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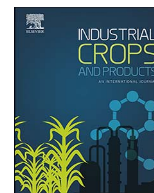

# Microencapsulation of *Peumus boldus* essential oil and its impact on peanut seed quality preservation

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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

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