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Short proceedings

Impregnation of Krill oil microcapsules into golden apple slices intercellular tissue†

César Antonio Ortiz Sánchez[1,*], Enrique Flores Andradre[1], Marisol Castillo Morales[1], Rafael Uzárraga Salazar[1], Nayeli Gutiérrez Casiano[1], Francisco Erik González Jiménez[1] and Ubaldo Richard Marín Castro[1,*]

[1] Facultad de Ciencias Químicas, Universidad Veracruzana, Prolongación Oriente 6, C.P. 94340, Orizaba, Veracruz, México; ibtrichard.castro@gmail.com; enflores@uv.mx; teregonzalez@uv.mx

* Correspondence: ibtrichard.castro@gmail.com, ceortiz@uv.mx

Abstract: Krill oil (KO) and the impregnation process to enrich food matrices with bioactive compounds have become relevant. The impregnation process of KO microcapsules into Golden apple tissue was evaluated. A KO emulsion was used at different temperatures to impregnate Golden apple slices. The results showed that an increase in temperature causes greater removal of water and penetration of the KO microcapsules. At 60 °C the volume changes of the samples were evident, obtaining the highest concentration of astaxanthin. Through the impregnation process, it is possible to produce a new food product with potential functional properties.

Keywords: Krill oil; emulsion; microencapsulation; functional food

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1. Introduction

In recent years, people have been interested in new and better food products that incorporate bioactive compounds in their formulation, encouraging the development of new techniques aimed at the development of functional foods.

Microencapsulation is a process where certain biomolecules are coated or protected by a material so they do not participate in detrimental reactions with the surrounding environment, some other benefits of microencapsulation include storage stability, controlled or retarded release of compounds, and volatile stabilization [1]. It has been shown that some functional compounds, such as oils, have beneficial health properties, however, their lipophilic nature makes it difficult to incorporate them into water-rich food matrices. One way to achieve the above is through impregnation, this process employs the osmotic pressure to introduce solutes from the hypertonic solution to the intercellular tissue of a food, partial elimination of the water contained is generated and at the same time is impregnated with a solute of interest (antioxidants, probiotics, oils, etc.), without affecting the integrity of a food [2]. Krill oil (KO) has been widely used and it has gained great relevance since it contains astaxanthin (AST), which is a biomolecule with great antioxidant power, additionally, it contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with proved beneficial effects on health [3]. The aim of this work was to evaluate the effect of temperature during the impregnation process of Krill oil microcapsules into Golden apple slices and generate a new food product with potential effects on human health.

2. Materials and Methods

2.1. Materials

Golden apples of commercial ripeness and sucrose were purchased in a local supermarket in Orizaba, Veracruz, Mexico. Whey protein concentrate was obtained from

Droguería Cosmopólita, Mexico. Krill oil was purchased from Natura Extracta, Mexico. Ethyl acetate was analytical grade.

2.2. Sample and emulsion preparation

A solution (30 % solids w/w) of whey protein concentrate (WPC) was homogenized at 5000 rpm for 10 min; emulsions were made at a proportion of 1:4 (KO:WPC) by adding drop-wise KO and dispersing it into the first solution. Then, the solids concentration of the emulsion was adjusted to 60 % (w/w) with sucrose. Finally, the emulsion was mixed for 30 min before being employed. Golden apples were manually peeled and axially sliced with dimensions of 36 mm diameter and 3.5 mm thick [2].

