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Potential of microencapsulation to protect ascorbic acid under different temperature and pH during heating process

Running head stability of microencapsulated ascorbic acid

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ABSTRACT

Ascorbic acid (Vitamin C) is an important nutritional component which increases quality and technological properties of food but is naturally unstable. In order to increase the stability of ascorbic acid under a variety of processing conditions, the potential of microencapsulation has been investigated in this work. Six microcapsule formulations were prepared containing gum Arabic (GA), maltodextrin (MD) and chitosan at ratios of 4:1:1, 1:4:1, 1:1:4, 2:2:2, 3:2:1, 3:1:2, respectively. Encapsulation efficiency was varied depending on microcapsules composition and ranged from 40.5% to 80.4%. Microcapsules with higher efficiency were then further characterized by SEM and DSC. Finally, the retention of encapsulated ascorbic acid was evaluated under different simulated process conditions in term of temperature (50, 70, 90 ºC) and pH (3, 4, 5) over time (0 to 200 min). The rate of loss for ascorbic acid varied widely between samples and those encapsulated were more stable at different temperature and pH especially at 50 ºC and pH 5.

Keywords: Ascorbic Acid, Encapsulation, Microcapsules Stability, Encapsulation Efficiency

1 INTRODUCTION

Ascorbic acid (AA) is a bioactive compound essential for human health and probably the vitamin most widely added to food products. This vitamin includes a group of compounds that exhibit the biological activity of ascorbic acid (SARTORI *et al*., 2015) and this is plentiful in fruits and vegetables such as guava, orange, capsicum, kiwi fruit, strawberry, brussel sprout and cauliflower (GUPTA *et al*., 2015). Ascorbic acid is a water-soluble antioxidant which is highly sensitive to oxidation and then environmental factors like temperature, pH, oxygen, metal ions (such as like Cu2+ and Fe3+), UV and x-ray which can cause a problem in food systems, for examples color changes in the processing stage (WILLIAMS *et al*., 1998).

Given the growing interest in fortification of foods, stable ascorbic acid would be a good asset to fortify foods and microencapsulation can help to stabilize this vitamin. Microencapsulation has long been an established technique in the field of pharmaceuticals and its application in the field of food industries have raised dramatically in recent decades. This technique is the creation of a coating to avoid chemical reactions and/or to stabilize sensitive compounds (such as ascorbic acid) and to control release of the ingredients (DESAI AND PARK, 2005; WILLIAMS *et al*., 1998). This technique offers several advantages such as protection from the action of oxygen and humidity, increased bioavailability, and controlled core release. Controlled release overcomes the issue of inefficient use related to loss of additives during the processing steps and product storage (SARTORI *et al*., 2015).

There is little study on encapsulated ascorbic acid retention in model systems under conditions similar to what are used for processing of food products. Therefore, the objectives of this study was

i) to investigate the effect of the encapsulating materials (maltodextrin, gum Arabic and chitosan) at different ratios on the encapsulation efficiency of ascorbic acid,
ii) to select the best ratio in coating materials based on encapsulation efficiency and characterization of the microcapsules and finally
iii) to evaluate the potential of microencapsulation technique to preserve vitamin C in fortified foods under different temperature and pH during thermal processing such as pasteurization.

2 MATERIALS AND METHODS

2.1 MATERIALS

Encapsulating agents used, gum Arabic (spray dried), maltodextrin (DE 16.-19.5), medium molecular weight) 450 kDa (chitosan (deacetylation degree 90% as well as L-ascorbic acid were purchased from Sigma Aldrich (Steinheim, Germany). Citric acid, sodium citrate, 2,6 dichlorophenol indophenol, ethanol and methanol, oxalic acid and sodium carbonate were purchased from Merck (Germany).

2.2 PREPARATION OF MICROCAPSULS

Six microcapsule formulations were prepared and the effects of different wall material composition including gum Arabic, maltodextrin and Chitosan at ratios of 4:1:1, 1:4:1, 1:1:4, 2:2:2, 3:2:1, 3:1:2 were tested on the encapsulation efficiency of ascorbic acid.

Microcapsules are prepared by dissolving a blend of wall materials at mentioned ratios in deionized water at 60oC to give a total of 25% soluble solids. Ascorbic acid was separately combined with wall materials solution to obtain 5:1 wall to core ratio and allow to mixing well. The final solution were then frozen (-18oC) for 24 h and dehydrated by sublimation in a FD-5003-BT freeze dryer. The operation conditions were as follows: process time of 24 h, pressure of 0.08 mbar and condenser temperature of -38°C.

