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Microwave drying and disinfestation of Brazil nut seeds

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ABSTRACT

Fungal contamination of Brazil nuts can cause health problems, which limits their worldwide consumption and leads to economic restrictions for the producing regions. In this study the contamination of Brazil nut seeds by aflatoxin-producing fungal strains was investigated. It was observed that of the 31 strains isolated from the inside of the seed coat, 39% showed aflatoxigenic potential, indicating a high level of contamination by microorganisms which produce aflatoxins, which have strong carcinogenic and hepatotoxic activity. The moisture content and water activity are determining factors for fungal growth. Measurements of the dielectric properties at microwave frequencies of the Brazil nut shell and the kernel reveals that the shell has a higher loss tangent (0.12) at 30 °C relative to the kernel (0.06) and high values for the penetration depth of both shell (20 cm) and kernel (35 cm) that are related to their chemical composition. Microwave heating was employed leading to reductions in the moisture content (46.4%) and water activity (20%). Furthermore, the effectiveness of microwave dielectric heating as a disinfestation process for contaminated nuts was investigated, showing that colonization inside the shell decreased by 61.67% and on the kernel by 81.75%, without damaging the organoleptic properties of the Brazil nut seeds.

1. Introduction

The Amazon Rainforest, spanning nine countries in Latin America, with most of its territory in Brazil (63.4%), is a unique region and the vast diversity of its fauna and flora has still not been fully described. This biome accounts for one third of the world's rainforests and provides a habitat for around 50% of the planet's biodiversity. The Brazil nut (*Bertholletia excelsa H.B.K.*), belonging to the family Lecythidaceae, is one of the most important extractive Amazonian species (Mori & Prance, 1990). The Brazil nut kernel is one of the most nutritious foods available to humans, due to its high content of proteins, carbohydrates, unsaturated lipids, vitamins (notably vitamin E) and minerals (Costa, Ballus, Teixeira-Filho, & Godoy, 2010; Yang, 2009). Due to its pleasant taste and high nutritional value, the Brazil nut has become a very popular food worldwide. It is commonly referred to as "vegetable meat", being a high-energy food which is rich in protein and micronutrients. It is also highly valued due to the presence of phytosterols, tocopherol, squalene, phenolic compounds and antioxidants such as selenium, an essential trace element associated with protection against the harmful effects of free radicals in the body as well as heavy metals (Hafidi, Pioch, Brochier, & Montet, 2008; Naozuka & Oliveira, 2007; Vonderheide et al., 2002). It can also aid in the prevention and reduction of chronic diseases by increasing the resistance of the immune system (Yang, 2009). The Brazil nut is collected exclusively from natural forests by extractive communities. It is then transported to processing plants and most of the produce is destined for exportation.. The fruit of the Brazil nut is considered to be one of the main non-wood forest products, which not only helps to preserve the Amazonian rainforest but also generates income for thousands of people living in this region (Mori & Prance, 1990). The drying process is one of the main Brazil nut processing stages and it represents a considerable challenge because if this step is not efficiently performed the moisture content (*mc*) and water activity (*Aw*) in the medium will favor fungal growth.

Fungal contamination of this food product is a common public

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health problem in many countries and fungal invasion in Brazil nut causes a major economic impact on the marketing of this product, leading to losses in the production chain. Brazil nut contamination is mainly associated with aflatoxins, which belong to a group of fungal toxins known as mycotoxins, which have strong carcinogenic and hepatotoxic activity (Arrus, Blank, Abramson, Clear, & Holley, 2005; de Mello & Scussel, 2009; Freitas-Silva & Venâncio, 2011; Martins, Klusczcovski, & Scussel, 2014; Pacheco & Scussel, 2007). The contamination of Brazil nuts seeds with aflatoxins produced by strains of *Aspergillus* section Flavi has been reported (Arrus et al., 2005; Baquião, 2012; Pacheco & Scussel, 2007). According to the report of the Codex Alimentarius Commission, the limit of aflatoxins in Brazil nuts for consumption is 10 μg kg^{-1}, and 15 μg kg^{-1} for shelled nuts destined for further processing. (FAO and WHO. Codex Alimentarius, 2012).

Many studies about the mycobiota of brazil nuts have been accomplished. The most commonly isolated species are *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nomius*, *Aspergillus niger*, *Aspergillus tamarii*, *Penicillium glabrum*, *Penicillium citrinum*, *and Fusarium oxysporum* (Baquião, 2012; Costa, Freire, Vieira, Andrade, & Mendes, 2009; Olsen, Johnsson, Möller, Paladino, & Lindblad, 2008; Reis et al., 2012). The contamination of the Brazil nut by aflatoxin is a deterrent factor for the export of this product to Europe and the United States of America, where strict aflatoxin limits are imposed by legislation (FAO and WHO. Codex Alimentarius, 2012). A large quantity of Brazil nuts has been returned by importing countries, because the product did not fulfill the requirements of the international market. This has led to the development of a Brazil nut monitoring program by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) aimed at contamination prevention and control by setting maximum levels of aflatoxins in Brazil nut, which are currently established by RDC Resolution No. 7 18/02/11 of ANVISA (Brazilian National Health Surveillance Agency).

