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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).

To evaluate the amount of encapsulated antioxidant, difference between the initial levels used and non-encapsulated concentration estimated was made. Encapsulation efficiency (EE) was expressed as the percentage of compounds encapsulated with respect to the initial amount of antioxidant used according to (Kaushik & Roos, 2007). To confirm these results, chloroformic extracts containing BHA and BHT were injected in the High Performance Liquid Chromatography (HPLC). Each experiment was performed in duplicate.

2.4.2. BHA and BHT detection and quantification by chromatography

To asses BHA and BHT levels in chloroform, the solvent was evaporated. Dry extract was redissolved in 250 μl of acetonitrile, homogenized in a vortex mixer and injected into the HPLC system by full loop injection technique (Hewlett Packard model 1100 pump, Palo Alto, CA, USA; Rheodyne manual injector with a 50 μl loop, Rheodyne, Cotati, CA, USA). Chromatographic separations were performed on a stainless steel C18 reversed-phase column (150 × 4.6 mm i.d., 5 μm particle size; Luna-Phenomenex, Torrance, CA, USA) connected to a precolumn Security Guard (20 × 4.6 mm i.d., 5 μm particle size, Phenomenex). Mobile phase was methanol: acetonitrile: water (35:35:30) for BHA and methanol: acetonitrile: water (40:40:20) for BHT, at a flow rate of 1.5 mL/min. Antioxidants were detected by UV (Hewlett Packard model 1100 programmable UV detector, Palo Alto, CA, USA) at 280 nm and quantified by a data module Hewlett Packard Kayak XA (HP ChemStation Rev. A.06.01, Palo Alto, CA, USA). BHA and BHT levels were calculated by comparing the area of the chromatographic peak of each sample with those of the standard calibration curve which were 1, 5, 10, 50, 100, 250, 500, 750, 1000 and 1500 ng/mL. Detection limits (signal-to-noise ratio = 3) were 0.4 ng/mL for BHA and 1 ng/mL for BHT.

2.5. BHA and BHT permanence in microcapsules at different time in vitro in the formulation and on peanut grains

Permanence of both antioxidants inside the capsules was evaluated *in vitro*, using the microcapsules produced under the two best yield conditions of microencapsulation: formaldehyde 10 min (F10) and without crosslinking agent (WCA), both made with 240 gelatin bloom. To estimate the concentration of antioxidant inside the capsules, the formulation was stored in glass flasks at 25 °C. Secondly, to estimate the permanence of encapsulated compounds on the peanut kernel system, natural peanut collected during the harvest season 2013–2014 from Córdoba, Argentina, with initial water content of 0.65 a_w were used throughout this study. Seeds were sterilized twice by autoclave at 120 °C for 20 min and then water activity (a_w) was adjusted by aseptically adding sterile distilled water to peanut in sealed containers. The containers were cooled to 4 °C for 48 h with periodic hand shaking during this time. The amount of water necessary to reach the different a_w levels was determined by calibration curves (water activity-mL water to be added/g substrate) previously made (data not show). Final a_w values were confirmed with an AquaLab Series 3 (Series 4, TE, USA) with an accuracy ± 0.003. Then, 0.1 g of microcapsules was placed into a flask containing 5 g of sterile peanuts conditioned at different water activities (a_w) (0.65, 0.75, 0.85 and 0.95). The flasks were incubated at temperatures 15, 25 and 30 ± 2 °C and the samplings were done at intervals from 3 up to 50 days intervals (depending on the crosslinking agent) to analysis the antioxidant contents.

2.5.1. BHA and BHT extraction from capsules stored alone and with peanut grains

To know the level of antioxidant inside the capsules, 10 mL of chloroform were added into the flask that contained 0.1 g of microcapsules and the suspension was shaken during 5 min twice to washing the sample. Powder particles were separated from chloroform by filtering through filter paper (Whatman No. 1). Finally, 10 mL of chloroform were added again to the capsules and the suspension was sonicated (Ultrasonic bath Elma D-78224 SINGEN/Htw, ultrasonic frequency 37 Khz) during 30 min to break the capsules. This last step allowed complete solubilization of both antioxidants with the solvent. Then, chloroform solution was filtering through filter paper (Whatman No. 1) and 1.5 mL were recovered and evaporated to dryness. Antioxidants were detected and quantified by HPLC as was described in Section 2.3. Each experiment was performed in duplicate.

