Effect of Processing Conditions on the Microbiological Quality of Cashew Nuts

This work evaluated the occurrence of pathogenic and indicator microorganisms in cashew nuts produced in small factories (cottage industries) in the state of Ceará, Brazil, as well as the occurrence of coagulase positive Staphylococcus and its correlation with the anacardic acid content. Ninety samples of nuts were collected from six different small factories (A, B, C, D, E, F) and examined for the MPN/g of coliforms at 35°C and 45°C and for E. coli, the population of coagulase-positive staphylococci, moulds and yeasts, Enterobacteriaceae, total aerobic mesophilic bacteria (TAMB), detection of Salmonella sp. and anacardic acid. It was found that 20% of the samples obtained from factories A, B, C and F showed TAMB values ranging from 4.0 to 7.0 log CFU/g. High mould and yeast counts (> 4.0 to 7.0 log CFU/g) were detected in 7, 20, 20 and 60% of the samples obtained from factories B, C, E and F, respectively. Coagulase-positive staphylococci were found in 27% of the samples from industry E, ranging from 1.5 to 2.8 log CFU/g, negatively correlated with the content of anacardic acid. Contamination by Enterobacteriaceae occurred in samples from three factories (7% of B, 60% of C and 33% of D), but at low levels (1.18 to 3.56 log CFU/g). Coliforms at 35°C were detected in 13% of the samples from industry C, 13% from D and 40% from E, with values of 23 MPN/g. Coliforms at 45°C were only confirmed in samples from E. E. coli was not detected in any of the samples. Salmonella sp. was detected in 18 samples (20%) from factories B, C, D and F. We suggest an improvement of the sanitary practices and the addition of a final heating step preceding packaging in order to reduce the microbial content of the nuts.

RESUMO

Este trabalho objetivou avaliar a ocorrência de microrganismos indicadores higiênico-sanitários e patogênicos em amostras de amêndoas de castanha de caju processadas em minifábricas de beneficiamento de castanha de caju localizadas no Estado do Ceará, bem como a correlação entre os teores de ácido anacárdico e as contagens de Estafilococos coagulase positiva. Para tanto, foram coletadas de 90 amostras de amêndoas de caju oriundas de seis diferentes minifábricas (A, B, C, D, E e F), que foram submetidas às análises de contagem de bactérias aeróbias mesófilas, bolores e leveduras, Enterobacteriaceae, Estafilococos coagulase positiva, determinação do NMP de coliformes a 35°C, a 45°C e de E. coli, pesquisa de Salmonella sp., além da determinação do teor de ácido anacárdico. Um total de 20% das amostras obtidas das minifábricas A, B, C e F possuíam contagens de bactérias aeróbias mesófilas variando de 4.0 a 7.0 log UFC/g. Elevadas contagens de bolores e leveduras (> 4.0 a 7.0 log UFC/g) foram detectadas em 7, 20, 60% das amostras coletadas das minifábricas B, C, E e F, respectivamente. Estafilococos coagulase positiva foi detectado em 27% amostras, variando de 1,5 a 2,8 log UFC/g. Os teores de ácido anacárdico e as contagens de Estafilococos coagulase positiva correlacionaram-se negativamente, sendo que, as baixas contagens desse microrganismo podem ter sido influenciadas pela presença dos ácidos anacárdicos. Contaminação por Enterobacteriaceae ocorreu em amostras de apenas três minifábricas (7% de B, 60% de C e 33% de D), porém em baixos níveis (1,18 a 3,56 log UFC/g). Coliformes a 35°C foram detectados em 13% das amostras da minifábrica C, 13% da D e 40% da E com valores de 23 NMP/g. Coliformes a 45°C foram confirmados em amostras da minifábrica E. E. coli não foi detectada em nenhuma das amostras. A presença de Salmonella sp. foi confirmada em 18 amostras (20%) oriundas das minifábricas B, C, D e F. Sugere-se a implementação das Boas Práticas de Fabricação durante e após o processamento das amêndoas, bem como a padronização de um tratamento térmico ao fim da linha de processamento, antes da etapa de embalagem.

PALAVRAS-CHAVE

Nut; Salmonella sp.; Staphylococcus; Enterobacteriaceae; Mould; Anacardic Acids.

Castanha; Salmonella sp.; Staphylococcus; Enterobacteriaceae; Bolores; Ácido Anacárdico.
1. INTRODUCTION

The cashew tree produces a pseudo fruit, usually referred to as cashew apple, which is attached to an external drupe (true fruit) by a stem. Inside the drupe, there is a single seed called the nut, surrounded by a pellicle that separates it from the inside of the shell. The cashew nut is highly valued as a food and is used widely in confectionery. It has a long history of cultivation in Central and South America, where it is grown by smallholder farmers and also on large commercial plantations. Worldwide, cashew nuts are an esteemed and highly priced food delicacy because of their pleasant taste and flavour (RAMOS, 1988).

