DECREASED TOXICITY IN SEEDS OF BRAZILIAN FRUITS AFTER SOLID-STATE FERMENTATION

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RESUMO – Sementes dos frutos do Cerrado araticum, cagaita e mangaba caracterizadas como tóxicas em estudos prévios de bioensaio, foram tratadas com microorganismos endofíticos de mandioca mediante fermentação semi-sólida para avaliar sua biodegradabilidade e a segurança alimen- tardo dos derivados industrializados. O tratamento com culturas mistas provocou a redução da toxicidade, em 80% (araticum), 78,3% (cagaita) e 81,7% (mangaba). Foram isoladas duas bactérias, B1 e B2 e dois bolores, F1 e F2, identificados preliminarmente como Coccus, Lactobacillus, Penicillium e Aspergilus, respectivamente. A fermentação com as bactérias B1 e B2 reduziu a toxicidade nas sementes de mangaba mas não afetou as sementes de araticum e cagaita. Já o fungo F1 provocou a degradação das toxinas nas sementes de cagaita e mangaba, enquanto F2 favoreceu a detoxificação das três sementes estudadas. Os resultados evidenciam a presença de toxinas com estrutura e biodegradabilidade diferentes, sendo as toxinas das sementes de mangaba as mais sensíveis e as de araticum, as mais resistentes.

ABSTRACT – Toxic seeds of fruits from Brazilian Central Plain araticum (Annona crassiflora), cagaita (Eugenia dysenterica) e mangaba (Hancornia speciosa), according to previous bioassay studies were treated with endophytic micro-organisms from cassava, using semi-solid fermentation, in order to evaluate biodegradability of those compounds and food safety of industrial derivatives from such fruits. Mixed culture fermentation caused the decrease of toxicity about 80% (araticum), 78.3% (cagaita) and 81.7% (mangaba). Two bacterial and two fungal strains were isolated, preliminarily identified as Coccus, Lactobacillus, Penicillium e Aspergilus, respectively. Fermentation with bacteria B1 and B2 reduced toxicity in mangaba seeds only, but did not show any effect on araticum and cagaita seeds. Mold F1induced degradation of cagaita and mangaba toxins, while mold F2 enabled detoxification of all three studied seeds. These results evidence the presence of toxins with different structure and biodegradability, being the mangaba seeds toxins the most sensitive and araticum toxins the most resistant.

PALAVRAS-CHAVE: toxinas alimentares; biodegradação; bioensaio; Cerrado.

KEYWORDS: food toxins; biodegradation; bioassay; Cerrado.
1. INTRODUCTION.

This work is part of a major research project aimed to identify and explain the structure and action mechanism of natural toxic compounds found in the seeds of edible fruits from the Brazilian central region: Araticum (Annona crassiflora), Cagaita (Eugenia dysenterica) and Mangaba (Hancornia speciosa) (Fonseca et al., 2013). Commonly, these fruits are consumed fresh, but in the last few years several derivatives such as ice-creams, jellies, deserts and fermented drinks began to appear in the markets, being appreciated by local buyers and tourists, and showing potential for production expansion and exportation. The risk of intoxication is low when fruits are consumed fresh, but it increases in industrial derivatives since seeds may be partially broken during pulp removal. Detectable amounts of cyanide compounds have been found in industrial derivatives of peaches, plums and cherries, such as liqueurs and canned fruits (Fernandez and Novelli, 1997).

Several researchers have described a reduction of toxicity due to cyanide in starch and other cassava products after natural fermentation (Vasconcelos et al., 1990; Sokari & Washukwu, 1993; Carvalho et al., 1996; Grizotto & Menezes, 2004). This fact could be important since fermented food may contain reduced amount of toxic compounds. Thus, toxin biodegradability studies arise as an interesting biotechnological alternative in order to reduce intoxication risks and assure food safety of industrial derivatives manufactured from fruits with toxic seeds.

Based on these facts, the objective of this work was focused on the study of the biodegradability of natural toxins found in the seeds of araticum, cagaita and mangaba fruits, using solid state fermentation with both mixed cultures and isolated endophytic micro-organisms (two bacteria and two fungi) obtained from cassava tissues and evaluating the eventual toxicity reduction with Artemia salina bioassay.

