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Microencapsulation of *Peumus boldus* essential oil and its impact on peanut seed quality preservation

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ABSTRACT

Argentinean peanut provides a significant source of food worldwide, therefore is essential to preserve the quality of seeds during the storage period in order to ensure the yield of this crop. In this study *in situ* effect of a formulation based on microencapsulated boldo (*Peumus boldus*) essential oil (EO) was evaluated on in-pod stored peanut (*Arachis hipogaea*) to preserve the seed quality. Statistically significant ($p < 0.05$) fungitoxic effects were observed at the end of the storage period. Seed damages caused by insects was very low during the 5 months, however, reductions of seed germinations were produced by the formulation. Low levels of residual boldo oil were recorded at the end of the assay. The application of boldo oil microcapsules is useful to preserve stored peanuts avoiding its deterioration, but not when it is intended for seed, but for another purposes as by-product elaboration.

1. Introduction

Peanut (*Arachis hypogaea*) is a world economically important culture, the Argentinean production in 2016–2017 is estimated to will reached 795.500 tn of grain peanut (BCBA, 2017). Of the total peanut produced, 75.4% is destined to exportation being the first worldwide exporter of this food (Agrovoz, Agricultura, 2016) and around of 2.0% is intended for seeds (Blengino, 2014).

Peanut has the peculiarity of having aerial flowers and underground fruits (Câmara, 1998). This unique mode of growth (hypogeum) makes the seeds more exposed to the infections, since in the soil inhabit numerous pathogens that can attack the seeds in the different maturation stages (Elwakil, 2003), thus causing a high economic impact estimated at USD 14.623.527 in the Argentinean peanut production area (Paredes et al., 2016). However, the increase in the level of fungal contamination occurs not only in the field, but also during the harvesting, drying, transportation, and storage of grains (Rossetto et al., 2005). The tropical climate, with high temperatures and relative humidity, combined with inadequate storage conditions adversely affects the conservation of the grains, leading to fungal and insect's development thus producing a loss of seed quality. The effect of these biological factors involve the reduction of germination rate, as well as result in loss of total carbohydrate, protein and fat content, and increase in moisture content, free fatty acids and other biochemical changes (Ameer Junaithal Begum et al., 2013). For this reason it is of great importance to preserve the peanut seeds quality during the storage stage.

Synthetic pesticides have been considered until now the only effective means available for controlling fungi and insects that spoil stored food. Local stockers only apply synthetic fumigants such as phosphine to control pest proliferation. However, this fumigant has a high inhalation toxicity. Exposures to high levels can cause bronchitis, pulmonary edema and death, while prolonged exposures can lead to motor speech disorders. In addition, it is a recalcitrant compound, meaning that its residues persist along the food chain (Ministerio de Trabajo y Asuntos Sociales, España, 2007). Consequently, the search for new alternatives such as botanical pesticides appears as more safe, effective and ecological option to be explored as integrated pest control.

The essential oils (EOs) are considered a powerful source of natural derivatives useful against stored product pests demonstrating their antifungal and insecticidal activities (de Medeiros et al., 2016; Prakash et al., 2013; Zabka et al., 2014). The inherent aroma and antimicrobial activity of EOs are commonly related to the chemical structure for their components, the concentration in which the components are present, and the interactions among them affecting their bioactive properties.

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Peumus boldus, commonly known as boldo, is an endemic plant from the central region of Chile, whose volatile oils have been thoroughly studied (Passone and Etcheverry, 2014), and to which different biological activities are attributed including antibacterial, antifungal, insecticidal, anti-inflammatory, antipyretic, hepatoprotective, anti-carcinogenic and antioxidant (Backhouse et al., 1994; Bonilla and Sobral, 2016; Gerhardt et al., 2009; Passone and Etcheverry, 2014; Silva Borges de Castro et al., 2016; Srivastava et al., 2011). Most of these biological effects can be attributed to its strong ability to scavenge free radicals (Srivastava et al., 2011).

However, the biological activity of these products can be lost through the volatilization of their components or their degradation (exposure to high temperatures, oxidation and sunlight and UV radiations) (Passone et al., 2013), making the commercial applications of these oils limited. Moreover, EOs have short residual activity which results in the need of repeated applications in order to obtain constant pesticide effect. As an alternative for specific applications, volatile oils can be prepared in a large number of formulations: liquid, semiliquid or solid forms to be used to control the release of active ingredients and to protect them from the external environment (Miró et al., 2010). Microencapsulation is one of the most efficient processes for this kind of products, showing long-lasting antifungal and insecticidal activities (Bonilla and Sobral, 2016; Estrada-Cano et al., 2017). A technique for microencapsulating boldo EO by complex coacervation method was recently developed in our laboratory (Girardi et al., 2016). The mean particle size obtained (4.33 ± 2.19 μm) in combination with the use of a power sprayer with a flow rate of 90 m s^{-1} assured the adherence of microcapsules to peanut seeds. Promising results have been reported by Bonilla and Sobral (2016) and Girardi et al. (2016) by applying encapsulated formulations of boldo oil for the control of fungi and insects in microcosm assays.

