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Research on the Protective Effect of Tert-butyl hydroquinone and Butylated caffeic acid on Tocopherols under High Temperature Deep-frying

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Abstract. In order to explore the protective effect of TBHQ (tert-butyl hydroquinone) and BCA (butylated caffeic acid) on tocopherols in oils and fats, various physicochemical indexes of oil samples and changes of tocopherols content during high temperature frying and Rancimat accelerated oxidation were determined. The results showed that the addition of appropriate amount of TBHQ and BCA in oils and fats greatly improved the antioxidant activity, and the added TBHQ and BCA could effectively reduce the loss of tocopherols during the heating process of fats and oils and played a certain role in the protection of tocopherols. In comparison, BCA had a stronger protective effect, especially for the protection of δ -tocopherol.

1. Introduction

As one of the main nutrients of human beings, fats and oils not only provide essential fatty acids and fat-soluble vitamins, but also give food a good taste and flavor [1]. However, during the long-term high-temperature frying process, a series of physical and chemical changes occur in the fat and oil. The color of the oil is deepened, the viscosity is increased, and the odor is generated. A large amount of peroxidation is generated by chemical reactions such as polymerization, oxidation, and hydrolysis. And lipid oxides, adversely affect the quality of frying oils and fried foods [2-5], and even produce some harmful components such as polycyclic aromatic hydrocarbons, endangering human health [6]. In order to prevent and delay the oxidation and rancidity of oils, it is currently considered that the most effective method is to add an appropriate amount of antioxidants to the oils [7, 8].

Tert-butyl hydroquinone (TBHQ) is the most widely used antioxidant in oils and fats. It has a good protective effect on oils and fats. According to domestic and foreign research, the antioxidant capacity of TBHQ is 2-5 times of propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and TBHQ inhibits carotenoid decomposition and stabilizes oils and fats Tocopherol and bacteriostatic effects [9]. Butylated acid (BCA), synthesized by the Department of Food Nutrition and Function of the School of Life Sciences, Shanghai University, it is a kind of fat-soluble antioxidant transformed by a natural product of caffeic acid. The new antioxidant is especially suitable for use in high temperature oil and fat food processing [10].

Vitamin E (VE), a generic term for tocopherols and tocotrienols [11, 12], is one of the four fat-soluble vitamins indispensable to the human body. It is mainly found in vegetable oils, animal oils, as well as fruits and vegetables, eggs, nuts, citrus peel, lean meat, and milk [13]. As an important natural antioxidant added to oil, it not only can prevent and delay the oxidation of oil, but also can improve the



nutritional value of oil, protect biofilm, delay human aging, enhance the body's immunity and so on [14-17]. However, as a natural phenolic antioxidant, tocopherol is chemically active. Researches at home and abroad have shown that tocopherol is prone to loss during high-temperature frying [18-22]. However, how to reduce tocopherol loss during oil heating, and how the tocopherol changes in the presence of other exogenous antioxidants, etc., need to be further studied. Therefore, this paper mainly studies the protective effects of TBHQ and BCA on α -tocopherol and δ -tocopherol during high temperature frying.

2. Materials and methods

2.1. Materials and reagents

α -tocopherol standard (purity >99.0%), δ -tocopherol standard (purity >99.0%), TBHQ: Shanghai Maclean Biochemical Technology Co., Ltd. BCA (purity > 99.0%): synthesized and prepared by the Food Nutrition and Function Research Laboratory of the School of Life Sciences, Shanghai University. Petroleum ether, acetonitrile, methanol: China National Pharmaceutical Group Shanghai Chemical Reagent Company.

Lard: Commercially available pig oil, wet tanning. Jinlong brand soybean oil: commercially available. Potatoes: Commercially available potatoes are peeled, sliced (3cm \times 3cm \times 2mm), washed, placed in a 50ml centrifuge tube, centrifuged at high speed (4000 r / min), dried and stored.

2.2. Instruments and equipments

HPLC Agilent 1100, MYP11-2 magnetic stirrer, 743 Rancimat grease oxidation tester, J-752 UV-Vis spectrophotometer.

