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Stability of food grade antioxidants formulation to use as preservatives on stored peanut

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ABSTRACT

The aim of this study was to microencapsulate butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) by complex coacervation using gelatin-gum arabic system as encapsulating agent under different reticulation conditions (with or without crosslinking agent). Morphology, encapsulation efficiency (%EE) and permanence period of compounds in formulation at 25 °C and in peanut grains stored at different temperatures (20, 25 and 30 °C) and water activities (0.65, 0.75, 0.85 and 0.95) were evaluated. Significant statistically differences were observed between crosslinking agents, where microcapsules reticulated with glutaraldehyde were 19% and 21% higher than microcapsules with formaldehyde and without crosslinking agent. Microencapsulation technique used gave about 80 %EE for both antioxidants, regardless of reticulation condition. Permanence of both antioxidants was between 14.4 and 4.6 μg/g in the formulation stored at 25 °C during 30 days and between 730 and 350 ng/g for BHA and BHT, respectively, in peanut system, after 40 days. This methodology is a promising technique for the addition of food grade antioxidants to peanut providing preservative effects for a storage period greater than 40 days, being and alternative for control of mould, mycotoxins and insects in peanut grains.

1. Introduction

Peanut (*Arachis hypogaea* L.) is an important food and feed commodity in Argentina. This product is important for the Argentinean economy, with a total production of 1.03 million tons in 2012/13 harvest season with an increase of 70% of the total production in the last 10 years (SIIA, 2014). Our country ranks seventh among the world's largest producers of peanuts shell and experts for marketing year 2013/14 are forecast up million tons due to the larger reached production (USDA, 2014). However, peanuts are considered to be a high-risk product for contamination with aflatoxins (AFs) since they are frequently contaminated with soil fungi, particularly *Aspergillus flavus* and *Aspergillus parasiticus*, and because of long peanut drying times and occurrence of rainy periods after uprooting (Fonseca, 2012). Mycotoxins can be produced in peanut grains in the field, during transport and storage where conditions are suitable for their production. Besides, postharvest losses of agricultural food commodities due to the deterioration by different storage insect pest, is a serious problem in peanut (Nesci, Montemarani, Pasone, & Etcheverry, 2011). *Oryzaephilus surinamensis* (L.) is the most widely distributed stored grain pest in the world (Muggleton, Llewellin, & Prickett, 1991) and they act as vector of aflatoxigenic fungi in stored peanut (Nesci et al., 2011). Therefore, constant interaction between substrate, biological and abiotic factors may promote a moldy substrate and toxin accumulation in stored grains (Magan & Lacey, 1984). Hence, control measures which effectively reduce the food losses due to insects, fungi and aflatoxins contamination, as well as, having antioxidant activity, would be adapted by peanut agroindustry.

Microencapsulation is a technology used in order to protect synthetic and natural components from the action of physicochemical and technological agents for the protection, stabilization, and slow release of food ingredients (Shahidi & Han, 1993). Different techniques such as complex coacervation, ionotropic external/internal gelation, molecular inclusion, extrusion, freeze drying, spray

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drying, and spray chilling/cooling have been used for the encapsulation of different antioxidants and essential oils (EOs) (Comunian et al., 2013; Inoue, Kawai, Kanbe, Saeki, & Shimoda, 2002; Rocha-Selmi, Favaro-Trindade, & Grosso, 2013; Xiao-Ying, Zhi-Ping, & Jian-Guo, 2011). Complex coacervation technique has a series of advantages considering that it is used as simple technology and generally it has an encapsulation efficiency of over 90% (Gouin, 2004). Some authors have reported microcapsules obtained through this method, which were stable at high temperature and they enable the controlled release of components (Dong et al., 2011; Jun-xia, Hai-yan, & Jian, 2011).

Some aims in the use of encapsulation in food industry are (Champagne & Fustier, 2007; Onwulata, 2012):

- To protect the active compounds from degradation by the environment (heat, air, wet, etc.)
- To control the release of active compounds from the encapsulating matrix under specific conditions (pH, temperature, etc.).
- To mask unpleasant flavors.
- To separate components to prevent them their react.

