Fatty Acid Composition and Flavor Characteristics of Palatase-catalyzed Modification of Butteroil

LIU Zhi-dong[1], CHEN Xue-zhong[1], GUO Ben-heng[2], LI Yun-fei[3], QU Yin-hong[4], DENG Yun[3], HUANG Hong-liang[1]

(1.East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, China)

(2.Technical Center, Bright Dairy Co. Ltd, State Key Laboratory of Dairy Biotechnology, Shanghai 200436, China)

(3.School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 200040, China)

(4.College of Food Science and Technology, Shanghai Ocean University, Shanghai 200040, China)

Abstract: Enzymatic hydrolysis of butter oil was carried out using Palatase 20,000 L as biocatalyst. The fatty acid components of butter oil and the hydrolysates of butteroil were analyzed using a combination of thin layer chromatography (TLC) and gas chromatography (GC). The flavor compounds were analyzed by SPME-GC-MS. Palatase 20,000 L -catalysis significantly changed the composition of fatty acids of the butter oil and contributed to releasing the flavor compounds, especially the key flavor compounds octanoic and decanoic acid ($P<0.05$). Therefore, Palatase 20,000L lipase-catalysis is a potential method for improving the nutritional value and flavor of butter oil.

Key words: butteroil; Palatase 20,000L; catalysis; fatty acid; flavor compound

Article No.: 1673-9078(2013)9-2186-2191

Palatase 20,000L 催化对黄油脂肪酸和风味特征的影响

刘志东[1]，陈雪忠[1]，郭本恒[2]，李云飞[3]，曲映红[4]，邓云[3]，黄洪亮[1]

（1. 中国水产科学研究院东海水产研究所，上海 200090）

（2. 光明乳业股份有限公司技术中心，乳业生物技术国家重点实验室，上海 200436）

（3. 上海交通大学农业与生物技术学院，上海 200040）（4. 上海海洋大学食品学院，上海 200040）

摘要： 研究了脂肪酶 Palatase 20,000 L 催化对黄油脂肪酸和风味物质的影响。采用薄层层析（TLC）和气相色谱(GC)相结合分析黄油及其酶解物的脂肪酸，采用顶空固相微萃取-气相-质谱（SPME-GC-MS）分析风味物质。Palatase 20,000 L 脂肪酶催化黄油显著改变了脂肪酸的组成，促进了风味物质（特别是主要风味物质：辛酸和癸酸）的释放($P<0.05$)。结果表明，Palatase 20,000 L 脂肪酶催化改变了黄油脂肪酸和风味物质的种类和含量。因此，Palatase 20,000 L 脂肪酶催化能够提高黄油的营养价值和应用领域。

关键词： 黄油；Palatase 20,000L；催化；脂肪酸；风味物质

Introduction

Butter is an important source of dietary fat and imparts excellent flavor and mouth feel and widely consumed all over the world. According to the EC Council regulation No. 2991/94, butter is a water-in-oil emulsion that consists of minimum 80% of milk lipid, maximum 16% of water and a maximum dry non-fat material content of 2%. Butter is mainly composed of triacylglycerols (TAGs), free fatty acids, glycolipids and some other minor components. However, with consumer focusing on products containing less and healthier lipid, the consumption of butter is decreased considerably in recent years[1]. It also has provided an impetus for research on new technologies for transforming lipid into value-added products in which fatty acid compositions in naturally occurring lipid are either released to generate

收稿日期：2013-05-01

基金项目：“863”科技计划项目（2012AA092304、2012AA092303）

作者简介：刘志东（1976-），男，博士，副研究员，研究方向：食品生物技术，水产品加工与利用

通讯作者：黄洪亮（1964-），男，研究员，研究方向：远洋渔业资源开发利用

key components that can be used to impart desirable functional properties, or converted to forms in which many of the original fatty acid compositions have been replaced by compositions with beneficial effects on human health [2]. Among the new technologies, lipase-catalyzed modification of lipid is receiving particular attention nowadays. Lipase-catalyzed modification of lipid involves the use of lipase to restructure TAGs by inducing the exchange of fatty acid among glycerol backbones. It has the advantages of moderate reaction conditions, remarkable selectivity, few by-products and mimicking natural pathways for controlled lipase-catalysis of milk lipids [3]. Thus, it is generally presented as an alternative and more environmental-friendly process than physical fractionation or chemical modification of milk lipids.

