

doi: 10.13241/j.cnki.pmb.2015.18.043

· 技术与方法 ·

氢火焰气相色谱仪内标法测定反式脂肪酸的条件的建立及验证*

陈哲¹ 张福庆¹ 陈兴才¹ 王兆琪¹ 程宇¹王明霞¹ 李省三¹ 李继媛¹ 苏莹¹ 孙国新² 姜峰阁^{1Δ}

(1 齐齐哈尔医学院 黑龙江 齐齐哈尔 161006; 2 黑龙江出入境检验检疫局 黑龙江 哈尔滨 150001)

摘要 目的:研究氢火焰气相色谱仪(GC112A)内标法测定反式脂肪酸的实验室最佳条件。**方法:**采用气相色谱内标-标准曲线法,通过测定反式脂肪酸标准品工作液的标准曲线、重现性和分离度来确定和验证 GC112A 的最佳色谱条件。**结果:**实验室最佳色谱条件:毛细管柱,PC-88(100 m× 0.25 mm× 0.2 μm);进样口温度:260 ℃;载气:高纯 N₂,压力 220 kPa,流速 41.0 mL/min;检测器(FID)温度:290 ℃,氢气压力 100 kPa,流速 21.0 mL/min;空气压力 160 kPa,流速 215.0 mL/min;检测器(FID)灵敏度:1010;手动进样,进样量:1.0 μL。**结论:**该方法避免了外标法(国标方法)的国产仪器局限性和操作者人工进样的不确定性,具有受仪器参数变化的影响较小的特点,适合在基层食品检验机构推广。

关键词:气相色谱仪;反式脂肪酸;反-9-十八碳一烯酸甲酯(C18:1-9t);反-9,12-十八碳二烯酸甲酯(C18:2-9t,12t)

中图分类号:O657.7+1;TS207 **文献标识码:**B **文章编号:**1673-6273(2015)18-3564-06

The Building and Validation of the Optimal conditions' Determination for Laboratory Hydrogen Flame Gas GC Method the Trans Fatty Acids by Internal Standard *

CHEN Zhe¹, ZHANG Fu-qing¹, CHEN Xing-cai¹, WANG Zhao-qi¹, CHENG Yu¹,WANG Ming-xia¹, LI Xing-san¹, LI Ji-yuan¹, SU Ying¹, SUN Guo-xin², LOU Feng-ge^{1Δ}

(1 Qiqihar Medical College, Qiqihar, Heilongjiang, 161006, China; 2 Heilongjiang CIQ, Harbin, Heilongjiang, 150001, China)

ABSTRACT Objective: To measure laboratory optimum conditions of trans-fatty acids in Hydrogen flame gas chromatograph (GC112A) internal standard method. **Methods:** We used gas chromatography internal standard - a standard curve method to testify and confirm the best chromatographic condition of GC112A by measuring trans-fatty acids standard working liquid standard curve, reproducibility and degree of separation. **Results:** Laboratory optimum chromatographic conditions: Capillary Column, PC-88 (100m× 0.25mm× 0.2 μm); Inlet temperature: 260℃; The carrier gas: Purity N₂, the pressure 220 kPa, flow rate of 41.0 mL/min; detector (FID) temperature: 290 ℃, hydrogen pressure of 100 kPa, flow rate of 21.0 mL/min; air pressure 160kPa, flow rate 215.0 mL/min; detector (FID) Sensitivity: 1010; manual injection, injection volume: 1.0μ L. **Conclusion:** This method avoids domestic equipment limitations and uncertainties of operator manual injections of the external standard method (GB method), which has a characteristics of less influence on instrument parameters change and is suitable for promoting in primary food inspection institution.