2.6. Data analyzed

Capsule size and %EE data were performed by analyses of variance (ANOVA) ($p < 0.01$). A posteriori tests were done in order to assess significant differences between the different treatments assayed. LSD test ($p < 0.05$) was used to establish significant differences among mean values of each treatment for capsule size and %EE using Statgraphics® Plus version 5.1 (Manugistics, Inc, Maryland, USA).

3. Results

3.1. Microcapsules size and morphology

The effect of single factor as well as their two, three and four way interactions on microcapsules sizes was determined by ANOVA with two interacting factors that significant influenced on capsules that contained BHA (Table 1). However, statistical analysis showed that crosslinking agent (CA), reticulation time (T), gelatin bloom (B), lyophilization process (L) and their two and three way interactions significantly affected ($p < 0.01$) the size of microcapsules with BHT. The major effects were produced by T, followed by B*T and CA*T.

LSD test showed that crosslinking agent used significantly affected ($p < 0.05$) size of lyophilized microcapsules that containing BHA (Table 2). The mean diameters of microencapsulated BHA were 14.8; 14.5 and 11.0 μm for formaldehyde (F), glutaraldehyde (G) and without crosslinking agent (WCA), respectively. Size of WCA-B240 microcapsules was 20% higher than those of WCA-B220. Moreover, no statistical difference was observed between WCA-B240

Table 1
ANOVA test. Effect of different factors (lyophilization process-L, crosslinking agent-CA, gelatin bloom-B, reticulation time-T and their interactions) on the BHA and BHT microcapsules sizes.

Factors	DF	BHA		BHT	
		MS	F	MS	F
L	1	60.9	0.2	1438.79	6.84*
CA	2	24.1	0.1	1479.07	7.03*
B	1	248.5	0.9	1890.85	8.98*
T	2	510.3	1.9	5849.46	27.79*
L*CA	2	786.2	2.9*	3115.90	14.81*
L*B	1	2.5	0.0	1427.80	6.78*
L*T	2	142.2	0.5	859.05	4.08
CA*B	2	319.4	1.2	2123.53	10.09*
CA*T	4	259.3	1.4	4900.07	23.28*
B*T	2	176.4	0.7	4946.27	23.50*
L*CA*B	2	326.5	1.2	1540.89	7.32*
CA*B*T	4	218.3	0.8	238.80	1.13
L*B*T	2	957.6	3.6*	196.78	0.94
L*CA*B*T	4	684.9	2.6*	2472.82	11.75*
Residual	762	262.9		210.45	

DF: degree of freedom. MS mean square. Values with asterisk are statistically significant ($p < 0.01$).

Table 2
Average size of BHA and BHT capsules after lyophilization process for the different encapsulation conditions.

Microcapsules size μm (mean ± SD)[a]				
Crosslinking agent	Bloom	Time	BHA[b]	BHT
Formaldehyde	220	10	13.3 ± 13.4 a	12.4 ± 9.8 a
		60	22.1 ± 32.0 a	9.3 ± 9.2 a
		120	14.8 ± 14.0 a	50.5 ± 17.5 c
	240	10	12.7 ± 12.9 a	10.7 ± 5.7 a
		60	13.2 ± 11.8 a	10.9 ± 10.3 a
		120	12.9 ± 9.4 a	17.6 ± 12.7 b
Glutaraldehyde	220	10	17.5 ± 16.7 b	15.7 ± 15.0 b
		60	12.9 ± 10.1 b	12.1 ± 11.6 b
		120	16.3 ± 21.2 b	16.3 ± 14.5 b
	240	10	11.8 ± 9.9 b	15.3 ± 12.7 b
		60	11.4 ± 9.6 b	16.6 ± 15.0 b
		120	17.2 ± 15.8 b	17.9 ± 15.6 b
Without crosslinking agent	220		9.8 ± 10.2 c	17.3 ± 15.0 b
	240		12.2 ± 13.3 a	11.5 ± 8.8 a