2.3. Methods

2.3.1. Rancimat experiment. The Rancimat oxidizer is set to a temperature of 80 °C, 100 °C, 120 °C, and air is continuously supplied at a gas flow rate of 20 L/h. Take 3 g of prepared lard in the reaction tube of Rancimat oil oxidized rancidity meter, then add the sample to be tested in the tube, 2 parallel samples in each group, and carry out blank control to calculate the protection coefficient of different antioxidants on oil Pf value:

$$Pf = \frac{IP_{\text{sample}}}{IP_{\text{control}}}$$

IP_{sample} : Oxidation induction time of antioxidant sample

IP_{control} : Oxidation induction time of blank sample

2.3.2. High temperature frying experiment. The experiment was divided into 7 groups: blank group, adding 0.02% α -tocopherol group, adding 0.02% δ -tocopherol group, adding 0.02% α -tocopherol and 0.01% TBHQ group, adding 0.02% δ -tocopherol and 0.01% TBHQ group, 0.02% α -tocopherol and 0.01% BCA group, adding 0.02% δ -tocopherol and 0.01% BCA group.

Put 500 g of soybean oil into a large beaker with a capacity of 1 L, add the above antioxidants to each beaker and mix well. Place the beaker on a magnetic stirrer and set the heating temperature to 180 °C \pm 5 °C, frying at a speed of 60 g potatoes/hour. Frying for 8 minutes each time every 10 minutes, continuous frying for 24 hours. No new oil is added during the experiment, sampling every 2 hours, and the samples were kept in a refrigerator at 2 °C for use.

2.3.3. Determination of Conjugated Double Bond Concentration. The oil sample was diluted to 1 g/mL with spectrally pure petroleum ether using a gradient dilution method. The absorbance of the sample at 233 nm was measured using a J-752 spectrophotometer (quartz cuvette 1 cm²). The conjugated double bond concentration (CD value) was calculated from the absorbance value.

$$CD = \frac{A}{C} P$$

A: Absorbance of the product at a wavelength of 233 nm;

C: Final dilution concentration of the sample (g/100 mL);

P: Quartz glass dish thickness (1 cm).

2.3.4. Determination of Acid Value. Refer to GB5009.229-2016 "National Food Safety Standard Determination of Acid Value in Food"

2.3.5. Determination of Iodine Value. Refer to GBT 5532-2008 "Determination of iodine value of animals and vegetables fats"

2.3.6. Determination of Tocopherol in Oil Samples. Accurately weigh 1 g of oil sample in a centrifuge tube, add 5 mL of methanol-acetonitrile (1:1, V / V) mixed solution, shake and mix for 5 min, ultrasonic bath for 10 min, and centrifuge at 4°C. After 10 min (8000 r/min), the layer was allowed to stand, and the supernatant was removed with a disposable plastic pipette, and the lower layer of the oil was discarded. The above steps were repeated twice, and the supernatants obtained twice were combined. It is dried by low pressure rotary evaporation and nitrogen blowing, and reconstituted with 200 µL of methanol. The sample was filtered through a 0.45 µm filter and the sample was stored in a 2 °C refrigerator for later use.

2.3.7. Determination of tocopherol content in oil samples from Rancimat accelerated oxidation process. The detection time of lard was determined on the 743 Rancimat oil oxidation stability tester. In the experiment, 5 g of prepared lard was accurately weighed into the reaction tube of the oil oxidation stability tester, and the air flow rate was adjusted to 20 L/h. The set temperature is 120°C. Each sample was sampled at 10 time points in sequence, and the sampling interval was determined according to the induction time of each sample. The collected samples were immediately cooled in a water bath and placed in a 2 °C refrigerator for refrigeration. According to the above high-performance liquid chromatography, the change tendency of the content of tocopherol in oil and fat was measured.

3. Results and analysis

3.1. Rancimat experiment results

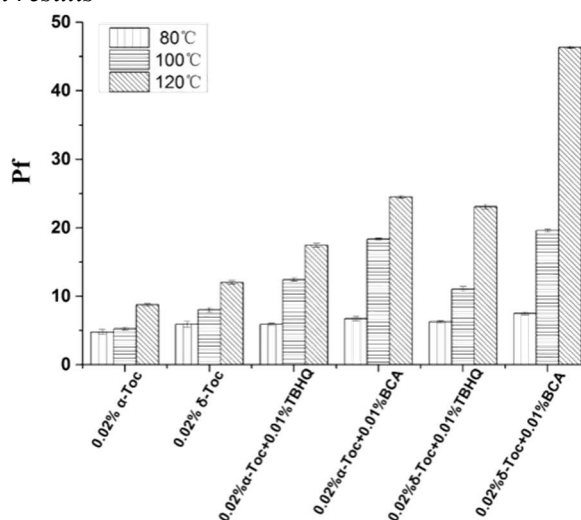


Figure 1. Pf values of tocopherols with BCA and TBHQ.

