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China - Peoples Republic of

Post: Beijing

National Food Safety Standard - Trans Fatty Acids

Report Categories:

FAIRS Subject Report

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Report Highlights:
On November 20, 2009, China notified the WTO of "National Food Safety Standard of the People's Republic of China for Determination of Trans Fatty Acids in Foods for Infants and Children" as SPS/N/CHN/150. The date for submission of final comments to the WTO is January 1, 2010. The proposed date of entry into force has not been specified.

Executive Summary:
On November 20, 2009, China notified the WTO of "National Food Safety Standard of the People's Republic of China for Determination of Trans Fatty Acids in Foods for Infants and Children" as SPS/N/CHN/150. The date for submission of final comments to the WTO is January 1, 2010. The proposed date of entry into force has not been specified.

Thanks go to the consortium of industry and 3rd country Embassies in Beijing for their assistance in

translating and reviewing this standard.

This report contains an UNOFFICIAL translation of National Standard on Determination of Trans Fatty Acids in Foods for Infants and Children.

General Information:

BEGIN TRANSLATION

ICS 67.100.10
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GB National Food Safety Standard
GB ××××—××××

Determination of Trans fatty Acids in Raw Milk and Dairy Product Foods for Infants and Young Children

(Draft for Comment)

Issued on xx-xx-xxxx Implemented on xx-xx-xxxx

Issued by the Ministry of Health

of the People's Republic of China

Foreword

Annex A of this present National Standard is informative annex.

This present National Standard was proposed by and is under the jurisdiction of Ministry of Health, the People's Republic of China.

1. Scope

This present National Standard specifies the method for determination of trans fatty acids in foods for infants and young children, raw milk and dairy products.

This present National Standard is applicable to the determination of trans fatty acids in foods for infants and young children, raw milk and dairy products.

The detection limit of this present National Standard is 30 mg/kg.

2. Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this present standard. Note: As for the dated references, all the amendments or revisions after them except the corrigenda are not applicable to this present standard. However, parties to agreements based on this present national standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. As for the references that are not dated, their most recent editions are applicable to this present national standard.

GB/T 6682 Water for analytical laboratory use - Specification and test methods

3. Principle

The fat in sample is extracted by organic solvents. The extract product reacts with methanol under alkali condition to get fatty acid methyl esters, from which cis and trans fatty acid methyl esters can be separated by gas chromatograph with hydrogen flame ionization detector. The external standard method is used for quantification.

4. Reagents

Unless otherwise specified, purity of all reagents used in this present method is analytically pure, and that of water used in the test is the third-graded specified in GB/T 6682.

4.1 Petroleum ether: Boiling range: 30-60 Celsius.

4.2 Ethyl ether.

4.3 Ethanol: 95% volume fraction.

4.4 Normal hexane: Chromatographically pure.

4.5 Ammonia: 25-28 %.

4.6 Potassium hydroxide.

4.7 Methanol.

4.8 Amylase: Activity unit 1.5 U/mg, on which the dose depends.

4.9 Anhydrous sodium sulfate.

4.10 Potassium hydroxide – methanol solution (4mol/L): Weigh 26.4 g of potassium hydroxide, and dissolve it into about 80 mL of anhydrous methanol. Cool to room temperature, and dilute to 100 mL with methanol. Add about 5g of the anhydrous sodium sulfate (4.9), stir well, and filter it. Store the filtrate.

4.11 Standard substance of fatty acid methyl ester: Methyl octadecanoate (C18:0), trans-9-methyl octadecanoate (C18:1 9t), cis- 9-methyl octadecanoate (C18:1 9c), trans-9, 12- methyl octadecadienoate (C18:2 9t,12t), cis-9, 12- methyl octadecadienoate (C18:2 9c,12c).

4.12 Standard stock solutions of trans fatty acid methyl esters, of which the concentrations are both 10.0 mg/ mL. Weigh 500 mg of standard trans-9-methyl octadecanoate and standard trans-9, 12- methyl octadecadienoate accurately (accurate to 0.1 mg), and dissolve and dilute them to 50 mL with normal hexane respectively. Store them in refrigerator at -18℃.

