Mycological analysis of *ambrosia* and creamy *dulce de leche*

Análise micológica de ambrosia e doce de leite pastoso

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ABSTRACT
Creamy *dulce de leche* is a product made from concentrated milk by heating, and adding sucrose. *Ambrosia* is a variety of *dulce de leche* prepared by adding eggs and sugar into milk. Aiming at identifying the genera of filamentous fungi isolated from creamy *dulce de leche* and *ambrosia*, 40 *ambrosia* and 20 creamy *dulce de leche* samples were analyzed in the present study. Filamentous fungi and yeast counts and the identification of genera of the isolated filamentous fungi were performed. Thirty-nine samples (65%) showed filamentous fungi and yeast counts that exceeded the acceptable limits. Six fungus genera were identified, and among them the *Penicillium* was the most frequent. These findings are a warning of the requirement for strict control on good manufacturing practices as well as on the inspection and monitoring of *ambrosia* and creamy *dulce de leche* trading.
Keywords. filamentous fungi, creamy *dulce de leche*, *ambrosia*.

RESUMO
O doce de leite pastoso é um produto obtido por concentração do leite, adicionado de sacarose, sob ação do calor. A ambrosia é um tipo de doce de leite preparado com leite, ovos e açúcar. Com o objetivo de identificar os gêneros de fungos filamentosos isolados de amostras de doce de leite pastoso e ambrosia, foram analisadas 40 amostras de ambrosia e 20 de doce de leite. Foram realizadas contagens de bolores e leveduras e identificação dos gêneros de fungos filamentosos isolados. Trinta e nove (65%) amostras apresentaram contagens de bolores e leveduras acima dos limites aceitáveis. Seis gêneros fúngicos foram identificados, sendo *Penicillium* o mais frequente. Os resultados obtidos são um alerta quanto à necessidade de rigoroso controle nas boas práticas de fabricação, bem como na inspeção e fiscalização do comércio da ambrosia e do doce de leite pastoso.
Palavras-chave. bolores, doce de leite pastoso, ambrosia.

INTRODUCTION

Dulce de leche is a product obtained from the concentration and heating of milk with the addition of sucrose, developing characteristic color and taste[1]. It may either have a pasty texture or be manufactured in tablets. *Ambrosia* is a kind of *dulce de leche* prepared by the addition of eggs and sugar to milk. Both *ambrosia* and pasty *dulce de leche* are largely consumed in South America, especially in Brazil, Uruguay and Argentina[2].

Hygiene procedures and food safety are constant concerns to consumers, as well as to institutions which are responsible for monitoring food quality. Foods can be contaminated by inadequate practices on the farm, during the manufacturing process, in the distribution centers, in the retail markets or in the consumers' homes[3]. However, there are few papers on *dulce de leche* in scientific bibliography. Most studies have been developed in Argentina or Brazil and focus either on the manufacturing process or on the physical and chemical characteristics of the product[4-8].

Filamentous fungi and yeasts counts provide information on general conditions of hygiene in food processing, storage and transportation and are important indicators of food spoilage[9]. Additionally, the control of the contamination caused by these microorganisms is necessary due to the possibility of mycotoxin production by some species. According to Vechia e Castilhos-Fortes[10], fungi of the *Aspergillus*, *Penicillium* and *Fusarium* genera are thought to be important toxin producers in food. Some heat and cold resistant mycotoxins can remain viable even in foods that have been subjected to technological processes and are a health hazard to the consumer. Aflatoxins are secondary metabolites synthesized by fungi of the *Aspergillus* genus and have been held responsible for the poisoning of different animal species, including man[11]. The MAPA – Ministério de Agricultura, Pecuária e Abastecimento (Ministry of Agriculture, Livestock and Supply) establishes limits for filamentous fungi and yeast occurrence in *dulce de leche* in Brazil[1]. The identification of filamentous fungi that are carried by highly manipulated dulces de leche, such as homemade *ambrosia* and pasty *dulce de leche* fractioned for sale, is important to evaluate the quality of these foods.