[a] Mean values based on fifteen data. SD: standard deviation.
[b] Values in the same column with different letters are significantly different according to LSD test ($p < 0.05$).

and F-B240 capsules. In relation to BHT lyophilized microcapsules, the kind of crosslinking agent used significantly affected ($p < 0.05$) their size with mean diameters of 18.6, 15.7 and 14.4 μm for F, G and WCA, respectively. However, size of BHT spheres prepared with F during 120 min (F120) was 78% higher than the reticulated during 10 and 60 min. Besides, mean size of microcapsules with F120 was three times larger when B220 was used. Capsules of BHA and BHT are shown in Fig. 1.

3.2. Encapsulation efficiency by absorbance

Encapsulation efficiency percentage (%EE) estimated for the different coacervation conditions assayed ranged between 74-84% and 78–90% for BHA and BHT, respectively. No statistical differences ($p < 0.05$) were found for %EE in either of the variables evaluated (time, crosslinking agent and gelatin blooms) (Table 3) (BHA 79.7; BHT: 82.7%). When it was analyzed the effect of reticulation time on %EE for both antioxidants, mean values estimated were 80.4, 78.9 and 84.3% at 10, 60 and 120 min, respectively. In general, microcapsules with 240 gelatin bloom had higher %EE (BHA: 81.8. BHT: 83.4%) than 220 bloom (BHA: 77.6. BHT: 81.9%). Taking into account the sort of crosslinking agent used, no significant differences were observed among them. On the other hand, a strong odor was conferrer to the microcapsules by the application of glutaraldehyde.

Table 3
ANOVA test. Effect of different factors (crosslinking agent-CA, gelatin bloom-B, reticulation time-T and their interactions) on the encapsulation efficiency percentage (%EE) of BHA and BHT.

Factors	DF	BHA		BHT	
		MS	F	MS	F
CA	2	0.32	0.01	9.96	0.24
B	1	124.44	3.13	13.64	0.33
T	2	9.52	0.24	42.88	1.05
CA*B	2	15.42	0.39	56.99	1.39
CA*T	2	86.45	2.17	19.72	0.48
B*T	2	14.48	0.36	16.37	0.40
CA*B*T	2	24.68	0.62	2.58	0.06
Error	14	39.78		40.97	

DF: degree of freedom. MS mean square. p (<0.01).

3.3. Permanence of BHA and BHT inside of microcapsules as affected by environmental factors

In view of the results obtain in the Sections 3.1 and 3.2 two microencapsulation conditions were chosen in order to perform the permanence experiment. Therefore, microcapsules containing 240 gelatin bloom with formaldehyde 10 min (F10) with and without (WCA) crosslinking agent were used to determinate the BHA and BHT levels inside the capsule at different times and environmental conditions. The %EE of these capsules was