4.13 Intermediate solution of trans fatty acid methyl esters, of which the concentrations are both 1.0

mg/ mL. Draw 10.0 mL of the stock solutions of two standard stock solutions of trans fatty acid methyl esters (4.12) respectively into one and the same 100 mL volumetric flask, and dilute to volume with normal hexane (4.4). The solution should be prepared fresh before use. It acts as the peak of standard curve.

4.14 A series of standard working solutions: Prepare fresh before use. Pipet 0, 2.0, 4.0, 6.0, 8.0, and 10.0 mL of the standard intermediate solution (4.13) in 10 mL volumetric flasks, and dilute them with normal hexane respectively. The concentrations of this series of solutions are 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL respectively.

4.15 Standard mixture of fatty acids methyl esters: Prepare the standard mixture of fatty acids methyl esters from the standard fatty acids methyl ester (4.11) and normal hexane, in which concentrations of each components are about 0.05 mg/mL to 0.5 mg/mL. It is used for identification of separation degree of cis and trans fatty acid methyl esters and for qualification.

4.16 Congo red solution: Weigh 1g of Congo red, dissolve and dilute to 100 mL.

5. Apparatus

5.1 Gas chromatography: with hydrogen flame ionization detector.

5.2 Rotary evaporator.

5.3 Constant temperature water bath.

5.4 Vortex shaker.

5.5 Centrifuge.

5.6 Mojonnier fat extraction bottle.

5.7 Shaker for Mojonnier fat extraction bottle.

5.8 Fat collection bottle.

5.9 Balance: accurate to 0.01 g and 0.0001 g.

6. Analytical steps

6.1 Pretreatment of sample

6.1.1 Starch-containing sample: Weigh about 1.5 g of solid sample that has been mixed well or about 5 g of solid sample (*sic.*) (accurate to 0.1 mg) into the Mojonnier fat extraction bottle, add about 0.1 g of amylase (enzyme activity 1.5 U/mg), and mix well. Add 8 ~ 10mL of water at 45℃, shake to mix well, and cork it; keep it in the water bath at 55 ℃ for 2 hours. Shake it every 10 minutes. To test whether the starch is hydrolyzed completely: Add two drops of iodine solution of about 0.1mol/L; if blue color does not appear, the hydrolysis is complete; otherwise the fat extraction bottle should be put into the water bath again until the blue color disappears. Take the Mojonnier fat extraction bottle out and cool it.

6.1.2 Starch-free sample: Weigh about 1.5 g of solid sample that has been mixed well or about 5 g of solid sample (*sic.*) (accurate to 0.1 mg) into the Mojonnier fat extraction

bottle, add 10mL of water at 45℃, and elute the sample into the small ball of the fat extraction bottle. Mix well until the sample disperses completely. Cool it. Liquid sample: Weigh about 10 g of sample that has been mixed well in Mojonnier fat extraction bottle directly (accurate to 0.1 mg).

6.1.3 Extraction of fat: Add 3.0mL of ammonia (4.5) into the Mojonnier fat extraction bottle, and mix well. Keep it into the water bath at 60℃ for 15 ~ 20 minutes. Cool to room temperature. Add 10mL of the ethanol (4.3) and 1 drop of the Congo red solution (4.16), and mix well. Add 25.0mL of ethyl ether (4.2), cork it, and shake for 1min on the Shaker for Mojonnier fat extraction bottle. Add 25mL of the petroleum ether (4.1), shake for 1 minute, and then centrifuge at 4000 r/min for demixing. Pour the supernatant into the fat collection bottle. This is the first extraction. Add 5mL of ethanol, 25mL of ethyl ether, and 25mL of petroleum ether into the residual sample solution, and carry out the second extraction as the same method mentioned above. Pour the supernatant, and merge it with that from the first extraction. Rotary evaporation is carried out with nitrogen gas at 60℃ to remove the solvent and maintain the residue, or fat.

