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Effect of micro-encapsulated antioxidant formulations on mycobiota, residual levels, sensory analyses and insect pest attack in stored peanuts

Daiana Garcia*, Natalia Soledad Girardi, María Alejandra Passone, Andrea Nesci, Miriam Etcheverry

Laboratorio de Ecología Microbiana, Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas Físico Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta 36 Km 601 (X5806JRA), Río Cuarto, Córdoba, Argentina
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

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ABSTRACT

The *in situ* effect of microencapsulated 2(3)-tert-butyl-4 hydroxyanisole (BHA) on stored peanuts (*Arachis hipogaea*) intended for human consumption was evaluated. Peanut were stored unshelled in flexible containers called "big bags" that were made of polypropylene raffia. 100 kg of peanuts were used in each big bag and stored in refrigerated cells (< 18 °C) for about 5 months in two different peanut processing companies during 2015/2016 period. Fungal populations, aflatoxin accumulation, BHA residues, acidity and fatty acid profile, sensory analyses, insect damage and environmental factors variation, were evaluated. At the end of the storage period, significant ($p < 0.05$) fungitoxic effects of the BHA formulation were observed in the order of 30 and 15% for the first and second company, respectively. *Cladosporium,* yeasts*, Penicillium, Fusarium, Alternaria* and *Aspergillus* were the main fungal isolates. No aflatoxins were found for both companies and years evaluated. In addition, taste of the peanuts was not significantly affected ($p < 0.05$) by formulation used and insect damage was always lower than 3%. However, different levels of BHA were detected throughout the experiment in the two companies, with final levels of 2.5 for the C1 and 275 ng BHA/g peanuts in C2. Formulation did not affect acidity and organoleptic properties of peanuts. These results show that BHA formulation could be used as part of alternative strategy for control of fungal contamination storage period.

1. Introduction

Peanut (*Arachis hypogaea* L.) cultivated in Argentina is known worldwide for its quality. In this country around 80% of peanut production is exported, accounting for 25% of annual global of these oil seed exports (USDA, 2015). Peanuts comprise 6 different products, with blanched ones being the most important for export (49%) followed by shelled peanuts (38%) and then peanut oil (10%) with Argentina being the main exporter of peanut confectionery (Blengino, 2015). Quality of peanuts and by-products, from the field to the table, should ensure fresh and long-lasting flavor, ideal texture, and outstanding nutrition without any risk for health. However, peanut storage in Argentina extends from 3 to 6 months, period during which its quality is susceptible to be decreased by chemical, physical and biological factors (Passone et al., 2014). One important chemical risk of this food is the presence of aflatoxins (AFs) due to contamination with toxigenic fungi, particularly *Aspergillus flavus* and *Aspergillus parasiticus*. These are frequently isolated form peanuts during storage (Bhattacharya and Raha, 2002; Gonçalez et al., 2008; Nakai et al., 2008). Mycotoxins are associated the presence of fungal inoculum on susceptible substrates. Usually, they can be produced in ripening peanuts in soil, especially under drought stress in the field and also during transport and storage where conditions are suitable for their production. Despite the absence of direct correlation between the extent of mould growth and mycotoxin production, prevention of fungal growth effectively minimizes the risk of mycotoxin accumulation (Garcia et al., 2009).

On the other hand, insect contamination in food commodities is an important quality control problem of concern for food industries (Nesci et al., 2011). Stored products of agricultural origin are attacked by > 600 species of beetles, 70 species of moths, and about 355 species of mites causing quantitative and qualitative losses (Rajendran, 2002). Major insect pests of stored peanuts include the groundnut bruchid *Caryedon serratus* (Olivier), *Oryzaephilus surinamensis* (L.), *Plodia interpunctella* (Hübner), and *Tribolium castaneum* (Herbst) (Rajendran and

* Corresponding author at: Laboratorio de Ecología Microbiana, Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas Físico Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta 36 Km 601 (X5806JRA), Río Cuarto, Córdoba, Argentina.
E-mail address: dgarcia@exa.unrc.edu.ar (D. Garcia).

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Sriranjini, 2008). In addition, interactions between biotic and abiotic factors may promote the formation of a moldy substrate and toxin accumulation in stored peanuts.