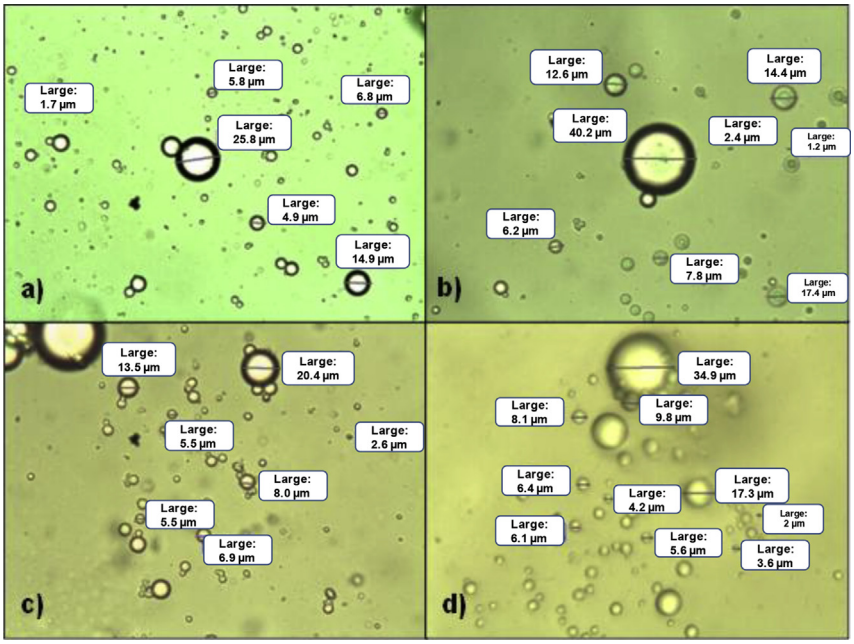

Fig. 1. Antioxidant lyophilized microcapsules performed with 240 gelatin bloom: (a) BHA without crosslinking agent (WCA); (b) BHA with formaldehyde10 min (F10); (c) BHT WCA and (d) BHT F10 (40×). Bars size: 1.2–40.2 μm.