At the different temperatures shown in Figure 1, the antioxidant coefficient of the oil added with different antioxidants in lard directly reflects the antioxidant effect of each antioxidant on the oil. Under the condition that the type and quantity of antioxidants are the same, the higher the Pf value with the increase of temperature, the stronger the protective effect of each antioxidant on oil. The 0.02% δ -Toc with 0.01% BCA group has the strongest antioxidant properties among several antioxidants, the Pf value reached 46.34. The order of strength of each antioxidant was 0.02% δ -Toc+0.01% BCA group > 0.02% α -Toc+0.01% BCA group > 0.02% δ -Toc+0.01% TBHQ group > 0.02% α -Toc+0.01% TBHQ group > 0.02% δ -Toc group > 0.02% α -Toc group, studies have shown that the addition of TBHQ and BCA can effectively protect the tocopherol in oil, thereby enhancing the oxidative stability of oil, in contrast, BCA has a better protection effect for tocopherol.

3.2. Conjugate double bond content changes with time

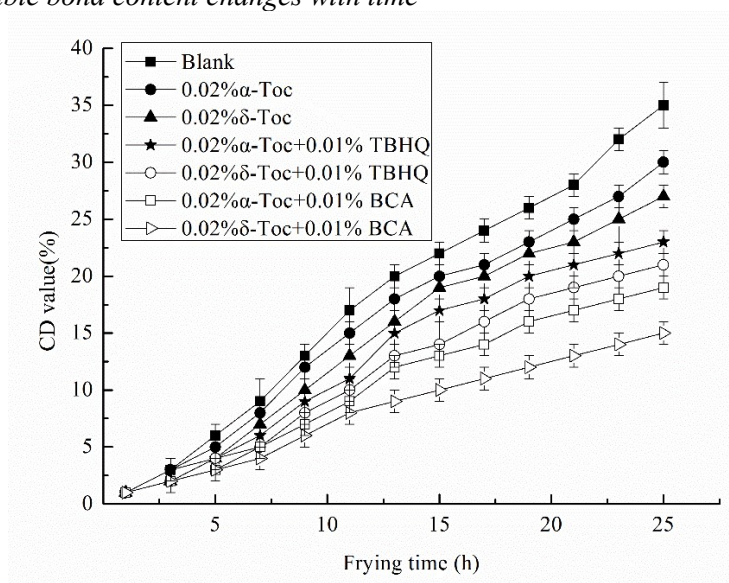


Figure 2. The content of conjugated double bonds of soybean oil during deep-frying

It can be seen from Fig. 2 that the content of conjugated diene in the oil and fat increases with the extension of the frying time, indicating that the oil is continuously oxidized during this process. In the initial stage of frying (0 h-8 h), the CD value is small, and the CD value of the oil with different antioxidants is not much different. As the frying time is prolonged, the CD value of the oil increases continuously, and the difference continues increasing. The CD value of the antioxidant group was significantly lower than that of the blank group, indicating that the added antioxidant exerted different antioxidant properties during the high temperature frying process.

The trend of CD values in each group was: blank group > 0.02% α -Toc group > 0.02% δ -Toc group > 0.02% α -Toc + 0.01% TBHQ group > 0.02% δ -Toc + 0.01% TBHQ group > 0.02% α -Toc + 0.01% BCA group > 0.02% δ -Toc + 0.01% BCA group. It shows that adding proper amount of tocopherol to oil and fat can effectively inhibit the conjugate of grease double bonds during high-temperature frying. When adding equal amount of tocopherol to fat and oil, 0.01% of TBHQ and BCA are added to inhibit the oil. The degree of yoke is enhanced, especially the addition of BCA, and the conjugate of the lipid double bond is well suppressed, and the oxidative stability of the oil is enhanced. The results are almost the same as those obtained by the above Rancimat accelerated oxidation experiment. The antioxidant activity of each antioxidant in the case of high temperature frying soybean oil at 180 °C is: 0.02% δ -Toc group + 0.01% BCA group > 0.02% α -Toc + 0.01% BCA group > 0.02% δ -Toc + 0.01% TBHQ group > 0.02% α -Toc + 0.01% TBHQ group > 0.02% δ -Toc group > 0.02% α -Toc group.