Key words: GC; Trans-fatty acids; Anti -9 - octadecadienoic a methyl ester (C18:1-9t); Anti -9,12 - octadecadienoic acid methyl ester (C18 :2-9t, 12t)

Chinese Library Classification (CLC): O657.7+1; TS207 **Document code:** B

Article ID: 1673-6273(2015)18-3564-06

前言

随着经济的发展和生活方式变化,各种氢化食品层出不穷,由于从此类食品中摄入过量的反式脂肪酸而引起的多种疾病的安全问题已成为 21 世纪全球面临最受关注的话题之一,引发了消费者对食品安全的担忧^[1]。反式脂肪酸含量的准确测定是判断食品安全性的重要条件,尤其对于反式脂肪酸风险的

评估,尤为重要。本实验采用气相色谱内标-标准曲线法对反-9-十八碳一烯酸甲酯(C18:1-9t)和反-9,12-十八碳二烯酸甲酯(C18:2-9t,12t)的实验室条件进行摸索。该方法避免了外标法(国标方法)的国产仪器局限性和操作者人工进样的不确定性,具有受仪器参数变化的影响较小的特点^[2-4]。并验证了该方法具有较高的准确性和较好的重现性,为各类食品检验机构进一步开展气相色谱法测定食品中的反式脂肪酸奠定了重要的基础。

* 基金项目:黑龙江省自然科学基金项目(201227);黑龙江省教育厅面上项目(12531819);大学生创新创业训练项目(201311230018)

作者简介:陈哲(1990-),女,本科,从事公共卫生实验教学,E-mail:732597922@qq.com,电话:18714305232

Δ 通讯作者:姜峰阁,E-mail:lfgygy@163.com

(收稿日期:2014-11-12 接受日期:2014-12-06)

1 材料与方法

1.1 仪器

GC112A 气相色谱仪(带氢火焰离子化检测器,上海精密仪器有限公司)、色谱柱 APEG-20M (25 m× 0.20 mm× 0.50 μm)和 PC-88(100 m× 0.25 mm× 0.2 μm)等。

1.2 试剂

正己烷(C₆H₁₄)(色谱纯);内标物乙酸丁酯(色谱纯);脂肪酸甲酯标准品:反-9-十八碳一烯酸甲酯(C18:1-9t)纯品和反-9,12-十八碳二烯酸甲酯(C18:2-9t,12t)纯品(瑞典 Larodan Fine Chemicals AB);内标物贮备液;反式脂肪酸甲酯标准储备液;反式脂肪酸甲酯标准工作液。

1.3 气相色谱分析条件

1.3.1 载气为高纯 N₂ 检测器(FID)灵敏度为 109;进样量为 1.0 μL,手动进样;柱温箱升温条件为初始温度 120 ℃,以 10 ℃/min 的速率升至 175 ℃,保持 10 min;以 5 ℃/min 的速率升至 210 ℃,保持 5 min;以 5 ℃/min 的速率升至 230 ℃,保持 5 min^[5-7]。

1.3.2 参数组合 可以选择的检测器温度、氢气压力和流速、空气压力和流速、分流比的参数组合^[8,9]见表 1。

1.3.3 调试方案 进样口温度、载气压力和流速的调试方案,见表 2。

表 1 GC112A 气相色谱仪参考色谱参数

Table 1 GC112A Chromatographic parameters of Gas chromatograph reference parameters

Condition 1	FID's temperature: 290 ℃, hydrogen pressure: 110 kPa,
	current speed:
	26.5 mL/min;
Condition 2	air pressure: 260 kPa, current speed : 427.0 mL/min
	split ratio: 16:1
	FID's temperature: 290 ℃, hydrogen pressure : 100 kPa, current speed :
Condition 3	21.0 mL/min;
	air pressure : 170 kPa, current speed : 274.0 mL/min
	split ratio: 13:1
Condition 3	FID's temperature: 290 ℃, hydrogen pressure: 100 kPa,
	current speed:
	21.0 mL/min;
Condition 3	air pressure: 160 kPa, current speed : 215.0 mL/min
	split ratio: 10:1