To prevent mycotoxin contamination, control of growth of mycotoxigenic moulds and pests is necessary. Both biological contaminant factors are commonly controlled using synthetic fungicides and insecticides (López et al., 2004; López-Malo et al., 2000). However, continuous and indiscriminate use of chemical preservatives in foods and feeds, can lead to toxic effects for consumers and to the development of resistances in microorganisms (López et al., 2004). On the other hand, butylated hydroxyanisole (BHA) is a food grade antioxidant that has been used extensively for many years as antioxidants to preserve and maintain freshness, nutritive value, flavor and color of food and animal feed products (JECFA, 1996). Besides, this antioxidant showed antifungal and insecticidal effects on stored peanuts (Nesci et al., 2011, Passone et al., 2007; Passone et al., 2008a, 2008b; Passone et al., 2009). However, analysis of antioxidant residual levels in stored peanuts shows a rapid reduction, probably due to the environmental and biological factor interactions (Passone et al., 2008c). Girardi et al. (2015) applied BHA microencapsulation technology to protect food grade antioxidants from the action of different environmental factors such as temperature and water activity (a_w). Besides, Garcia et al. (2016) showed that 20 mM of this BHA formulation completely inhibited *Aspergillus* section *Flavi* development, and therefore, AFs accumulation, with 95% mortality of the studied pest (*Oryzaephilus surinamensis*). While a lower dose (10 mM) decreased growth of both organisms and toxin levels with 50% mortality for the insect. However, results of both studies were obtained at a laboratory scale where environmental factors are more controlled. The aim of this work was evaluate the *in situ* effect of microencapsulated BHA on stored peanuts intended for direct consumption, stored in big bags for five months, assessing: a) total fungal population; b) aflatoxin B_1 accumulation; c) antioxidant residue levels; d) acidity and fatty acid profile; e) sensory analysis; f) insect damage; g) environmental variations, in two different peanut processing companies of south of Córdoba, Argentina, during 2015/2016.

2. Materials and methods

2.1. Estimation of mould populations in peanut samples

Subsamples of 10 g of each sample were finely milled and diluted with 90 mL peptone-water (0.1%) and shaken during 20 min at room temperature (25 ± 2 °C). After that, serial decimal dilutions until 10^{-3} were made. An aliquot of 0.1 mL of each dilution per sample was spread in duplicate on the surface of two solid media: dichloran rose Bengal (DRBC) and dichloran glycerol 18% (DG18) (Pitt and Hocking, 1997; Samson et al., 2010). Plates were incubated in darkness for 5–7 days at 25 ± 2 °C. The colonies were counted and populations expressed as colony-forming units per gram (CFU/g) of peanut. Macro and microscopic identification of fungal genera were made according to (Samson and Frisvad, 2004; Samson et al., 2010). Samples were analyzed in triplicates.

2.2. Aflatoxin B_1 analyses in peanut samples

2.2.1. Aflatoxin B_1 extraction

1 kg of each sample was mix in a blender with 2 L of water during 3 min. 75 g of the mixture was homogenized also in blender with 75 mL of methanol (99.8%) for 2 min. They were filtered twice with filter paper (Whatman N° 1) and transferred to 2 mL vials. Aflatoxin concentration of the samples was determined by high performance liquid chromatography (HPLC).

2.2.2. Aflatoxin B_1 detection and quantification

Aflatoxin B_1 was detected and quantified by using a HPLC system (Agilent, Hewlett Packard, series 1100, separation module) and a C_{18} column (5 µm LiChrospher 100 RP-18 Merck 125 mm length and 4 mm diameter Darmstadt, Germany) and a pre-column (5 µm Hypersil ODS Agilent, length and 4 mm diameter). Water: methanol: acetonitrile (66.6: 16.7: 16.7) mixture was used as the mobile phase at a flow rate of 1 mL/min and an injection volume of 30 µL. Fluorescence Detector module (G1321A, series N° E40505866) was used for fluorescence detection (λexc 365 nm; λem 435 nm). A post column photochemical derivatization system (Kobra Cell, corriente 100 mA.) was used. Mycotoxin was quantified on the basis of the HPLC fluorimetric response compared with that of a range of mycotoxin standards. Detection (LOD) and quantification (LOQ) limits of the analytical method were 1.5 ng/g and 4.5 ng/g, respectively.

2.3. Extraction and quantification of BHA residues in peanut samples

Extraction, detection and quantification of BHA in peanut samples were made according to Passone et al. (2008c): subsamples of 5 g of peanut kernel were shaken with 10 mL of acetonitrile on an orbital shaker for 10 min. After that, the grains were separated from the extract by filtration by repeating this procedure three times. At the last extraction, samples were sonicated (Elma D-78224 SINGEN) during 15 min. An aliquot of 1 mL was taken from each extraction and combined to obtain a final volume of 3 mL. Each sample was analyzed in triplicate.