In Brazil, the cashew nut agribusiness is an important source of income. Brazil is the second world producer, with 29% of the world trade (SOBECK, 2001). Ceará State contributes with 34% of the Brazilian production (IPLANCE, 2004), and the nuts are an important export product. This industry has helped to improve the development of the state, playing economic and social roles in rural areas (PAULA PESSOA and LEITE, 1998).

Small-scale factories (cottage industries), located in the Northeast of the country, process cashew drupes to obtain the crude nuts, which are exported and optionally roasted. These small factories decrease the percentage of blemished and broken nuts, reaching a final yield of 85% and adding value to the processed nut (PAULA PESSOA et al., 1994). This enhanced yield is partially due to the manual stages of the process, which is totally mechanized in the larger industries. The overview of cashew nut processing is shown in Figure 1.

![Figure 1. Overview of cashew nut processing in cottage industries.](image)

2. MATERIAL AND METHODS

2.1 Sampling

From January to October 2003, 90 samples of cashew nuts were purchased from six different cottage industries (A, B, C, D, E and F) in Ceará State, Brazil. Each month, three portions of 500 g were randomly sampled from the industries at the end of processing. The final heating stage varied between the factories, as did the sampling days. When employed, the temperature and time settings were not controlled. Women responsible for the grading did not wear any protective clothing and recycled buckets were used to hold the processed nuts. The samples were put into sterile screw-capped glass bottles and immediately taken to the laboratory.

The cottage industries help to decrease poverty in some rural areas of the North-eastern region (LOPES NETO, 1997). However, manual peeling and grading also open the possibility to microbial contamination through improper sanitization, compromising the hygienic-sanitary quality of the final product, especially when Good Manufacturing Practices (GMP) regulations are not employed (SILVA NETO, 2000). Furthermore, the presence of deteriorative microorganisms could cause economic losses to both processors and consumers (COTTAM, 2001).

Although the cashew nut is widely marketed and consumed, information on its microbiological quality is very scarce. At the end of processing, values for Aw (water activity) can reach low levels making microbial growth difficult. However, contamination by Salmonella sp. can represent a risk since raw cashew nuts are frequently used in the production of other food products, such as ice cream and confectionery (ICMSF, 1998).

Anacardic acids are phenolic compounds present in cashew nut oil that could have some inhibitory action against Gram-positive bacteria, yeasts and fungi (TORQUATO et al., 2004). KUBO, I., MUROI, H. and HIMEJIMA, M. (1993) reported a list of different anacardic acids with distinct lateral chains that were effective against the growth of Staphylococcus aureus, Propionibacterium acnes, Streptococcus mutans and Brevibacterium ammoniagenes. This antimicrobial activity has not previously been studied for cashew nuts and might help to reduce the contamination problem.

This work evaluates the presence of Salmonella sp. and hygienic-sanitary indicator microorganisms, such as coliforms, moulds and yeasts, Enterobacteriaceae and total aerobic bacteria, as well as the occurrence of coagulase positive Staphylococcus and its correlation with the anacardic acid content, in cashew nuts processed in cottage industries which do not employ GMP or HACCP ( Hazard Analysis and Critical Control Point), in the state of Ceará, Brazil.
2.2 Microbiological and chemical analyses

Representative 25 g portions were aseptically weighed and homogenised with 225 mL sterile buffered peptone-physiological saline solution (0.1% w/v neutral peptone, 0.85% w/v sodium chloride) using a Stomacher 400 laboratory blender (Seward Ltd., UK). Three tenfold serial dilutions were prepared with the same diluent. The enumerations of the total aerobic mesophilic bacteria (TAMB), moulds and yeasts, coliform bacteria, E. coli, coagulase-positive staphylococci and Enterobacteriaceae were carried out using the diluted sample. Detection of Salmonella sp. in 25g of sample was also carried out. All the determinations followed the standard methods of the American Public Health Association (A.P.H.A., 2001). The aerobic plate count was determined using plate count agar (PCA). The plates were inoculated by pour plating and incubated at 35°C for 48 h. Enumeration of moulds and yeasts was undertaken using spread-plates on acidified potato dextrose agar (PDA) and incubated at 25°C for 72 h. Coliform bacteria were estimated employing a three-tube Most Probable Number (MPN) technique. Positive tubes from MPN were streaked onto eosine methylene blue (EMB) agar and than incubated overnight at 35°C. Typical isolates were confirmed based on their IMVC pattern for confirmation of E. coli strains.