3.3. Changes in acid value over time during fat frying

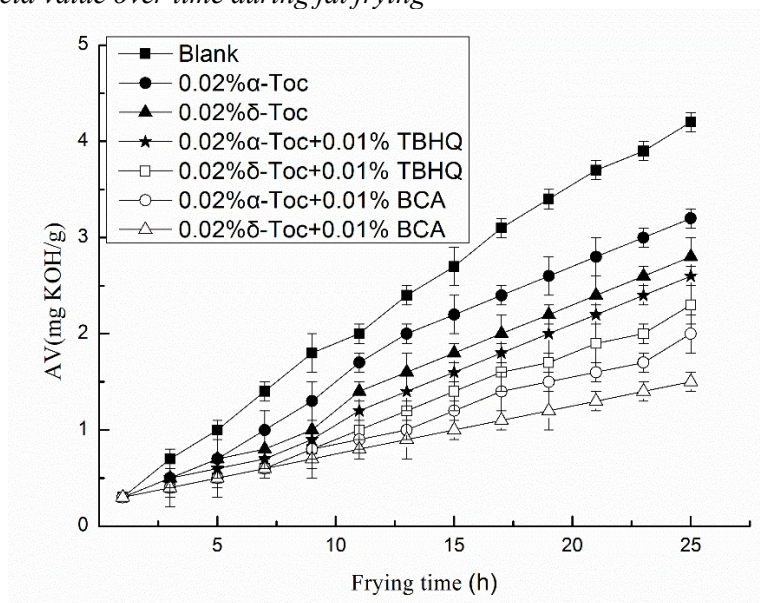


Figure 3. Acid values of soybean oil against time under $180^{\circ}\text{C} \pm 5^{\circ}\text{C}$ during deep-frying

It can be seen from Fig. 3 that with the extension of the frying time, the acid value of the seven frying oils tends to increase, indicating that the degree of oxidative rancidity of the oil increases. Due to the addition of antioxidants, the acid value of soybean oil during frying is smaller than that of the blank group, indicating that the added antioxidant can effectively inhibit the free fatty acids produced during the frying of the oil, thereby protecting the oil. During the whole frying process, the acid value of soybean oil was compared as follows: Blank group > 0.02% α -Toc group > 0.02% δ -Toc group > 0.02% α -Toc + 0.01% TBHQ group > 0.02% δ -Toc + 0.01% TBHQ group > 0.02% α -Toc + 0.01% BCA group > 0.02% δ -Toc group + 0.01% BCA group. Among them, the addition of 0.01% TBHQ and BCA to the oil and fat can effectively inhibit the production of free fatty acids during frying, thereby enhancing the oxidative stability of the oil, especially on the basis of adding δ -tocopherol. Adding 0.01% BCA, the acid value of the oil is the smallest, and the free fatty acid content is the least, which effectively prevents the oxidation of the oil. During soybean frying, the antioxidant activity sequence was: 0.02% δ -Toc group + 0.01% BCA group > 0.02% α -Toc + 0.01% BCA group > 0.02% δ -Toc + 0.01% TBHQ group > 0.02% α -Toc + 0.01% TBHQ group > 0.02% δ -Toc group > 0.02% α -Toc group, the results are the same as the antioxidant sequences of the respective antioxidants obtained by the conjugated double bond value and the Pf value.

3.4. Changes in iodine value over time during fat frying

It can be seen from Fig. 4 that the iodine value of fats and oils decreases with the prolongation of high temperature frying time, indicating that the unsaturated fatty acid content of soybean oil is decreasing during high temperature frying. During the whole frying process, the iodine value of the oil added to the antioxidant group was significantly lower than that of the blank group, indicating that the addition of the antioxidant can effectively protect the unsaturated fatty acid in the oil, thereby protecting the oil. The antioxidant activity of each antioxidant was in the order of: 0.02% δ -Toc group + 0.01% BCA group > 0.02% α -Toc + 0.01% BCA group > 0.02% δ -Toc + 0.01% TBHQ group > 0.02% α -Toc + 0.01% TBHQ group > 0.02% δ -Toc group > 0.02% α -Toc group, adding tocopherol to fat and fat added 0.01% TBHQ and BCA, effectively protecting soybean oil during high temperature frying. Unsaturated fatty acids, especially the addition of 0.01% BCA based on the addition of δ -tocopherol, have the lowest reduction in iodine value, and the ability to protect unsaturated fatty acids of soybean oil is the strongest.

This result is the same as the antioxidant sequence of each antioxidant obtained by the Pf value, the conjugated double bond value, and the acid value, and further indicates that the addition of BCA and TBHQ can delay the oxidation of the oil and improve the oxidative stability of the oil.