During the harvesting of Brazil nut pods, the nuts are processed under the environmental conditions of the Amazon region, with temperatures generally greater than 30 °C and relative humidity greater than 70%. These conditions affect the moisture content (*mc*) and water activity (*Aw*) of the Brazil nut and provide conditions for aflatoxigenic fungi to produce aflatoxins (Arrus et al., 2005; de Mello & Scussel, 2009; Martins et al., 2014; Pacheco & Scussel, 2007). Therefore, an efficient drying process that maintains the organoleptic features of the nuts must be applied in order to avoid fungal infestation. In order to inhibit fungal growth many aspects must be considered during harvesting and post-harvest procedures, and some steps involved in the production chain are traditionally carried out by hand. Several strategies of disinfection have been suggested, including physical methods (conventional drying to the safe moisture levels, mechanical sorting, thermal inactivation, UV and gamma ray irradiation and modified atmospheres in food storage), chemical treatments (hydrogen peroxide, ammonia, ethylene, organic acids and anti-fungal compounds) and biological control methods (use of atoxigenic strains) (Basaran, Akgul, & Oksuz, 2008.; Bhatnagar-Mathur, Sunkara, Bhatnagar-Panwar, Waliyar, & Sharma, 2015; De Mello & Scussel, 2009; Gunterus, Roze, Beaudry, & Linz, 2007; Waliyar et al., 2015; Yan et al., 2015). Fungicides and preservatives have long been used to reduce losses caused by fungal contamination. Nevertheless, the use of such products can cause health and environmental problems due to, for instance, their residual toxicity and carcinogenicity (Freitas-Silva & Venâncio, 2011).

The conventional drying process may require long application times, for example, 36 h in a rotary dryer (Gonçalves et al., 2010). In addition, if this step is not properly performed there are no assurances concerning subsequent infestation during storage and transportation due to poor dehydration and even due to rehydration given the high air moisture content. Conventional drying can be performed by natural air convection (in the shade or in sunlight) and by hot air. However, artificial drying can improve the performance in terms of reducing the moisture content, reaching around 9.7% after 48 h (Gonçalves et al., 2010). Clearly, a crucial aspect of the drying process is the drying rate, which for any technology is associated with the rate of the relative increase in temperature. In this regard, the energy transfer to the material and mass transfer of the water out of the material under drying to the surrounding environment are important factors. In drying processes, microwave heating has been widely applied in the food industry since it provides the fastest means available for the transfer of energy into biological materials (Ahmed & Ramaswamy, 2007, Chap. 29). Microwave-based food processes, which include the tempering of frozen foods, pre-cooking of bacon and pasteurization, offer many advantages, such as high energy efficiency, a significant reduction in the process time, space saving and selective heating, while providing foods with high nutritional quality (Ahmed & Ramaswamy, 2007, Chap. 29; Gunasekaran, 1999; Tang, Feng, & Lau, 2002). Moreover, dielectric heating also has a wide variety of applications in organic and medicinal chemistry (Kappe & Standler, 2005; Leadbeater, 2010). Microwave drying is based on a unique volumetric heating mode involving the application of electromagnetic radiation at 915 and 2450 MHz and it has been increasingly used in a large variety of materials making it a very promising drying technology (Gunasekaran, 1999; Tang et al., 2002). In addition, sterilizing effects on materials heated by microwave irradiation and the control of aflatoxins by microwave detoxification have been reported (Luter, Wyzlouzil, & Kashyap, 1982; Basaran & Akhan, 2010; Fang, Hu, Xiong, & Zhao, 2011; Feng, Yin, & Tang, 2012; Pluyer, Ahmed, & Wei, 1987).

Microwave heating is a macroscopic effect achieved through the interaction of electromagnetic fields with matter and accordingly, in this regard, the characterization of any material medium is carried out based on its intrinsic dielectric properties. These are determined through the empirical measurement of simple or complex materials, such as reaction solutions or food products (Gunasekaran, 1999; Kappe & Standler, 2005; Leadbeater, 2010; Tang et al., 2002). The dielectric properties of any substance are defined by their complex relative permittivity $\hat{\varepsilon} = \varepsilon' - j\varepsilon''_{ef}$, with ε' being the relative dielectric constant, which describes the capacity of a material to store electromagnetic energy when subjected to an external electrical field. The imaginary part, $\varepsilon''_{ef} = (\varepsilon'' + \sigma/\omega\varepsilon_0)$, is the dielectric loss factor of the substance. The conductivity is σ and ε'' is the imaginary part of the relative permittivity that accounts for the dielectric relaxation process (Gabriel, Gabriel, Grant, Halstead, & Mingos, 1998; Leadbeater, 2010; Metaxas & Meredith, 1993), and these parameters indicate the ability of the material to dissipate electromagnetic energy, due to an applied electrical field, generating heat. Two important parameters to describe the dielectric response of materials are the loss tangent and penetration depth (Gabriel et al., 1998; Metaxas & Meredith, 1993). The loss tangent is defined by $\tan\delta = \varepsilon''_{ef}/\varepsilon'$ and this parameter is associated with the rate of the temperature increase within the material. The penetration depth (D_p), the depth at which the amplitude of the electrical field is damped to $1/e = 0.369$ of its initial value at the surface of the material, is related to the loss tangent by $D_p = \frac{c}{\omega}\sqrt{\frac{2}{\varepsilon'}}[\sqrt{1+\tan^2\delta} - 1]^{-1/2}$ and therefore it is frequency and temperature-dependent. This denotes the region of the sample where the electrical field effectively penetrates, thus specifying the actual volume of the sample where the heating effectively occurs by dielectric heating rather than convection (Gabriel et al., 1998; Metaxas & Meredith, 1993). Hence, the penetration depth is an important parameter in determining the non-uniform volumetric