Extracts were analyzed using HPLC system (Waters 2696 separations module, Waters, Milford, USA). Chromatographic separations were performed on a C18 reverse phase stainless steel column (5 µm LiChrospher 100 RP-18 Merck125 mm length and 4 mm diameter, Darmstadt, Germany), connected to a pre- column (20 × 4.6 mm id, 5 µm particle size, Phenomenex). Mobile phase used was methanol:acetonitrile:water (35:35:30), at a flow rate of 1.5 mL/min. BHA was detected by UV (Waters 2998) at 280 nm and quantified using a software integrator (Empower, Milford, MA, USA). Antioxidant levels were calculated by comparing the area of the chromatographic peak of samples with those of standard calibration curve which were 0.1, 1, 10, 100, 250, 500, 750 and 1000 ng/mL. Limits of detection and quantification were 0.4 ng/g and 1.2 ng/g.

2.4. Determination of acidity and fatty acid profile in peanut samples

Methodology based on AOAC 940 and ISO 660 for determination of free fatty acid content was used. For this purpose, extraction was carried out by pressing the oil, using a hydraulic press. Between 4 and 20 g of oil were placed in an Erlenmeyer, to which were added 30 mL of 99.5% ethyl alcohol, 30 mL of sulfuric ether and 4 drops of phenohphthalein (1%). After that mix was stirred and titrated with a solution of 0.1 N of sodium hydroxide (NaOH), until the solution color changed to wards pink. Percentage of acidity was calculated as:

$$Acidity\ (\%) = \frac{\text{Volume of NaOH consumed} \times \text{Normality of NaOH} \times 28.24}{\text{Oil weight (g)}} \quad (1)$$

Fatty acid profile was determined by gas chromatography. For this, 10–12 drops of the oil previously obtained by pressing were placed in a test tube and 2 mL of a 1 M KOH-methanol solution was added. The mixture was shaken in a vortex for 30 s and placed in a thermostatic bath until solution became clear (about 5 min at 70 °C). Subsequently, 6 mL of n-hexane was added, shaken manually for 20–30 s and centrifuged during 5 min at1800 rpm. Supernatant was recovered and fatty acids quantified by gas chromatography. Results were expressed as percentage of oleic, linoleic acid, and ratio of Oleic acid/Linoleic acid (O/L).

2.5. Sensory analysis in peanut samples

A peanut subsample of 500 g was roasted at 100 °C for 1 h and

allowed to cool removing then peanut skin with a blanched machine (RAUMAK Smart Machine). Peanuts were then fried in vegetable oil at 60 °C for 2 min and when cooled, the sensorial analysis was performed tasting 100 grain of each sample. This analysis was always done by the same person for the two companies. Results were expressed as percentage of grains with bad taste.

2.6. Insect damage in peanut samples

A peanut subsample of 500 g was heated in an oven at 100 °C during 1 h. After that, peanut was cooled at room temperature and the skin was removed from the grains with a blanched machine (RAUMAK Smart Machine). Grains presenting insect damage (any sign of activity -perforations, tunnels, cobweb- of the insect in the grains and/or presence of eggs, cocoons, larvae) were selected and weighed separately expressing the results of grain with insect damage in % w/w.

2.7. Measurement of substrate a_w and temperature

Temperature data of peanut grains were registered at each sampling time using distance-reading thermometers. At each sampling period, water contents of peanut seeds were determined by triplicate measurement of a_w with AquaLab Water Activity Meter 4TE (Decagon Devices, Inc.) with an accuracy of the equipment ± 0.003.