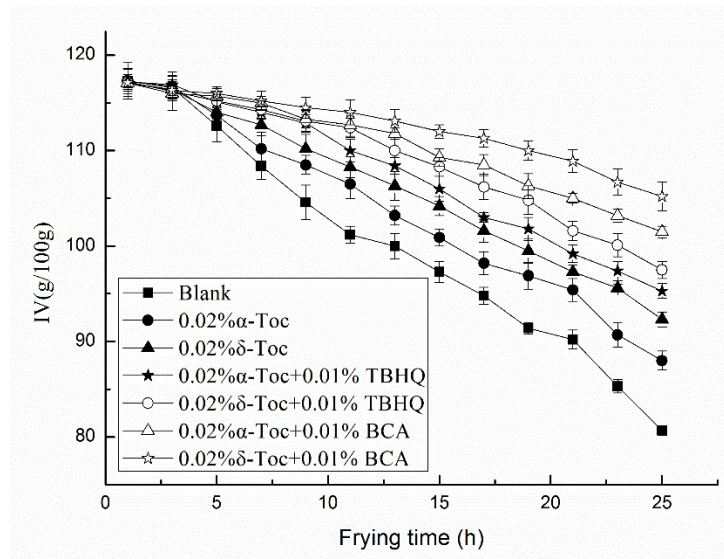


Figure 4. Iodine value of soybean oil against time during deep-frying under $180^{\circ}\text{C} \pm 5^{\circ}\text{C}$

3.5. Attenuation of Tocopherol Content in Soybean Oil

In order to further detect the change of tocopherol content with frying time, we used HPLC to determine the spectrum of tocopherol in the oil sample during high temperature frying experiment. After integrating the spectra, the peak area of each target in the sample taken at 0 min was 100% respectively, and the series peak area was normalized, and the normalized value was plotted against the sampling time to obtain the decay curve of tocopherol content [23] as the picture shows.

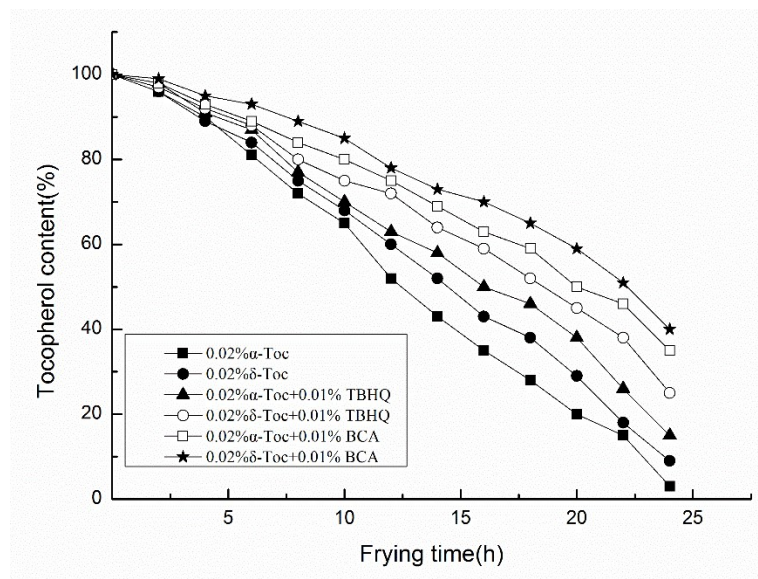


Figure 5. Attenuation Rule of Tocopherol Content in Soybean Oil during deep-frying

As can be seen from Figure 5, during the high temperature frying process, the tocopherol content is continuously decreasing as the frying time. Among the oil samples with only the antioxidants α -tocopherol and δ -tocopherol, the tocopherol content decreased the fastest. After 24 h high-temperature frying, the content of α -tocopherol and δ -tocopherol was attenuated to the 5%, 8%, while adding 0.01% TBHQ to the oil sample, the rate of tocopherol decay is slowed down. After 24 h high temperature frying, the content of α -tocopherol, δ -tocopherol was attenuated by 17% and 28%. Compared with the oil sample with tocopherol alone, the content of α -tocopherol and δ -tocopherol increased by 12% and 20% after 24 h high temperature frying. While adding 0.01% of BCA to the same amount of tocopherol, the content of tocopherol decays more slowly. After 24 h of high temperature frying, the content of α -tocopherol and δ -tocopherol still reaches 32%. 40% respectively, compared with the oil sample added with tocopherol alone, the content of α -tocopherol and δ -tocopherol increased by 27% and 32% after 24 h high temperature frying. The presence of TBHQ and BCA effectively reduces the loss of tocopherols during high temperature frying, and the protective effect of BCA on tocopherols is more obvious, especially for a stronger protective effect on δ -tocopherol.