2.3 ENCAPSULATION EFFICIENCY (EE)

The encapsulation efficiency was determined as a difference between total mass of ascorbic acid detected in the capsules and that detected at the surface using 2,6-dichlorophenolindophenol (DCPIP) method according to AOAC official method (1984) as modified by Benassi & Antunes (1988) in which the metaphosphoric acid was substituted by oxalic acid. For total ascorbic acid measurement, microcapsules were macerated in oxalic acid (1% w/w) and after centrifugation at 2000 rpm (5 min), ascorbic acid content of supernatant was estimated. Ascorbic acid content at the surface was estimated by mixing microcapsules with methanol and then centrifuged at 2000 rpm for 5 min. Supernatant was titrated by standardized solution DCPIP. The encapsulation efficiency was calculated through the following equation:

$$\%EE = (\text{Total acid} - \text{Surface acid}) / \text{Total acid} \times 100 \quad (1)$$

Microcapsules with higher efficiency were then selected for further analyzes as described below.

2.4 MORPHOLOGICAL CHARACTERIZATION OF THE MICROCAPSULES BY SCANNING ELECTRON MICROSCOPY (SEM)

The external structure of freeze dried powders was tested by scanning electron microscopy (Eiko IB3, Tokyo, Japan). Microcapsules were mounted on the SEM stub by adhesive tape and coated with 20 nm thickness gold at 0.05-0.07 torr for 4 min. Then, microscope images were observed at 15 kV and a vacuum of 9.0 ×10-5 torr.

2.5 THERMAL CHARACTERIZATION BY DSC

Thermal characteristics of the microcapsules were determined using a Differential Scanning Calorimeter (SPA 449, NETZCH). The standard ascorbic acid was also analyzed in addition to the encapsulated samples. Appropriate amount of sample was weighed into an aluminum pan and hermetically sealed. The DSC instrument was calibrated with indium (m.p = 156.6 oC, ΔH = 28.5 J/g). An empty, hermetically sealed aluminum pan was used as reference. Nitrogen (99.999% purity) was the purge gas flowing at 30 ml/min. Samples were scanned over a temperature range of 25–250 oC at a heating rate of 5 oC/min.

2.6 STABILITY OF THE ENCAPSULATED ASCORBIC ACID AT DIFFERENT TEMPERATURE AND PH DURING HEATING TIME

Microencapsulated ascorbic acid (0.5 g) was poured into 50 ml buffer citrate solution (sodium citrate + citric acid, 0.1 M) at three pH level (3, 4, 5) and heated for 200 min at three temperature (50, 70 and 90o C) with occasional orbital shaking. Aliquot samples were taken every 20 min and residual ascorbic acid was measured by titration according to AOAC (1984) as described before. The analyses were carried out in triplicate. The stability of standard ascorbic acid in free form was compared with encapsulated samples at the same conditions.

2.7 STATISTICAL ANALYSIS

The analyses were run by a completely randomized design, and the process was repeated triplicate. The data obtained were analyzed statistically through the analysis of variance (ANOVA) and factorial tests using SAS (Statistical Analysis System) version 9.1 and the differences at ($p< 0.05$) were considered as significant.

3 RESULTS AND DISCUSSION

3.1 ENCAPSULATION EFFICIENCY

The influence of wall materials ratio on encapsulation efficiency was presented in Figure 1. Encapsulation efficiency was varied depending on the microcapsules wall composition and ranged from 80.4% to 40.5% with statistical difference ($p < 0.05$). The ratio of 4:1:1 GA/MD/chitosan exhibited the highest encapsulation efficiency (80.4%) as compared to all other wall materials combinations while the 1:1:4 ratio (larger amount of chitosan in coating materials) exhibited significantly low efficiency (40.5%).

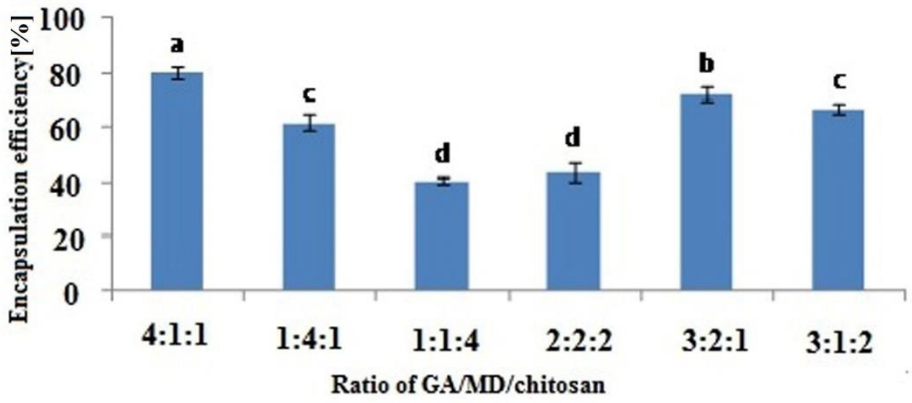

Figure 1 - Influence of the wall material formulation on encapsulation efficiency of ascorbic acid (Different letters show significant differences at $p<0.05$).