2.8. Preparation of antioxidant formulations

The industrial grade antioxidant 2(3)-tert-butyl-4 hydroxyanisole (BHA) obtained from Eastman Chemical Company (Kingsport, Tennessee, United State) was used as core material. BHA had a purity of 98.5% containing as trace elements sulphated ash 100 μg/g, citric acid 2,5 μg/g, arsenic 3 μg/g, and heavy metals 10 μg/g. Contaminant compounds of industrial grade antioxidants did not exceed levels allowed by JECFA (1996). Gelatin (type A, gel strength 240 bloom) and gum arabic were used as wall material. All other chemicals used were of analytical grade. Microcapsules were made by complex coacervation following the methodology proposed by Girardi et al. (2015): 25 mL of gelatin and gum arabic solution 5% p/v were prepared at 50 °C in a thermostatic bath (Decalab SRL). The pH of gum arabic solution was adjust to 6 with sodium hydroxide 1 M (NaOH). 450 μL of core material (BHA 70% p/v in peanut oil) were added into the gum arabic solution, forming an emulsion by magnetic stirring (Auto Science, AM-5250B). Then, a gelatin solution was added and the mix was stirred at 400 rpm during 10 min at 50 °C. After that, pH was adjusted to 4 with hydrochloric acid 1 M (HCl) solution and the stirring was continued for 10 min. Subsequently, pH was adjusted to 9 with NaOH 1 M and stirring another 10 min. Then, temperature was lowered until 10 °C in an ice bath and 5 mL of formaldehyde was added during 10 min, to firm the gelatin-gum arabic coating. Microcapsules obtained were washed twice with distilled water and frozen at −80 °C during 3 h and freeze-dried with a chamber (L-T8-A-B3-CT, RIFICOR) pressure < 0.05 mbar and −45 °C for 72 h. Finally, lyophilized samples were ground using a mill CT 193 Cyclotec™ to obtain a fine powder.

2.9. Pilot-scale experiments

Trials were carried out in two different storage companies in the south of Córdoba, Argentina, during 2015 (company 1, C1) and the assay was repeated during 2016 (company 2, C2) during 5 (C1) and 4 (C2) month respectively. Four flexible containers called “big bags”, made of polypropylene raffia of a high resistance and tenacity, were used in this study. Each big bag size was 90 × 90 × 90 cm with a capacity for 150 kg of peanuts. 400 kg of bulk peanuts, free of aflatoxins and conventionally cultivated and harvested in 2014–2015 and 2015–2016 for direct human consumption, were distributed in the four experimental units (big bags). Two big bags were treated with 10 mM of BHA formulation (F-BHA) using a solid particulate spray equipment (Stihl SR 450) for a homogeneous distribution of the microcapsules. The other two experimental units were used as control. All big bags were stored in cells with controlled temperature inside the establishment (where usually peanut is stored in the companies). Besides, water activity of peanuts from each experimental unit, at each sampling period were checked in triplicate with an AquaLab Water Activity Meter 4TE (Decagon Devices, Inc.) with an accuracy of ± 0.001. Three samples of 2.5 Kg were taken of each big bag monthly to evaluate the biological and physicochemical characteristics of the grains. For this, a coarse grain changer was used (Gama, 150 × 20 cm) and 5 samples were taken from different positions within the big bag.

2.10. Data analysis

For each company, results of the total fungal counts, BHA residues, acidity, fatty acid profile and sensory analysis were evaluated by ANOVA. Data were analyzed through the program InfoStat version 2012. InfoStat Group, FCA, National University of Cordoba, Argentina. URL http://www.infostat.com.ar. To establish significant differences among treated and untreated samples, we tested the least significant Fisher difference (LSD) ($p < 0.05$).

3. Results

As mentioned results correspond to two different peanut processing companies, called company 1 (C1) and company 2 (C2). The work started in the year 2015 in C1 and was repeated in 2016 in C2.

3.1. Effect of BHA formulation on total fungal population

3.1.1. Fungal count assessment in C1

Fungal counts were significantly affected by time (F: 24.66), treatment (F: 13.94) and their interaction (F: 3.15) according to the ANOVA test ($p < 0.05$). Table 1 shows the amounts fungal population (Log_{10} CFU/g) present on peanuts stored in big bags during the 5 months' experimental period. At the beginning of the assay, fungal populations detected in control and treated stored peanuts F-BHA were similar (in the order of 3.71 and 4.00 Log10 CFU/g, for control and F-BHA, respectively). After 30 days, reductions in total fungal counts were observed in both treated (51%) and untreated (38.27%) stored peanuts without significant difference ($p < 0.05$). However, after 4 months of storage, a significant reduction ($p < 0.05$) between 23.8 and 41% was

Table 1
BHA formulation effect on total fungal level recorded in peanut kernel stored during 5 and 4 months for C1 and C2, respectively (Log_{10} CFU/g).