Therefore, the aim of this work is to evaluate the *in situ* application of boldo EO formulation on peanut pods stored in big bags for seed purpose during a period of five months, monitoring (i) the total fungal population, (ii) the damage caused by insects, (iii) the seed germination power, (iv) the oil permanence on the substrate and (v) the environmental variations.

2. Materials and methods

2.1. Collection and characterization of the essential oil

The spice used in this study (*Peumus boldus*) was collected in December-March period 2014 in the O'Higgins Region (VI), Chile. Dried leaves (0.3373 ± 0.004 a_W) of boldo were purchased from a local market located in La Paz town, Córdoba, Argentina. A portion (100 g) of each plant material part was submitted for 3 h to water-distillation, using an extractor of EOs by steam distillation at laboratory scale (Figmay S.R.L.) (yield 2.0%). The obtained EO was dried over anhydrous sodium sulfate and, after filtration, stored in a sterilized vial at 4 °C for up to 1 week until testing. Chemical characterization of this EO was previously performed in our laboratory (Girardi et al., 2016).

2.2. Preparation of coacervate microcapsules

Microcapsules were made by complex coacervation following Girardi et al. (2016). Boldo EO was used as the core material, while gelatin and gum arabic were used as the wall material. 25 mL of gelatin and gum arabic aqueous solution 5% w/v were prepared at 50 °C in a thermostatic bath (Decalab SRL). pH of gum arabic solution was adjust to 6 with sodium hydroxide 1 M (NaOH). 2 mL of boldo EO 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. After that, pH was adjusted to 4 with hydrochloric acid 1 M (HCl) and the stirring was continued for 10 min. Subsequently, pH was adjusted to 9 with NaOH 1 M and stirring others 10 min. After that temperature was lowered to 10 °C in an ice bath and 5 mL of crosslinking agent was added to compact the gelatin/gum arabic coating. Then, microcapsules were washed twice with distilled water and were stored at −20 °C until the lyophilization step. For lyophilization process, microcapsules were frozen at −80 °C during 3 h and then were lyophilized (L-T8-A-B3-CT, RIFICOR). Finally, samples were ground (CT 193 CyclotecTM Sample Mill) to obtain a powder with a particle size of 1000 μm and stored at −20 °C.

2.3. Effect of boldo oil formulation on in-pod peanut seeds

2.3.1. Big bag assay. Storage conditions

Trials were conducted in a storage company located in the south of Córdoba, Argentina (PRODEMAN, SA). Two-hundred kilograms of in-pod peanuts destined for seeds were distributed in four flexible and air permeable containers (called "big bag") manufactured of polypropylene raffia of high resistance and tenacity and used to carry out the study from July to November 2015. One peanut portion (100 kg) was sprayed with the boldo EO formulation by using a dosing equipment (Stihl SR 450) at the same time the big bag was filled; while the other experimental unit did not received any treatment (control). Boldo EO formulation was applied at the dose of 3 μL g^{-1} because it showed favourable results in the microcosm assay previously performed. The two experimental units were placed alongside a 60 t stockpiled cell to store in-pod peanuts intended for seed. Three points of each big bag were sampled at each collection time by using a compartment-sampling spear, which enabled samples to be taken from different depths. Samples (1 kg) were collected in polyethylene bags (to minimize water loss), sealed, transferred immediately to the laboratory and kept at low temperature (−20 °C). After each storage period, each sample was analysed to evaluate the biological and physicochemical characteristics of the seeds.

2.3.2. Estimation of mycoflora populations

A sample of 10 g was taken from each treatment, milled and shaken for 30 min with 90 mL of 1 g/L peptone: distilled water. Serial decimal dilutions until 10^{-3} were performed. An aliquot of 0.1 mL of each dilution was spread on surface of two general counting media: DRBC (dicloran- rose bengal- chloramphenicol agar) and DG18 (dicloran 18% glycerol agar) (Pitt and Hocking, 1997). Plates were incubated in darkness at 25 ± 1 °C for 5–7 days. Colonization of peanut seeds was expressed as colony forming units per gram of peanut seeds (CFU/g). The macro and microscopic identification of fungal genera was done according to Samson and Frisvad (2004) and Samson et al. (2010). Moreover, the total fungal mycoflora was again evaluated thirty days after to apply the seed fungicide (Options Advance FS 800 cc/100 kg of seeds + Micro Grower 100 cc/100 kg of seeds) intended for germination test. The impact of seed fungicide was evaluated at all sampling period.