The present work aimed to identify the genera of filamentous fungi isolated from pasty *dulce de leche* and *ambrosia* samples marketed in Pelotas city, Rio Grande do Sul, Brazil.

MATERIAL AND METHODS

Twenty samples of pasty *dulce de leche* sold by local supermarkets and 40 *ambrosia* samples, 20 of which from supermarkets and 20 others from street markets, were obtained in Pelotas city, Rio Grande do Sul, Brazil. The collected samples were referred to a laboratory in their sales packages and immediately processed. Twenty-five grams of each sample were weighed and analyzed according to the Official Analytical Methods for Microbiological Analysis for Animal Product and Water Control[9] so that filamentous fungi and yeast counts could be performed.

After the counts, spread on potato dextrose agar (Acumedia, Baltimore, MD, USA) of at least one colony showing a distinct morphology for each plate was done. After growth at 25 ± 2 ºC, the cultures were kept in cool storage awaiting identification.

The isolated filamentous fungi were identified according to Lacaz et al.[12] and Sidrim & Rocha's[13] suggested procedures by observing macroscopic characteristics of the colonies (color, texture and topography) as well as microscopic characteristics from micro cultivations kept at room temperature for a seven-day period and then viewed by lacto phenol cotton blue at a 10 and 40 fold increase.

RESULTS

Of the 40 *ambrosia* samples analysed, 30 (75 %) presented filamentous fungi and yeast counts above the limit allowed by MAPA for *dulce de leche*, which is 1.0×10^2 CFU/g[1]; 15 of these samples came from supermarkets and 15 from street markets. Of the pasty *dulce de leche* samples, nine (45 %) had results exceeding the limit allowed. Only 21 (35 %) out of a total of 60 studied samples had filamentous fungi and yeast counts within acceptable limits (Table 1).

Six fungal genera were identified in *ambrosia* and pasty *dulce de leche* samples (Table 2). Yeast occurrence was observed in 9 (22.5%) out of 40 *ambrosia* processed samples, and pasty *dulce de leche* had yeasts in 3 (15 %) out of 20 studied samples.

DISCUSSION

Ambrosia and pasty *dulce de leche* for human consumption were studied as to their contamination level by filamentous fungi and yeasts and the kind of filamentous fungi found. A great number of the analysed samples had filamentous fungi and yeast counts that exceeded the limits

Table 1. Mould and yeast counts from *ambrosia* and pasty *dulce de leche* samples marketed in the city of Pelotas, RS

Ambrosia		Pasty *dulce de leche*
Street markets (CFU/g)	**Supermarkets (CFU/g)**	**Supermarkets (CFU/g)**
5.0×10^1	2.0×10^1	1.0×10^2
5.0×10^1	1.0×10^1	4.0×10^1
8.5×10^1	6.0×10^1	$<1.0 \times 10^1$
7.0×10^1	1.0×10^2	$<1.0 \times 10^1$
6.5×10^3*	$<1.0 \times 10^1$	$<1.0 \times 10^1$
5.0×102	**5.0×10^3**	$<1.0 \times 10^1$
1.0×10^3	**7.1×10^3**	**2.4×10^2**
2.2×10^4	**1.1×10^3**	**1.1×10^2**
$<1.0 \times 10^1$	**1.2×10^3**	**1.3×10^2**
5.5×10^2	**6.0×10^2**	**2.1×10^3**
5.7×10^3	**9.2×10^2**	**6.0×10^2**
3.5×10^3	**1.4×10^3**	**1.1×10^3**
9.2×10^2	**7.3×10^3**	**9.7×10^3**
2.8×10^2	**2.9×10^3**	**2.7×10^3**
3.4×10^3	**9.4×10^3**	**1.0×10^3**
3.8×10^4	**1.4×10^2**	7.0×10^1
6.7×10^2	**6.6×10^2**	2.1×10^3
2.4×10^2	**7.3×10^2**	$<1.0 \times 10^1$
8.2×10^3	**3.5×10^2**	1.1×10^3
2.1×10^3	**1.6×10^2**	$<1.0 \times 10^1$

** The values in bold exceed the limits determined by Brazilian law (BRASIL, 1997)*

established by Brazilian law, and potentially mycotoxigenic fungus genera were identified.