Dairy products have highly nutritional values and remarkable flavor characteristics. The production of milk flavors has taken a great proportion of the international flavors industry. Bovine milk lipids are substrates for various lipolytic enzymes (endogenous, exogenous: microbial or animal). The trade named “lipolyzed milk lipids” gathers an important number of dairy flavor ingredients produced by enzymatic hydrolysis of milk lipids. As the world-wide development trend of reduced-fat food products in which fat cannot play its well established role of flavor source and reservoir, the importance of such flavor concentrates has gradually increased. Milk TAGs present a wide range of molecular weights (470~890 g/mol) which correspond to acyl carbon numbers ranging from 24 to 54. Milk TAGs’ structure is precisely described by their fatty acid composition, as well as the regiodistribution of these acyl moieties on the glycerol backbone. Up to now, 416 types of fatty acids have been identified in bovine milk TAGs. However, only 12 fatty acids are present in amount greater than 1%. During the process of hydrolysis, most of the short-chain fatty acids on the positions of sn-1 and sn-3 of triacylglycerol of milk fat and other volatile compounds, which have strong odor and taste flavor correlating with cheese or other dairy products, are released. After proper flavor treatment process, the lipolyzed milk lipid could be made into different kinds of natural milk flavors. Lipase-catalyzed butter provides a more intense butter oily flavor than butter itself, as well as cheese-like aromas. Thus, modified butter by lipase catalysis is often used in food products in situations [4,5]. Several researches have focused on TAGs’ hydrolysis or structure, i.e. the modification of the acyl moieties composition and/or distribution on these TAGs using lipases. Recent publications were orientated toward the use of continuous process, cost-effective catalyst (TL-IM) and have assessed globally the physical, nutritional but also sensory and oxidative stability of milk structured lipids. These modification of the molecular structure of milk lipids impact their functional properties (as flavor reservoir, as texturizer) and their nutritional properties. Although obvious correlations exist between the fatty acid composition and lipase-catalysis some lipids, there is limited information on the relationship of the content and kind of fatty acid composition, flavor compounds and lipase-catalyzed butteroil.

Hence, the objective of this work was to determine effect of lipase catalysis on the fatty acid composition and flavor compounds of butteroil, and to better understand the underlying mechanisms of this process. This research could be useful for application of butter oil and its hydrolysates in food and elsewhere.

1 Materials and methods

1.1 Material

Butter was obtained from Bright Dairy and Food Co., Ltd., (Shanghai, China), containing 95% fat, 3.2% protein and 0.4% carbohydrates. Butter was stored in closed plastic containers at -20 ℃ until further analysis. Palatase 20,000 L (Activity: 20,000 μ/g)was obtained from Novozmes (Denmark). Free fatty acids standards and fatty acid methyl esters (FAMEs) were purchased from Sigma Chemical Co (St. Louis, MO, USA).

1.2 Preparation of butteroil

The melted butter was continuously stirred and the oil phase above the serum phase was allowed to separate for 3 h. The oil phase was clarified by filtering through several layers of cheese cloth into a glass beaker [6].

1.3 Lipase catalysis of butteroil

Butteroil is high in complex lipids, but extremely low in protein and carbohydrate. Lipase-catalysis butteroil was carried out according to the methods described by Sun T, et al. and Wanasundaraun[7,8]. Butteroil (99.0% lipid) was catalyzed with Palatase

20,000 L (1%, *m/V*) at 45 ℃ in 0.25 M Tris buffer (pH 7.5) in the flat-bottomed flask. The mixture was agitated with a magnetic stir bar at 500 r/min. A preliminary experiment determined the amount of 1 M KOH necessary to neutralize free fatty acids released by reaction was added to the mixture. Lipase was inactivated by heating at 95 ℃ for 10 min. The hydrolysates were evaporated by a rotary evaporator at 45 ℃ for 30 min and stored at-20 ℃ until further analysis.

1.4 Flavor compounds analysis

Flavor compounds in the lipase- catalyzed butteroil were analyzed by SPME-GC-MS using a modified method based on that of Ivan Kurtovic et al. (2011) [9].

