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Lipase-mediated enrichment of handmade butter with unsaturated fatty acids in solvent-free system

(Manteiga de garrafa enriquecida com ácidos graxos insaturados usando lipase em sistema livre de solvente orgânico)

"Nota/Note"

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Abstract

This work aimed to investigate the use of microbial lipase to modify the nutritional proprieties of the handmade "bottle butter" used in the Brazilian Northeast culinary. In the transesterification reactions was used a commercial lipase from Candida rugosa plus oleic and linolenic fatty acids. The results demonstrated that total unsaturated fatty acids content increased 10%, with similar decreasing in total saturated fatty acids. Myristic, lauric and palmitic fatty acids, known as causing agents of coronarian diseases, were clearly substituted by oleic and linolenic fatty acids. Therefore, these results point out for the possibility to modify the fatty acids composition of local handmade butters by enzymatic method in solvent- free system to produce a healthier product for human dietary use.

Key-words: *transesterification, lipase, bottle butter, fatty acids.*

Resumo

Este trabalho teve como objetivo investigar o uso de uma lipase microbial para modificar as propriedades da "manteiga de garrafa" usada na culinária do Nordeste Brasileiro. Na reação de transesterificação foi usada uma lipase comercial de Cândida rugosa e os ácidos graxos oleico e linolênico foram utilizados para modificação da manteiga. Os resultados demonstraram que houve um aumento de 10% no conteúdo total de ácidos graxos insaturados com similar decréscimo no total de ácidos graxos saturados. Ácidos graxos como mirístico, láurico e palmítico, conhecidos como agentes causadores de doenças coronarianas, foram claramente substituídos pelos ácidos graxos, oleico e linolênico. Isto também demonstra que é possível modificar a composição dos ácidos graxos da "manteiga de garrafa" pelo método enzimático, usando sistemas livres de solventes orgânicos, para tornar o produto mais saudável à dieta humana.

Palavras-chave: *transesterificação, lipase, mateiga de garrafa, ácidos graxos.*

Introduction

Nutritional advice recommends the reduction of dietary saturated fatty acids (SFA) intake, since they can increase the risks of coronary artery disease (CAD) (KATAN et al., 1995).

Increasing of the plasmatic cholesterol is related to high levels of lauric (12:0), myristic (14:0) and palmitic (16:0) acids (DENKE and GRUNDY, 1992; STORM et al., 1997; HU et al., 2001). However, stearic acid (18:0) does not cause the same effects,

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probably because it is weakly absorbed (KRITCHEVSKY, 1994; JONES et al., 1999).

On the other hand, diets enriched with oleic acid (18:1n-9) have been demonstrated to reduce the risks of CAD, probably due to the decreasing of the LDL oxidation (WOOD et al., 1993; REAVEN and WITZTUM, 1996; MANGIAPANE et al., 1999)**.** In the same way, diet enrichment with n-3 fatty acid induced reduction of cholesterol levels, improving all lipidics parameters (MORISE et al., 2004).

Transesterification is useful for modifying the physical properties of fats and oils through the exchange of fatty acids between triglycerides. Lipases (EC. 3.1.1.3) have been investigated intensively with regard to the modification of oils rich in high value unsaturated fatty acids such as oleic acid, linoleic acid, linolenic acid and some others (WARWEL et al., 1999) through transesterification processes.

A dietary common component in the Brazilian´s Northeast culinary is the handmade butter known as bottle butter, which corresponds to the liquid lipidic fraction produced after milk cooking process during local cheese production. This fraction is immediately bottled and it is extensively used as taste enhancer. Despite its general use, the fatty acid composition is unknown.

In the present study, we first describe the fatty acids composition of "bottle-butter" and use lipase from *Candida rugosa* in transesterification reactions between triglycerides of "bottle butter" and free oleic acid or linolenic acid in a solvent-free system.

Materials and Methods

Reactants

The reactants used to achieve that were: lipase from *Candida rugosa*, 1,3-regioespecific (860 U/mg), provided by Novo Nordisk Co. (Denmark); oleic and linolenic acids purchased from Sigma Chemical Co; and "bottle butter" purchased from a local producer. The identification of FAME was determined by means of the retention times by comparing them to authentified FAME, using a standard fatty acid methyl esters mixture (Sigma Chemical Co L9405). All other chemical were of analytical grade.

Transesterification reactions

The transesterification reactions were carried out according to Brown et al. (2008) in solvent-free system. They were performed in screw-capped glass tubes under constant magnetic stirring at 40°C. The reaction mixture contained: 0.85 g of lipase, 3.16 g of oleic acid or linolenic acid, 8.33 g of "bottle butter" and 0.25 mL of 100 mM sodium phosphate buffer pH 8.0. Aliquots were withdrawn at different intervals (0.4 and 8h) and submitted to analytical procedures.