1.4 验证实验

1.4.1 绘制标准曲线 取反式脂肪酸甲酯标准工作液,分别用 1 μL 进样,以(两种反式脂肪酸甲酯的峰面积与内标物的峰面积比)AC18:1-9t/A 内标和 AC18:2-9t,12t/A 内标为纵坐标 Y,标准工作液(两种反式脂肪酸甲酯的质量与内标物的质量比)mC18:1-9t/m 内标和 mC18:2-9t,12t/m 内标为横坐标 X,绘制两条标准曲线。当标准曲线的决定系数达到 R²=0.999 以上时,即

表 2 进样口温度、载气压力和流速调试方案

Table 2 The debugging scheme of the inlet temperature, carrier gas pressure and current speed

Scheme	Inlet temperature (℃)	Carrier gas pressure (kPa)	Current speed (mL/min)
1	240	330	72
2	250	330	72
3	250	220	41
4	240	220	41
5	260	330	72
6	260	220	41

可判定用内标物来预测反式脂肪酸的实际效果较佳^[10-14]。

1.4.2 计算回收率 取标准品工作液,各 1 μL 进样,做 3 次平行试验。用测定回收率的方法评价准确度,一般要求回收率应达到 95%~105%。用相对标准偏差的方法评价重复性,要求相对标准偏差(relative standard deviation, RSD)小于 5%即可^[15-18]。进入检测器的待测组分 i 的含量 m_i(质量或浓度)与检测器的响应信号(峰面积 A_i 或峰高 h_i)成正比。

1.4.3 计算分离度 分离度反应两个相邻色谱峰的分离程度^[19]。取已配制好浓度为 0.8 mg/mL,内标物浓度为 1 mg/mL 的标准品工作液平行测定 3 次,取平均值^[20,21],得出正己烷与乙酸丁酯的分离度。

2 结果及讨论

2.1 色谱柱的选择

当选用长度为 100 m 规格的 PC-88(100 m× 0.25 mm× 0.2 μm)时,两种反式脂肪酸得到了很好的分离,获得理想的分离效果和峰形(见图 1)。

2.2 最佳色谱条件的选择

按照表 2 中的六个调试方案,对应表 1 各色谱参考条件反复摸索,每个方案的组分出峰情况如表 3 所示:

由表 3 可知,序号为 12 和 18 的两个调试方法所获得的组分峰形较好,分离效果理想,为可参考的调试方案。取已配制好浓度为 0.8 mg/mL 的两种反式脂肪酸甲酯标准品工作液各 3 份,分别 1 μL 进样,通过计算平均回收率对两个可参考调试方案进一步摸索,结果见表 4。

根据上表回收率获得最佳的色谱条件,详列表 5。

2.3 最佳工作条件的验证

2.3.1 分离度 在仪器最佳工作条件下(具体参数如见表 5),正己烷与乙酸丁酯的分离情况如图 2,反-9-十八碳一烯酸甲酯和反-9,12-十八碳二烯酸甲酯的分离情况如图 3,平行测定 3 次,取平均分度。

结合 N2000 色谱工作站中图谱,浓度为 0.8 mg/mL 的标准品工作液(内标物浓度为 1 mg/mL)中正己烷与乙酸丁酯的分离度(R)为 3.65,C18:1-9t 与 C18:2-9t,12t 分离度(R)为 6.22,R 均大于 2.5^[6],则本实验对正己烷与乙酸丁酯和 C18:1-9t)与 C18:2-9t,12t 皆具有理想的分离效果。

