

## THE OCCURRENCE OF MOLDS, YEASTS AND MYCOTOXINS IN PRE-COOKED PIZZA DOUGH SOLD IN SOUTHERN RIO GRANDE DO SUL

Beatriz Helena Pinho<sup>1\*</sup>; Eliana Badiale Furlong<sup>2</sup>

<sup>1</sup>Departamento de Patologia and <sup>2</sup>Departamento de Química, Fundação Universidade Federal do Rio Grande, Rio Grande, RS, Brasil

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### ABSTRACT

The quality of pre-cooked pizza dough was investigated by assessing the occurrence of molds, yeasts and mycotoxins. Random sampling of commercial pre-cooked pizza cakes was done in different stores in the cities of Rio Grande and Pelotas, RS, between 1996 and 1997. The products were analysed on the sampling day and after storage at room (22-30°C) or refrigerated temperature (7°C) following the shelf life stated by the manufacturer (25,30 and 45 days). The results showed that mold and yeast contamination was frequently above the maximum limits ( $10^3$ CFU/g<sup>-1</sup>) established by Brazilian guide lines, even in samples kept at refrigerated temperatures up to the end of shelf life. Although no mycotoxin contamination was detected, a strain of the *Penicillium* genus, isolated from various samples, produced ochratoxin A at refrigeration temperatures.

**Key words:** pre-cooked pizza dough, fungi, mycotoxins

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### INTRODUCTION

The bakery industry has been increasing and varying its production during the last few years, and pizza has become very popular. They are sold ready to use, frozen, chilled or pre-cooked in many different commercial establishments. The large consumption of this food is not only due to its flavor but also because it is practical, easy to prepare, has high nutritional value and is cheap.

The dough of pre-cooked pizzas, being a product based on cereals and having intermediary moisture content, is a potential substrate for fungal development, caused by contamination occurring after baking during packaging and inappropriate storage (13).

These fungi can grow and affect the nutritional and sensory properties of the product and if the species are toxigenic, they may produce mycotoxins (4). The occurrence of mycotoxins has been observed worldwide in wheat, peanut, corn, beans and grains, besides meat and milk. In addition, mycotoxins are not

completely eliminated by processing (2,6, 10).

The aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> and ochratoxin A are the most frequent mycotoxins present in grains and foods because they are produced by ubiquitous fungal genera like *Aspergillus* and *Penicillium*. The cancerous and nephrological effects of these toxins reinforce the need for a regular search for their occurrence. Due to their peculiar blue and green fluorescence, they can be detected by relatively simple techniques (10,12,19)

In the specific case of pre-cooked pizza doughs, which are baked, the fungi may be inactivated, even though they can contaminate the food again after processing. The mycotoxins may come from the raw material or be produced in the food when contaminated during post baking handling (4, 10, 13).

The aim of the present work was to study the contamination levels in pre-cooked pizza dough by an assessment of the molds and yeasts present and the identification of the more frequent genera present. The occurrence of the following mycotoxins: aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, ochratoxin A and zearalenone was also determined.

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\* Corresponding author. Mailing address: Fundação Universidade Federal do Rio Grande, Laboratório de Micotoxinas, Rua Alfredo Huch, 475, CEP 96201-900, Rio Grande, RS, Brasil.

## MATERIALS AND METHODS

### Sampling

The pre-cooked pizza doughs were acquired from commercial establishments in the cities of Pelotas and Rio Grande in the State of Rio Grande do Sul, Brazil. The three brands chosen had expiration dates within 25,30 and 45 days, according to the manufacturers. Sample collection was performed in the fall, winter and spring of the year of 1996 and the summer of 1997.

Sets of 8 discs of pre-cooked pizza crusts were formed at each sample collection for brands A, B and C. This procedure was repeated three times for each brand comprising 9 experiments and totalizing 72 discs, which constituted the analytical samples. The determinations on the samples were performed at time zero (To), a period between the first and the fifth day after manufacture, and after storage at refrigerated temperature (RFT) (7°C) and at room temperature (RT) (22-30°C) for up to two days before the end of the corresponding shelf life for to each brand.

### Mycological Determinations

The enumeration of molds and yeasts was performed after using

pour plate technique in acidified potato dextrose agar (PDA) according to A.P.H.A(1984). After enumeration, the most frequent fungal colonies found in the pre-cooked pizza crusts were isolated and kept in Sabouraud agar until the identification procedures were completed.

The results of the enumeration using PDA were compared with those obtained using a Petrifilm YM 3M in one experiment (15).

The identification of the most frequent genera in PDA was effected from the macroscopical and microscopical characteristics of the colonies and microcultiive (11, 15,16).