		T0	T1	T2	T3	T4	T5
C1	Control	3,71 ± 0,69 a	2,29 ± 1,10 a	3,29 ± 0,55 a	2,95 ± 0,82 a	2,37 ± 0,94 a	2,36 ± 0,33 a
	F-BHA	4,00 ± 0,56 a	1,96 ± 1,25 a	2,13 ± 0,83 b	2,83 ± 0,43 a	1,41 ± 0,58 b	1,80 ± 0,74 b
C2	Control	5,00 ± 0,05a	5,63 ± 0,37a	4,13 ± 0,34a	4,46 ± 0,33a	3,81 ± 0,40a	–
	F-BHA	5,81 ± 0,48a	4,84 ± 0,37a	3,81 ± 0,78a	4,78 ± 0,18a	3,25 ± 0,32a	–

C1 and C2 are the different companies (company 1 and company 2). T0: initial time. T1, T2; T3, T4 and T5 refers to the months of sampling. F-BHA: BHA formulation. Data with different letters for each time are significantly different according to the LSD test. ($p < 0.05$).

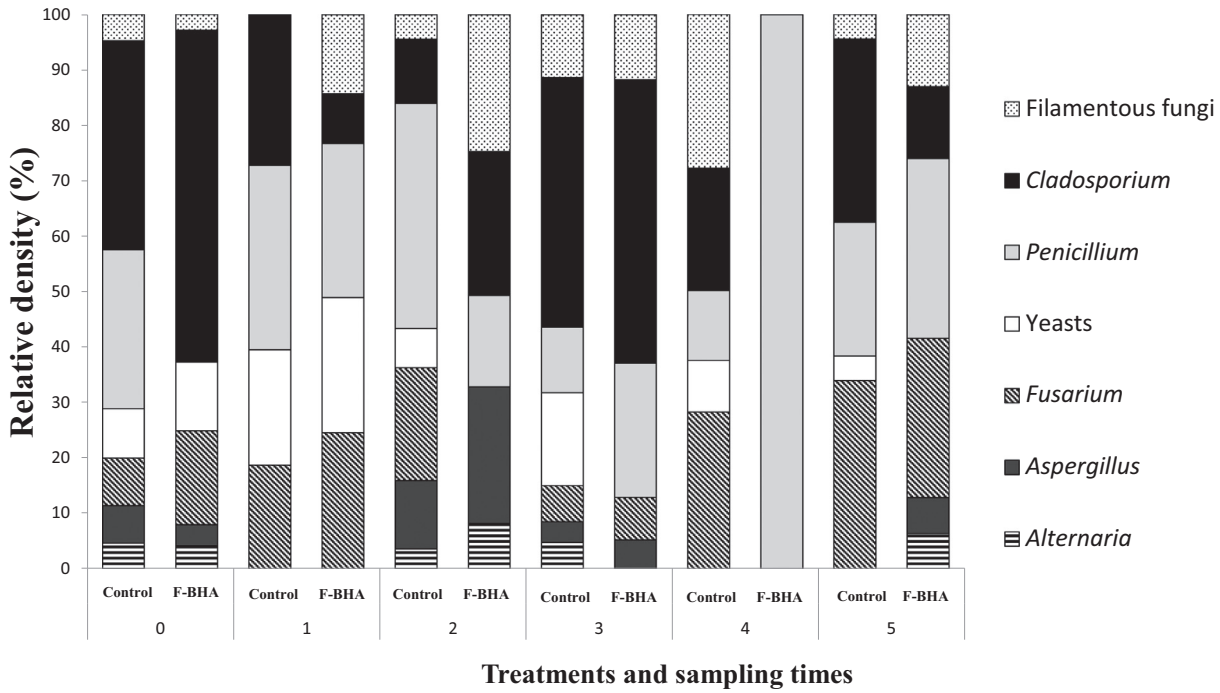

Fig. 1. Relative density (%) of fungal genera isolated in peanut kernel stored in big bags treated with BHA formulation and control in Company 1 during 2015.

observed in treated peanuts compared to the controls. In general, the main genera of fungi isolated from treated and untreated grains in all the samplings were *Penicillium* spp., *Cladosporium* spp., *Fusarium* spp., yeasts, *Aspergillus* spp. and *Alternaria* spp. (Fig. 1). At the beginning of storage period, *Cladosporium* spp. were the highest component populations followed by *Fusarium* spp. and yeast. However, there was no a clear trend of fungal population throughout time or due to the treatment.

3.1.2. Fungal count assessment in C2

In the second company, the fungal populations were significantly affected ($p < 0.05$) by time (F: 23.89) and the interaction between time and treatment (F: 4.02) according to the ANOVA test. The fungal populations (Log_{10} CFU/g) present in peanut kernel stored for 4 months are shown in Table 1. Initial fungal population ($t = 0$) in the control and the F-BHA treatments were 5.00 and 5.81 Log10 CFU/g, respectively. However, at the end of the storage period a reduction in total fungal level was observed in both treated (44.06%) and untreated (23.8%) peanuts without statistically significant differences between them ($p < 0.05$). Fig. 2 shows the most frequently isolated fungal genera. *Penicillium* spp., and yeasts were the fungi mainly isolated in this company. *Cladosporium* spp., *Fusarium* spp. and *Aspergillus* spp. represented as minor populations of the total fungal load.