2.3.3. Insect damages

A peanut sample of 500 g was heated to 100 °C during 1 h, and then cooled at room temperature. The skin of the grain was removed with a blancher equipment. Those grains that exhibited insect damages were selected, weighed and the results were expressed as percentage.

2.3.4. Effect on germination power

A sample of 100 peanut seeds from each treatment were placed on plastic trays containing sterile sand moistened with sterile distilled water according to the validated methodology for seeds testing by ISTA (2014). The trays were incubated at 25 ± 5 °C during 10 days. The percentage of normal seedlings (equivalent to germinated seeds-GS), anomalous seedlings, hard seeds, fresh seeds and dead seeds in each treatment was determined and the results expressed as percentage. The samples were analysed in quadruplicate form, before and after of the

Table 1
Effect of boldo EO formulation and fungicide on total peanut mycoflora.

Fungicide	Treatment	Fungal counts (Log_{10} CFU g^{-1})					
		t0	t1 (30 d)	t2 (60 d)	t3 (90 d)	t4 (120 d)	t5 (150 d)
WF	Control	4.5 ± 0.1b	4.1 ± 0.7a	4.8 ± 0.4a	3.9 ± 0.4bc	4.4 ± 0.4b	4.3 ± 0.2a
	Boldo	4.4 ± 0.2b	3.4 ± 0.2ab	4.0 ± 0.1a	4.5 ± 0.3ab	5.0 ± 0.3a	3.1 ± 0.1b
F	Control	5.2 ± 0.4a	3.2 ± 0.4ab	4.2 ± 0.2a	5.0 ± 0.1a	4.5 ± 0.2ab	3.9 ± 0.1a
	Boldo	2.9 ± 0.1c	2.5 ± 0.5b	3.6 ± 1.3a	3.8 ± 0.1c	2.6 ± 0.2c	3.1 ± 0.1b

WF: without fungicide treatment, F: with fungicide treatment. Data with different letters for each treatment are significantly different according to LSD test ($p < 0.05$).

fungicide application (Options Advance FS 800 cc/100 kg of seeds + Micro Grower 100 cc/100 kg of seeds).

2.3.5. Extraction and quantification of the boldo residual oil

Boldo residual components were extracted and quantified according to Passone and Etcheverry (2014) with some modifications. At the end of the storage time, boldo EO components from a seed sample (55–60 g) obtained from the treated big bag were determined in triplicate. Each subsample was placed in a sealed flask (500 mL) and was maintained at 50 °C during 1 h. For extraction of oil components, peanut seeds was exposed to the solid phase microextraction (SPME) using polydimethylsiloxane (PDMS/DVB) fiber cover of 100 µm (Supelco) during 15 min.

The detection analyses were performed by gas chromatography coupled to a mass spectrometer (GC/MS Clarus 560, Perkin Elmer) equipped with a DB5 column (30 m, 0.25 mm ID, 0.25 µm particle Perkin Elmer). The injection volume was 1 µL and the fiber was remained in the injector port for 10 min. The sample was injected in a Splitess mode. To control the equipment and data acquisition the Turbo Mass program was used. Working conditions were: initial temperature 70 °C (15 min) ramp: 10 °C/min, final temperature 240 °C. The mobile phase used was Helio (31.8 psi). The temperature of the injector was maintained at 250 °C. Ionization was performed in the mass spectrometer vacuum with electron impact ionization energy −70 eV. The chromatogram was obtained in 'scan' mode from $m/z = 50$ to $m/z = 350$ (scan time 0.2 s, inter-scan time: 0.1s), with a solvent delay of 1 min. The identification of the components of the EO was performed by comparison with spectra libraries NIST MS Search 2.0.

For quantification of the residual oil, an external calibration curve of eucalyptol (MW: 154.25 g/mol; CAS: 470-82-6) and limonene (MW: 136.23 g/mol; CAS: 5898-27-2) were used. The quantification curve in the range of sample concentrations (0.00303085–1.21155 µg/µL of eucalyptol; $R^2 = 0.984$; 0.008232–1.0976 µg/µL of limonene, $R^2 = 0.9626$) was performed. Each concentration level of standard solution was analysed by GC/MS in triplicate. Quantification was performed by reporting the measured integration areas in the calibration equation of the corresponding standards. The detection (LOD) and quantification (LOQ) limits of the analytical method were 0.05 and 0.30 ng/g and 0.02 and 0.82 ng/g for eucalyptol and limonene, respectively. The results were expressed as percentage of boldo EO released, considering the initial EO present in the microcapsules and the chemical composition.

