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National Food Safety Standard - Determination of Polar Components (PC) in Edible Oils

食品安全国家标准

食用油中极性组分（PC）的测定

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National Food Safety Standard - Determination of Polar Components (PC) in Edible Oils

1 Scope

This Standard specifies the determination method for polar components (PC) in edible animal and vegetable oils.

This Standard is applicable to the determination of polar components (PC) content in various edible animal and vegetable oils.

Method I Preparative Flash Column Chromatography

2 Principle

Through the separation of preparative flash column chromatography, oils and fats samples are divided into two parts: non-polar components and polar components. The non-polar components are firstly eluted; then, after the evaporation of the solvent to dryness, weigh it. After deducting the remaining part of non-polar components from oils and fats samples, polar components can be obtained.

3 Reagents and Materials

NOTE: unless it is otherwise stipulated, all reagents used in this Method are analytically pure; water is Grade-1 water stipulated in GB/T 6682.

3.1 Reagents

3.1.1 Ether ($C_4H_{10}O$), before usage, place it in low-temperature environment for several hours, so that its temperature can be controlled between 10 °C ~ 18 °C.

3.1.2 Petroleum ether, boiling range: 30 °C ~ 60 °C. Before usage, place it in low-temperature environment for several hours, so that its temperature can be controlled between 10 °C ~ 18 °C.

3.1.3 Acetone (C_3H_6O), before usage, place it in low-temperature environment for several hours, so that its temperature can be controlled between 10 °C ~ 18 °C.

3.1.4 Trichloromethane ($CHCl_3$).

3.1.5 Glacial acetic acid ($C_2H_4O_2$).

4.3 Rotary evaporator.

4.4 Balance: division value shall at least reach 0.001 g.

4.5 Vacuum constant-temperature drying oven.

4.6 Constant-temperature drying oven.

4.7 Glass desiccator (filled with color-changing silica gel desiccant).

4.8 Thin-layer chromatographic glass chromatography cylinder: can be matching with thin-layer chromatographic plate (3.3.2).

4.9 10 mL glass beaker.

4.10 10 mL disposable plastic syringe.

4.11 1,000 mL small mouth glass collection bottle.

5 Analytical Procedures

5.1 Preparation of Samples

5.1.1 Removal of impurity

Test samples shall be liquid, limpid and precipitation-free; they shall also be thoroughly mixed. If samples are not limpid, or if samples have precipitation, then, place oils and fats in 50 °C constant-temperature drying oven. Heat up the oils and fats to 50 °C; thoroughly shake it, so that the possible crystallization of oils and fats can be dissolved. At this moment, if the oils and fats samples become limpid and precipitation-free, then, they can be considered as samples. Otherwise, place the oils and fats in 50 °C constant-temperature drying oven; use filter paper to filter the insoluble impurities; take the filtered limpid liquid oils and fats as the samples. In order to avoid oxidation of oils and fats, the filtration process shall be completed as soon as possible.

In terms of samples whose freezing point is higher than 50 °C, or, samples which contain oils and fats whose freezing point is higher than 50 °C, place the oils and fats in constant-temperature drying oven, which is around 10 °C higher than the freezing point. Heat up and thoroughly shake the oils and fats, so that the possible crystallization of oils and fats can be dissolved. If filtration is still needed, place the oils and fats in constant-temperature drying oven, which is around 10 °C higher than the freezing point. Use filter paper to filter the insoluble impurities; take the filtered limpid liquid oils and fats as the samples. In order to avoid oxidation of oils and fats, the filtration process shall be completed as soon as possible.

5.1.2 Drying and dehydration

Method II Column Chromatography

8 Principle

Through the separation of column chromatography, oils and fats samples are divided into two parts: non-polar components and polar components. The non-polar components are firstly eluted; then, after the evaporation of the solvent to dryness, weigh it. After deducting the remaining part of non-polar components from oils and fats samples, polar components can be obtained.