3.2. Effect of BHA formulation on aflatoxin detection

For both companies, no aflatoxins accumulation was detected in peanut grains during the storage period.

3.3. BHA residue detection

3.3.1. BHA residue detection in C1

Levels of BHA residues detected on stored peanuts were significantly affected ($p < 0.05$) by the storage time (F: 11.86) according to the ANOVA test (data not shown). Amounts of the antioxidant detected on peanut grains treated with the formulation during the 5 months of storage are shown in Fig. 3. At the initial time, BHA residue detected was 90.43 ng/g peanuts. However, after 2 months of storage, BHA levels increased significantly ($p < 0.05$) reaching a value 334.8 ng/g peanut. The antioxidant decreased significantly ($p < 0.05$) until 2.5 ng/g peanuts at the end of storage. No BHA levels or interference were detected in control big bags.

3.3.2. BHA residue levels detection in C2

In the second company, the ANOVA test showed that levels of BHA residues in the peanut samples were affected by the storage time (F: 57.20) (data not shown). The antioxidant amounts recorded at initial time of the assay, after microcapsules application, was 591.92 ng/g peanut (Fig. 4). After 2 months of storage, BHA residues increased significantly ($p < 0.05$) reaching 1433.04 ng/g peanut. After this time, level of the antioxidant decreased significantly ($p < 0.05$) until 275.02 ng/g peanut at the final of storage. No BHA levels or interference were recorded in control big bags.

3.4. Effect of BHA formulation on acidity and fatty acid profile

Acidity index in peanut oil stored in both companies were significantly affected ($p < 0.05$) by storage time (F: 145.51 for C1 and F: 122.25 for C2) and by treatments (F: 5.92 for C1 and F: 3.25 for C2) according to the ANOVA test. In both cases, peanut oils showed an initial acidity around 0.16% regardless of the origin (company, control or treated big bags). Acidity value in peanut oil on both, control and F-BHA, increased parallel and significantly ($p < 0.05$) until reaching a final percentage 0.56% after 4 month of storage. No statistically significant differences ($p < 0.05$) were observed between oleic acid/linoleic acid (O/L) ratio for the both, control and F-BHA, as well as, between the different storage times.

Oleic acid percentages observed during the different samplings in C1, were higher than 79.66% and linoleic acid values lower than 5.05%, yielding therefore, a high O/L ratio (> 15.88). Also, for C2 percentages of oleic acid were higher than 76.71% whereas linoleic acid was lower than 7.42% during the assay, with an average O/L ratio of 11.24%.