Therefore, there was a significant decrease ($p < 0.05$) in capsulation efficiency by decreasing the GA concentration in the wall composition. Gum Arabic was an important biopolymer chosen for encapsulation because of its recognized film forming and emulsifying properties (MADENE *et al.*, 2006). It has the ability to create a strong protective matrix around the core material. Then, this resulted in higher encapsulation efficiency values with increasing the gum concentration in coating material (McNAMEE *et al*. 1998).

Despite of low cast of maltodextrin, the increase in its content in the wall matrix reduced the encapsulation efficiency of ascorbic acid ($p<0.05$) probably due to its low viscosity even at concentrated solutions and low film forming capacity. However, the coating effect of maltodextrin in wall materials composition seems to be better than chitosan, since its effect on encapsulation efficiency reduction was less than chitosan content. The greatest effect on encapsulation efficiency was observed with changes in the amount of chitosan in the composition of the wall materials. Thus, at the ratio of 1:1:4 of GA/MD/chitosan, the lowest efficiency was observed (40.5%). This could be due to complex structures formed between macromolecular chains of gum Arabic and chitosan that

has a negative influence on the formation of microcapsules (TURGEON *et al*., 2007). Similarly, Dima *et al.* (2013) showed that encapsulation efficiency for coriander oil using only gum Arabic was more than chitosan and gum Arabic combination (82.6% and 79.1 %, respectively).

3.2 SURFACE MORPHOLOGY STUDY BY SCANNING ELECTRON MICROSCOPE (SEM)

The morphology of the microcapsules prepared with wall composition of GA/MD/chitosan at 4:1:1 ratio was studied by scanning electron microscopy (Figure 2). Overall, the particles exhibited larger size and resembled flake-like structure with sharp edges and brittle texture. The pores were clearly noticed in microcapsules due to sublimation of smaller ice crystal during freeze drying as indicated earlier (ANANDHARAMAKRISHNAN *et al.*, 2010). During freeze-drying, ice supported the frozen structure, and once ice removed by the sublimation, the GA/MD/chitosan encapsulates retained the pore structure.

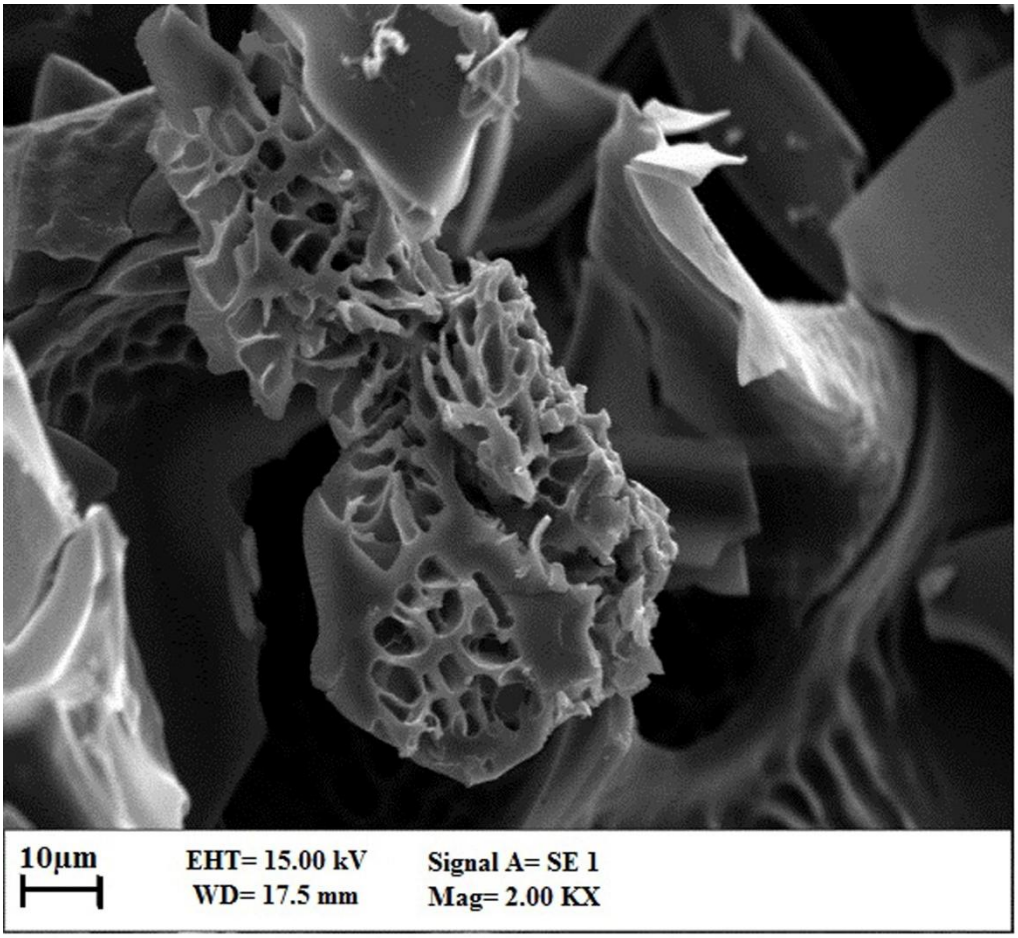

Figure 2 - SEM micrograph of microencapsulated powder.