9 Reagents and Materials

Unless it is otherwise stipulated, all reagents used in this Method are analytically pure; water is Grade-1 water stipulated in GB/T 6682.

9.1 Reagents

9.1.1 Column chromatography adsorbent: silica gel 60, SiO_2, amorphous silica gel (grain size: 0.063 mm ~ 0.200 mm); average pore size: 6 nm; pore volume: 0.74 mL/g ~ 0.84 mL/g; specific surface area: 480 m^2/g ~ 540 m^2/g; pH: 6.5 ~ 7.5; moisture content: 4.4% ~ 5.4%.

9.1.2 Sea sand, chemically pure.

9.2 Preparation of Reagents

9.2.1 Non-polar components eluent: same as 3.2.1.

9.2.2 Thin-layer chromatographic developer: same as 3.2.3.

9.2.3 Thin-layer chromatographic color developing agent: same as 3.2.4.

9.3 Materials

Thin-layer chromatographic plate: same as 3.3.2.

10 Instruments and Equipment

10.1 Glass chromatographic column: inner diameter: 21 mm; length: 450 mm; the lower part has a PTFE piston valve. There is a layer of sand core sieve plate in the chromatographic column in the upper part of the piston valve. Moreover, this layer of sand core sieve plate can effectively prevent the adsorbent (9.1.1) from leaking out of the chromatographic column. In addition, after 20 mL of petroleum ether is vertically added, open the piston valve to the maximum; all the petroleum ether shall drain within

of the eluent, so as to collect the eluent.

11.3.3 Use a transfer pipette to accurately transfer-take 20 mL of sample solution (11.3.1) into the already-filled glass chromatographic column. During this process, avoid the sample solution's interference with the sea sand layer on top of the chromatographic column. Open the piston valve on the lower end of the glass chromatographic column; discharge the eluent inside the chromatographic column, till the liquid level of the eluent in the chromatographic column drops to the top of the sea sand layer. During this process, collect the outflowing eluent in the 250 mL flask (containing non-polar components) below it.

11.3.4 In 2 ~ 3 times, add a total of 200 mL of non-polar eluent into the glass chromatographic column, so as to continue to elute the non-polar components. Collect all the eluent in the same 250 mL flask. During this period, adjust the piston valve on the lower end of the glass chromatographic column, so that the 200 mL of eluent can completely pass through the glass chromatographic column within 80 min ~ 90 min.

After the elution is completed, use a transfer pipette or a burette to absorb the non-polar eluent to rinse the substance that is attached to the entrance of the solvent on the lower end of the glass chromatographic column. Combine the eluent in the same 250 mL flask.

11.3.5 After completing the operation in 11.3.3 and 11.3.4, immediately use 150 mL of ether to elute the polar components that are adsorbed by the chromatographic column. Collect the eluent in another 250 mL flask. After the elution is completed, discard the silica gel inside the glass chromatographic column.

11.3.6 Place the 250 mL round-bottom flask, which contains non-polar components eluent, into the rotary evaporator, with the water bath temperature of 60 °C. Under the condition of normal pressure, evaporate most of the solvent. Then, under the condition of negative pressure, conduct rotary evaporation of the small amount of the remaining solvent to near dryness. Then, take out the flask; wipe the water on the outer wall of the flask. Then, place the 250 mL round-bottom flask into 40 °C vacuum constant-temperature drying oven. Under the negative pressure of 0.1 MPa, start drying for 20 min ~ 30 min. After the drying ends, place it into the glass desiccator to cool it down to room temperature. The residue shall be non-polar components. Then, weigh it (m_1, accurate to 0.001 g). ($m_1 - m_0$) shall be the mass of the non-polar components.