2.3.6. Water activity and temperature determination

Temperature changes were monthly measured by introducing distance-reading thermometers in each big bag. The water content of peanut seeds from each sampling were determined by duplicated with water activity (a_w) measuring equipment (Aqua Lab, Succession 3, TE Decagon Devices, Inc.).

2.4. Data analyses

Statistical analyses were performed through the program InfoStat version 2012. InfoStat Group, FCA, National University of Cordoba, Argentina. http://www.infostat.com.ar URL. Means data on total fungal populations and germination seeds were determined by analyses of variance (ANOVA). To establish significant differences, the test of Fisher's Least Significant Difference (LSD) ($p < 0.05$) was performed.

3. Results

3.1. Effect of boldo EO formulation on peanut seed mycoflora

According to ANOVA Test fungal counts were significantly affected ($p < 0.05$) by the treatment-T (F 58.6), fungicide-F (F 24.8), storage time-t (F 11.7) and the treatment*fungicide-T*F (F 24.3) and time*-treatment*fungicide-t*T*F (F 7.0) interactions, being the main effect that produced by the microencapsulated boldo EO.

Fungal counts were similar in both big bags (WF-C and WF-B) at the first sampling, but at the end of storage period (150 d), the fungitoxic effect of the boldo oil formulation became statistically significant ($p < 0.05$) (Table 1). Although, the seeds treated with the fungicide were analysed 1 month after it application, fungal levels in both WF-C and F-C were similar. Meanwhile, peanut seeds that received the combined treatment of EO formulation and fungicide (F-B) showed a fungal load between 21.9 and 44.2% lower than the F-C during the first month of storage. In the subsequent sampling periods (60–90 d), fungal counts registered in seeds treated with fungicide (F-C and F-B) remained in the order of 4.0 Log_{10} CFU g^{-1}. However, from the fourth month to the end of storage, reductions of the total mycoflora in F-B treated seeds were in the order of 20.5 and 42.2% in relation to F-C.

The fungal genera most commonly isolated from treated and untreated peanut seeds with (a) and without fungicide (b) are shown in Fig. 1. A high frequency of fungi classified as *Cladosporium, Fusarium, Alternaria, Aspergillus* and *Penicillium genera* and yeasts were registered in peanut seeds, regardless of treatment applied. The fungal genera that showed a relatively low frequency of isolation were all included in the filamentous fungi group.

A poblational susseccion in F-C and F-B seeds was observed after the fourth month of storage, where *Penicillium*, *Cladosporium* and *Fusarium* species, that showed a high incidence in the order of 2.0 Log_{10} CFU g^{-1}, were replaced by *Aspergillus* species with counts in the order of 3.0 Log_{10} CFU g^{-1}. A similar fungal diversity was observed in seeds exposed to boldo microcapsules and fungicide. However, some variations were observed througth the storage time. Yeast isolates were only observed in F-B seeds at the first sampling; meanwhile in WF-C and WF-B it was one of the prevalent fungi along all the storage period, with mena counts of 2.3 and 5.0 Log_{10} CFU g^{-1}, respectivelly It is notable, that in this seeds the level of contamination with storage fungi was low in the order of 3.0 and 4.5 Log_{10} CFU g^{-1}.

3.2. Insect damages

The percentage of damages caused by insects were very low (up to 0.4%) during the five months of storage, both in control and treated peanut seeds (Table 2).