Brazilian Journal of Technology

3.3 THERMAL CHARACTERIZATION BY DSC

Thermal behaviors of the free and encapsulated ascorbic acid were investigated to verify the formation of coating materials around the core. After coating, the total or partial disappearance of thermal events (melting point) corresponding to guest molecules is generally taken as a proof of complex formation (PRALHAD & RAJENDREKUMAR, 2004).

In Figure 3, thermograms of free ascorbic acid and the ascorbic acid/coating materials complex were reported. Ascorbic acid thermogram showed a sharp endothermic peak at (190 °C) corresponding to its melting point while the curve corresponding to encapsulated powder did not show any sharp endothermic peak in the range of the ascorbic acid degradation.

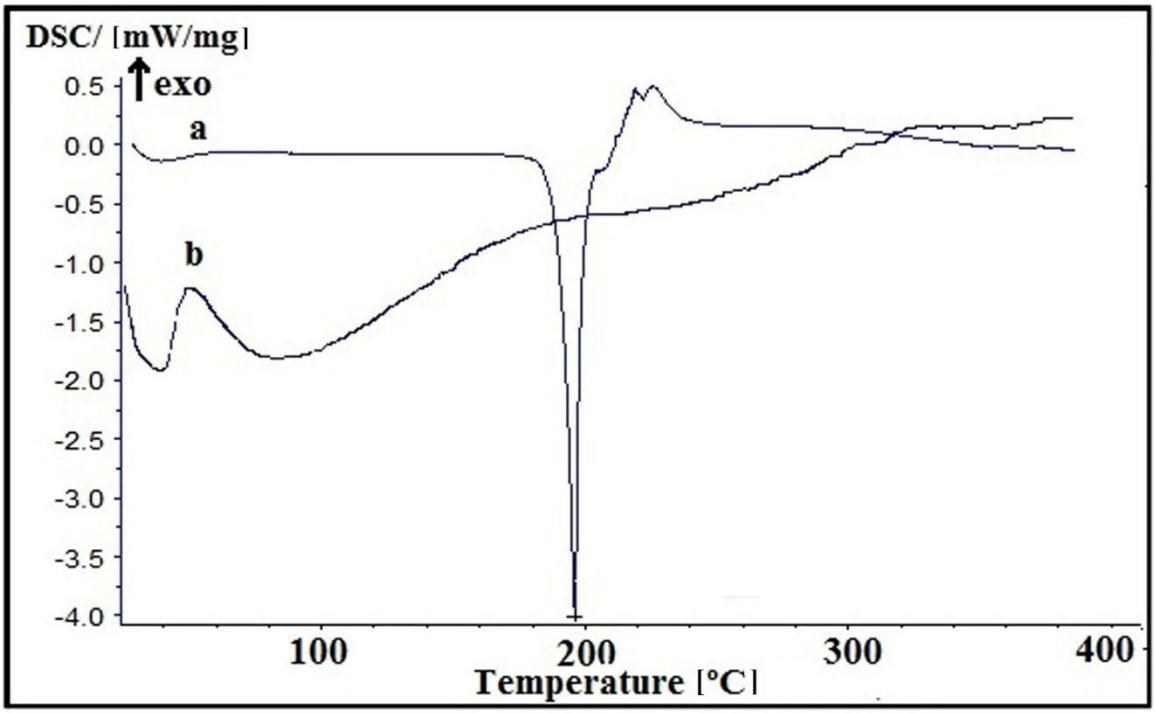

Figure 3 - DSC thermograms of (a) free ascorbic acid and (b) microencapsulated ascorbic acid.

Regarding that after encapsulation, the core material will be in the final system, either free or partially or completely encapsulated, therefore, the disappearance of the endothermic DSC signal corresponding to the core material is a strong evidence of the inclusion of ascorbic acid inside the wall materials. Bastianini *et al.* (2017) observed the same trend in capsulation of ascorbyltetraisopalmitate (VC-IP) with cyclodextrine. They reported that in the DSC curve of the cyclodextrin/VC-IP complex, only the signals of the cyclodextrin were present, suggesting a good interaction between the matrix and the VC-IP. Similar observation was reported by Devi & Kakati

(2013) during DSC analysis of ascorbic acid encapsulated within gelatin and sodium alginate microparticles. They did not show any characteristic peak of ascorbic acid in the thermogram of ascorbic acid loaded microparticles which was an indicator of ascorbic acid dispersion in the microparticles.