### Determination of Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, ochratoxin A and zearalenone

A thin layer chromatographic method (19) was used for the simultaneous detection of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, ochratoxin A and zearalenone. The toxins were extracted using methanol and 4% KCl (9+1), followed by clarification with ammonium sulfate and partition with chloroform. Confirmation was accomplished by chemical reaction (8,17), elution with different solvents and co-chromatography.

The determinations were performed on the pre-cooked pizza cakes at time zero and after storage at refrigerated temperature (7°C) and at room temperature (22 - 30°C) for up to two days before the corresponding expiration date of each brand and on the isolated fungal cultures, incubated in Sabourand agar under the same conditions as the pizza doughs.

The detection limits of the method were 1.7; 6.5 and 60 ng.g<sup>-1</sup>

for aflatoxins, ochratoxin A and zearalenone respectively (3).

## RESULTS AND DISCUSSION

The enumeration of mold and yeast was done using a PDA media because it is convenient for screening a large number of species, and the observations can be made over an extended period of time on a single colony. (3,7). This was observed during previous studies where bread was used as a model (3).

As for Beuchat *et al.* (16) no differences between the enumeration on Petrifilm and on PDA during the test experiment. The latter was chosen because of its lower cost and the ease of recovering fungi for identification procedures. The average results of the enumeration of molds and yeasts on PDA for the three studied brands are presented in Table 1.

**Table 1:** Enumeration of molds and yeasts for pre-cooked pizza dough before and after being stored

Samples	TO CFU.g <sup>-1</sup>	RFT CFU.g <sup>-1</sup>	RT CFU.g <sup>-1</sup>
A1	1.3X10 <sup>4</sup>	7.2X10 <sup>4</sup>	2.9X10 <sup>6</sup>
A2	1.6X10 <sup>4</sup>	1.4X10 <sup>4</sup>	2.5X10 <sup>6</sup>
A3	4.3X10	9.6X10 <sup>3</sup>	2.8X10 <sup>6</sup>
B1	2.0X10 <sup>2</sup>	2.7X10 <sup>6</sup>	6.8X10 <sup>3</sup>
B2	9.2X10 <sup>2</sup>	4.1X10 <sup>3</sup>	1.51X10 <sup>4</sup>
B3	1.2X10 <sup>2</sup>	4.7X10 <sup>4</sup>	9.5X10 <sup>4</sup>
C1	7.9X10	4.8X10 <sup>3</sup>	Mold
C2	1.1X10 <sup>2</sup>	1.6X10 <sup>4</sup>	1.2X10 <sup>4</sup>
C3	1.4X10 <sup>2</sup>	2.1X10	Mold

TO: zero time

RFT: refrigerated temperature (7°C)

RT: room temperature (22-30°C)

A, B, C: brands of pre-cooked pizza cakes

1, 2, 3: periods of sample collection

According to the Brazilian guideline, the maximum tolerated level for molds and yeasts in baked products is 5x10<sup>3</sup> CFU.g<sup>-1</sup> (Ministry of Health, SVS portaria 451, 19/09/97). According to DINAL (Divisão Nacional de Alimentos), another national organization food control, the maximum level for molds and yeasts is 10<sup>4</sup> CFU.g<sup>-1</sup> (5). The most contaminated sample was brand A, with respect to molds and yeasts, at the moment of sampling.

All the brands of pre-cooked pizza doughs showed values higher than the limits established by the national control boards to molds and yeasts, after storage at room and refrigerated temperatures. In general, the samples stored at refrigerated temperatures showed lower values of CFU.g<sup>-1</sup>. This was due to refrigeration temperatures slowing down the exogenous or endogenous degradation reactions.

An interesting proposal was made by Rayman *et al.* (18), for the adequacy of baked products. According to their criteria,

in terms of molds and yeasts, the limit between acceptable quality and marginal quality is  $2 \times 10^3$  CFU. g<sup>-1</sup> and the limit separating marginal quality from unacceptable quality is  $5 \times 10^4$  CFU. g<sup>-1</sup>.

According to the above mentioned proposal (18) the pre-cooked pizzas doughs, stored at room temperature, were not recommended for consumption. Storage at refrigeration temperature was not sufficiently effective to avoid the samples being considered of marginal quality with respect to their molds and yeasts counts.

The identification of the genera was effected from the macroscopical and microscopical characteristics of the colonies and the microculture showed that the fungi belonging to the genera *Penicillium* and *Aspergillus* were more frequent. Others genera present were identified as *Mucor sp*, *Geotrichium sp* and yeasts. These fungal genera are present in the environment and easily contaminated food.