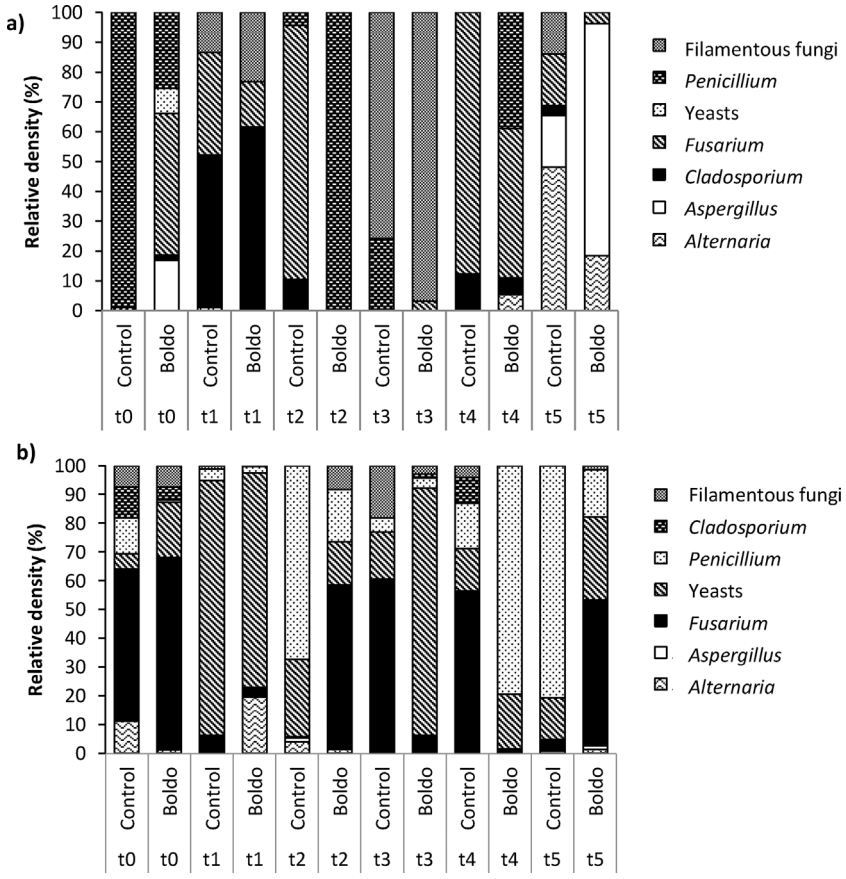

Fig. 1. Fungal genera isolated from treated (boldo oil formulation) and untreated (control) peanut seeds, with (a) and without fungicide (b).

Table 2
Effect of boldo oil formulation on insect damage.

Treatment	Insect damage (%)				
	t1 (30 d)	t2 (60 d)	t3 (90 d)	t4 (120 d)	t5 (150 d)
C	n.d.	0.4	n.d.	n.d.	n.d.
B	n.d.	n.d.	n.d.	0.2	n.d.

C: control, B: with boldo oil formulation.

3.3. Effect on germination power

Results regarding to germination power (GP) of peanut seeds treated and untreated with boldo EO formulation, with and without the fungicide addition, during a storage period of 150 days were analysed by ANOVA test. This parameter was significantly affected ($p < 0.05$) by F (F 87.2), T (F 57.5), t (F 9.5) and t*T (F 12.9) and t*F (F 5.1) interactions.

Table 3 shows GP percentages of stored peanut seeds. Germination of control seeds with fungicide (F-C) was up to 14% higher than the control seeds without fungicide (WF-C) during all the storage period. During the first month of storage, the GP of seeds treated with oil formulation was similar to its respective control. However, after 60 d of storage the germination of seeds in contact with microencapsulated boldo oil tended to reduce respect to the control. From the fourth month, this parameter was significantly lower ($p < 0.05$) than untreated seeds, both without (30.1%) and with (15.4%) fungicide treatment.

The percentage of normal (equivalent to germinated seeds-GS) and anomalous seedlings, hard, fresh and dead seeds for each treatment of in-pod peanut stored during five months, before (a, b) and after (c, d) the fungicide application are presented in Fig. 2. A reduction of fresh seeds was observed in all treatments as the storage time increased. A high number of anomalous seedlings was observed in the peanut samples without fungicide (16.9%) in comparison with the fungicide treated seeds (6.9%), regardless of boldo oil formulation application and sampling period. Moreover, the seeds in contact with microencapsulated oil both with and without fungicide, showed the highest

Table 3
Effect of boldo oil formulation on germination power (%) of peanut seed.

Treatment	Germination power (%)					
	t0	t1 (30 d)	t2 (60 d)	t3 (90 d)	t4 (120 d)	t5 (150 d)
WF-C	67.3 ± 6.4b	75.3 ± 7.5a	90.5 ± 2.5a	74.8 ± 5.4b	80.5 ± 2.9b	80.3 ± 3.1b
WF-B	67.5 ± 6.8b	76.5 ± 4.7a	76.3 ± 6.7b	71.8 ± 6.7b	60.8 ± 4.6c	60.5 ± 4.5c
F-C	81.8 ± 2.4a	75.5 ± 6.6a	89.8 ± 2.4a	86.0 ± 1.6a	90.3 ± 4.4a	91.3 ± 5.6a
F-B	84.0 ± 4.4a	78.5 ± 4.4a	85.8 ± 1.5a	80.0 ± 7.1ab	76.8 ± 3.2b	74.0 ± 3.7b

WF-C: without fungicide treatment-control, WF-B: without fungicide treatment-boldo, F-C: with fungicide treatment-control, F-B: with fungicide treatment-boldo. Data with different letters for each treatment are significantly different according to LSD test ($p < 0.05$).

