Rice is a basic food of the Brazilian people and it is thus very important to have information about the occurrence of mycotoxins in this cereal. The aim of this study was to verify the effect of cooking on the levels of aflatoxin B\textsubscript{1} (AFB\textsubscript{1}) and ochratoxin A (OTA). The mycotoxins were determined according to the method of SOARES; RODRIGUEZ-AMAYA (1989) adding cyclohexane to provide partition in the cleaning of the extract (SIMIONATO et al., 2003). To verify the effect of cooking on the mycotoxin levels in the samples, they were artificially contaminated with AFB\textsubscript{1} and ochratoxin A separately and together. The samples were submitted to normal cooking, cooking in an excess of water and in a microwave oven. The highest mycotoxin reductions were found when cooked in excess water (89.1% for AFB\textsubscript{1} and more than 86.7% for OTA), followed by normal cooking (82.3% for AFB\textsubscript{1} and 83.1% for OTA) and finally the microwave oven (72.5% for AFB\textsubscript{1} and 82.4% for OTA). In the samples artificially contaminated simultaneously with AFB\textsubscript{1} and OTA, the rates of reduction were lower than when contaminated separately. Ochratoxin A and AFB\textsubscript{1} were also detected in the cooking water when cooked in an excess of water.
1. INTRODUCTION

Some fungi, frequently found in grains, have the capacity to produce secondary metabolites capable of causing toxic, mutagenic, teratogenic or carcinogenic alterations in animals and humans. These chemical substances are named mycotoxins.

The aflatoxins, secondary metabolites of the fungi Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius, are those which can cause the most severe harm to humans and animals due to their high toxicity and wide occurrence, also presenting carcinogenic, mutagenic and teratogenic properties. Aflatoxin B₁ is the most toxic, being considered as carcinogenic for humans by the International Agency for Research on Cancer (IARC, 1993) followed by AFG₁, AFB₁, and AFG₂ (SOLFRIZZO, 1998).

The toxic action verified in animals is called hepatocellular carcinoma (HCC), which is considered as liver failure with destruction of the parenchymal cells and proliferation of the bilious canals. In humans, the toxic action is chronic and has been correlated with the incidence of liver cancer in sites with high aflatoxin contamination (OLIVEIRA; GERMANO, 1997).

Aspergillus ochraceus and Penicillium verrucosum are the main ochratoxin A (OTA) producers (MARTH, 1992). OTA is nephrotoxic and considered by the IARC as possibly carcinogenic to man (IARC, 2003). It has been described as the causal agent of endemic nephropathy in the Balkans, due to the high level of OTA found in the food in that region and also in the blood of those with the disease. The disease is characterized by a progressive reduction in renal function, on rare occasions accompanied by sodium retention or hypertension (FINK-GREMMELS, 1995).

The presence of aflatoxins has been detected in many foods, especially in peanuts and derived products, corn, barley, almonds, walnuts and spices (SOARES; RODRIGUEZ-AMAYA, 1985; SOARES; RODRIGUEZ-AMAYA, 1989; PURWOCO et al., 1991; ELLIS et al., 1991; TABATA et al., 1993; DHAVAN; CHAUDARY, 1995; EL-GOHARY, 1996; CALDAS et al., 1998; SABINO et al., 1999; FURLONG et al., 1999) and the presence of ochratoxin A in corn, barley, wheat, rice, coffee and wine (SOARES; RODRIGUEZ-AMAYA, 1989; STUDER-ROHR et al., 1995; FURLONG et al., 1999; TRUCKESE et al., 1999; VISCONTI et al., 1999).

Rice together with corn, are the second most produced cereals in the world, wheat being the first. The annual World production of these two cereals is estimated at 500 million tons and Brazil is one of the few non-Asian countries in which rice is cultivated and used as a basic food item, being in seventh place in World production, with 10 million tons annually. China heads this list with an annual production of 180 million tons (SINDARROZ, 2003).

The occurrence of mycotoxins in rice has been reported by investigators from several countries (JAYARAMAN; KALYNASUNDARAN, 1990; PURWOKO et al., 1991; TABATA et al., 1993; DHAVAN; CHAUDARY, 1995; RESNIK et al., 1995; EL-GOHARY, 1996; PATEL et al., 1996). In Brazil, although the reports of mycotoxin occurrence are few, a low frequency (<6%) of AFB₁ and OTA has been verified, with concentrations below 50µg/kg (SOARES; RODRIGUEZ-AMAYA, 1989; CALDAS et al., 1998; FURLONG et al., 1999; LIMA et al. 2000; SIMIONATO et al., 2003). LARROZA-NUNES et al. (2003) analyzed 56 samples of rice and detected one sample of parboiled rice and one sample of type 1 rice contaminated with 128 and 104µg/kg of the ochratoxin A, respectively. Aflatoxins, zearalenone, deoxynivalenol and toxin T-2 were not found in the samples.