3.4 THE EFFECT OF TEMPERATURE, PH AND TIME ON STABILITY OF FREE AND ENCAPSULATED ASCORBIC ACID

3.4.1 Effect of Temperature

The influence of temperature on the stability of encapsulated ascorbic acid was presented in Figure 4. Temperature showed a significant negative effect ($p < 0.05$) on the stability of ascorbic acid in both free and encapsulated forms, whereas it did interact with pH and time.

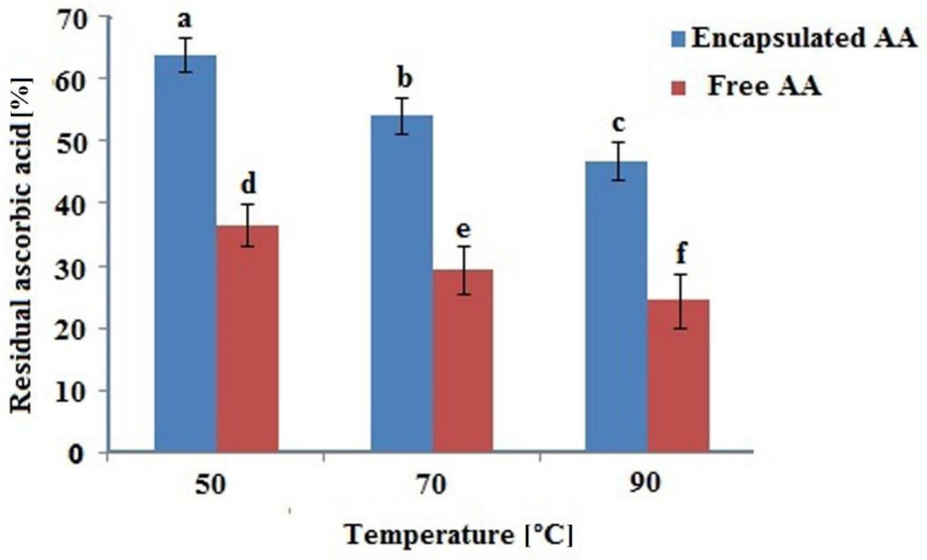

Figure 4 - Effect of temperature on stability of encapsulated and free ascorbic acid (AA). Different letters show significant difference at $p < 0.05$.

As expected, the losses were higher as the temperature was increased. Analysis of variance indicated that retention of free ascorbic acid was significantly different ($p<0.05$) at all temperatures so that the mean of residual ascorbic acid after 200 min thermal process at 50, 70 and 90 ºC were 36.5, 29.3 and 24.3%, respectively. However, the retention of ascorbic acid was notably higher (approximately twice) in capsulated samples (63.8, 54.1 and 46.8% at 50, 70 and 90ºC, respectively).

At 70 ºC and especially 90 ºC, most of the ascorbic acid was easily destroyed in free form which clearly shows that at such high temperatures the oxidation is rapid.

Interestingly, the thermal stability of the ascorbic acid at high temperature was markedly improved by encapsulation technique. This indicates that ingredients combination in the walls of the capsules provides enhanced protection of the ascorbic acid against degradation by temperature and this effect on stability may be of practical significance especially for some products which fortified with vitamin C before thermal process.

Similar observation was reported by Wills & Silalahi (1990) that studied the loss of vitamin C at 2, 20, 30 and 50°C over 12 weeks. The loss of vitamin C at 2°C was low but the rate of loss increased exponentially as the temperature was increased. It was reported that 50 % of vitamin had degraded at 20°C by 6 weeks whereas at 50°C a loss of 50% occurred after 3 weeks. Comunian *et al.* (2013) studied the stability of encapsulated ascorbic acid stored at 37°C and 20°C and observed approximately 57 to 80% and 32 to 44% retention of the original ascorbic acid after 30 days storage at 20°C and 37°C, respectively.

3.4.2 Effect of pH

According to Figure 5, the rate of oxidation was pH dependent and a maximum retention of ascorbic acid was observed at pH 3 (mean value of about 35%) in its free form while the distraction was more with the rise in pH up to 5 (mean residual amounts of ascorbic acid at pH 4 and 5 were about 30% and 25%, respectively).