Synthetic phenolic compounds such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been used extensively for many years as antioxidants to preserve and maintain the freshness, nutritive value, flavor and color of food and animal feed products (JECFA, 1996). In general are used as food preservatives in meats, butter, cereal, bakery products, beer, dehydrated potatoes, chewing gum, and cereals, between others foods and feeds. From a human health perspective, the Codex Alimentarius (2006) and the US Food and Drug Administration (FDA) allow the use of phenolic antioxidants in foods and they are regarded as safe (GRAS) chemicals. The maximum usage level of single or multiple antioxidants approved by the more restricted legislation mentioned above, is 200 μg/g of peanut oil. Furthermore, as was reported in previous works, these antioxidants have insecticide, antifungal and antiaflatoxigenic effects on peanut storage agroecosystem (Nesci et al., 2011; Passone, Doprado, & Etcheverry, 2009; Passone, Resnik, & Etcheverry, 2007a, 2007b; Passone, Resnik, & Etcheverry, 2008), where environmental variations play an important role. However, these works showed that antioxidant levels decreased with time in peanut food system, probably due to the interaction with physical and biological factors (Passone, Funes, Resnik, & Etcheverry, 2008). For this, in order to protect the active components of BHA and BHT from external factors, the aims of this work were: i) to encapsulate these compounds by complex coacervation method ii) to analyze the encapsulation efficiency and iii) to study antioxidants permanence inside of microcapsules and the formulation stability on peanut grain.

2. Materials and methods

2.1. Materials

Industrial grade antioxidants 2(3)-tert-butyl-4 hydroxyanisole (BHA) and 2.6-di(tert-butyl)-*p*-cresol (BHT), obtained from Eastman Chemical Company, were used as core material. BHA had a purity of 98.5% containing trace elements: sulphated ash 100 μg/g, citric acid 2.5 μg/g, arsenic 3 μg/g, and heavy metals 10 μg/g. BHT had a purity of 99% containing as contaminants ash 200 μg/g, arsenic 3 μg/g and heavy metals 10 μg/g. Contaminant compounds industrial grade antioxidants did not exceed the levels allowed by JECFA (1996). Gelatin (type A, gel strength 220 and 240 bloom) and gum arabic were used as wall material. All the other chemicals used in this work were of analytical grade.

2.2. Preparation of coacervate microcapsules

Microcapsules were made by complex coacervation following Luzzi and Gerraughty (1967) and Vahabzadeh, Zivdar, and Najafi (2004) with some modifications. Twenty five mL of gelatin (bloom 220 and 240) and gum arabic solution 5% p/p were prepared at 50 °C in a thermostatic bath (Decalab SRL). pH of gum arabic solution was adjust to 6 with sodium hydroxide 1 mol/L (NaOH). 450 μL of core material (BHA or BHT 0.7 g/mL and 0.5 g/mL in peanut oil, respectively) were added into the gum arabic solution, forming an emulsion by magnetic stirring (Auto Science, AM-5250B). Then, gelatin solution was added and the mix was stirred at 400 rpm during 10 min at 50 °C. Next, pH was adjusted to 4 with hydrochloric acid (HCl) 1 mol/L solution and the stirring was continued during 10 min. Then two kinds of microcapsules were made:

1 microcapsules without crosslinking agent (WCA), solution was cooled at 10 °C in an ice bath and was stored at −20 °C until lyophilization.
2 microcapsules with crosslinking agent (CA), pH was adjust to 9 with NaOH 1 mol/L and stirring during 10 min. After that temperature was lowered to 10 °C in an ice bath and 5 mL of formaldehyde (F) or glutaraldehyde (G) was then added to compact the gelatin-gum arabic coating. Crosslinking times were 10, 60 and 120 min at room temperature. Finally, microcapsules were washed twice with distilled water and were stored at −20 °C until the lyophilization step.

Finally, microcapsules were frozen at −80 °C during 3 h and then were lyophilized (L-T8-A-B3-CT, RIFICOR). Then the samples were ground (CT 193 Cyclotec™ Sample Mill) to obtain a fine powder.

2.3. Morphology of coacervate microcapsules

Size and morphology of microcapsules were evaluated following the methodologies proposed by Chang, Leung, Lin, and Hsu (2006). Size of microparticles before and after lyophilization was examined by optical microscopy at 40 × magnification (Carl Zeiss, 37081). Diameters of fifteen capsules were registered for each treatment. The images were captured and size of microcapsules was estimated by using Motic Images Plus 2.0 (2005) software.

2.4. Efficiency of encapsulation technique

Efficiency was adapted from Kaushik and Roos (2007) as follow: 1 g of microcapsules was added to 20 mL of chloroform in glass flask and shaked with an orbital shaker for 5 min. Powder particles were separated from chloroform extract by filtering through filter paper (Whatman No. 1). BHA and BHT present on the surface of the microcapsules (without encapsulated) were estimated from chloroformic extracts by spectrophotometric and chromatographic techniques.

2.4.1. Detection and quantification by spectrophotometry

Absorbance of antioxidant chloroformic extract was measured with a spectrophotometer (Spectrum SP 2100UV) at 280 nm using pure chloroform as blank. This wavelength was found to correspond to the maximum absorbance of compounds over the spectrum of wavelengths from 200 to 600 nm. A standard curve was building by measuring absorbance of BHA and BHT dissolved in chloroform at different concentrations (w/v) (0–80 and 0–150 μg/mL for BHA and BHT, respectively).