The use of heat treatment with the double aim of processing the food and partially or totally reducing mycotoxin contamination, has been investigated by several research groups. Particularly in the case of peanuts and corn, the conventional practice of toasting has proved to reduce the contamination level by more than 40% (LEE et al., 1969; WALKING, 1971; SCOTT, 1984; SYLOS, 1986; SARAJEWIA, 1990; SYLOS; AMAYA-FARFAN, 1992; TAHA et al., 2001), and when toasted in a microwave oven, a reduction of above 90% was found (LUTTER et al., 1982; PLUYER et al., 1987; PRADO; OLIVEIRA, 1996).

The reduction of aflatoxin contamination in rice was also evaluated, in normal cooking, cooking with excess water and in a pressure cooker, the latter proving to be the most effective, showing a reduction of up to 75% of the initial contents (REHANA et al., 1979).

The effect of heat treatment on OTA in rice and oat bran was evaluated by TRENK (1971). For rice, artificially contaminated with 400µg/kg, the author verified thermal OTA destruction using heat processes with no addition of water, the addition of water, and the addition of 20% acetic acid solution. The heat treatment parameters were 0.5, 1.0 and 3.0 hours in an autoclave at 121°C. The most significant reduction occurred in the sample with no water addition, only 11.27 % of the initial content remaining after 0.5 hour at 121°C.

The purpose of this study was to assess the effect of different types of cooking on the contents of aflatoxin B₁ and ochratoxin A in rice.

2. MATERIAL AND METHODS

2.1 Material

Polished type 1 rice samples, free of contamination, were artificially contaminated with AFB₁ and OTA. The contamination levels employed were 20µg/kg for AFB₁ and 30µg/kg for OTA both when added separately and together. Adequate amounts of the standards dissolved in benzene were dripped on to 50g portions of sample held in 600mL beakers, taking care to avoid contaminating the wall. After 24 hours, the samples were submitted to the three types of cooking.
2.2 Cooking method

The artificially contaminated rice samples were submitted to three types of cooking. Each experiment was repeated six times. The cooking times used for the heat treatments were established as being those required for the rice to present a proper texture for ingestion.

**Normal cooking:** this means the normal domestic way of cooking rice in Brazil. Approximately 50g of artificially contaminated rice were cooked in 100mL water on a gas stove. Nine minutes were required to cook the rice after it had boiled.

**Cooking with excess water:** The same procedure described above was carried out but with twice the amount of water (200mL). After cooking, the excess water was drained off and analyzed separately.

**Microwave oven cooking:** About 50g contaminated rice was transferred to a special pan used to cook rice in a microwave oven and 100mL water added. The total cooking time was 9 minutes at medium potency (0.45kW).

2.3 Analyses

Aflatoxins and ochratoxin A were determined using the method of Soares; Rodriguez-Amaya (1989), with the addition of cyclohexane for partitioning during the cleaning of the extract (Simionato et al., 2003). Recovery varied from 94 to 104% for AFB₁ and 80 to 105% for OTA. The quantification limits were 1 and 4 μg/kg for AFB₁ and OTA, respectively (Simionato et al., 2003). The mycotoxins were separated and quantified by visual comparison of the intensity of fluorescence in the samples with that of known standards on the same plates, when submitted to UV light at 365nm. The residual cooking water obtained from the samples cooked in excess water was also analyzed by the same methodology.

### TABLE 1. Effect of cooking in excess water on the initial contents of aflatoxin B₁ and ochratoxin A in artificially contaminated rice samples*

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Initial level (μg/kg)</th>
<th>Contents after cooking (μg/kg)**</th>
<th>Contents in Residual water (μg/L)</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁</td>
<td>20</td>
<td>2.2 (ND – 5.7)</td>
<td>1.7 (ND – 4.3)</td>
<td>89.1</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>30</td>
<td>&lt;4.0 (ND – &lt;4.0)</td>
<td>&lt;4.0 (ND – &lt;4.0)</td>
<td>&gt;86.7</td>
</tr>
<tr>
<td>Aflatoxin B₁ +</td>
<td>20</td>
<td>2.2 (&lt;1 – 6.2)</td>
<td>2.1 (&lt;1 – 4.3)</td>
<td>89.1</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>30</td>
<td>4.8 (&lt;4 – 6.5)</td>
<td>4.8 (&lt;4 – 7.3)</td>
<td>84.0</td>
</tr>
</tbody>
</table>

* Quantification limit of 1μg/kg for AFB₁ and 4μg for ochratoxin A
** means of six determinations. Numbers in brackets are the contamination ranges. ND – not detected

Confirmation of the identity of aflatoxin B₁ was carried out using the trifluoracetic acid (TFA) reaction, effected directly on the chromatographic plate. For ochratoxin A, a 20% solution of aluminum chlorite was sprayed onto the plate after the chromatographic run, followed by heating at 100°C and observation under ultraviolet light at 365nm (Scott, 1984).