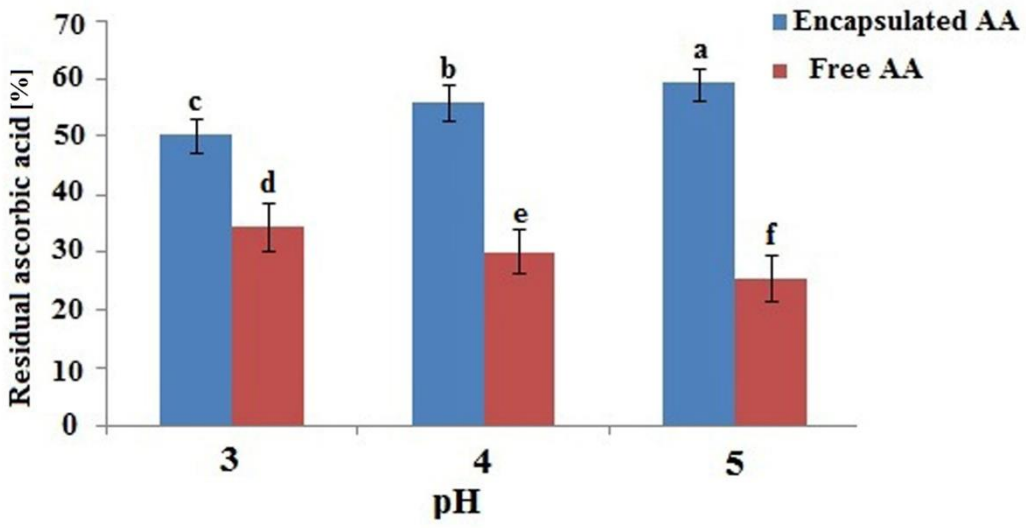

Figure 5 - Effect of pH on stability of encapsulated and free ascorbic acid (AA). Different letters show significant difference at $p<0.05$.

The highest stability at pH 3 can be ascribed to dominance of the fully protonated form of ascorbic acid at a pH under its first pKa (4.3) (Herbig & Renard, 2016). In some researches, ascorbic acid showed great thermal stability at the low pH of citrus fruits and it was not affected by the treatments (Sanchez-Moreno et al., 2003). On the other hand, degradation of encapsulated ascorbic acid at different pH was also observed but with an inverse trend. So that, the mean of residual amount of ascorbic acid was more at pH 5 (59%) and decreased by lowering the pH to 3 (retention of ascorbic acid was 55 and 50% at pH 4 and 3, respectively).

These observations can be due to the lack of resistance of the wall compounds to acidic conditions. Although maltodextrin and gum Arabic are stable to acidic conditions but chitosan is degraded via hydrolysis at the same condition because acid acts as a catalyst which splits the polymer chains. Also, because of the presence of amino groups in the chitosan structure, the pka is about 6.5, which cause it is protonated in acidic pH and hence be soluble in acid and water. Therefore, at lower pH, due to the greater destruction of the capsules wall, especially by heating, ascorbic acid is more abandoned and exposed to the environment oxidizing agents, especially oxygen (Nguyen et al., 2008; Ilina & Varlamov, 2004).

3.4.3 Effect of heating time

According to Figure 6 a direct relation was observed between time and ascorbic acid destruction. A significant ($p< 0.05$) decrease in ascorbic acid content was observed in the samples after 200 min heating time but encapsulated samples was less affected and the loss of ascorbic acid in microcapsules was lower than the free form. After 120 min of heating time, the microcapsules still maintained about 50% of the initial concentration of ascorbic acid while in free form a decrease of 50% was observed after only 20 min heating which proving the protective effect of wall materials on ascorbic acid retention.

The decrease of ascorbic acid content over time has been recently studied in a number of papers. Reports on vitamin C stability in some fortified foods and beverages stored for 12 months at 23 °C showed that its retention ranges was from 60 to 97% with the highest retention in solid foods and the lowest one in carbonated beverages (Steskova et al., 2006). Comunian et al. (2013) showed that ascorbic acid in its free form in solution was completely degraded after 15 and 30 days of storage at 37 °C and 20 °C, respectively. However, more than 50% of initial ascorbic acid concentration maintained in microcapsules after 30 days at 20 °C.

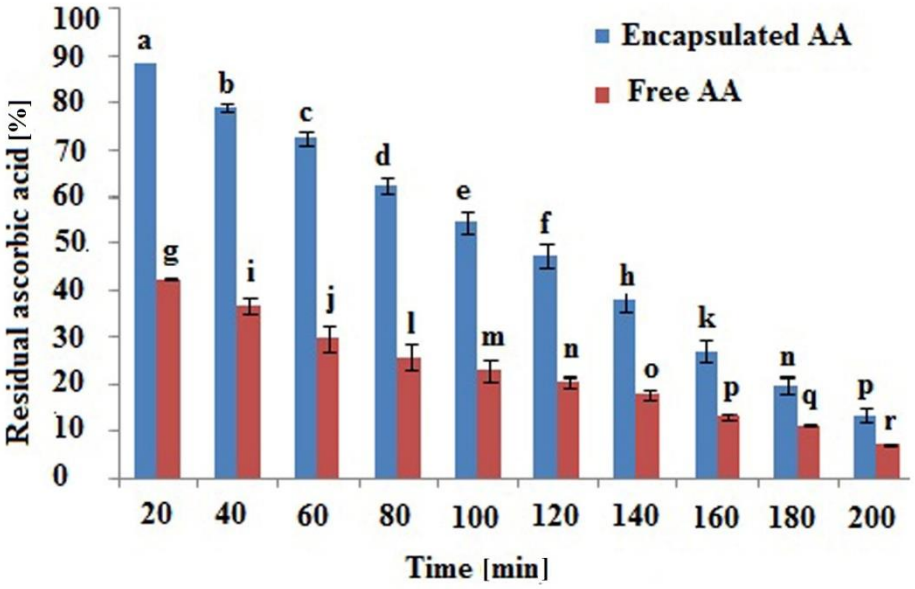

Figure 6 - Stability of encapsulated and free ascorbic acid (AA) over heating time. Different letters show significant difference at $p< 0.05$.