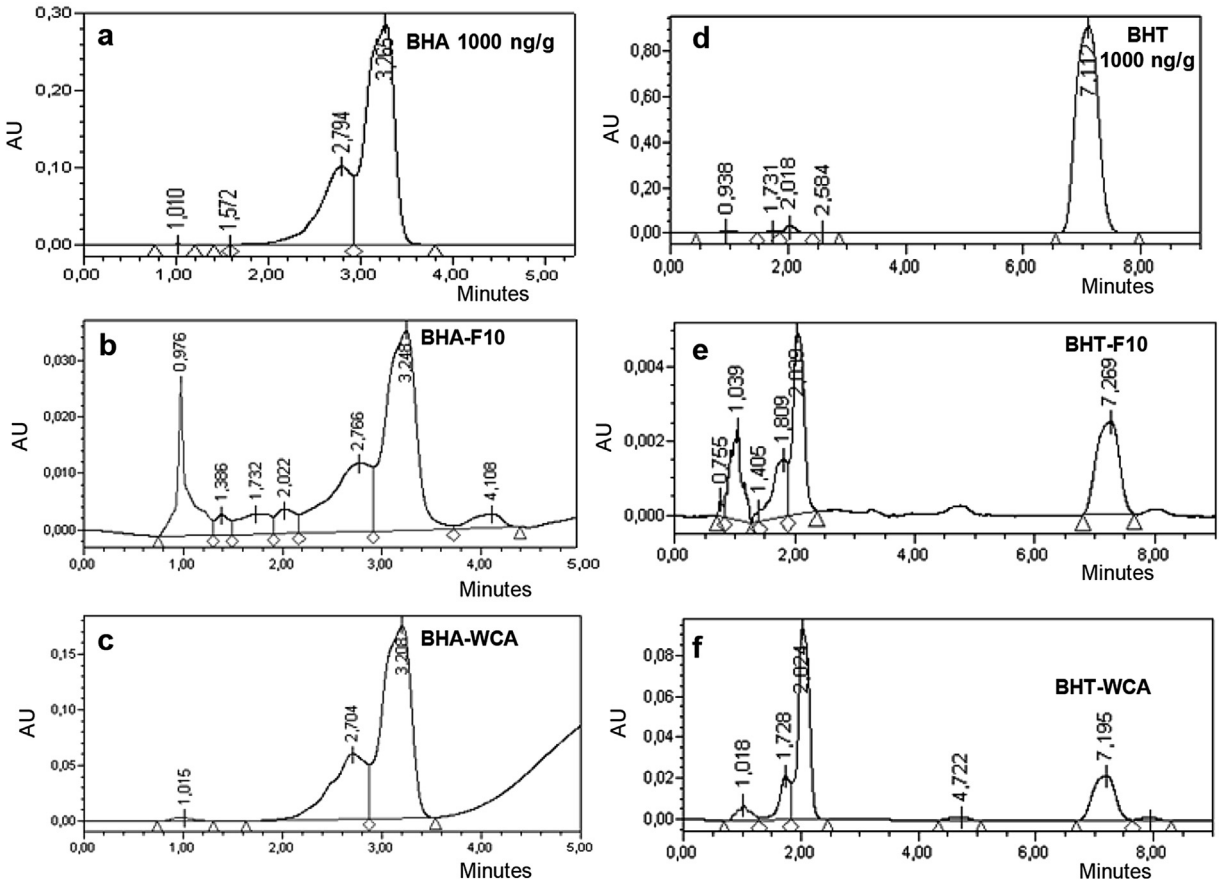

Fig. 2. HPLC chromatogram of a) 1000 ng/mL standard of BHA; b) BHA microcapsules with formaldehyde 10 min (F10); c) BHA microcapsules without crosslinking agent (WCA) and d) 1000 ng/mL standard of BHT; b) BHT-F10 microcapsules and c) BHT-WCA microcapsules. b) c) e) and f) corresponding to the last sampling of the storage peanut.

previously confirmed by HPLC where for both microencapsulation condition and antioxidants %EE was higher than 99% (data not show). Fig. 2 shows two chromatograms of BHA and BHT standard (1000 ng/mL) and two chromatograms of the antioxidants permanence in the microcapsules at the end of the assay of incubation for the both microencapsulation conditions.

3.3.1. Assay on antioxidant formulation

Fig. 3 shows Mt/MT as a function of time when formulations were stored at 25 °C. Mt is the mass of antioxidants inside of microcapsule at time t and MT is the sum of the antioxidant mass accumulated along the storage period.

In general the permanence of BHA inside the microcapsules showed the same behavior regardless of crosslinking agent assayed (Fig. 3 A1). Levels of BHA gradually decreased around of 64% for WCA and 50% for F10 microcapsules during the first 16 days, but a pick was observed at 23 days. At the end of the storage time (30 days), levels of antioxidant inside the capsules decreased in order of 99% and 40% for WCA and F10 microcapsules, respectively. But, still the concentrations inside the capsules were around of 201 and 14,425 ng/g for WCA and F10 spheres, respectively.

On the other hand, BHT levels inside the capsules were stable during the first 20 days in the order of 7000 ng/g, regardless of microencapsulation conditions evaluated (Fig. 3 A2). However, low decrease of antioxidant levels (43% and 28% for WCA and F10, respectively) was observed at 30 days of storage. Mean level of BHT inside the spheres subjected to different reticulation conditions at the end of the storage period was estimated at 4600 ng/g.

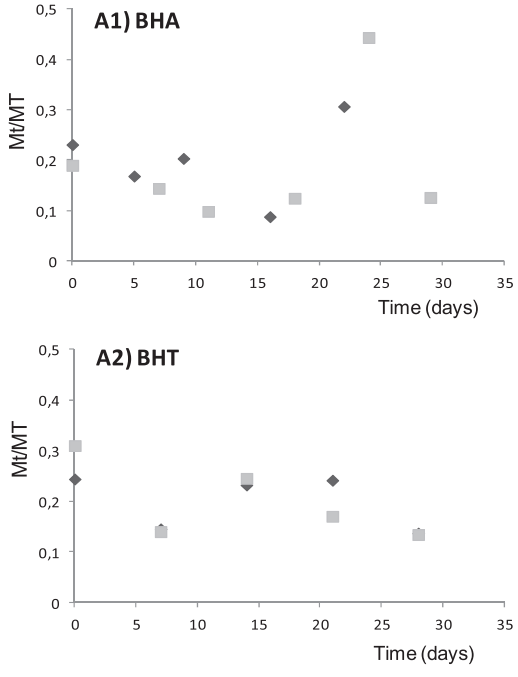

Fig. 3. Permanence of antioxidants in the formulated; microcapsules were ◆ WCA ■ and F10 stored at 25 °C.