3.6. Rancimat accelerates the oxidation process of tocopherol content attenuation

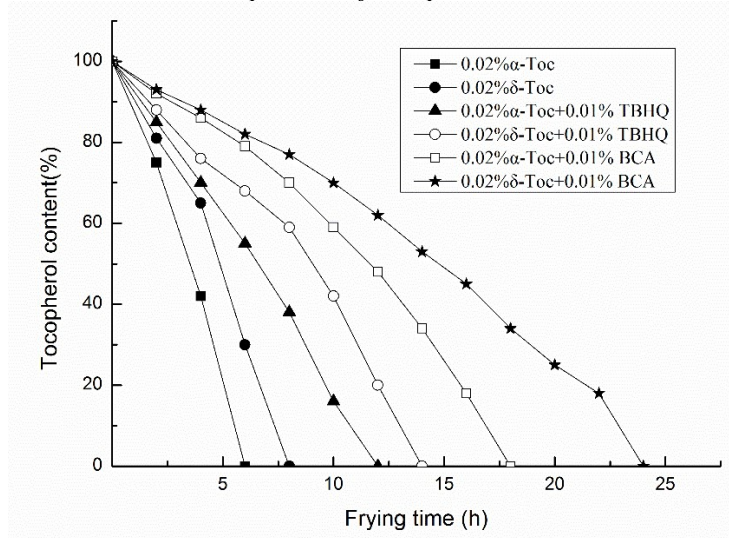


Figure 6. Attenuation curve of Tocopherol Content in lard under Rancimat accelerates oxidation

In order to further reveal the law of the change of tocopherol content with time in the heating process of fat and oil, the temperature of the Rancimat oil oxidation stability meter is set to 120 °C, the gas flow rate is 20 L/h. The curve of the decay of tocopherol content can be seen from Fig. 6. In the accelerated oxidation process of 20 L/h at 120 °C, the attenuation rate of tocopherol in the oil samples with only α -tocopherol and δ -tocopherol was the fastest, and the content was 0 after 6 h and 8 h, respectively; adding 0.01% of TBHQ on the basis of adding the same amount of tocopherol, the decay rate of tocopherol is obviously slowed down, and it changes to 0 after 12 h and 14 h respectively; adding 0.01% of BCA on the basis of adding the same amount of tocopherol in the oil sample, the tocopherol content was attenuated more slowly. Among the oil samples with 0.01% BCA added to the addition of δ -tocopherol, the tocopherol content became 0 after 24 h. The protective effect of TBHQ and BCA on tocopherol during oil heating process was further explained, and BCA had stronger protective effect on δ -tocopherol. This result is consistent with the conclusions obtained during the high-temperature frying experiment, which fully proves the protective effect of BCA and TBHQ on tocopherols under high temperature heating and frying conditions.

4. Conclusion

In this paper, the following conclusions are obtained by simulating the high temperature frying experiment and the Rancimat accelerated oxidation experiment:

(1) During the continuous high-temperature frying process for 24 hours, the acid value and conjugate double bond value of soybean oil increased gradually with the extension of frying time, and the iodine value gradually decreased, but the oxidation stability of the oil sample added with antioxidant was significantly enhanced. And antioxidant effects of various antioxidants: 0.02% δ -Toc+0.01% BCA group >0.02% α -Toc+0.01% BCA group >0.02% δ -Toc+0.01% TBHQ group >0.02% α -Toc +0.01% TBHQ group >0.02% δ -Toc group >0.02% α -Toc group.

(2) The protection coefficient of various antioxidants on oils and fats under the conditions of 80 °C, 100 °C and 120 °C was measured by Rancimat oil oxidative rancidity. It was found that 0.01% of the tocopherol was added to the fats and oils, the oxidative stability of fats and oils can be significantly enhanced, and the higher the temperature, the stronger the oxidative stability of the oil.

(3) Using HPLC method to detect the change trend of tocopherol content during the high temperature frying experiment and the accelerated oxidation process of Rancimat at 120 °C, it was found that the addition of TBHQ and BCA can effectively protect the tocopherol in oil, especially for δ -tocopherol. In contrast, the protective effect of BCA for δ -tocopherol is more obvious. The results of this study can provide a theoretical basis for the application of BCA in vegetable oils and the protection of tocopherols.

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