1.5 TLC separation

The fractions of butteroil and the hydrolysates of butteroil was separated by thin layer chromatography (TLC) on precoated silica gel 254 plate (20×20 cm, 0.25 mm thickness) (Merck, Darmstadt, Germany). The plates were then activated at 100 ℃ for 30 min. After spotting the sample, the plates were developed using solvent systems consisting of n-hexane/diethyl ether/glacial acetic acid (either 60:40:1, 70:30:1, 80:20:1, or 90:10:1, *V/V/V*). The corresponding bands on the plates were scrapped into test-tubes and extracted from the silica gel using the method of the Bligh-Dyer (1959) [10]. After phase separation, the chloroform extracts were evaporated until dryness. The lipid components were extracted four times with 20 mL diethyl ether protected, filtered, and the solvent was evaporated under a gentle stream of nitrogen until dryness. The different fractions were quantified by weight and used for further analysis the composition of fatty acids. Each fraction was repeated three times to assure reproducibility of results, and each measurement was made by comparison with the standard.

1.6 Fatty acid analysis

Butteroil and the hydrolysates of butteroil were converted into fatty acid methylation (FAMEs) according to AOCS, and their composition was determined by gas chromatography (Beaconsfield, UK) equipped with programmed temperature vaporizer inlet, flow splitter and hydrogen flame ionization detector connected to HP Chemstation (Rev.A.0401) software for peak area and fatty acid percentage calculation [11,12].

1.7 Statistical analysis

Data were expressed as means ± standard deviations (SD). Statistical analysis was performed using One-Way ANOVA procedure of SPSS (11.0), using Duncan test with a significant level of $P < 0.05$.

2 Results and discussion

2.1 Flavor compounds analysis

The desirable flavor of butter mostly results from the presence of fatty acids, especially some short chain and free fatty acids. These flavor compounds are found in substantial quantities, both as free fatty acids and as part of TAGs in butter (Table 1).

Table 1 Flavor compounds in butter oil and its hydrolysates

Compound	Butteroil	THOBO	Odour description
2-pentanone	0.05±0.01	-	Ether, fruity
C4:0	-	11.12±0.06*	Sharp
C6:0	-	18.64±0.24**	Pungent, musty, cheesy,
2-heptanone	0.12±0.02	0.12±0.08	Cheesy, rancid, sweaty
2-nonaone	-	0.085±0.014	Fresh sweet, weedy earthy, herbal
2-hendecanoe	-	0.085±0.013	Fruity, Spicy
C8:0	-	24.81±0.27**	Sweaty, cheesy
C10:0	-	23.57±0.32*	Unpleasant rancid sour
C10:1	-	0.59±0.21*	Waxy green fruity
C12:0	-	10.29±0.15	Mild fatty coconut bay oi
C14:0	-	5.38±0.16*	Waxy fatty soapy coconu
C16:0	-	2.20±0.13	Low heavy waxy
Others	99.77±0.03	3.11±0.18	

Note: All values were means of three measurements and expressed as means±standard deviation of triplicates; THOBO denotes the hydrolysates of butteroil. * $P<0.05$, ** $P<0.01$.

Moreover, some fatty acids (C4:0 and C6:0) were the dominant flavor compounds in the lipase-catalysed butter oil. These fatty acids are responsible for the flavor enhancement of the hydrolysates of butteroil. The hydrolysates of butteroil displayed a more complex flavor profile, including several short chain fatty acids and ketones, which contribute butter flavor to this final product. Another distinctive characteristic of the hydrolysates of butteroil is the relatively high level of octanoic acid, which was not detected in the control butteroil. Patalase 20,000L lipase-catalysis released both C8:0 and C10:0 as the dominant flavor compounds. These two fatty acids significantly contributed to the odour and flavor of many dairy products. This is in agreement with the sensory characteristics of butter oil

(Buttery and fatty) as Patalase-catalyzed modification of butter oil (Fatty, fruity, rancid and buttery) was very similar. SPME-GC-MS results showed a potential use of Palatase 20,000 L lipase for the generation of desirable flavor in dairy products, which would be different from the flavor profiles produced by dairy product in itself. This is most likely due to the high specificity of Palatase 20,000L for the precursors of some flavor compounds of butter oil. Small amounts of fatty acids (acetic acid and C4:0) were present, due to a combination of the effects of heat and lipase exposure.

In some previous analysis of the flavor compounds of butter oil, 2-heptanone made a considerable contribution, although its contribution greatly varied among the different samples analyzed [1]. The discrepancy regarding 2-heptanone, between our result and these previous studies, is likely due to the source of butter. Some low molecular weight flavor compounds (2-pentanone and 2-heptanone) also dominated the flavor of butteroil. These flavor compounds have previously been reported as the major flavor compounds in butter, and are responsible in part for the desirable flavor of butter.