2.3. Impregnation process

Each golden apple plate was weighed and immersed into the sucrose-emulsion solution at 35, 45, and 60 °C keeping a 1:20 (w/w) plate-solution ratio for 120 min. Samples were withdrawn in regular time intervals, and the excess of solution from the surface was blot-dried using paper towels. The method reported by Azuara et al. [4] was employed to evaluate the kinetics of the process; water loss (WL in g/g fresh fruit) and solids gain (SG in g/g fresh fruit) were calculated with the next equations:

$$WL=s_1 t WL_\infty/(1+s_1 t) \quad (1)$$

$$SG=WL-ML \quad (2)$$

Where t represents the time of the impregnation process, s_1 is a constant related to the water lost, WL_∞ is the amount of water lost when equilibrium was reached and, mass lost is ML. WL_∞ and s_1 can be estimated with equations 3 and 4:

$$WL_\infty = 1/[p(1-(SG/WL)_m)] \quad (3)$$

$$S_1= 1/[bWFL_\infty(1-(SG/WL)_m)] \quad (4)$$

Constants p and b are the slope and intercept respectively of the t/ML vs t plot, subindex m means that WL and SG were calculated at the last point of each experiment using equations 5 and 6 where M_0 and M_f are the initial and final sample weight (g) respectively, as well as X_0 and X_f are the initial and final moisture content (wet basis).

$$WL/M_0=(M_0X_0-M_fX_f)/M_0 \quad (5)$$

$$SG/M_0=(M_0(X_0-1)-M_f(X_f-1))/M_0 \quad (6)$$

2.4. Analysis of samples impregnated with KO microcapsules

Initial and final moisture contents were obtained using AOAC method (23.003:2003)[5]. For AST content apple plates were withdrawn at 90 and 180 min of the process, grounded, and mixed manually with 15 mL of ethyl acetate for 10 min, then subjected to vortexing for 10 min at 5000 rpm, solvent phase was recuperated and its absorbance was recorded at 480 nm using a UV-Vis spectrophotometer (Thermo Scientific), astaxanthin concentration (µg/g) was determined with the next equation:

$$AST = AV10^4/ w A_{(1\%, 1cm)} \quad (7)$$

Where A is the absorbance in nm; V corresponds to the total extract volume in mL; w stands for sample weight in grams; $A_{(1\%, 1cm)}$ is the astaxanthin specific absorbance in ethyl acetate (2150 nm)[6].

3. Results and Discussion

The kinetics of WL during the osmotic dehydration/impregnation process are shown in Figure 1A, as can be seen, there is a greater WL as temperature increases, a similar behavior was observed by Jiménez-Hernández et al. [7] in mango impregnated with inulin

and piquin-pepper oleoresin, they argue that cell swelling and an increase in thermal energy due temperature enhance water removal. WL of apple plates were 0.713±0.006, 0.696±0.011, and 0.723±0.002 g water/g fresh fruit, respectively, after 180 min of impregnation. Flores-Andrade et al. [8] reported a WL value of 0.75 during the vacuum impregnation of *L. rhamnosus* microcapsules into apple slices, they observed that WL appeared to increase exponentially over the time process because the differences in osmotic pressure between the fruit and the sucrose solution.