2.3.2 标准曲线 在仪器最佳工作条件下(具体参数如见表 5),用标准工作液进样 1 μL,色谱测定结果列于表 6 中,绘制出

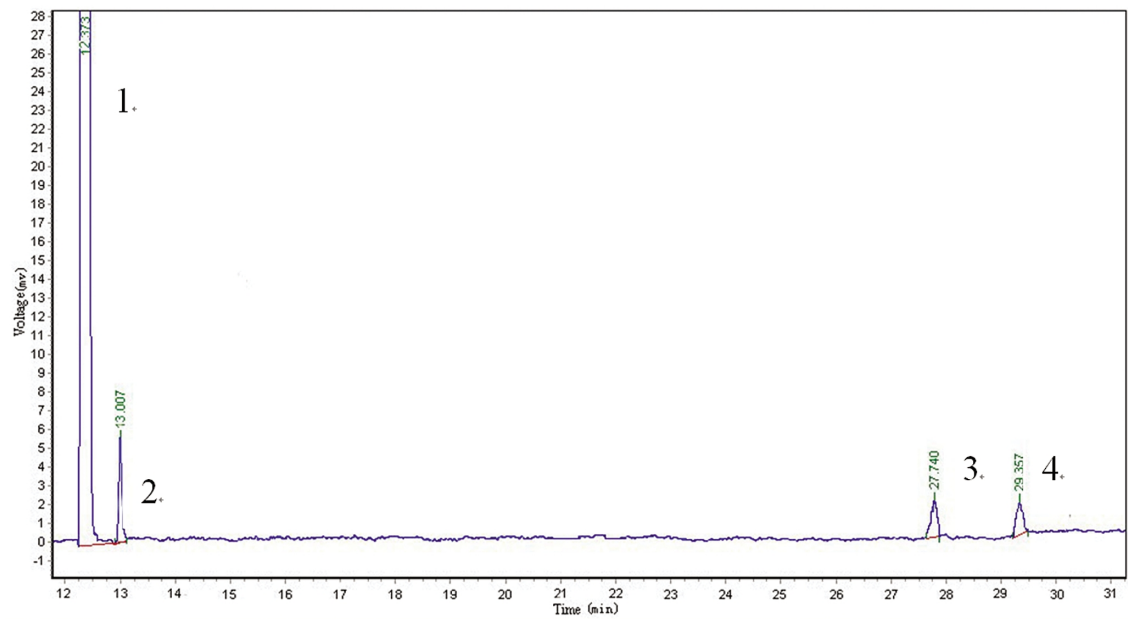


图 1 反式脂肪酸标准工作液色谱分离图谱(色谱柱 PC-88)

Fig. 1 The spectral separation of trans fatty acid's standard working fluid

Note: 1: hexane 2: internal standard(butyl acetate) 3: C18:1-9t 4: C18:2-9, 12t

表 3 不同条件和方案的调试结果

Table 3 Debugging results of the different conditions and schemes

Number	Debugging method	Hexane	Butyl acetate	C18:2-9t, 12t	Degree of separation
1	condition 1 scheme 1	★	☆	▲	※
2	condition 1 scheme 2	★	★	★	※
3	condition 1 scheme 3	☆	☆	▲	2.37
4	condition 1 scheme 4	☆	★	▲	※
5	condition 1 scheme 5	★	★	☆	※
6	condition 1 scheme 6	★	☆	▲	※
7	condition 2 scheme 1	☆	☆	★	2.77
8	condition 2 scheme 2	★	☆	★	※
9	condition 2 scheme 3	☆	☆	▲	3.23
10	condition 2 scheme 4	☆	☆	▲	2.75
11	condition 2 scheme 5	★	★	★	※
12	condition 2 scheme 6	☆	☆	☆	3.47
13	condition 3 scheme 1	★	★	▲	※
14	condition 3 scheme 2	★	☆	☆	※
15	condition 3 scheme 3	☆	☆	▲	2.78
16	condition 3 scheme 4	☆	★	★	※
17	condition 3 scheme 5	★	★	▲	※
18	condition 3 scheme 6	☆	☆	☆	3.59

Note: ★: tailing or not good peak shape ☆: the peak shape ▲: no peak ※: unable to calculate.