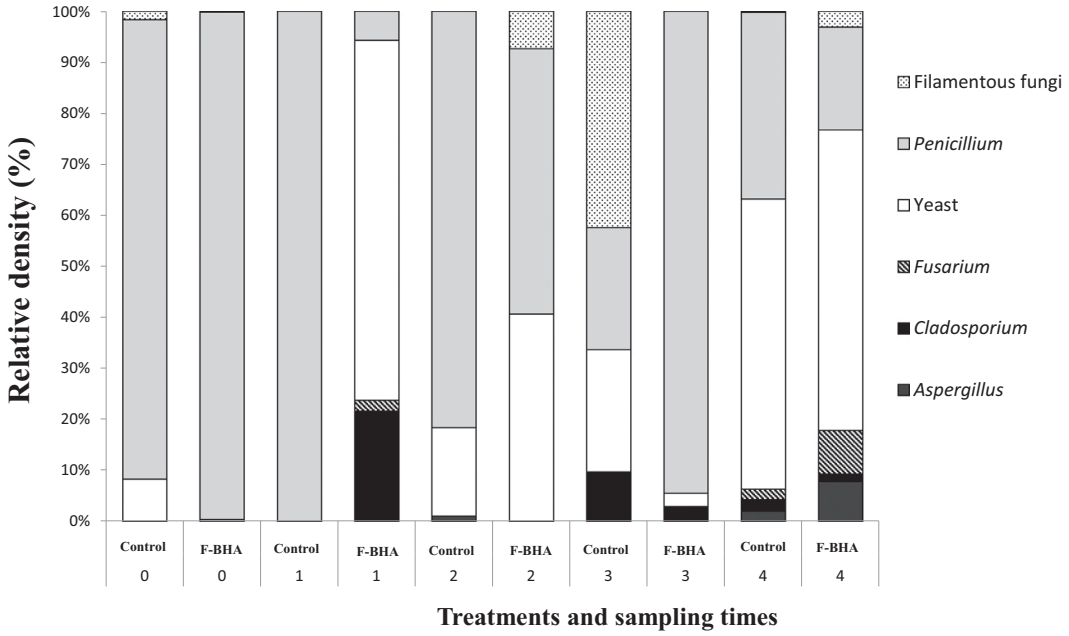

Fig. 2. Relative density (%) of fungal genera isolated in peanut kernel stored in big bags treated with BHA formulation and control in Company 2 during 2016.

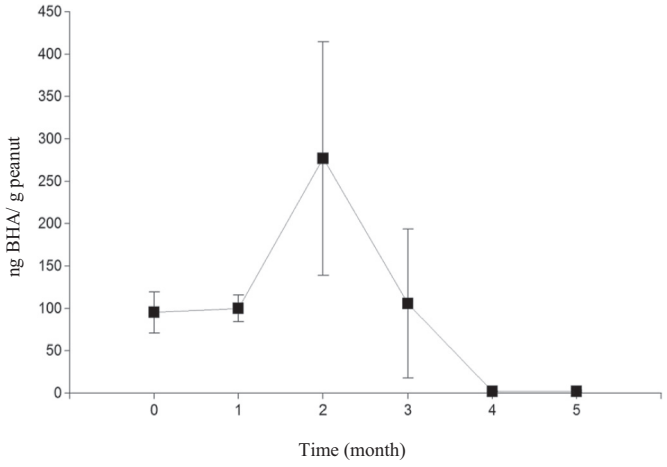

Fig. 3. BHA residue levels detected on peanut kernels treated with micro-encapsulated BHA stored in big bags for 5 months in Company 1.

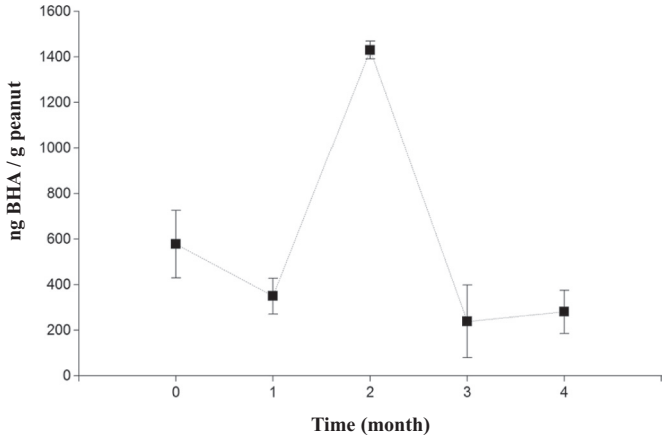

Fig. 4. BHA residue levels detected on peanuts kernels treated with micro-encapsulated BHA stored in big bags for 4 months in Company 2.

3.5. Sensory analysis

For the two peanut processing companies, the taste of peanuts was not significantly affected ($p < 0.05$) by treatment or storage time according to ANOVA test (data no shown). In both cases, between 0.0 and 2.0% of the peanut presented bad taste, regardless of the company, treatment and storage time.

3.6. Peanut kernel insect damage

Damage caused by insects in peanuts was not significantly affected ($p < 0.05$) by treatment or storage time according to the ANOVA test. Percentage of injury was lower than 0.27% for C1 and 2.7% for C2 during the whole storage period without significant differences ($p < 0.05$) between the control and antioxidant treatment.

3.7. Variations in temperature and a_w of peanut samples

During evaluation on C1, peanuts showed a water availability average of 0.62 a_w during the first sampling. However, after 30 days, the stored peanuts reached a value around 0.65 a_w, decreasing to 0.58 aw at the end of the storage period. With respect to temperature, 12 °C were recorded at start of storage but after 3 months a decrease in this parameter was found (Control: 8 °C; F-BHA: 7 °C). From this time until the end of the assay the temperature was maintained between 8 and 9 °C. For C2, peanuts showed an average of 0.66 a_w at the start of storage period, maintaining this level over the whole experimental period. Temperature in stored was initially at 9 °C, but this increased to 14 and 22 °C over the two months' storage period.