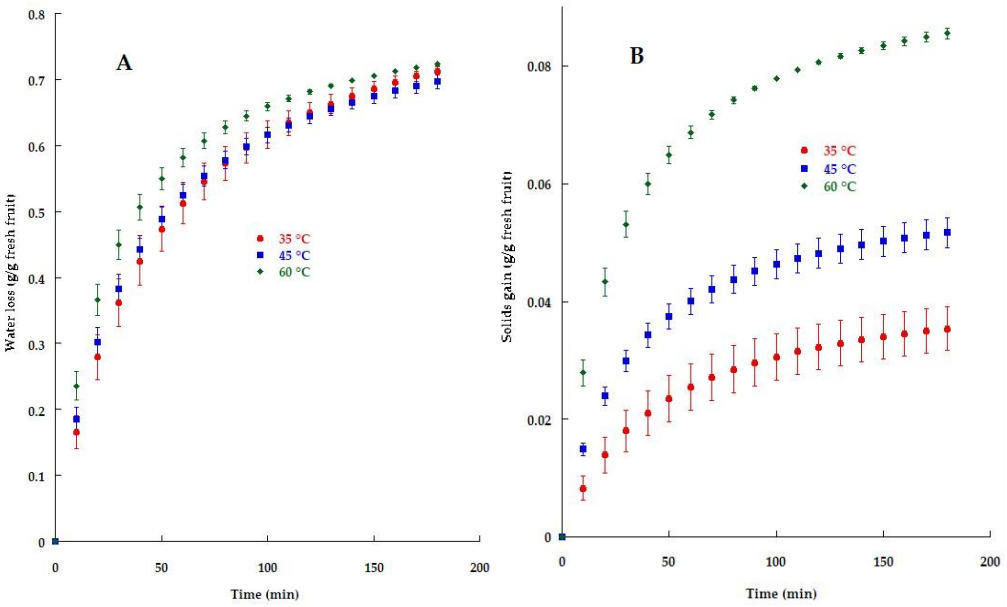

Figure 1. Water loss (A) and solids gain (B) kinetics during KO microcapsules impregnation of apple slices at different temperatures.

Solid gain kinetics can be observed in Figure 1B, there is a remarkable difference in SG when temperature increases, some reasons for that phenomenon are changes in cell membrane permeability and structural changes (compaction in the surface layers) that lead to a reduction of SG as well as WL.

Ahmed et al. [9] explained that during the solute impregnation process a cell submerged in a hypertonic medium will lose water due to plasmolysis, which starts with dehydration of protoplasm caused a cell shrinkage, leading to a plasmalemma separation from the cell wall; finally, the volume between the plasmalemma and cell wall gets filled with the osmotic solution due to the permeability of cell wall.

SG values for 35, 45, and 60 °C were 0.035±0.003, 0.051±0.002 and 0.085±0.001 g water/g fresh fruit respectively; closer values were reported by Jiménez-Hernández et al. [7] who obtained 0.03 and 0.05 g water/g fresh fruit when the temperature of impregnation process was 30 and 40 °C respectively; they observed that over the process time particle size of the impregnation solution has influence, large particles block pores on the fruit surface diminishing solid gain and producing lower internal resistance which favors WL by diffusion. Salazar López et al. [2] explained that fruit cells change their shape and reduce their size due to liquid loss, they observed that the impregnation of oleoresin into pineapple tissue was a consequence of the internal gas/liquid replacement in open pores by the external liquid phase as a result of hydrodynamic mechanisms promoted by pressure changes.

Figure 2 shows changes in volume and moisture content over the impregnation process, three stages can be observed, during stage one, the water contained in the extra and intercellular tissue is lost which causes that apple plates reduced their size; in the second stage, intracellular water is released leading to a protoplasm and vacuole size reduction, during this stage solute gain increases due the new intercellular spaces created among

cells with a consequent porosity increase; finally the structure of the food starts to collapse and a volume reduction occurs.

According to Chiralt and Fito [10] cell water loss not only influences volume changes but also the cell membrane since the lipid bilayer shows lyotropic and thermotropic mesomorphism, so a rupture of the bilayer structure of the membrane is observed and solutes diffuse freely to all parts of the tissue not only to the open cellular spaces. Volume changes are also affected by the kind of cellular packaging, in high porosity materials total volume reduction could be lower than a compact structure because a smaller reduction of the gas phase volume mitigates the total volume change.

Nieto et al. [11] reported a decrease in shape and volume in apple slices during glucose and sucrose dehydration, they observed that at short times of the osmotic process the steep loss of water resulted in a decrease of the initially fully turgid cell volume and folding and deformation of cell walls, other modifications at intercellular level are cell lumen deformation and shrinkage. As the osmotic process advances, slow relaxation of stressed cell walls may occur and the shape of the cells may become spherical.

Changes in volume may also be attributed to a reduction in the internal gases of the food material, they are mainly located in pores and apple tissue accounting for around 20 % of the total food material [8].