Counts of coagulase-positive staphylococci and Enterobacteriaceae were determined using a ready-to-use system, Petrifilm™ S. aureus Count Plates and Enterobacteriaceae Count Plates (3M, Minneapolis, MN, USA), respectively. Representative colonies of S. aureus were collected and subjected to the coagulase test.

Plate counts were converted to log CFU to facilitate comparisons.

For Salmonella sp. detection, tetrathionate broth (TTB) and selenite-cystine broth were used for selective enrichment, after pre-enrichment in lactose broth. The selective media used were brilliant green (BG) agar, Salmonella-Shigella (SS) agar and Hektoen enteric (HE) agar. Cultures selected after preliminary and secondary biochemical screening tests were confirmed by the agglutination test using polyvalent O serum.

Simultaneously, samples were analysed for their anaerobic acid content, according to AGOSTINI-COSTA and JALES (2001).

2.3 Statistical analysis

Microbiological and chemical analyses were done using three complete replications. All the microbiological data were submitted to regression analysis using the Micorcal™ Origin™ (Micorcal Software, INC, Northampton, USA) to determine the correlation coefficients between the content of anaerobic acid and the counts of coagulase-positive staphylococci.

3. RESULTS AND DISCUSSION

The microbiological quality of the nuts is presented in Tables 1 and 2. It was found that 20% of each batch of samples obtained from factories A, B, C and F had TAMB values ranging from 4.0 to 7.0 log CFU/g, which is considered to show poor quality. High mould and yeast counts were detected in samples from B (7%), C (20%), E (20%) and F (60%), respectively. Coagulase-positive staphylococci were only found in samples from F (27%), ranging from 1.50 to 2.78 log CFU/g. Contamination by Enterobacteriaceae occurred in samples from three factories (7% of B, 60% of C and 33% of D), but at low levels (1.18 to 3.56 log CFU/g). Coliforms at 35°C occurred in 13% of the samples from C, 13% from D and 40% from E, with values ranging from 4 to 23 MPN/g. Coliforms at 45°C were only confirmed in samples from E, with values of 4 MPN/g. E. coli was not detected in any of the samples. Percentages of 20, 60, 20 and 20 were positive for Salmonella sp. in samples from factories B, C, D and E respectively.

The highest TAMB level (6.04 log CFU/g) was found in samples from A. King and Jones (2001) reported that this contamination does not necessarily imply deterioration problems for the nuts, since such microorganisms cannot grow at such low activity water (Aw) in this kind of food. However, according to PIXTON (1982), most stored agricultural products, including cashew nuts, are hygroscopic and will absorb moisture from the surrounding atmosphere until they reach equilibrium, and thus the storage environment is an important concern in preserving this food. KRISHNASWAMY et al. (1973) observed the occurrence of 100 species of insects attacking cashew trees and fruits and this infestation could be responsible for the bacterial contamination of the nuts. CANDLUSH et al. (2001) also found TAMB in cashew nuts.

The highest level of contamination (5.78 log CFU/g) by moulds and yeasts was found in samples from E. According to FREIRE and BARGUÍL (2001) contamination of cashew nuts by moulds may occur early in the field, and deterioration could develop during prolonged storage. FREIRE, KOZAKIEWICZ and PATERSON (1999) detected 37 different species of mould in samples of cashew nut and FREIRE and OFFORD (2002) encountered yeasts such as Rhodotorula and Pichia. The ICMSF (1998) sets fungal tolerance standards for flour, cereal and packaged nut products in the range from 2 to 4 log CFU/g. Many studies have shown that fungal contamination is very common in different kinds of nut, such as pistachio, areca, cashew and hazel nuts (MOJTAHEDI et al., 1979; RAISUDIN and MISRA, 1991; PITT et al, 1993; OZILGEN and OZDEMIR, 2001) and this contamination is undesirable mainly due to the possibility of mycotoxin production. This toxin could pose a threat to humans, since they may be mutagenic, carcinogenic and teratogenic (POHLAND, 1993).

The low values for coagulase-positive staphylococci were considered satisfactory from the safety point of view, since the production of toxin only occurs at higher counts (above 6.00 log CFU/g) (ADAMS and MOSS, 1997). In foods that undergo thermal treatment, staphylococci are good indicators of poor personal worker hygiene (ICMSF, 2000). This bacterium was
also found in 20% of walnut kernel samples analysed by RIYAZ-UL-HASSAN et al. (2003), indicating that even low moisture content foods are susceptible to careless handling causing contamination by this microorganism.

In general Enterobacteriaceae counts are used as an indicator of hygienic quality rather than of faecal contamination and give more information about the general microbiological quality than about possible health risks posed by the product (HUI, PIERSON and GOHRAM, 1994). Samples contaminated with Enterobacteriaceae reflect poor hygienic conditions during processing, where intense manipulation and contact with different surfaces takes place. The members of the Enterobacteriaceae are relatively sensitive to heat and when they are found in thermally processed foods, indicate that the environmental hygiene of the industry is deficient.