3.3.2. Assay on antioxidant formulation in peanut

Figs. 4 and 5 show Mt/MT of microencapsulated BHA and BHT, respectively, as a function of time when the formulation were applied on peanut grains and stored at different temperatures and a_w. Level of BHA inside of WCA microcapsules began to reduce between 10 and 15 days around of 23.2 and 50% at 20 and 25 °C, respectively, regardless of grain a_w (Fig. 4, a–c). However, at the highest temperature assayed (30 °C) a minor stability of all microcapsules was observed as increased the substrate a_w. Under this temperature, levels of BHA were between 28 and 90% higher at the lower a_w (0.65; 0.75; 0.85) at the last sampling period. In general, final permanence levels of the antioxidant was around 687.4–1597.7 ng/g. When it was analyzed BHA levels inside the microcapsules reticulated with F10, a stable behavior at all storage temperature and a_w assayed was observed (Fig. 4, d–f). The antioxidant levels that remained in the capsules were in order of 814, 706 and 641 ng/g at 20, 25 and 30 °C, respectively, during all incubation period, with levels between 464.5 and 1624.7 ng/g at the end of period incubation. In some treatments, levels of BHA inside the particles at the first sampling were lower than in the following.

Permanence analysis of BHT inside the microcapsules showed that in both kinds of spheres (F10 and WCA) antioxidant levels were reduced with time between 24-91% and 29–88% respectively, at the end of incubation period (Fig. 5) with levels of 71.0–856.6 and 15.6–505.1 ng/g, respectively, after the 80 days. Moreover, this behavior was not affected by any of the environmental factors assayed, except for WCA microcapsules at 30 °C. Under, this incubation condition, BHT permanence inside WCA capsules showed major stability along the sampling periods with mean levels of 736, 582, 844 and 326 ng/g at 0.65; 0.75; 0.85 and 0.95, respectively.

4. Discussion

Currently there is a great worldwide interest in finding new and safe antimicrobial compounds to prevent oxidative deterioration of stored and to minimize the damage caused by organisms such as fungi and insects. In previous studies carried out in our laboratory, the potentiality of BHA and BHT as antifungal, antiaflatoxigenic e insecticide in the peanut food system was demonstrated (Nesci et al., 2011; Passone, Funes, et al., 2008; Passone et al., 2007a, 2007b, Passone, Resnik, et al., 2008; Passone et al., 2009). However, the use of encapsulated antioxidants instead the free compounds can overcome the disadvantage of instability, decrease the unpleasant taste and to extend the bioavailability and shelf life of the compounds (Fang, Li, Liu, & Wu, 2010; Munin & Edwards-Lévy, 2011). Consequently, some researchers have recently focused on studying the microencapsulation of compounds with antioxidant properties such as lycopene, ascorbic acid, lutein and others (Comunian et al., 2013; Da Rosa et al., 2013; Medina-Torres et al., 2013; Rocha-Selmi et al., 2013; Xiao-Ying et al., 2011). In our research, both antioxidants were encapsulated by complex coacervation with gelatin-gum arabic system by using different gelatin blooms and reticulation conditions. The mean size of the particles varied from 10 to 22 μm and 9–50 μm for BHA and BHT, regardless of reticulation condition. These results are in agreement with other studies published in the literature, which cite variations between 1 and 500 μm for microcapsules produced by complex coacervation (Favaro-Trindade, Pinho, & Rocha, 2008). Similar particle diameters have been reported by Xiao-Ying et al. (2011), Rocha-Selmi et al., 2013 and Comunian et al., (2013). They observed size of particles ranged from 0 to 20; 61–144 and 51–83 μm for lutein, lycopene and ascorbic acid microcapsules, respectively, using the same