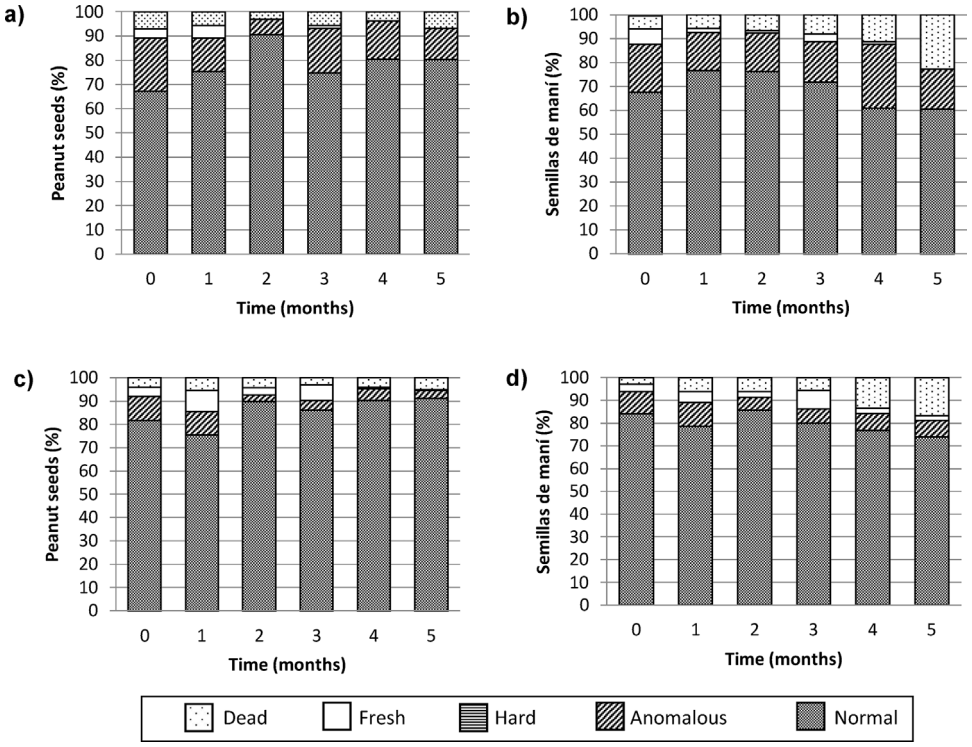

Fig. 2. Percentage of normal and anomalous seedlings, hard, fresh and dead seeds. (a) WFC, (b) WFB, (c) FC and (d) FB.

number of dead seeds (9.23%).

3.4. Boldo EO residual

The residual level of boldo EO estimated in-pod peanut seeds at the end of storage period was of 5.45 × 10^{-3}% respect to the initial dose (Fig. 3).

3.5. Temperature and water activity

The determination of physical properties of the samples revealed marked differences in a_W and temperature among the first and the subsequent months of storage in both big bags (Fig. 4a–b). The highest a_W values (mean = 0.70 ± 0.03) were registered at the first month of storage, regardless of seed sources. Water activity levels in peanut samples from control and treated big bags significantly decreased during the second month of storage and thereafter remained relatively constant until the end of assay, reaching similar values in the order of 0.57 ± 0.03. Meanwhile, temperatures registered in peanuts from the two big bags showed a constant and gradual increase from 15.25 ± 0.96 °C in July to reach 23.50 ± 1.29 °C in November.

4. Discussion

In this work the ability of microencapsulated boldo oil to preserve in-pod stored peanut seeds under actual storage conditions was evaluated during a period of 5 months.

The seeds that did not receive any treatment showed an initial fungal load in the order of 4.5 Log_{10} CFU g^{-1}. In general, fungal count tended to reduce throughout time, reaching reductions up to 28.6% after the fifth month, in parallel with progressive reductions of a_W values (about of 0.57 ± 0.03), despite the gradual increase of temperature from 15.50 ± 0.71 to 24.50 ± 0.71 °C. Fungal genera that showed the highest isolation frequency in this study were *Penicillium*, *Cladosporium* and *Fusarium*. Mycological population successions of peanut stored in three systems (big bags, wagons of conditioning and drying and stockpiled warehouse) during 5 months were analysed in different trials carried out in our laboratory (Passone et al., 2014). The most common fungi identified included *Penicillium*, *Aspergillus*, *Eurotium* and *Fusarium* sp. Nevertheless, in the present work, it is noteworthy the high level of yeast fungi registered in untreated seeds throughout the assay, as opposed to the mycoflora present in the seeds that received the addition of fungicide treatment.