C18:1-9t 和 C18:2-9t, 12t 两条标准曲线如图 4。
2.3.3 回收率 在仪器最佳工作条件下 (具体参数如见表 5), 得到平均回收率。C18:1-9t 平均回收率在 95.96%~102.92%之间, 相对标准偏差(RSD)在 0.86%~2.44%之间, RSD 值均小于 5%^[7]; C18:2-9t, 12t 平均回收率在 96.11%~104.58%之间, 相对标准偏差(RSD)在 1.31%~2.29%之间, RSD 值均小于 5%, 本实验反式脂肪酸的标准品回收率为 95.96%~104.58%。

3 结论

表 4 可参考调试方法的调试结果

Table 4 Debugging results of the referable debugging method

Number of the watch 4	Sample	the amount standard (1×10^{-3} mg)	the detection value (1×10^{-3} mg)	rate of recovery (%)	average recovery rate (%)
12	C18:1-9t	0.80	0.67	83.75	87.92
		0.80	0.79	98.75	
		0.80	0.65	81.25	
	C18:2-9, 12t	0.80	0.74	92.50	83.75
		0.80	0.59	73.75	
		0.80	0.68	85.00	
18	C18:1-9t	0.80	0.78	97.50	99.17
		0.80	0.83	103.75	
		0.80	0.77	96.25	
	C18:2-9, 12t	0.80	0.76	95.00	100.83
		0.80	0.82	102.50	
		0.80	0.84	105.00	

表 5 GC112A 气相色谱仪最佳工作条件

Table 5 GC112A The best working condition of gas chromatograph

Parameters	
Capillary column:	PC-88(100 m× 0.25 mm× 0.2 μm)
Inlet temperature:	260 ℃;
Carrier gas:	high purity N ₂ , pressure: 220 kPa, current speed: 41.0 mL/min
FID's temperature:	290 ℃, hydrogen pressure : 100 kPa, current speed : 21.0 mL/min; air pressure : 160 kPa, current speed: 215.0 mL/min
FID's sensitivity:	10 ¹⁰
Split ratio	10:1
Sample size:	1.0 μL, manual injection
Temperature conditions of column oven:	initial temperature: 120 ℃, at a rate of about 10 ℃/min to 175 ℃, keep 10 min; at a rate of about 5 ℃/min to 210 ℃, keep 5 min; at a rate of about 5 ℃/min to 230 ℃, keep 5 min