2.2 Fatty acid composition of butter oil and its hydrolysates

It has been generally recognized that butter oil consists of about 15 major fatty acids, with perhaps 12 or so minor (trace quantity) fatty acids (Table 2). Although 14 categories of fatty acids are outlined, it was generally appreciated that many other fatty acids are present in small or trace quantities. The major fatty acids in butter oil and the hydrolysates of butter oil were C16:0, C18:0, C18:1, C14:0 and C12:0. However, the distribution of these fatty acids was not evenly or randomly. Meanwhile, the samples both contained unsaturated fatty acids (C18:2 and C18:1). The significant differences of butter oil and its hydrolysates were that C6:0 and C16:1 just existed in butteroil, while C20:1 only presented in the hydrolysates of butteroil. Moreover, some fatty acids distribution patterns were similar to between butteroil and the hydrolysates of butteroil. The samples had high amounts of total saturated fatty acids (which consisted mainly of C16:0, C14:0), accounting 36.6~38.0% and 15.2~15.9% for total lipids, respectively. It can be found that the significant differences (P< 0.05) in the composition and positional distribution of fatty acids among the samples. The percentage of C16:0 and C14:0 was higher (P< 0.05) in butteroil, whereas C16:0 and C18:1 was higher (P< 0.05) in the hydrolysates of butteroil. This phenomenon may Palatase 20,000L-catalysis reaction mainly occurred at specific positions of triacylglycerol that lead to the contents of some kinds of fatty acids significantly increased. The fatty acid composition and distribution were almost the same in butteroil and the hydrolysates of butteroil, except for C6:0, C16:1 and C20:1. These results are coincided with Bourlieu, et al (2009) [13] reported which the predominant fatty acids are C16:0 (30%), C18:1 (20%) and C14:0 (12%). Moreover, C14:0/C16:0, C16:1/C18:0, C14:1/C18:1ω-9 and C18:1ω-9/C18:2ω-6 were differenced significantly (P<0.05). These data are valuable based on nutritional and food science purposes. Precise and repeatable values are not highly correlated due to such variables as stage of lactation, feed source, cattle breed, etc.

Table 2 The composition of fatty acids of butter oil and its hydrolysates

Fatty acids	Content/%	
	Butteroil	THOBO
C6:0	1.56±0.11	n.d.
C8:0	1.26±0.24	1.01±0.36
C10:0	3.88±0.19	2.88±0.24
C12:0	5.86±0.21*	4.65±0.41
C14:0	15.90±0.35*	15.20±0.27*
C14:1	0.87±0.09	0.84±0.34
C15:0	1.62±0.13	1.46±0.31
C16:0	38.00±0.38**	36.60±0.20*
C16:1	1.64±0.31	n.d.
C17:0	0.81±0.22	0.75±0.18
C18:0	14.20±0.34*	13.00±0.26*
C18:1	13.90±0.26*	17.50±0.34*
C18:2	0.53±0.27	2.05±0.37
C20:1	n.d.	4.04±0.28

Note: All values were means of three measurements and expressed as means ± standard deviation of triplicates; THOBO denotes the hydrolysates of butteroil; n.d., not detected. * P<0.05, ** P<0.01.

2.3 Fatty acid composition of the different fractions

It is of great importance to know the composition the fatty acids at all the three positions (i.e. sn-1, sn-2 and sn-3) of TAGs. Generally, if the distribution of fatty acids

at all the three positions (i.e. sn-1, sn-2 and sn-3) of TAGs is evenly, the content of fatty acids at each position of TAG is 33.33 %. However, in fact the distribution of fatty acids at all the three positions of TAGs follows by some rules, but it is not evenly. Therefore, lipase-catalyzed lipid provides an effective means for producing tailor-made lipids with desired physical characteristics, chemical properties, and/or nutritional benefits.