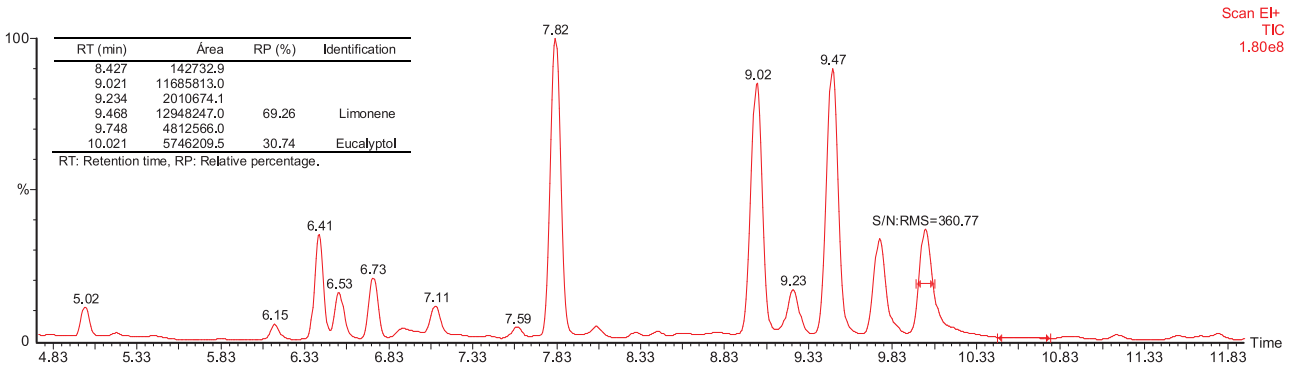

Fig. 3. Chromatogram of peanut seeds treated with formulated Boldo oil obtained by GC–MS.

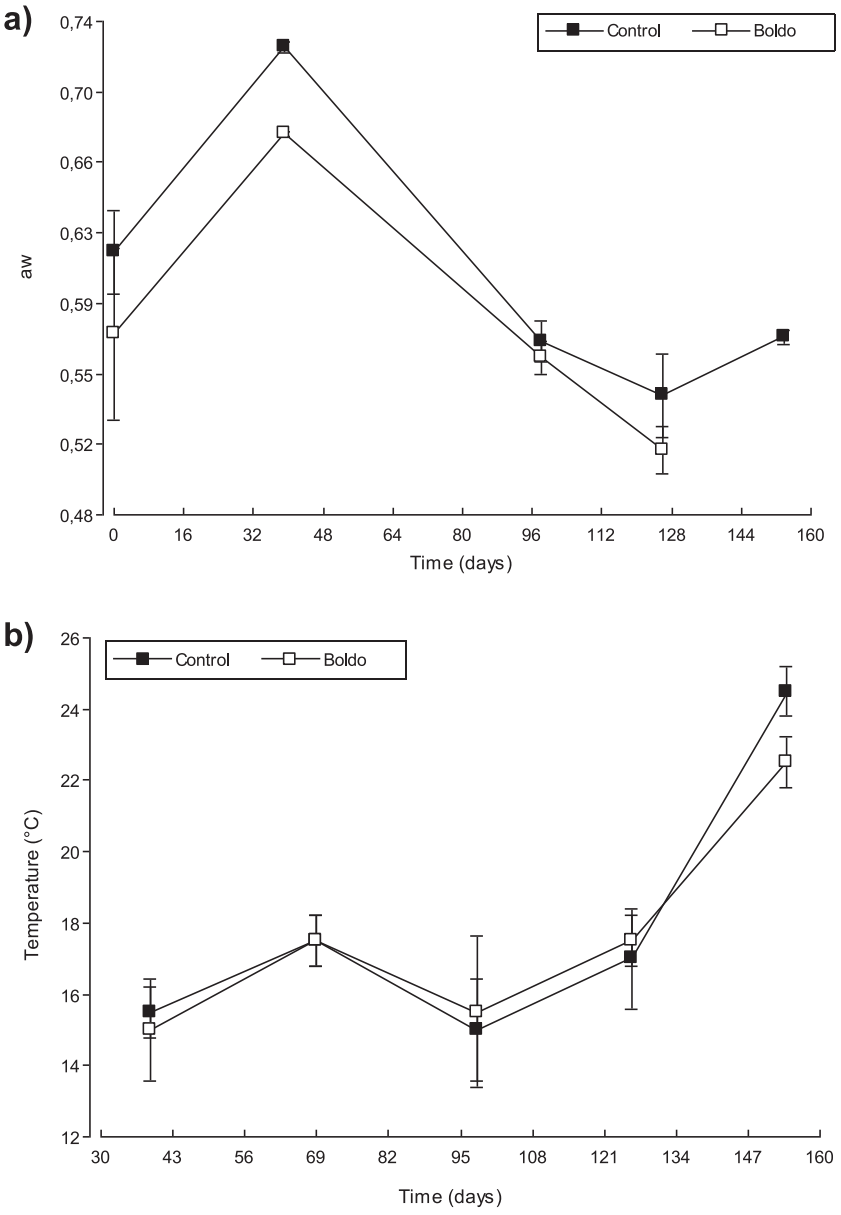

Fig. 4. Environmental changes – (a) a_W and (b) temperature levels registered in-pod peanut seeds stored in big bags during 5 months.