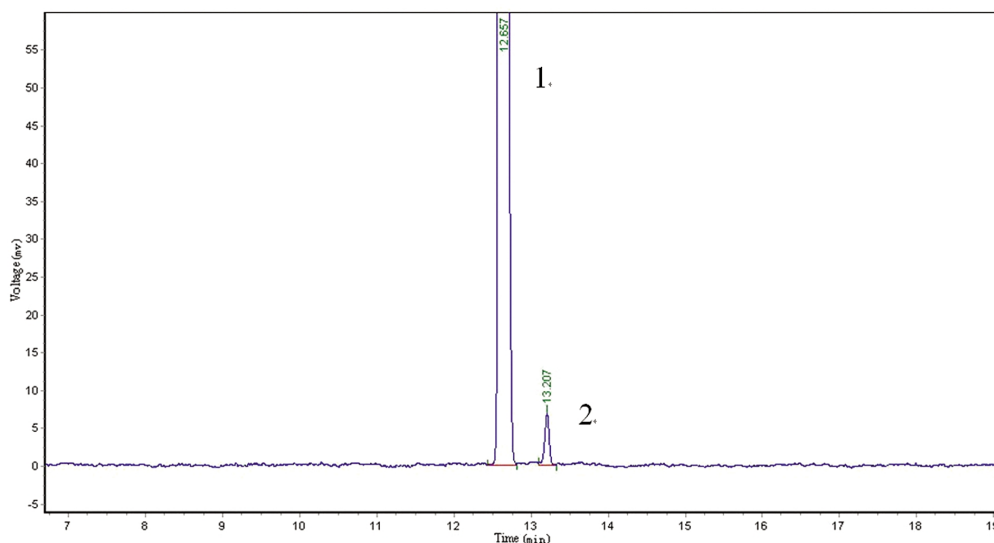


图 2 正己烷与乙酸丁酯的色谱分离图谱

Fig. 2 The spectral separation of hexane and butyl acetate

Note: 1: hexane 2: butyl acetate

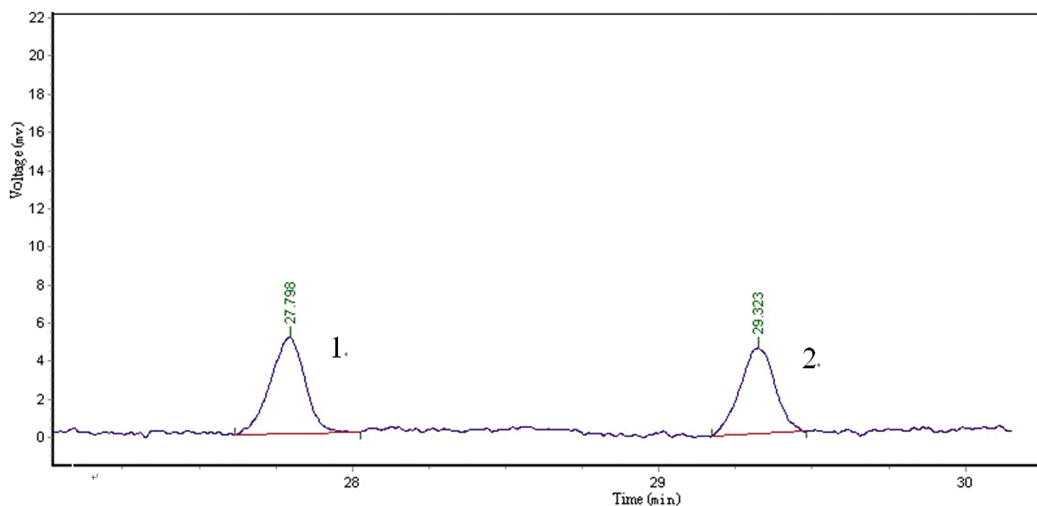


图 3 C18:1-9t 与 C18:2-9t, 12t 的色谱分离图谱

Fig. 3 The spectral separation of C18:1-9t and C18:2-9t, 12t

Note: 1: C18:1-9t 2: C18:2-9t, 12t

表 6 两种反式脂肪酸甲酯标准曲线的结果数值

Table 6 The results of numerical of two kinds of trans fatty acid methyl ester's standard curve

Sample	Number	Mass ratio ($m_{\text{sample}}/m_{\text{internal standard}}$)	Peak area ratio ($A_{\text{sample}}/A_{\text{internal standard}}$)
C18:1-9t	1	0	0
	2	0.8	0.807
	3	1.2	1.226
	4	1.6	1.584
	5	2.0	2.023
	6	2.4	2.401
C18:2-9t, 12t	1	0	0
	2	0.8	0.805
	3	1.2	1.189
	4	1.6	1.547
	5	2.0	2.003
	6	2.4	2.325

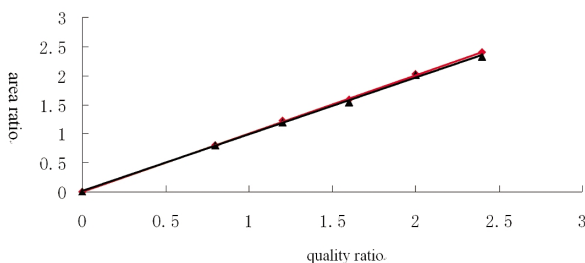


图 4 两种反式脂肪酸甲酯标准曲线

Fig. 4 The standard curves of two kinds of trans fatty acid methyl ester
representative the standard curve of C18:1-9t

equation of a curve: $y=1.0013x+0.0051$ coefficient of determination:
 $R^2=0.9997$

representative the standard curve of C18:2-9t, 12t

equation of a curve: $y=0.975x+0.0115$ coefficient of determination:
 $R^2=0.9990$

