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# Effect of vacuum on the impregnation of *Lactobacillus rhamnosus* microcapsules in apple slices using double emulsion

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ABSTRACT

Mass transfer during the incorporation of probiotics protected within a double emulsion $W_1/O/W_2$ using hypertonic solutions were analyzed. Osmotic dehydration (OD) and vacuum osmotic dehydration (VOD) were used to impregnate the fruit with probiotics. Fresh apple tissue was soaked in osmotic solutions at 40, 50 and 60 °Brix, using a ratio of 1:9 (w/w) emulsion: sucrose solution. The results showed that the survival of probiotics decreased with increasing osmotic pressure of the solution. Higher impregnation of microorganisms was promoted by the application of a period (20 min) of vacuum treatment. Afterwards the atmospheric pressure was restore. The number of viable cells in the osmodehydrated apple was in the range $10^6$–$10^8$ CFU/g d.b. Scanning electron microscope (SEM) images revealed the presence of microcapsules of 1–2 μm into apple tissue.



## 1. Introduction

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits on the host (AAVV, 2001). The international trend or scientific consensus suggests that probiotic food should contain $10^7$–$10^8$ CFU/g to exert a health benefit (Champagne et al., 2011). The beneficial effects of these strains on human health are three: the improvement of gut health, the lowering of blood cholesterol and the improvement of the defense mechanisms. One of the most studied probiotic strain is *Lactobacillus rhamnosus* LC705, which is originated from cheese. This strain has been used in the treatment of gastrointestinal infections and has been shown to adhere to human intestinal cells Caco-2 in vitro studies (Lehto and Salminen, 1997), to reduce the fecal azoreductase activity in the elderly people (Ouwehand et al., 2002) and urinary aflatoxine B–N7 which is one of the biomarkers for increased risk of liver cancer in young men (El-Nezami et al., 2006). In addition *L. rhamnosus* LC705 diminished caries associated salivary microbial counts in young adults (Ahola et al., 2002). To provide health benefits in prevention and treatment of certain disorders or digestive diseases, the suggested concentration for *L. rhamnosus* has been reported in the range of $6 \times 10^9$ to $10 \times 10^{11}$ CFU x 2/d of a dose (Aureli et al., 2011). In order to act as probiotic, the bacteria should be delivered alive to the intestine of their host. However, the recommended level of probiotic bacteria is not always delivered in commercial products because there are numerous factors affecting its survival, such as tritatable acidity, pH, hydrogen peroxide, dissolved oxygen, temperature, water activity and the presence of other cultures (Krasaekoopt et al., 2003). Gastric acidity and bile salts are major obstacles for the survival of probiotic bacteria after ingestion (Bezkorovainy, 2001). Therefore, novel approaches are demanded to improve the viability of microorganism.

Encapsulation methods have been used for protecting probiotics. As the population of microorganisms may be affected by encapsulation process itself, therefore it is important to select an adequate method for protecting them. The double emulsion may

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serve as a suitable wrapper to encapsulate and protect probiotic bacteria during food processing, storage and passage through the human gastrointestinal tract. Water-in-oil-in-water (W/O/W) emulsions are compartmentalized liquid dispersions in which oil globules, containing small aqueous droplets that are dispersed in an aqueous continuous phase. The presence of inner aqueous reservoirs separated from a continuous phase by an immiscible oil phase enables many potential applications, such as the entrapment of hydrophilic compounds, the partitioning of incompatible substances, the performance improvement of active compounds, the sustained release of chemical substances and the survival of microorganisms.

The active research in the area of osmotic dehydration and vacuum impregnation of fruit and vegetables is continuing all over the world. The former has been used as pretreatment to many processes used to reduce water activity of food materials and to improve nutritional, sensorial and functional properties. In order to make OD and VOD more attractive in economical and industrial terms, the incorporation of probiotics microencapsulated in double emulsions within the fruit was proposed. This application showed that probiotics microorganisms enter into the matrix structure of apple for the production of functional foods. In this study, $W_1$ was used to entrap the microorganism, oil phase was used to protect it and $W_2$ was used to solubilize the emulsion in the sucrose osmotic solution. Fruits and vegetables have been proposed as carriers for probiotic microorganisms, since they would create more convenient products for consumers. For this purpose, technologies such as vacuum impregnation are used for incorporating probiotics in the porous structure of some foods (Betoret et al., 2003; Fito et al., 2001). The double emulsions protect the microorganisms within the apple cells, making this approach potentially useful to elaborate products. It is hope that this study will contribute to a better understanding of incorporation of encapsulated probiotics within the fruit.

The objective of this work was to study the impregnation of apple slices of *L. rhamnosus* encapsulated in a double emulsion during osmotic dehydration (OD) and vacuum osmotic dehydration (VOD) and to evaluate the mass transfer during the impregnation.