4. Discussion

Peanut storage environment represents an ecosystem in which quality and nutritive changes could occur due to the interactions between physical, chemical and biological factors, among which fungal spoilage, mycotoxin contamination and insects' pest are of paramount interest. In this work, application of F-BHA to peanuts intended for direct human consumption, stored in big bag systems during 4–5 months, was evaluated in two different processing companies. Stored peanuts in untreated big bags showed a decrease in fungal

populations of 1.4 and 1.8 logarithmic units, for C1 and C2 respectively, through time until the end of the experiment. However, for C2 peanut treated with F-BHA showed a reduction of 2 logarithmic units over time compared with the control. Garcia et al. (2016) showed that BHA formulation maintained antifungal and insecticide effect in the stored peanuts microcosm. However, this study was carried out under lab conditions where environmental factors are more controlled and, in most cases, the effects cannot be equivalent to natural ecosystems where other factors may influence the efficacy of the treatment (Garcia et al., 2011). Consequently, there was a need to confirm that formulations based on BHA treatment efficacy was maintained in pilot scale in an *in situ* assay. The fungal populations and genera isolated were maintained through time in C1, with the highest frequency of the genus *Cladosporium* spp., *Penicillium* spp., and *Fusarium* spp. However, for the second company, *Penicillium* spp. and yeasts were the main fungi which appeared in all samples. In both companies, *Aspergillus* spp. were detected after 2 months but at low population levels. These result agree with Passone et al. (2014) who also found *Penicillium* and *Fusarium* spp. (among other genera) as the most common fungi in harvested peanuts stored for 5 months, in three storage systems including, big bags.

On the other hand, initial fungal populations in both peanut companies were below 5 logarithmic units. This may depend on the peanuts used in this work were subjected to a rigorous selection process, by which they acquired low initial mycobiota, satisfactory for peanuts destined for human consumption.

Decreased of fungal populations observed in controls during the storage period, could be related to the storage in big bags system inside refrigerated cells, where good storage conditions were maintained. Besides, the two main factors affecting grain preservation, temperature and humidity (Roberts, 1972; Smith and Davidsoll Jr., 1982), were maintained at suitable values for the 5 and 4 months' storage for C1 and C2 respectively ($a_w < 0.66$; $T < 18\,^{\circ}C$). This circumstance and the low density of aflatoxin-producing fungi, may also explains the absence of toxin and the lack of insect damage observed in grains during this period.

Residual amounts of BHA detected was low during the initial assay due to antioxidant remaining within microcapsules. However, levels detected for the next samplings were lower than expected with cumulative amounts observed of 881.68 and 143.8 ng/g peanut for C1 and C2 respectively, while the initial encapsulated dose was 1080 ng/g peanut. Probably, storage conditions (low a_w and temperature) did not favor release of BHA, especially in C1 when temperature never exceeded 9 °C, since it was previously shown that released of this antioxidant from the microcapsules increased under high temperature and a_w conditions (30 °C and 0.95 a_w) (Girardi et al., 2015). In spite of this, levels of antioxidant released by the capsules increased significantly until 60 days, but after this time a decrease of the antioxidant release was observed. After the second month of storage, a BHA release peak was observed for C1 and C2. This increase in antioxidant level released to the peanuts together with storage conditions could be responsible of the decrease in the total fungal populations, especially for C1.

Free fatty acid content (acidity) was used to detect level of oxidation of the stored grains. Final recorded of acidity after 5–4 month of storage, for C1 and C2, respectively (< 0.56%) for both big bags (treated and untreated), remained below the maximum level allowed in the markets (1.5%) during storage period (Resolución no 1075/ANEXO XIII). These low levels of oxidation of fat corresponded to a high O/L ratio (> 15.88) in peanut kernel, a characteristic “high oleic peanuts”. Presence of higher amounts of oleic acid makes kernels less exposed to oxidative rancidity because of the monounsaturated fatty acid (Treadwell et al., 1983).

Finally, sensory analysis showed that storage conditions were appropriate for the preservation of peanut characteristics during the period evaluated. In addition, BHA formulation did not alter organoleptic characteristics of peanut, suggesting be an appropriate methodology for antioxidant addition to products intended for human consumption.

In conclusion, F-BHA together with good storage conditions preserved product quality during the evaluated period, controlling fungal development, insect damage and oxidative deterioration of stored peanuts, without affecting the peanut organoleptic characteristics. Therefore, results indicate that BHA formulation could be used as part of alternative strategy for control of fungal contamination, including aflatoxigenic species, in stored peanuts for up to 4 months.

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Conflicts of interest

The authors declare no conflict of interest.

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