本实验利用基层食品检测机构及县区 CDC 实验室常用的国产氢火焰色谱仪,采用气相色谱内标 - 标准曲线法进行反式脂肪酸的分离测定。该方法避免了外标法(国标方法)的国产仪器局限性和操作者人工进样的不确定性,具有受仪器参数变化影响较小的特点,适合在基层食品检验机构推广。

参 考 文 献(References)

- [1] 田雨. 反式脂肪酸与人体健康 [J]. 中华预防医学杂志, 2011, 12(10): 894-898
Tian Yu. The trans fatty acid and health of human body [J]. Chinese Journal of Preventive Medicine, 2011, 12(10): 894-898
- [2] 柯润辉, 尹子波, 张英, 等. 气相色谱法测定焙烤食品中反式脂肪酸含量[J]. 食品研究与开发, 2010, 31(12): 165-168
Ke Run-hui, Yin Zi-bo, Zhang Ying, et al. Determination of the trans fatty acids' content in the baked food by gas chromatograph[J]. Food Research and Development, 2010, 31(12): 165-168
- [3] GB 10765-2010. 婴儿配方食品[S]. 北京: 中国标准出版社, 2010

- GB 10765-2010. Infant formula [S]. Beijing: China Standard Press, 2010
- [4] GB 10767-2010. 较大婴儿和幼儿配方食品[S]. 北京: 中国标准出版社, 2010
- GB 10767-2010. Older infants and infant formula food [S]. Beijing: China Standard Press, 2010
- [5] 赖晓英, 武德银, 伍颢, 等. 反式脂肪酸的危害及其检测方法 [J]. 现代食品科技, 2011, 23(2): 73-76
- Lai Xiao-ying, Wu De-yin, Wu Yang, et al. The harm and detection method of the trans fatty acids [J]. Modern Food Science and Technology, 2011, 23(2): 73-76
- [6] Brouwer I A, Wanders A J, Katan M B. Effect of animal and industrial trans fatty acids: a quantitative review[J]. American Journal of Public Health, 2010, 4(3): e9434
- [7] 阮辉, Neil H. Mermelstejn. 反式脂肪酸分析[J]. 中外食品, 2010, (3): 95-97
- Ruan Hui, Neil H. Mermelstejn. Analysis of the trans fatty acids[J]. Chinese and Foreign Food, 2010, (3): 95-97
- [8] 郭林, 李靖靖, 宁建中. 反式脂肪酸测定方法的研究 [J]. 现代化工, 2011, 31(3): 87-92
- Guo Lin, Li Jing-jing, Ning Jian-zhong. Study on detection method of the trans fatty acids [J]. Modern Chemical Science and Thechnology, 2011, 31(3): 87-92
- [9] Icha R, Mozaffarian D. Trans fatty acids: effects on cardiometabolic health and implications for policy [J]. Prostaglandins Leukot Essent Fatty Acids, 2008, 79(3/5): 147-152
- [10] Asp N, Bryggelsson S. Health claims in the labelling and marketing of food products: the Swedish food sector's Code of Practice in a European perspective[J]. Scandinavian Journal of Food and Nutrition, 2007, 51(3): 107-126
- [11] Vyth E L, Stefhuis I H, Roodenburg A J, et al. Front-of-pack nutrition label stimulates healthier product development: a quantitative analysis[J]. International Journal of Behavioral Nutrition and Physical Activity, 2010, 7(4): 1186-1479
- [12] Oh K, Hu FB, Manson J E, et al. Dietary Fat Intake and Risk of Coronary Heart Disease in Women: 20 Years of Follow-Up of the Nurses Health Study[J]. Am J Epidemiol, 2005, 161(7): 672-679
- [13] Ray Cook. Thermally induced isomerism by deodorisation [J]. Inform, 2012, 13(1): 71-76
- [14] 马雪婷, 曹栋. 气相内标 - 标准曲线法测定二亚油酰磷脂酰胆碱 (DLPC) 中 亚油酸[J]. 哈尔滨商业大学学报(自然科学版), 2007, 23(5): 592-598
- Ma Xue-ting, Cao Dong. Detemination of DLPC's linoleic acid GC internal standard-the standard curve method [J]. Journal of Harbin University of Commerce (Natural Science Edition) , 2007, 23(5): 592-598
- [15] Jorge Salmeron, Frank B Hu, JoAnnE Manson, etal. Dietary fat intake and risk of type 2diabetes in women[J]. American Journal of Clinical Nutrition, 2011, 73: 1019-1026
- [16] Stender S, Dyerberg J. The Influence of Trans Fatty Acids on Health [M]. 4th ed. Danish Nutrition Council, 2010: 19-26
- [17] 王颖, 宁文吉, 李扬, 等. 奶油制品中常见的 9 种反式脂肪酸的检测方法[J]. 职业与健康, 2012, 11(22): 2751-2752
- Wang Ying, Ning Wen-Ji, Li Yang, et al. Methods for the detection of 9 kinds trans eatty acids in common cream products [J]. Occupation and Health, 2012, 11(22): 2751-2752
- [18] Kohyama k, Nishinari K. Dependence of the specific volume of konjac glucomannan on pH. Gums Stab [J]. Food Ind, 1989, 5: 459-462
- [19] Ratnayake W M. Overview of methods for the determination of trans fatty acids by gas chromatography, silver-ion thin-layer chromatography, silver-ion liquid chromatography, and gas chromatography/mass spectrometry[J]. J AOACInt, 2009, 87:523-539
- [20] Ulberth F, Haider H J. Determination of Low Level trans unsaturation in fats by Fourier transform infrared spectroscopy [J]. J Food Sci, 1992, 57(6): 1444-1447
- [21] Mossoba MM, Yurawecz MP, Delmonte P, et al. Overview of infrared methodologies for trans fat determination[J]. J aoac Int, 2010, 87(2): 540-544