2. MATERIALS AND METHODS

2.1 Fermentation

Mixed cultures were prepared blending in a mixer 500 g of cassava pulp and 500 mL of distilled sterilized water and then filtered through a piece of aseptic gauze. This mixture was left still standing at 30°C during 48 hours to promote proliferation of endophytic micro-organisms. 5 mL of this mixture were added to 5 g of each toxic seed previously crushed.

Micro-organism isolation from cassava was performed according to Carvalho et al. (1996), after sequentially washing the samples with current water, ethanol 70% (1 minute), sodium hypochlorite 2% (6 minutes) and sterilized distilled water. Cassava roots were then cut into small pieces of 0.5cm, placed in Petri dishes containing Tryptone Soya Agar and Potato Dextrose Agar and then incubated at 28°C during 7 days. Isolated bacteria were conserved in glycerol 20% at -20°C while fungi were transferred to Petri dishes containing Potato Dextrose Agar and conserved at 4°C. 5 mL of culture broth containing isolated bacteria or fungal mycelia from each culture were added to 5 g of each toxic seed previously crushed.

Fermentation of seeds with both mixed and isolated cultures was performed in Petri dishes during 5 days and all experiments were performed in triplicate. Blank experiments were performed with seeds treated with distilled water during 5 days.

2.2 Bioassay

In order to evaluate seed toxicity before and after fermentation, Artemia salina bioassay was performed according to the method described by Garcia-Rodriguez et al. (2009). Concentrated water extracts (0.5g/mL) were prepared using the inner kernels of the seeds and then filtered through Millipore membranes of 0.45 micrometer pores. The cultures were prepared using 0.2 g of Artemia salina eggs placed in 200 mL of 3.5% marine salt solution under constant air
bubbling and illumination for 48 hours until outbreak. New born nauplia were caught individually with a Pasteur pipette and transferred to test tubes containing 1 mL of 3.5% marine salt solution (ten Artemia individuals per tube). Seed extracts were added in different triplicate concentrations to test tubes containing Artemia cultures, in amounts not greater than 100 μL. In each experiment, three test tubes containing 1 mL of Artemia salina cultures were used as control, which were treated with 100 μL of distilled water each since all seed extracts were prepared using distilled water with no further toxin purification. After 24 hours incubation, living and dead individuals were counted to calculate survival percentage.

2.3 Statistics
All the data presented in the results are the mean values of Artemia salina survival out of three independent experiments. Mean comparison of individual variables was performed using Tuckey test with STATISTICA 7.0 software (Statsoft, Inc., Tulsa, USA, 1997).

3. RESULTS AND DISCUSSION

3.1 Fermentation with mixed cultures
Seeds of araticum, cagaita and mangaba treated with cassava mixed cultures showed a significant toxicity reduction expressed as the increase of Artemia salina survival values from 0% to 80% in araticum, 6.7% to 85% in cagaita and 3.3% to 85% in mangaba (figure 1 A,B and C), together with the increase of lethal dose from 0.029 mg/mL to 116.5 mg/mL in the case of araticum, as shown in figure 1A.

Figure 1: Dose-response curves of toxic extracts of araticum (A), cagaita (B) and mangaba (C) seeds fermented with mixed cultures from cassava, on Artemia salina survival.

Since survival values of Artemia salina cultures treated with fermented seeds of cagaita and mangaba were higher than 80% even at the highest concentrations, it was not possible to calculate LD_{100} nor LD_{50} values, which indicates the efficiency of mixed cultures in the reduction of toxicity of those two seeds (figure 1 B and C).

3.2 Fermentation with isolated micro-organisms
Two bacterial strains (B1 and B2) and two filamentous molds (F1 and F2) were isolated from cassava mixed cultures, being preliminarily identified as Coccus, Lactobacillus, Penicillium and Aspergillus, respectively.

