible and commercial vegetable oil (also known as canola oil) produced from the seed of any of several cultivars of rapeseed bred to be low in erucic acid from the Brassicaceae family of plants.

Canola oil having a relatively low amount of saturated fatty acid, a substantial amount of monounsaturated fatty acid, with roughly a 2 : 1 mono- to poly-unsaturated fatty acids ratio (Lin *et al*., 2013).

The unique composition of fatty acids of rapeseed oil differentiates it from other vegetable oils. Rapeseed oil has a substantial amount (8 ± 12%) of linolenic acid ($C_{18:3}$) compared to other vegetable oils, such as soybean, sunflower, olive and corn, which contain approximately 8.0%, 0.2%, 0.8% and 0.7% of linolenic acid, respectively (Economou *et al*., 1991). The high content of unsaturated fatty acids (on average 38%) in rapeseed oil influences its stability (Bandoniene *et al*., 2000).

Blending oil with canola oil was lower in acid value and less changed than the other vegetable oils (Fig. 5). The lowering of the acid value of the branding oil seems to be due to the different chemical composition of unsaturated fatty acids. This was also demonstrated in the canola oil dilution experiment of Fig. 3.

Mostafa *et al*. (2013) also reported that when blended with 7 kinds of vegetable oils, linolenic acid increased and linoleic acid decreased when mixed with canola oil. Increase of unsaturated fatty acid will increase antioxidant activity. In other studies, blending of 7 vegetable oils has been reported to improve antioxidant capacity (Neff *et al*., 1993; Bhatnagar *et al*., 2009).

Bhatnagar *et al*. (2009) found that coconut oil and seven mixed plant oils increased the content of monounsaturated fatty acids (8% - 36%), polyunsaturated fatty acids (4% - 35%), total tocopherol (111 ㎎/㎏ - 582 ㎎/㎏), which showed a DPPH scan scavenging ability of 5% - 33%.

The higher the canola oil, the higher the viscosity. Because the viscosity of canola oil is higher than the viscosity of acid oil, the viscosity increases with blending. The viscosity of the edible oil measured at 24 degrees was reported as 78 for rapeseed, 52 for corn, 54 for soybeans and 56 for coconut (Noureddini *et al*., 1992).

In this study, branding oil containing 5 - 10% canola oil was more viscous than other edible fats. It was found that the manufacturing process of branding oil was required.

In this study, branding with canola oil was found to be stable against rancidity. Blending oil of canola oil and other edible oils have been reported to improve the stability of nutritional and edible oils. Blending of canola oil and sesame oil and/or rice bran oil enhanced the antifungal effect, total polar compound, carbonyl and acid value, and conjugated diene value on fried potato chips (Farhoosh and Kenari, 2009). Blending oil with canola oil and pumpkin seeds or olive oil enhanced frying potato stability, carbonyl value, acid value, total polar compound and total tocopherol content (Roiaini *et al*., 2015).

The absolute viscosity of the fluid is an important property required for fluid flow and heat transfer unit operation. This is a very important factor in the

manufacture and distribution of oil, such as pumping, flow measurement, heat exchange, sterilization, and refrigeration (Singh and Heldman, 2001).

Chromaticity was changed by diluting canola oil. The difference in the chromaticity of branding is also a characteristic of sancho oil and canola oil. The difference in chromaticity may also vary depending on the state of the seed before extraction and the extraction method. Joo *et al*. (2017) also reported that the values of L, a, and b changed as sesame oil was mixed with cooking oil such as corn and soybean oil. In this study, the chromaticity difference of the brand is considered to be the difference in color due to the inherent properties of sancho oil and canola oil, as well as the canola oil used for the experiment, which has degumming and deoxidation.

In this study, we confirmed that canola oil and blending oil inhibit rancidity and increase the availability of sancho oil. However, this study did not show the safety results of blending oil. Therefore, further study on safety as well as biological activity of blending oil is desired.

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