3.4.4 Effect of temperature and pH interaction

The effect of the pH and temperature interaction on encapsulated ascorbic acid degradation was presented in Figure 7. At all three pH, raising the temperature caused more damage to the vitamin. It can be seen that the impact of temperature on destruction of ascorbic acid was more pronounced at pH 4 and especially pH 3 compare to pH 5.

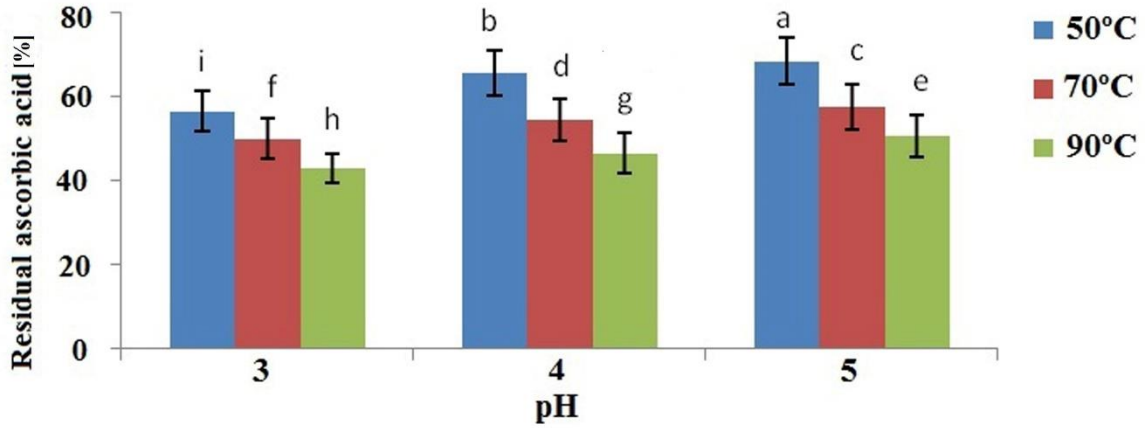

Figure 7 - Effect of temperature and pH on distraction of encapsulated ascorbic acid (AA). Different letters show significant difference at $p< 0.05$.

However, the most retention of ascorbic acid was observed at 50 ºC and pH 5 due to less damage to the walls of the microcapsules and consequently less vitamin release in the environment.

Farhang et al. (2012) encapsulated ascorbic acid by way of nanoliposomes prepared whit milk phospholipides, obtaining 28% and 4.3% retention of the ascorbic acid after 1 week at 4 °C or room temperature, respectively. Also, they showed that in spite of the lower physical stability of the liposomes at pH 3 compared with pH 7, degradation of ascorbic acid was approximately similar.

3.4.5 Effect of temperature and time interaction

The influence of temperature and time interaction on encapsulated ascorbic acid degradation was presented in Figure 8. It was observed that heating time affected the ascorbic acid content of all samples, as the heating time increases, the ascorbic acid content decreases. Temperature increase from 50 to 70ºC and 90 resulted in more destruction of ascorbic acid in the capsules. These findings highlight the gradual breakdown of the capsules structure over heating time which easily leads to ascorbic acid release into the water and then degraded by heat. Vitamin C degradation over heating time was more severe in free form because vitamin C is water-soluble and heat labile.