Figure 2 shows the effect of isolated micro-organisms on the toxicity of the seeds, expressed as survival values of the Artemia salina cultures, treated with equal concentrations of seed extracts (50
mg/mL). Both bacterial strains partially degraded toxins present in mangaba seeds, as noted by the increase of the survival of Artemia salina cultures from 40% in the unfermented control seeds to 75% and 71.6%, respectively, but they did not show any effect on toxicity of cagaita and araticum seeds, were Artemia survival remained 0% even after the treatment with fermented seeds. Two facts may explain these results: Bacteria could have metabolic routes affected by the toxins of cagaita and araticum, or they are simply more water and nutrient demanding, not finding in the seeds environment the ideal conditions to their proliferation.

Nevertheless, isolated fungi showed a more marked effect on seeds toxicity. Mangaba seeds treated with both F1 and F2 strains elevated Artemia survival from 40% (control) to 91.6%, indicating a significant degradation or inactivation of the toxins present in those seeds. A less but also significant effect was observed on cagaita seeds toxicity, by the elevation of Artemia survival from 0% (control) to 47.5% (seeds fermented with F1 strain) and to 83.3% (seeds fermented with F2 strain). Although mold F1 did not affect the toxicity of araticum seeds, the F2 strain drastically reduced their toxicity, increasing Artemia survival from 0% (control) to 83.3%. This better response could be explained by the fact that generally, fungi are more cosmopolitan and adaptable than bacteria and they are also water and nutritionally less demanding.

Figure 2: Effect of isolated bacteria and fungi on the seeds toxicity, expressed as Artemia salina survival. Control data refers to cultures treated with unfermented seeds.

Although cyanide is commonly present in the seeds of some fruits such as peaches, plums and cherries (Fernandez & Novelli, 1997), that toxin does not seem to be responsible for the toxicity observed in the seeds of araticum, cagaita and mangaba, since specific Gignard test showed to be negative in all three (Fonseca et al., 2013), suggesting that other toxic compounds rather than cyanide could be present in the seeds of these fruits. Some researchers have reported the presence of tetrahydrofurane related toxins in seeds of commercial annonaceous fruits (Colom et al., 2006; Kim et al. 2001; Pardhasaradhi et al., 2005). Since those toxic compounds known as acetogenins can inhibit electron transport chain in mitochondrial respiration, it could be affecting oxygen dependant metabolism in most of studied micro-organisms, so avoiding biodegradation process in araticum seeds by B1, B2 and F1 isolated strains. Acetogenins are also known as antibacterial agents (Rabelo et al.,
insecticides and even antitumoral (Costa et al., 2013). Thus, the presence of these compounds in araticum seeds probably avoids or limits bacterial metabolic activity.

The structure of the toxic compounds in the seeds of cagaita and mangaba was not completely elucidated yet, but preliminary experiments point at them as thermolabile water soluble compounds, probably indicating a protein or even enzymatic structure. Moreover, previous studies showed the presence of tannins and other phenolic compounds in cagaita seeds (Rocha et al., 2011; Roesler et al., 2007). Such compounds can have antibacterial activity, which could also explain the low effect of both bacterial strains on the toxicity of these seeds. It was suggested the presence of enzymes such as peroxidase and poliphenol-oxidase in the mangaba seeds (Moura et al., 2015). Those enzymes can express a low toxicity and could be easily degraded by both bacteria and fungi.

The results presented here could be indicating the presence of toxins with different structures and action mechanisms, and consequently, with different sensitivities to biodegradation. From the food safety point of view, these findings could be useful during manufacturing of industrial derivatives of araticum, cagaita and mangaba, suggesting that in the event of seed breaking during pulp removal, fermented products could contain reduced amounts of toxic compounds.

4. CONCLUSIONS

1- Mixed cultures with endophytic micro-organisms from cassava were highly efficient reducing the toxicity as can be seen in high survival values of Artemia salina cultures.

2- Isolated bacteria were able to reduce toxicity only in mangaba seeds, but were inefficient on toxicity of araticum and cagaita.

3- Toxin biodegradation by fungi was more effective, being the mold F2 (apparently an Aspergillus), the only capable to reduce de toxicity of all three studied seeds.

4- These results could be useful during manufacture of industrial derivatives from araticum, cagaita and mangaba, suggesting that in the case of eventual seed breaking, fermented products might contain less amounts of toxins.

5. REFERENCES


6. ACKNOWLEDGEMENTS

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