### 3. RESULTS AND DISCUSSION

Polished type 1 rice samples, artificially contaminated with AFB₁ (20μg/kg), ochratoxin A (30μg/kg), and with aflatoxin B₁ (20μg/kg) and ochratoxin A (30μg/kg) together, were submitted to normal cooking, cooking with excess water and microwave oven cooking. For the three processes the cooking time was 9 minutes, the time necessary for the final product to present an adequate texture. For the samples cooked with excess water, the residual water was separated for further analysis.

For the samples artificially contaminated with AFB₁, the highest reduction was found for the rice cooked in excess water (89.1%) followed by that cooked by normal cooking (82.3%) and finally by the microwave oven cooked sample (72.5%). Cooking in excess water was also the most effective way of reducing contamination with ochratoxin A, showing an average reduction greater than 86%. Normal cooking presented a average reduction of 83.1% and the microwave oven, 82.4% (Tables 1, 2 and 3).

In the process using excess water, the presence of micotoxins was detected in the residual water. In the samples contaminated with AFB₁, approximately 8.5% of the initial concentration of toxin was detected in the water, and in the samples contaminated with ochratoxin A, the residual water contained about 11.0% of the initial concentration of the toxin, indicating a possible migration of toxin to the water (Table 1).
TABLE 2. Effect of normal cooking on the initial contents of aflatoxin B₁ and ochratoxin A in artificially contaminated rice samples*.

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Initial level (µg/kg)</th>
<th>Contents after cooking (µg/kg)**</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁</td>
<td>20</td>
<td>3.5 [&lt;1 – 5.7]</td>
<td>82.3</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>30</td>
<td>5.1 [&lt;4 – 9.1]</td>
<td>83.1</td>
</tr>
<tr>
<td>Aflatoxin B₁</td>
<td>20</td>
<td>3.8 [&lt;1 – 6.2]</td>
<td>81.0</td>
</tr>
<tr>
<td>+ Ochratoxin A</td>
<td>30</td>
<td>7.0 [4.3 – 10.4]</td>
<td>76.6</td>
</tr>
</tbody>
</table>

* Quantification limit of 1 µg/kg for AFB₁ and 4 µg for ochratoxin A
** means of six determinations. Numbers in brackets are the contamination range.

TABLE 3. Effect of the microwave oven cooking on the initial contents of aflatoxin B₁ and ochratoxin A in rice samples artificially contaminated*.

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Initial level (µg/kg)</th>
<th>Contents after cooking (µg/kg)**</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁</td>
<td>20</td>
<td>5.5 [&lt;1 – 12.3]</td>
<td>72.5</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>30</td>
<td>5.3 [&lt;4 – 9.1]</td>
<td>82.4</td>
</tr>
<tr>
<td>Aflatoxin B₁</td>
<td>20</td>
<td>4.5 [&lt;1 – 8.2]</td>
<td>77.6</td>
</tr>
<tr>
<td>+ Ochratoxin A</td>
<td>30</td>
<td>7.2 [&lt;4 – 14.6]</td>
<td>75.9</td>
</tr>
</tbody>
</table>

* Quantification limit of 1 µg/kg for AFB₁ and 4 µg for ochratoxin A
** means of six determinations. The numbers in brackets are the contamination ranges.

In the samples artificially contaminated with both toxins the greatest reductions were obtained with the excess water cooking, followed by normal cooking and microwave oven cooking (Tables 1, 2 and 3).

From the results obtained in this study, microwave oven cooking was shown to be less effective in reducing contamination with the mycotoxins studied than cooking on a gas stove.

REHANA et al. (1979), studied the effect of normal cooking, cooking in excess water and cooking under pressure, on the concentrations of AFB₁, in artificially and naturally contaminated rice samples. Cooking under pressure for 5 minutes (proportion rice: water was 1:1) resulted in average reductions in contamination of 70.7 and 70.5% for the artificially and naturally contaminated rice samples, respectively. Normal cooking for 30 minutes (proportion rice: water was 1:4) resulted in an average reduction of 47.5% for the naturally contaminated samples and 49.0% for the artificially contaminated samples. Cooking in excess water (proportion rice: water was 1:8) resulted in higher toxin losses, with average reductions of 75.7 and 74.5% for the artificially and naturally contaminated rice, respectively. REHANA et al. (1979), also detected the presence of AFB₁ in the residual water for this kind of treatment.

4. CONCLUSIONS

The cooking of rice in an excess of water was the most effective method in reducing the levels of aflatoxin B₁ (89.1%) and ochratoxin A (>86%) in artificially contaminated rice samples than normal cooking and microwave cooking.

Microwave cooking was less efficient in reducing the levels of AFB₁ (77.6%) and ochratoxin A (75.9%) than cooking in excess water and normal cooking.

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REFERENCES