Fatty acid contents of the different fractions in butter oil and the hydrolysates are depicted in Table 3. In butteroil and the hydrolysates, the kinds and contents of fatty acids were different. No significant differences ($P > 0.05$) existed in the fatty acid distribution in butteroil and the hydrolysate. The major fatty acids were C16:0 and C18:1. From this work it can be concluded that there is potential for better commercial utilization of the hydrolysates of butteroil. The lipase-catalyzed butteroil is a good basis for potentially industrial extraction and further utilization of butteroil. Table 3 also presents the content of fatty acids (S, M, D and T) of TAG isolated from butteroil and the hydrolysates of butteroil according to their degree of unsaturation on the acyl chain-length of the fatty acids moieties. The theoretical contents of the different type of the fatty acids (S, M, D and T) were calculated from the relative percentages of each TAG species based on the data in Table 1. This result is agreement with previous studies [14].

Table 3 The composition of fatty acids of the different fractions of butter oil and the hydrolysates (%)

Fatty acids	FFA in butteroil	TAG in butteroil	MAG in THOBO	DAG in THOBO	FFA in THOBO	TAG in THOBO
C6:0	2.34±0.17	1.33±0.18	n.d.	n.d.	n.d.	n.d.
C8:0	1.44±0.13	0.78±0.10	n.d.	n.d.	n.d.	n.d.
C10:0	4.48±0.24	3.49±0.31	n.d.	n.d.	n.d.	n.d.
C11:0	n.d.	0.29±0.08	n.d.	n.d.	n.d.	n.d.
C12:0	5.82±0.27	5.11±0.19	n.d.	n.d.	n.d.	n.d.
C13:0	n.d.	n.d.	n.d.	n.d.	0.75±0.21	n.d
C14:0	15.00±0.24	14.50±0.11	2.82±0.31	3.32±0.23	1.07±0.19	0.55±0.27
C14:1	0.80±0.17	0.54±0.12	n.d.	n.d.	n.d.	n.d.
C15:0	1.54±0.18	1.46±0.21	n.d.	0.37±0.08	n.d.	n.d.
C16:0	37.00±0.19	37.50±0.15**	33.50±0.38**	17.70±0.35	32.50±0.27*	26.40±0.11
C16:1	1.84±0.13	1.73±0.20	3.81±0.12	6.62±0.27	n.d.	2.53±0.13
C17:0	0.78±0.27	0.75±0.12	n.d.	0.35±0.08	n.d.	0.31±0.09
Trans-C18:1	n.d.	n.d.	n.d.	0.33±0.09	n.d.	n.d.
C18:1	13.70±0.34*	15.00±0.18	25.00±0.23*	37.10±0.42**	14.20±0.29	36.30±0.26*
C18:2	0.97±0.18	1.80±0.21	4.32±0.18*	5.24±0.21	42.90±0.42*	13.50±0.23
α-C18:3	n.d.	n.d.	n.d.	n.d.	n.d	6.15±0.34
γ-C18:3	n.d.	0.46±0.09	n.d.	n.d.	4.97±0.23	n.d.
C20:1	n.d.	n.d.	n.d.	n.d.	n.d.	0.72±0.19
C20:1	n.d.	n.d.	4.87±0.24	13.30±0.32	n.d.	0.62±0.08
C20:4	n.d.	n.d.	n.d.	0.41±0.13	n.d.	n.d.
C22:1	n.d.	n.d.	n.d.	4.13±0.21	n.d.	n.d.
C22:1ω3	n.d.	n.d.	n.d.	n.d.	n.d.	1.99±0.28
C22:2	n.d.	n.d.	n.d.	0.47±0.12	n.d.	n.d.
C24:1	n.d.	n.d.	n.d.	2.76±0.20	n.d.	n.d.
Proportion	0.41±0.02	98.78±0.15	2.05±0.24	18.98±0.51	16.89±0.21	78.97±0.18
S	82.70±0.41	80.47±0.49	62.02±0.53	30.52±0.24	37.93±0.38	38.16±0.36
M	16.34±0.45	17.27±0.31	33.68±0.24	64.24±0.35	14.20±0.27	39.68±0.45
D	0.97±0.18	1.80±0.21	4.32±0.18	5.24±0.21	42.90±0.42	13.50±0.23
T	n.d.	0.46±0.09	n.d.	n.d.	4.97±0.23	6.15±0.34

Note: FFA denotes free fatty acid, MAG denotes monoacylglycerol, DAG denotes Diacylglycerol, S, M, D and T denote a saturated fatty acids, a monoene unsaturated fatty acids, a diene unsaturated fatty acids and a triene unsaturated fatty acids, respectively; Means ± SD of triplicates; THOBO denotes the hydrolysates of butteroil; n.d., not detected. * $P<0.05$, ** $P<0.01$.