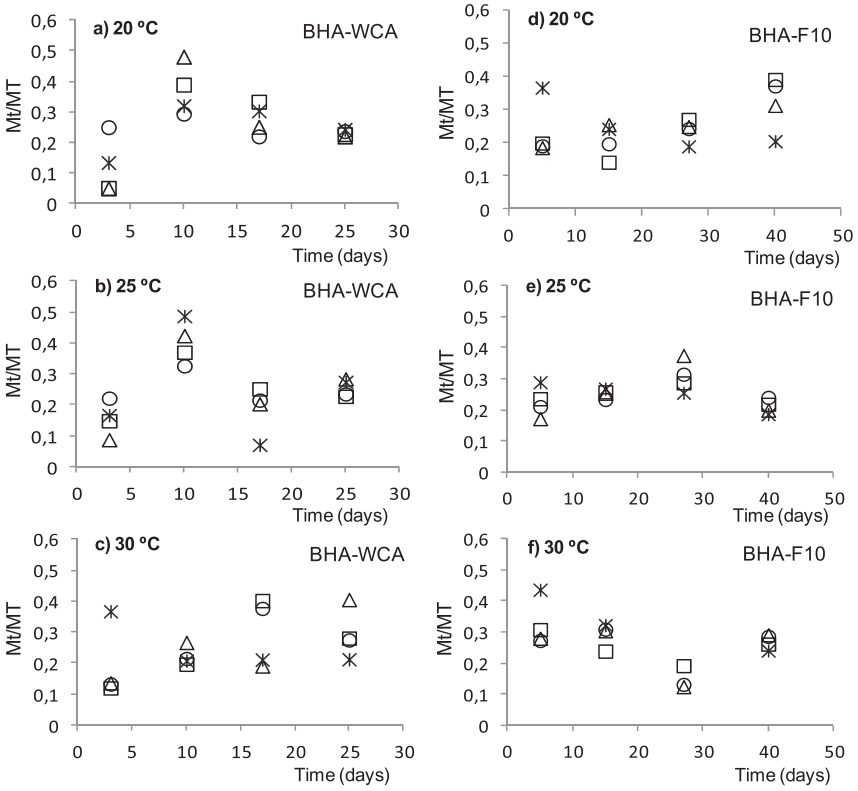

Fig. 4. Permanence curve of BHA inside the capsules: Mt/MT vs. time (days); stored at different water activities and temperatures in contact with peanut. ○ 0.65 a_w; □ 0.75 a_w; △ 0.85 a_w and ⁎ 0.95 a_w.

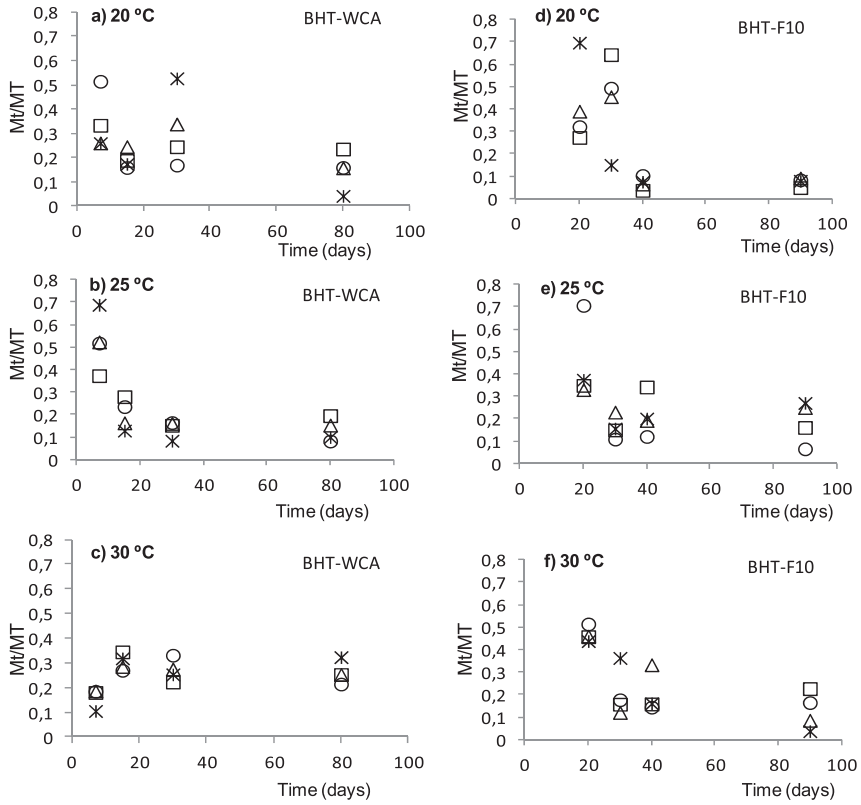

Fig. 5. Permanence curve of BHT inside the capsules: Mt/MT vs. time (days); stored at different water activities and temperatures in contact with peanut. ○ 0.65 a_w; □ 0.75 a_w; △ 0.85 a_w and ⁎ 0.95 a_w.