(上接第 3563 页)

- [13] Dimitrouli M, Geurtsen W, Lü hrs AK. Comparison of the push-out strength of two fiber post systems dependent on different types of resin cements[J]. Clin Oral Investig, 2012, 16(3): 899-908
- [14] Sharafeddin F, Alavi AA, Zare S. Fracture resistance of structurally compromised premolar roots restored with single and accessory glass or quartz fiber posts[J]. Dent Res J (Isfahan), 2014, 11(2): 264-271
- [15] Naumann M, Koelpin M, Beuer F, et al. 10-year survival evaluation for glass-fiber-supported postendodontic restoration:a prospective observational clinical study[J]. J Endod, 2012, 38(4): 432-435
- [16] Smith RJ, Light RA, Sharples SD, et al. Multichannel, time-resolved picosecond laser ultrasound imaging and spectroscopy with custom complementary metal-oxide-semiconductor detector [J]. Rev Sci Instrum, 2010, 81(2): 024901
- [17] Kapusta P, Szarek-Łukaszewska G, Stefanowicz AM. Stefanowicz et al. Direct and indirect effects of metal contamination on soil biota in a Zn-Pb post-mining and smelting area (S Poland)[J]. Environ Pollut, 2011, 159(6): 1516-1522
- [18] Hikasa T, Matsuka Y, Mine A, et al. A 15-year clinical comparative study of the cumulative survival rate of cast metal core and resin core restorations luted with adhesive resin cement [J]. Int J Prosthodont, 2010, 23(5): 397-405
- [19] Roy P, Schaate A, Behrens P, et al. Post-synthetic modification of Zr-metal-organic frameworks through cycloaddition reactions [J]. Chemistry, 2012, 18(22): 6979-6985
- [20] Biacchi GR, Basting RT. Comparison of fracture strength of endocrowns and glass fiber post-retained conventional crowns [J]. Oper Dent, 2012, 37(2): 130-136