The absence of E. coli in the samples analysed indicates that contamination with coliforms was possibly due to equipment and/or utensils. According to PASCUAL (1989), E. coli possesses a lower resistance to the external environment, when compared to other microorganisms from the Enterobacteriaceae family, including Salmonella sp., thus the lack of E. coli does not necessarily imply in the lack of the pathogen Enterobacteriaceae.

Enterobacteriaceae was not sensitive enough to recover injured Salmonella sp. or coliforms from the nuts.

The determination of anacardic acids and its correlation with the counts of coagulase-positive staphylococci was investigated. The anacardic acid content was shown to be negatively correlated with the counts of coagulase-positive staphylococci, as shown in Figure 2. The regression analysis trend lines were carried out for both a linear fit, resulting in $R = -0.91$, and for a polynomial fit, with $R^2 = 0.88$. As the anacardic acid content increased, so the counts of coagulase-positive staphylococci decreased. The highest level of anacardic acid may have reduced the level of staphylococci bacteria. A higher content of staphylococci bacteria was expected since the samples exhibited low microbial quality as a result of careless handling, as evidenced by the presence of other microorganisms. LIMA et al. (2000) confirmed the antimicrobial activity of anacardic acids against Staphylococcus aureus and against yeasts such as Candida albicans and Candida utilis. MUROI and KUBO (1993) tested some specific anacardic acids against cariogenic bacteria and concluded that acids containing 12 carbon atoms were more effective. Anacardic acid has been applied against methicillin-resistant S. aureus (MRSA), to study the potential for developing this phytochemical for use in the treatment of MRSA infections (MUROI and KUBO, 1996).

**TABLE 1.** Percentage of the cashew nut samples contaminated with total aerobic mesophilic bacteria (TAMB), moulds and yeasts and coagulase-positive staphylococci.

<table>
<thead>
<tr>
<th>Log CFU/g</th>
<th>TAMB Moulds and yeasts</th>
<th>Coagulase-positive staphylococci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1.0</td>
<td>1.0-4.0</td>
</tr>
<tr>
<td>A</td>
<td>53</td>
<td>27</td>
</tr>
<tr>
<td>B</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>80</td>
</tr>
<tr>
<td>D</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>E</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>F</td>
<td>73</td>
<td>27</td>
</tr>
</tbody>
</table>

**TABLE 2.** Percentage of the cashew nut samples contaminated with coliforms at 35°C, coliforms at 45°C, Salmonella sp. and Enterobacteriaceae.

<table>
<thead>
<tr>
<th>Coliforms at 35°C</th>
<th>Coliforms at 45°C</th>
<th>Salmonella sp./25g</th>
<th>Enterobacteriaceae (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>13 (23)</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>D</td>
<td>13 (9 - 23)</td>
<td>20</td>
<td>67</td>
</tr>
<tr>
<td>E</td>
<td>40 (4 - 15)</td>
<td>13 (4)</td>
<td>100</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
</table>

Numbers in brackets indicate values of MPN/g

Samples contaminated with Salmonella sp. are considered inappropriate for consumption according to Brazilian legislation (BRASIL, 2001) and reflect severely deficient conditions of hygiene during cashew nut processing.

Samples from E and F were positive for coliforms and for Salmonella sp., respectively, the negative for Enterobacteriaceae reflects the fact that the method employed to search for Salmonella sp. or coliforms from the nuts.

In terms of the ICMSF (1998) specifications, our results indicate that samples from factory A were of poor hygienic quality, which must have been caused by inadequate hygienic practices after the heat treatment. Samples from B, C, D, E and F were affected by the hygienic practices, allowing for contamination by pathogenic bacteria. According to KING and JONES (2001), cashew nuts possess low Aw values, ranging from 0.341 to 0.537, making them inappropriate for microbial growth. However, once installed, pathogenic microorganisms...
could constitute a risk if the nuts were added as an ingredient to confectionery products. Personal hygiene and education in sanitation practice is needed in order to improve the microbial safety of cashew nuts. Good post processing sanitary practices are important to prevent microbial contamination and the Food Regulations must be enforced. Hazard Analysis at Critical Control Points could be one of the best ways to assure the lowest risk of contamination by food borne pathogens. We also suggest the addition of a final heating step before packaging in order to reduce the microbial content of the nuts. The time/temperature combinations need to be determined by way of further evaluations, since elevated temperatures may compromise the nut texture. Nuts should also be packed in sealed polyethylene (nylon) bags after production before being shipped, instead of exposing them in open containers. In order to standardize the optimum anacardic acid value which might enhance the microbial quality of the nuts.

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