3 Conclusions

Lipase-catalyzed modification of butter oil changed their fatty acid composition and distribution. This biotreamtent increased the total amount of FFAs in butter oil and contributed to butter flavor directly or indirectly, by acting as precursors for the formation of butter flavor compounds. These results may be valuable for producers and industry in their effort to enhance the nutritional quality of butter oil by lipase-catalyzed modifications and to find a focus for future studies.

References

[1] Fereidoon Shahidi. Bailey's industrial oil and fat products. (6th Edition) [M]. A John Wiley & Sons, Inc., Publication. 2005

[2] Argov N, Lemay D G, German J B. Milk fat globule structure and function: Nanoscience comes to milk production [J]. Trends in Food Science and Technology, 2008, 19: 617-623

[3] Holms H C, Cowan D. The evolution of enzymatic interesterification in the oils and fats industry [J]. European Journal of Lipid Science and Technology, 2008, 110:679-691

[4] Maduko C, Park Y, Akoh C. Characterization and oxidative stability of structured lipids: infant milk fat analog [J]. Journal of the American Oil Chemists' Society, 2008, 85(3): 197-204

[5] Ivan Kurtovic, S N Marshall, M R Miller, et al. Flavour development in dairy cream using fish digestive lipases from Chinook salmon (Oncorhynchus tshawytscha) and New Zealand hoki (Macruronus novaezealandiae) [J]. Food Chemistry, 2011, 127: 1562-1568

[6] C Bourlieu, F Rousseau, V Briard-Bion, et al. Hydrolysis of native milk fat globules by microbial lipases: Mechanisms and modulation of interfacial quality [J]. Food Research International, 2012, 49: 533-544

[7] Sun T, Pigott G M, Herwig R P. Lipase-assisted concentration of n-3 polyunsaturated fatty acids from viscera of farmed Atlantic salmon (Salmo salar L.) [J]. Journal of Food Science, 2002, 67(1): 130-136

[8] Wanasundara U N, Shahidi F. Lipase-assisted concentration of n-3 polyunsaturated fatty acids in acylglycerols from marine oils [J]. Journal of American Oil Chemist's Society, 1998, 75(8): 945-951

[9] Van Aardt M, Duncan S E, Marcy J E, et al. Aroma analysis of light-exposed milk stored with and without natural and synthetic antioxidants [J]. Journal of Dairy Science, 2005, 88(3):881-890

[10] Bligh E G, Dyer W J. A rapid method of total lipid extraction and purification [J]. Canadian Journal of Biochemistry Physiology, 1959, 37: 911-917

[11] AOCS. Official methods and recommended practices of the American Oil Chemist's Society Champaign, IL: AOCS. 1998

[12] Bourlieu C, Bouhallab S, Lopez C. Biocatalyzed modifications of milk lipids: applications and potentialities [J]. Trends in Food Science and Technology, 2009, 20: 458-469

[13] Aparicio R, Aparicio-Rufz R. Authentication of vegetable oils by chromatographic techniques [J]. Journal of Chromatography, 2000, 881: 93-104

[10] Fraga M J, Fontecha J, Lozada L, et al. Silver ion adsorption thin layer chromatography and capillary gas chromatography in the study of the composition of milk Fat triglycerides [J]. Journal of Agricultural Food Chemistry, 1998, 46 (5): 1836-1843

[11] Bligh E G, Dyer W J. A rapid method of total lipid extraction and purification [J]. Canadian Journal of Biochemistry Physiology, 1959, 37: 911-917

[12] AOCS. Official methods and recommended practices of the American Oil Chemist's Society Champaign, IL: AOCS. 1998

[13] Bourlieu C, Bouhallab S, Lopez C. Biocatalyzed modifications of milk lipids: applications and potentialities [J]. Trends in Food Science and Technology, 2009, 20: 458-469

[14] Aparicio R, Aparicio-Rufz R. Authentication of vegetable oils by chromatographic techniques [J]. Journal of Chromatography, 2000, 881: 93-104

[15] Fraga M J, Fontecha J, Lozada L, et al. Silver ion adsorption thin layer chromatography and capillary gas chromatography in the study of the composition of milk Fat triglycerides [J]. Journal of Agricultural Food Chemistry, 1998, 46 (5): 1836-1843