methodology. Size of microcapsules produced by this technique can be affected by different process conditions such as stirring speed, solution viscosity, core polymers ratio, among others (Inoue et al., 2002). In this work, according ANOVA test, BHA microcapsules size was no affected by any of the reticulation variables assayed. Similarly, Prata, Zanin, Ré, and Grosso (2008) found no differences among the diameters of microcapsules contained vetiver EO which were performed with glutaraldehyde, trasglutaminasa and without crosslinking agent. Although, in our study, liophylization process alone and in combination with the other reticulation variables (gelatin bloom, crosslinking agent and time) significantly influenced size of BHT microcapsule; these variations did not alter microencapsulation efficiency.

Encapsulation efficiency is a significant indicator to evaluate the quality of the microencapsulation process. On the one hand, coacervation technique selected in this work gave a high %EE for the both antioxidants studied (BHA-79.7% and BHT-82.7%). The distinctive characteristic of complex coacervation is that allow a great charge capacity (Gouin, 2004). Similar %EE, higher than 60%, has been recently reported by using this methodology. In these works, different compounds such as lutein, lycopene and ascorbic acid were encapsulated with %EE of 80, 61 and 98%, respectively (Comunian et al., 2013; Rocha-Selmi et al., 2013; Xiao-Ying et al., 2011). On the other hand, the reticulation variables assayed did not significantly affect the %EE. Solomon, Sahle, Gebre-Mariam, Asres, and Neubert (2012) showed similar results of %EE when evaluated the reticulation effect on citronella EO microcapsules by using different formaldehyde concentrations.

In this work, the permanence of antioxidants compounds was evaluated in the formulated and in microcapsules added to a substrate (peanut). For both assays, the antioxidant levels inside the microcapsule were highest in that reticulated with formaldehyde. This behavior could be due to the effect of crosslinking agent that when it is used increase the reticulation among molecules giving a major stiffness to the microcapsules and therefore obstructing the compound release. Similarly, Chang et al. (2006) observed that release rate of camphor oil microcapsules decreased as concentration and glutaldehyde exposure time increased. Besides, data of the present work showed that levels of BHA microencapsulated in the formulation in the capsules added to peanut were better maintained at 20 and 25 °C where antioxidant was released more slowly. Nevertheless, the permanence of BHT inside the capsule was indifferent to the temperature effect. According to results obtained by Rocha-Selmi et al. (2013) an increase in temperature did not lead to an increase in aspartame rate release, showing that microcapsules were relatively resistant to high temperature (80 °C). However, Yang et al., (2014) observed that after 30 days, vanilla oil still existed inside the microcapsules and the cumulative release rate were about 40, 55 and 75% at 25, 50 and 100 °C, respectively. Furthermore, as confirmed by the permanence experiment carried out on microcapsules added to peanut, stability and storage period of BHA and BHT were greatly enhanced and a durable controlled released effect and long residual action were achieved by microencapsulation. In this regard, after 40 days of storage, both antioxidants still remained inside the microcapsules that were reticulated with formaldehyde in levels ranging to 464–1624 and 50–501 ng/g for BHA and BHT, respectively. In a previous study, pure BHA and BHT were applied on peanut and the antioxidant residues were reduced in the order of 70–100 % at 35 days of incubation (Passone, Funes, et al., 2008).

Considering the proposed aims and results obtained, it can be concluded that i) this is the first work that attempts to encapsulate the food grade antioxidants (BHA and BHT); ii) the formulation using 240 gelatin bloom and formaldehyde as crosslinking agent

during 10 min was the suitable, because it showed high %EE and ensured the stability of BHA and BHT inside the capsules, regardless of temperature and a_w conditions assayed. This result allow us to assume that antifungal and insecticidal microencapsulates compounds may exert a protective effect on peanut for a storage period greater than 40 days. Further studies are being conducted in order to evaluate the functionality of antioxidant microcapsules as aflatoxin and insect inhibitors in peanut food system.

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