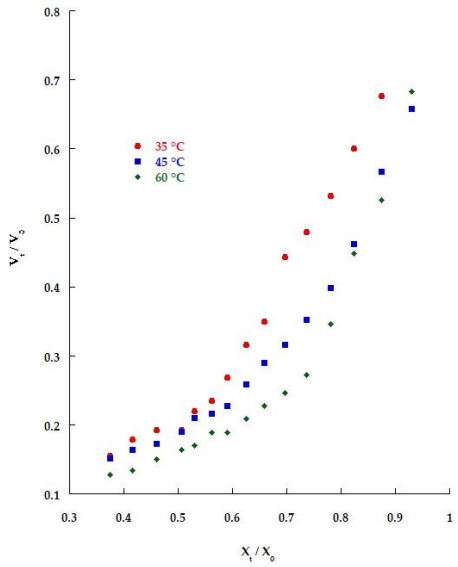

Figure 2. Volume changes during KO microcapsules impregnation of apple slices at different temperatures.

Figure 3 shows the amount of astaxanthin impregnated into apple plates, it can be seen that as the temperature and time process increased AST content did it as well, being 60 °C the one with the greatest AST content (1.4 μg/g).

Ertek et al. [12] observed that process temperature positively affects the total soluble solid content during the impregnation of strawberries with phenolic extracts. They argue that the most important factors that trigger mass transfer are concentration difference and process temperature.

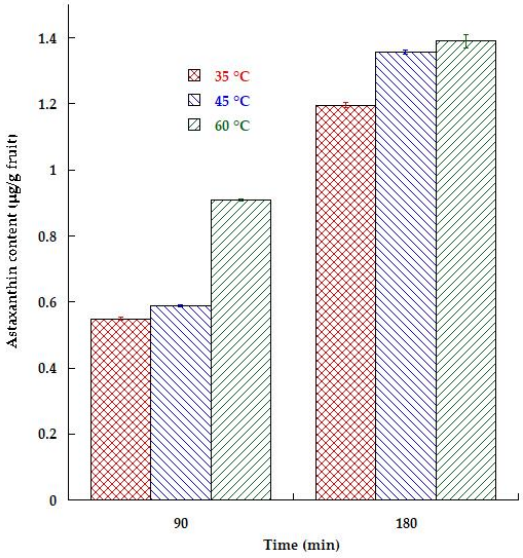

Figure 3. Astaxanthin content in impregnated apple slices at different temperatures and time process.

Salazar et al. [2] reported 8.0 ±0.4 g/kg of piquin pepper microcapsules impregnated in pineapple rings, later on, Rascón et al. [13] reported 109 CFU/g of *L. rhamnosus* impregnated in banana tissue. Differences in the amount of bioactive molecules incorporated into cellular tissue could be attributed to the porosity of the food material, when sample porosity is low cells are densely packed, and intercellular spaces are reduced, which difficult the solute uptake [10]. Apple pore diameter has been reported to be around 12 µm and the intercellular spaces in the range of 172 – 2297 µm, due to these characteristics it can host different bioactive compounds [14].

Osorio Gutierrez et al. [15] evaluated the impregnation process on apple slices, they compared the effect of an emulsion or a solution of sucrose-jamaica for solids intake, their results showed that solids intake was significantly increased when an emulsion was employed, therefore the final amount of bioactive compounds impregnated was higher. At the end of the 120 min they incorporated 4.33 and 0.6 g solids/100 g of dried fruit using an emulsion and a sucrose-jamaica solution, respectively. They also observed that, regardless of the osmotic solution used, a smooth surface was observed in the food and their cells had a reduction in shape and size because of the loss of water.

KO microcapsules impregnation into apple tissue can bring a new approach for developing a food product rich in antioxidants as well as essential fatty acids.

4. Conclusions

Impregnation of Krill oil microcapsules using osmotic dehydration is a feasible method that can help to incorporate lipophilic biomolecules into a hydrophilic medium. Temperature of the impregnation process positively augmented solids gain and water loss leading to a higher astaxanthin incorporation into apple tissue. The information about the process evaluated in this work can help in the development of enriched food matrices with possible health benefits for the human population. Further studies need to be done to evaluate the final product in terms of sensory acceptance and storage stability.

Supplementary Materials: No material.

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