6.1.4 Preparation of fatty acid methyl ester: Dissolve the fat mentioned above with 10mL of normal hexane (4.4), and take 3.0mL into a 10mL tube with lid; add 0.3mL of the potassium hydroxide – methanol solution (4.10). Cork it, and shake vigorously on the vortex shaker for 2 minutes. Centrifuge at 4000 r/min for 5 minutes, and transfer the supernatant into GC test sample bottle. This is the test sample solution.

6.2 Determination

6.2.1 Reference condition for chromatography

Chromatographic column: Capillary column in which the filling material is cyanpropyl aryl polysiloxane, the length is 100m, the inside diameter is 0.25 mm, and the membrane thickness is 0.2μm; or other chromatographic column with equivalent performance.

Injector temperature: 250℃; Carrier gas: (N2)

Detector temperature: 300℃;

Splitting ratio: 10: 1;

Injection volume: 1.0μL.

Temperature programming:

Heating rate (℃/min)	Target temperature (℃)	Retention time (min)
Initial temperature	120	0
10	175	10
5	210	5
5	230	5

6.2.2 Drawing of standard curve.

Under the optimal working condition of apparatus, inject the series of standard

working solutions (4.14) respectively. Draw the standard curve, in which y-axis is the peak area, and x-axis is the concentration of standard working solution.

6.2.3 Identification of trans fatty acid methyl ester:

Inject the standard mixed solution of fatty acid methyl ester (4.15) for identification of separation degree of cis and trans fatty acid methyl ester and qualification. The positions of peaks of trans methyl octadecanoate and trans methyl octadecadienoate should comply with Figures 1, 2 and 3 of Annex A.

6.2.4 Determination of test sample solution

Inject the test sample solution in the gas chromatograph. For the position of peak of trans fatty acid methyl ester in test sample solution, please refer Figure 2 of Annex A. Determine the total peak area in the areas of C18: 1t and C18: 2t; consult the standard curve to obtain the mass fraction of trans methyl octadecanoate and trans methyl octadecadienoate in the test solution.

7. Calculation and expression of result

The total content of trans fatty acids in test sample, X, of which the unit is milligram per hundred grams (mg/100g), should be calculated as formula (1):

$$X = X_1 + X_2 \quad \text{.....(1)}$$

in which:

X——the total content of trans fatty acids, of which the unit is milligram per hundred grams (mg/100g);

X_1—— the content of trans octadecanoic acid in test sample, of which the unit is milligram per hundred grams (mg/100g);

X_2 —— the content of trans octadecadienoic acid in test sample, of which the unit is milligram per hundred grams (mg/100g).

The content of trans octadecanoic acid and trans octadecadienoic acid in test sample are represented by X_1 and X_2 respectively, and expressed as mass fraction (mg/100g); they should be calculated as formula (2):

$$X_{(1or2)} = \frac{c_i \times V \times n \times M_{ai}}{m \times M_{bi}} \times 100 \quad \text{.....(2)}$$

In which:

$X_{(1or2)}$—— the content of trans octadecanoic acid or trans octadecadienoic acid in test sample, of which the unit is milligram per hundred grams (mg/100g);

V —— the constant volume of the test sample solution, of which the unit is milliliter (mL);

N—— the dilution times of test sample;

M—— mass of sample, of which the unit is gram (g);

c_i—— the mass concentration of trans methyl octadecanoate or trans methyl octadecadienoate in the test sample solution, of which the unit is milligram per milliliter (mg/mL);

M_{ai}—— the molecular weight of trans octadecanoic acid or trans octadecadienoic acid;

M_{bi} ——the molecular weight of trans methyl octadecanoate or trans methyl octadecadienoate.

The calculation result should be expressed as the arithmetic mean of two individual determinations under repeated condition, and should be accurate to one decimal place.

8. Precision

The absolute difference of results of two individual determinations under repeated condition should not be over 10 % of the arithmetic mean.