Analytical procedures

Reaction aliquots of 50 µL were mixed with 1 mL chloroform until complete homogenisation and 20 µL of this chloroform extract were applied on silica gel G60 plates (Merck Darmstadt, Germany). Triglycerides were separated by TLC using hexane:ethyl ether:acetic acid (80:20:1, v/v) as solvent phase and visualised after spray dying with 0.15% (w/v) diclorofluorescein in ethyl alcohol. Triolein was used as triglyceride standard. The identified spots were scraped from the plates and lipids extracted with cloroform:ethanol (FOLCH et al., 1957) and transmethylation was carried out (BERRY et al., 1965). The resulting extract was submitted to gas chromatography (model 14B, Shimadzu, Japan) with capillary column (SUPELCOWAX 60mx0.25mmx0.25µm) using hydrogen as carrier gas at a flux of 2 mL/min. Temperature programme ranged from 130°C to 245°C at 2.5°C/min in order to achieve baseline separations for the complex mixture of all cis/trans-isomers. Temperature for injection and detection was 250ºC. Peaks in gas chromatography were assigned by comparison of their retention times with those of known standards. Peak areas and percentages were calculated using CLASS-CR10 WORKSTATION SHIMADZU.

Results and Discussion

"Bottle butter" is widely consumed in Northeast of Brazil because of its desirable flavour comparing to vegetal oils. However, the SFA (saturated fatty acids) concentrations in lipids from animal origin are commonly very high. Triglycerides of "bottle-butter" were enriched with oleic and linolenic acid by transesterification using *Candida rugosa* lipase, which is 1.3-positionally specific. These lipase-catalysed transesterification reactions and could change fatty acids composition of those butters to produce a "healthy" product, enriched with unsaturated fatty acids. Changes in fatty acids composition of "bottle butter" triglycerides are shown in Table 1.

Table 1 - Modification of fatty acids composition of "bottle butter" triglycerides by transesterification with oleic acid catalysed by *Candida rugosa* lipase in solvent-free system. results expressed in % of total fatty acids.

		Incubation time (hour)		
	Fatty acids	Zero	4	8
Saturated	10:0	1.09	0.63	0.65
	12:0	2.01	1.71	1.62
	14:0	9.71	9.88	8.84
	16:0	36.85	34.71	29.58
	18:0	15.07	14.74	18.33
Monounsaturated	16:1n-7	1.87	1.66	1.21
	18:1n-9	23.09	25.04	26.28
	18:1n-7	3.24	3.92	5.34
Polyunsaturated	18:2n-6	1.23	1.52	2.10
	∑ saturated	67.78	64.29	60.84
	∑ unsaturated	32.22	35.23	35.34

As expected, the triglycerides from "bottle butter" are mainly composed by saturated fatty acids (67.78%), especially palmitic and stearic acids, which count together around 50% of total saturated fatty acids.

Results from Table 1 show that transesterification between oleic acid and bottle butter was efficiently catalysed by *C. rugosa* lipase, since it could be detected in an increase of 9.6% (32.22 to 35.34%) of unsaturated fatty acids in the triglycerides. Moreover, a similar decrease of SFA was also detected (67.78 to 60.84%). The saturated palmitic fatty acid in "bottle butter" triglycerides was notably replaced by oleic acid, probably because these fatty acids occupy the sn-1 position of trigliceryde molecule and during lipase transesterification process the substitution of palmitic acid by oleic acid in the triglycerides became more effective.

When the transesterification was performed with linolenic acid (18:3n-3), it was observed that its incorporation in the triglycerides was higher than oleic acid, showing a elevation of 0.35% at zero incubation time to 3.30% after 8h and could reach 6.09% after 24h incubation (data not shown). Possibly this elevation was due to replacement of saturated fatty acid 10:00 (capric) which occupy sn-3 position and presented a reduction of 55.84%.

Probably, such effect results from the lipase preference, since Warwel et al. (1999) also described a similar preference of *Candida cylindracea* lipase for unsaturated fatty acids containing cis-9 double bond, such linoleic and linolenic acids. It could be observed that the increase in the enzyme selectivity for

incorporation of cis-9 unsaturated fatty acids was firmly related to the increase in the degree of unsaturation of "bottle butter" triglycerides. Similar result was reported by Rangheard et al. (1989). However, when using linolenic acid for incorporation, similar levels of enrichment with unsaturated fatty acids (9.4%) were achieved only in prolonged reaction time (24h). This effect does not constitute a particular phenomenon, since Selmi et al. (1998) reported that an immobilised lipase of *Rhizomucor miehei* catalysis the esterification of linolenic acid more slowly than oleic acid in solvent-free system.

The results described here showed that nutritional properties of "bottle butter" could be efficiently modified by *Candida rugosa* lipase 1.3-positionally specific, in solvent-free system, offering an alternative to a healthier bottle butter for human diet.

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