The preparation of the homemade *ambrosia*, as well as the fractionation of the *dulce de leche* for sale in small portions, result in higher product handling and exposure to potential sources of contamination, which may be related to the filamentous fungi and yeast counts observed.

The *ambrosia* sold at street markets is a non-industrialized product that has often no official inspection and no guarantee of having been manufactured according to Good Manufacturing Practices (GMP). These processing conditions can affect the product's microbiological quality, as demonstrated by Sousa et al.[14] in a study on homemade buffalo *dulce de leche* in which eleven out of a total of 18 analysed samples had filamentous fungi and yeast counts over 10^3 CFU/g. Conversely, counts within legal standards are expected to be found in inspected products, as observed by Esser et al.[15] upon analyzing *dulce de leche* (samples from different counties in the State of Minas Gerais), when they found that only 6.6 % of the samples were exceeding acceptable limits.

Supermarkets display mostly industrialized goods that have been inspected and manufactured following adequate and hygienic-sanitary procedures. However, the sheer fact that a product has undergone inspection does not guarantee that it is consumption-appropriate when acquired by the consumer. These products are often manipulated, fractioned and repacked by stores, which expose them to contamination. Timm et al.[16], upon analysing pasty *dulce de leche* samples from supermarkets that were fractioned upon retail sale found filamentous fungi and yeast counts that

Table 2. Fungal genera isolated from ambrosia and pasty dulce de leche samples from supermarkets and street markets in the city of Pelotas, Rio Grande do Sul

Genus	Samples			Total Nº (%)
	Ambrosia		Pasty *dulce de leche*	
	Street markets Nº (%)*	Supermarkets Nº (%)	Supermarkets Nº (%)	
Penicillium	11 (55)	10 (50)	6 (30)	27 (45)
Cladosporium	3 (15)	3 (15)	0 (0)	6 (10)
Aspergillus	4 (20)	2 (10)	0 (0)	6 (10)
Rhizopus	1 (5)	0 (0)	1 (5)	2 (3.3)
Alternaria	0 (0)	0 (0)	1 (5)	1 (1.7)
Paecelomyces	0 (0)	0 (0)	1 (5)	1 (1.7)
Sterile mycelium	0 (0)	5 (25)	6 (30)	11 (18.3)

** (Nº) Number of isolates; (%) percentage in relation to the number of studied samples*

varied from 1.4 x 10^1 to 6.0 x 10^4 CFU/g. Only one sample had filamentous fungi and yeast counts within acceptable values according to Brazilian standards. Castro et al.[17] also obtained counts exceeding legal limits for both inspected and non-inspected *dulce de leche* samples.

The fungi isolated from the analyzed samples belong to genera that promote food deterioration[3]. In addition, some species belonging to the *Aspergillus* and *Penicillium* genera can produce mycotoxins[18,19]. The *Penicillium* genus was the most frequently found in the analysed pasty *dulce de leche* samples. *Aspergillus* ssp. grows in high sugar concentrations[10], which can be considered an important factor in the occurrence of this genus in the *ambrosia* analysed samples. In spite of the fact that the toxin occurrence in *dulce de leche* was not the focus of this study, this possibility cannot be overruled. Taniwaki et al.[20] isolated *Aspergillus* ssp. and *Penicillium* ssp. strains in cheese samples and found two citrinin- and patulin-producing species. According to Gava[21], spores and mycelium fragments can contain high toxin concentrations, which makes the *Aspergillus* occurrence in *dulce de leche* a public health hazard.

CONCLUSION

The *ambrosia* and pasty *dulce de leche* marketed in the city of Pelotas had filamentous fungi and yeast counts that exceeded the admissible limit, including species that are potentially mycotoxigenic, which justifies the adoption of a stricter control on the good manufacturing practices, on the inspection and also on the monitoring of the food trade.

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