Annex A

(Informative annex)

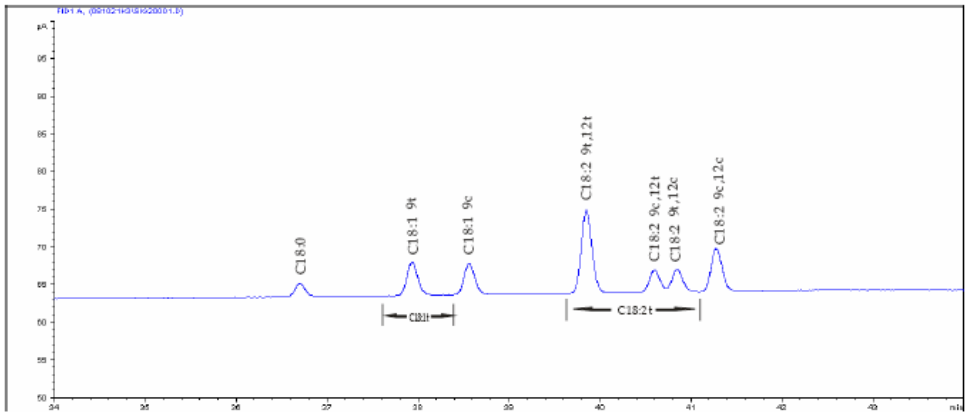

Figure1: Chromatogram of mixed standard solution of trans fatty acids

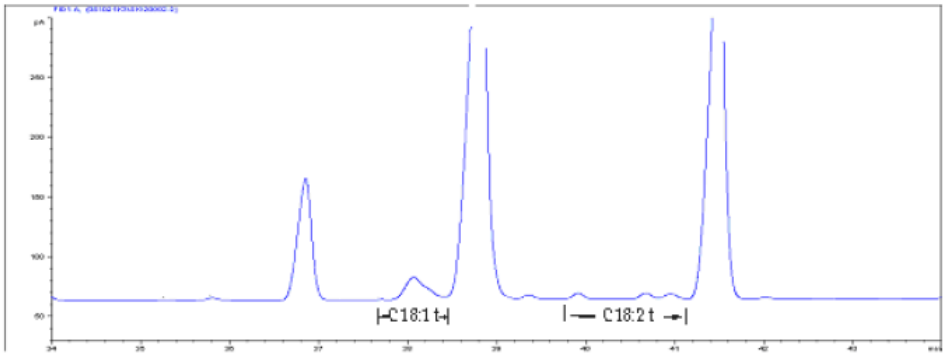

Figure 2: Chromatogram of test sample solution.

Note: C18: 1t is the area of retention time of peak of trans methyl octadecanoate; C18: 2t is that of trans methyl octadecadienoate.

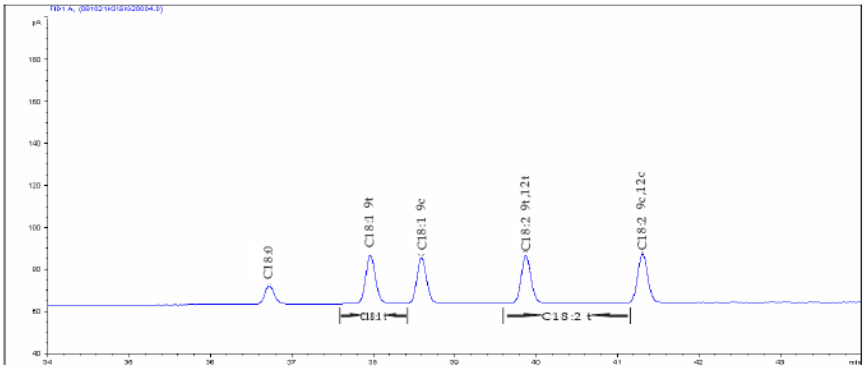

Figure 3: Chromatogram of mixed standard solution of trans fatty acids