## 2. Materials and methods

### 2.1. Materials

Granny Smith variety apples and refined sugar were obtained from a local market. The hydrophobic emulsifier was Grindsted PGPR 90 (fatty acid esters and polyglycerol polyricinoleate) and the hydrophilic emulsifier was Panodan SDK (esters of mono and diglycerides diacetyl tartaric acid), both purchased from Danisco Mexico, Inc. Whey protein concentrate (WPC 80) with a protein content of 80% dry basis (Amfer Foods, Mexico) was used as wall material. The oil phase used was grapeseed oil (Primex SA de CV, Mexico). The probiotic microorganism used was *L. rhamnosus* LC705 (Danisco, Niebüll, Germany) obtained from a lyophilized strain and agave inulin (Bestground, Guadalajara Mexico) was used as prebiotic. MRS broth and agar (Becton Dickinson, Mexico, DF) for activation and viable cell count was used.

### 2.2. Culture preparation

*L. rhamnosus* LC705 was rehydrated using 1% (w/v) inoculum in sterile MRS broth, and incubated for 24 h at 37 °C under anaerobic conditions. Cells were harvested by centrifugation at 900*g* for 10 min (R 320 Universal Centrifuge Hettich, USA). The supernatant was decanted, and the cells were re-suspended in 30 mL of MRS broth with 3.3% (w/v) inulin, obtaining a cell load of $9.98 \times 10^8$ CFU/mL.

### 2.3. Probiotic bacteria enumeration

The probiotic bacteria count was performed according to the methodology proposed by Homayouni et al. (2008), with slight modifications. Sample (1 g or mL) was diluted with isotonic saline (0.9%) and 100 μL aliquots of the dilutions were spread on MRS agar plates in triplicate. All plates for counting of *L. rhamnosus* were incubated at 37 °C for 48 h under anaerobic conditions. The mean was expressed as colony forming units per gram or mL of sample (CFU/g or mL).

### 2.4. Double emulsion preparation

The double emulsion water in oil in water ($W_1/O/W_2$), was prepared according to the methodology proposed by Pimentel-González et al. (2009) with some modifications. The emulsification process was made at 25 °C in two stages. In the first stage, water in oil emulsion ($W_1/O$) was formed with an aqueous phase of MRS broth ($W_1$) containing $9.98 \times 10^8$ CFU/mL; the oil phase was 62 g of grape seed oil. The total concentration of emulsifier in the $W_1/O$ emulsion was 8 g (1 part of hydrophilic emulsifier to 4 parts of lipophilic emulsifier). In the second stage, 30 g of the $W_1/O$ emulsion was dispersed in 70 g of an aqueous solution of whey protein concentrate (WPC) at the 14% (w/w). It was homogenized at 2800 rpm for 5 min (Silverson Homogenizer, Chesham, Bucks, England). At this stage, a double emulsion ($W_1/O/W_2$) was formed, wherein the microorganism was entrapped in the internal aqueous phase.

### 2.5. Particle size determination

After preparation of the emulsion $W_1/O/W_2$, the droplet size was measured using a laser diffraction particle size analyzer (LS 230 Beckman-Coulter, Bedford Hills, NY, USA). The measuring range of this equipment is from 0.04 to 2000 μm. All measurements were made in triplicate.

### 2.6. Microscopic observation of the double emulsions

Microscopic observation of the double emulsion was performed with an optical microscope with a digital camera (Motic BA-210, Motic China Group Co. Ltd., Hong Kong, China) coupled to an image analysis system (Motic Images Plus 2.0). Analysis was carried out on freshly prepared emulsion.

### 2.7. Solution preparation and osmotic process

Osmotic solutions were prepared using a mixture of emulsion: sucrose water solution with 1:9 (w/w) ratio. The final sucrose concentrations were 40, 50 and 60% with a probiotic load of $9.33 \times 10^7$ (CFU/mL). For the osmotic process, the apples were axially sliced with dimensions of 36 mm diameter and 3.5 mm thick. Each plate was weighed and immersed into the solutions of sucrose-emulsion at 35 °C maintaining a 1:20 (w/w) fruit-solution ratio. For osmotic dehydration at atmospheric pressure (OD), samples were removed from the solution at 20, 35, 50, 80, 140, 200, 260 and 320 min. In the VOD procedure, a period of 20 min of vacuum treatment was applied (114 mbar). Subsequently, the atmospheric pressure was restored and samples were maintained immersed in the solution until osmotic set times were reached. At the end of the osmotic process the excess of solution was removed with desiccant paper. The samples were weighed and the moisture content of the samples was determined using a vacuum oven at 70 °C for 24 h. The content of *L. rhamnosus* probiotic cells in the slices was measured at 35, 140 and 260 min of osmotic process.