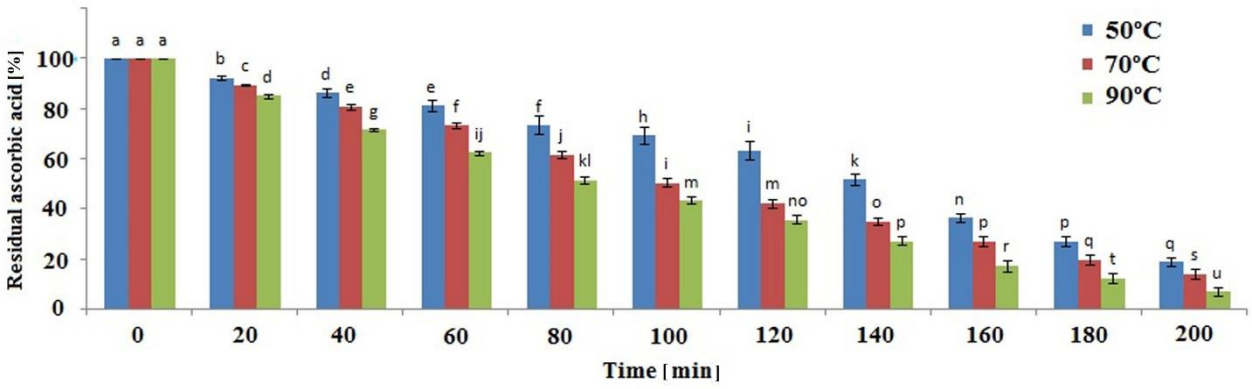

Figure 8 - Effect of temperature and time on distraction of encapsulated ascorbic acid (AA). Different letters show significant difference at $p< 0.05$.

3.4.6 Effect of time and pH interaction

The influence of pH and time interaction on degradation of encapsulated ascorbic acid was presented in Figure 9. At any time, pH increase from 3 to 5 resulted in more residual ascorbic acid in the capsules. The effect of pH reduction in the destruction of ascorbic acid was more pronounced at longer times. These findings highlight the instability of microcapsules wall at low pH because of chitosan dissolution and then the breakdown of the capsules structure which leads to more liberation of ascorbic acid and its exposure to oxidation process. However, it was observed that by encapsulation

more than 50% of mean ascorbic acid content was still protected after 100 min heating at all three pH.

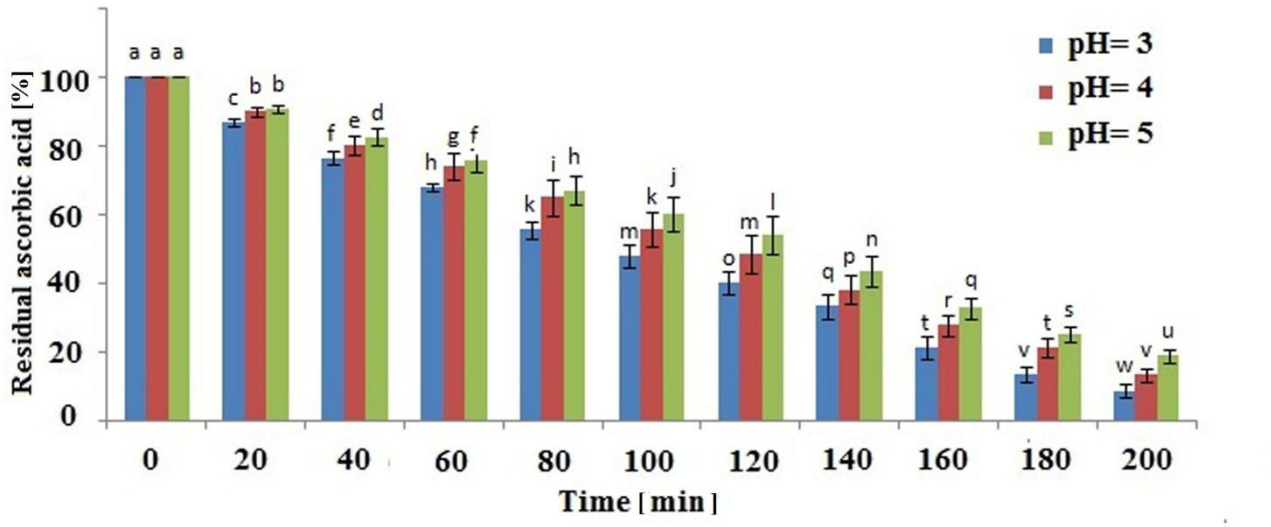

Figure 9 - Effect of pH and time on the stability of encapsulated ascorbic acid (AA). Different letters show significant difference at $p< 0.05$.

4 CONCLUSION

This study investigated the effect of wall materials (GA, MD, chitosan) with six combinations on encapsulation efficiency of ascorbic acid. It is concluded that higher ratio of gum Arabic gave enhanced encapsulation efficiency while the increase in the concentration of maltodextrin and especially chitosan did not offer enhanced efficiency. Thermal study at 50 °C, 70 °C and 90 °C showed that ascorbic acid degraded faster at higher temperature but its content in the encapsulated samples is still approximately twice as much as the free state. The loss of ascorbic acid in the microcapsules of all treatments increased with reduction in pH at the three selected temperature because of the breakdown of the microcapsules wall in the lower pH and it was greatest at 90 ºC and pH 3. Finally, based on the retention of ascorbic acid remaining after the thermal treatments at different pH values, it has been demonstrated that this micronutrient can be effectively preserved by this combination of ingredients in wall formulation in fortified foods after pasteurization process especially canned vegetables and fruit juices with pH higher than 3.

DISCLOSURE STATEMENT

The author(s) report no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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