These results suggest that more care should be taken in the handling of these products after they are baked, that storage should be done under refrigeration and also that the established shelf life period should be reviewed. Another aspect that might be studied is the type of package to be recommended for this kind of product. All these parameters determine the fungal recontamination of baked and cooked products.

The study of mycotoxins in pre-cooked pizza doughs is justified due to their thermostability, allowing for their presence in the raw material used in the pizza dough formulation. Although the fungi and their spores may be destroyed by the thermal treatment during baking, the product can be recontaminated in the next steps by toxigenic fungi present in the environment (3,10, 14). In these specific cases the probability of mycotoxin occurrence was high because the number of forming colony units was high.

During screening for aflatoxin (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>), ochratoxin A and zearalenone it was shown that 22% of the samples were probably contaminated, although this was not confirmed during the confirmatory test. These results imply that the raw material used in the manufacture of the products did not present at detectable levels of toxins after formulation. The high number of samples with false positive results may be caused by fluorescent components in the formulation or have been formed during baked. We can't discard the possibility of contamination by some other mycotoxins not researched here.

A previous survey of mycotoxins in pre formed loaves conducted in our laboratory showed the occurrence of ochratoxin A in moldy pre-form loaves during the shelf life period (3). This suggested the possibility that an extended storage under unsuitable conditions could lead to the occurrence of mycotoxins if there were toxigenic fungal species present.

Considering the above mentioned information, three types fungal colonies frequently found in pre-cooked pizza dough, belonging to the *Penicillium* genera and denominated I, II and III, were submitted to a screening chemical test for toxicities (14). The isolated colonies were incubated in Sabourand agar

under the same conditions (time and temperature) as the commercial products. They were then submitted to the multimethods proposed by Soares, taking care to follow the methods specified to determine mycotoxins (19). The results are presented in Table 2.

**Table 2.** Screening of mycotoxins in fungal colonies isolated from pre-cooked pizza cakes.

Colony	RFT (25 days)	RFT (45 days)	RT (25 days)
I	*ochratoxin A	*ochratoxin A	*ochratoxin A
II	-	Aflatoxins B <sub>1</sub> e B <sub>2</sub>	-
III	*ochratoxin A	*ochratoxin A and Aflatoxins B <sub>1</sub> e B <sub>2</sub>	-

I, II, III: *Penicillium* fungal colonies  
 RFT: refrigerated temperature (7°C)  
 RT: room temperature (22-30°C)  
 \* confirmed presence of ochratoxin A

The choice of the incubation conditions was according to the shelf life of the brand from which the colonies were isolated. Only one colony incubated at room temperature produced ochratoxin A after 25 days, colonies II and III produced the toxins at refrigerated temperatures after 45 and 25 days respectively. This is a characteristic of many toxigenic *Penicillium* species mentioned by other authors (7, 10, 15). Unfortunately the species was not identified in this work.

## CONCLUSIONS

The number of colony forming units of molds and yeasts increased during storage at the temperatures studied, being more intense when the samples were stored at room temperature.

The storage of the pre-cooked pizza dough at refrigerated temperatures was not effective in keeping the number of colony forming units in the samples within the limits established by the legislation during the shelf life period.

The samples of pizza dough were not contaminated with aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, ochratoxin A or zearalenone at the time of sample collection or after storage during the shelf life.

Fungal colonies belonging to the *Penicillium* genus produced ochratoxin A under the time and temperature conditions established in this work.

## RESUMO

### Ocorrência de bolores, leveduras e micotoxinas em massa de pizza pré-fabricada comercializada no Rio Grande do Sul

A qualidade de massa de pizza pré-fabricada foi avaliada através da determinação de bolores, leveduras e micotoxinas.

Entre 1996 e 1997, fez-se uma amostragem ao acaso de discos de pizza pré-cozidos, em diferentes estabelecimentos comerciais das cidades de Pelotas e Rio Grande, RS, Brasil. Os produtos foram analisados no dia da amostragem e após o armazenamento em temperatura ambiente (22-30°C) ou de refrigeração (7°C), seguida o prazo de validade indicado pelo fabricante (20, 30 e 45 dias). Os resultados indicaram que contaminação por bolores e leveduras estavam frequentemente acima dos limites ( $10^3$  UFC/g), estabelecidos pelos padrões brasileiros, mesmo nas amostras mantidas em refrigeração. Embora nenhuma micotoxina tenha sido detectada, uma cepa do gênero *Penicillium*, isolado de várias amostras, produziu ocratoxina A em refrigeração.

**Palavras-chave:** massa de pizza pré-cozida, fungos, micotoxinas

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