In this work, the effect of boldo oil microcapsules sprayed on in-pod peanut stored *in situ* was evaluated at fungal mycoflora, insect infestation and seed germination levels.

In regard to the first biological parameter evaluated, it was registered that microencapsulated boldo EO did not produce a homogeneous inhibitory effect on peanut seed mycoflora during the 5 months of storage, showing from reductions of 27.7% to stimulations in the order of 14.4%. However, when the fungicide was added to the seeds treated with EO formulation, fungal counts decreased up to 26.7% at the end of storage period. Some *in vitro* studies evaluated the behaviour of microencapsulated EOs on food spoilage fungi. Recently, Estrada-Cano et al. (2017) compared the inhibition ability of free and β-ciclodextrin-microencapsulated EOs of clove (*Eugenia caryophyllata*) and Mexican oregano (*Lippia berlandieri*) on *Fusarium oxysporum*. The highest fungal inhibition was observed with the application of microencapsulated clove EO. However, in the study conducted by Matos-Chamorro et al. (2010), non-encapsulated oregano (*Origanum vulgare*) EO showed the best results on filamentous fungi and yeast present in peel of tomatoes by using the agar diffusion method. Similar results were reported by Dima et al. (2014), who applied the same methodology to compare the impact between free and β-ciclodextrin microencapsulated coriander (*Coriandrum sativum*) oil on some indicator microorganisms. Although the microencapsulation processes reduces the immediate effect of the oils, the choosing of this innovative technology continues to be a promising option. On the on hand, this protection confer to oils a gradual release, as has been demonstrated by Girardi et al. (2016, 2017) in previous *in vitro* studies added to the residual boldo oil levels registered in the present work at the end of the storage period. On the other hand, this behaviour "gradual release" allow the oil bioactivity maintenance over time according to results obtained with the encapsulation of clove, thyme (*Thymus vulgaris*) and cinnamon (*Cinnamomum zeylanicum*) in Ca-alginate microspheres (Soliman et al., 2013), thus protecting them from the negative effect of exposure to environmental factors.

The second biological aspect considered in this work was the capacity of the boldo oil formulation to control insect pests in peanut agroecosystem. However, it was not possible to corroborate this effect due to the low infestation levels recorded in both big bags (treated and untreated).

Finally, boldo oil microcapsules produced a negative effect on the third biological factor analysed, "seed germination". Germination

reductions in parallel to increase of dead seeds were registered in treated peanut samples. This undesirable effect was previously registered in microcosm assays when the formulation was applied directly on shelled peanut seeds (Girardi et al., 2016). Therefore, the change in the application methodology employed in this work, which consisted in spreading the microcapsules on in-pod peanut, it was not sufficient to avoid the adverse effect on GDe Lira Guerra et al. (2015) evaluated the allelopathic effect of seven EOs (*Cymbopogon martinii*, *Cedrus atlantica*, *Copaifera officinalis*, *Zingiber officinale*, *Eucalipto staigeriana*, *Juniperus communis* and *Ocimum basilicum*) at the dose of 1000 ppm on peanut seeds, without findings inhibitory effects. However, *in vitro* studies conducted by Verdeguer et al. (2011) evidenced the phytotoxic effects of boldo EO on *Amaranthus hybridus* and *Portulaca oleracea*, attributing this activity to the high content of oxygenated monoterpenes that it possesses. On the other hand, the fungicide treatment reduced the quantity of anomalous seedlings, increasing the germination percentages at the end of the storage period.

This is the first work that reports the effect of microencapsulated EOs on mycoflora, insects and peanut seeds germination under real storage conditions. In conclusion, boldo oil formulation showed antifungal effects that were enhanced in combination with the seed fungicide application, thus preserving the health of peanut seed and avoiding its deterioration by pathogenic microorganisms. However, significant negative effects were recorded at germination level. Therefore, the data analyses suggest that the application of microencapsulated boldo oil is useful to prevent peanut deterioration by food spoilage microorganisms, but when it is destined to other purposes as by-products elaboration. The results of this work open the door to the search of new natural products to be applied in the peanut seed preservation.

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