11.3.7 Place the 250 mL round-bottom flask, which contains polar components eluent, into the rotary evaporator, with the water bath temperature of 60 °C. Under the condition of normal pressure, evaporate most of the solvent. Then, under the condition of negative pressure, conduct rotary evaporation of the small amount of the remaining solvent to near dryness. Then, take out the flask; wipe the water on the outer wall of the flask. Then, place the 250 mL round-bottom flask into 40 °C vacuum constant-temperature drying oven. Under the negative pressure of 0.1 MPa, start drying for 20 min ~ 30 min. After the drying ends, place it into the glass desiccator to cool it down to

Appendix B

Thin-layer Chromatographic Verification of Separation Effect of Polar Components and Non-polar Components of Preparative Flash Column Chromatography

B.1 Thin-layer Chromatogram of Polar Components and Non-polar Components

See Figure B.1.

B.2 Elution of Polar Components

If polar components need to be eluted, after completing the operation in 5.3, immediately conduct further elution treatment of the flash separation preparative chromatographic column, which adsorbs polar components. Please see the specific parameters below:

a) Mobile phase: polar components eluent;

b) Flow rate of mobile phase: 25 mL/min;

c) Fraction collector: use another clean 1,000 mL small mouth glass collection bottle as the collection bottle. Start from elution, collect under the full-collection mode.

d) UV detector monitoring wavelength: 200 nm;

e) Initiate the real-time monitoring system; obtain elution chromatogram;

f) Temperature of solvent temperature control system: 10 °C;

g) Elution time: 20 min.

Take another clean 500 mL flask. Pour all the eluent collected in the 1,000 mL small mouth glass collection bottle into the 500 mL round-bottom flask. Then, place the 500 mL round-bottom flask, which contains the collected eluent, into the rotary evaporator, with the water bath temperature of 60 °C. Firstly, under non-vacuum condition, evaporate most of the solvent. Then, under the condition of negative pressure, conduct rotary evaporation of the small amount of the remaining solvent to near dryness. Then, place the 500 mL round-bottom flask into 40 °C vacuum constant-temperature drying oven. Under the negative pressure of 0.1 MPa, start drying for 20 min ~ 30 min. Then, place it into the glass desiccator to cool it down to room temperature. The residual shall be polar components.

If the amount of the eluent is excessive, collect and concentrate it in several times. In other words, firstly collect a part of the eluent in the 500 mL round-bottom flask, rotate-

Appendix C

Thin-layer Chromatographic Verification of Separation Effect of Polar Components and Non-polar Components

C.1 Thin-layer Chromatogram of Polar Components and Non-polar Components

See Figure C.1.

C.2 Thin-layer Chromatographic Verification of Polar Components and Non-polar Components

Use trichloromethane to respectively prepare the separately prepared polar components and non-polar components into solutions with the mass volume concentration of around 10%. Use 2 spotting capillaries to respectively absorb around 2 μL of polar components solution and around 2 μL of non-polar components solution. At a distance of around 3 cm from the lower edge of the thin-layer chromatographic plate, respectively conduct sample spotting and evaporation of the solvent. Add a proper amount of thin-layer chromatographic developer to the thin-layer chromatographic glass chromatography cylinder; add the thin-layer chromatographic plate, which has spotted sample, for developing. After around 35 min, when the liquid level of the developer spreads to a distance of around 17 cm from the upper end of the thin-layer chromatographic plate, take out the thin-layer chromatographic plate; at room temperature, evaporate the solvent to dryness. In the mode of spraying, uniformly spray the thin-layer chromatographic color developing agent onto the surface of the silica gel coating of the thin-layer chromatographic plate; at room temperature, evaporate the solvent to dryness. Then, place the thin-layer chromatographic plate into 120 °C ~ 130 °C constant-temperature drying oven; heat it up, till the spots begin to develop colors. In accordance with the separation degree of polar components and non-polar components on the thin-layer chromatographic plate, verify the separation effect. The result is shown in Figure C.1.

In terms of sample with a high content of polar components, since it contains a small amount of strongly-polar substances (generally speaking, the content will not exceed 1% ~ 2 %) that cannot be eluted under the elution conditions of the column chromatography in this Standard, the recovery of polar components in the sample will be incomplete.

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