Development of probiotic-enriched dried fruits by vacuum impregnation

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Abstract

In this study an attempt is made to combine the beneficial effects of probiotics with fruit and vegetables by applying the vacuum impregnation process. Apple cylinders were impregnated either with commercial apple juice containing *Saccharomyces cerevisiae*, and with whole milk or apple juice containing 10^7 or 10^8 cfu/ml of *Lactobacillus casei* (spp. rhamnosus). Impregnated apple samples contained around 10^7 cfu/g. In order to increase stability and to assure fruit preservation, impregnated apple samples were air dried at 40 °C to a water content of 0.037 kg water/kg dry matter and stored at room temperature for two months. The content of *L. casei* viable cells in dried and stored product was greater than 10^6 cfu/g. This concentration level of probiotics is similar to that in commercial dairy products.

Keywords: Probiotics; Lactobacillus; Vacuum impregnation; Enriched dried fruits

1. Introduction

Nowadays diet is perceived to be the most important factor contributing to health (Cathro & Hilliam, 1993). Development of foods that promote health and well being is one of the key research priorities of food industry (Klaenhammer & Kullen, 1999). This trend has favored consumption of foods enriched with physiologically active components (PAC) such as prebiotics, probiotics, vitamins and minerals, dietary fiber, fish oils and plant sterols. Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health (Salminen, Ouwehand, Benno, & Lee, 1999) or as live microbial feed supplements which beneficially affects the host by improving its intestinal microbial balance (Fuller, 1989). Probiotic bacteria for human nutrition usually belong to lactic acid bacteria or bifidobacteria groups. The beneficial effects of these strains on human health and well being are documented in experimental and clinical studies (Reddy, Roth, Eigel, & Pierson, 1988), offering to consumers three main health benefits: the improvement of gut health, the lowering of blood cholesterol and the improvement of the body's natural defense mechanisms. Scientific evidences suggest that probiotic bacteria, consumed at high levels (10^9–10^{11} cfu/day), can decrease the incidence, duration, and severity of some intestinal illnesses (Sanders, 1999). Dairy probiotic products should have a microbial level higher than 10^6 cfu/ml at the end of their shelf life (Ouwehand, Kirjavainen, Shortt, & Salminen, 1999). Currently, industrial probiotic foods mainly belong to dairy products, but lactose intolerance and the cholesterol content are two drawbacks related to their consumption. Nevertheless, some technological advancements have made achievable to alter the structural characteristics of fruit and vegetables matrices by modifying food components in a controlled way (Chiralt et al., 1999; Fito & Pastor, 1994; Fito & Chiralt, 2000; Fito et al., 2001). Technologies such as vacuum impregnation (VI) are being used for the incorporation of PAC into the structure of fruit and vegetables (Fito et al., 2001).

The objective of this work is to study feasibility of VI to incorporate two different microorganisms (*Saccharomyces cerevisiae* and *Lactobacillus casei*) into apple

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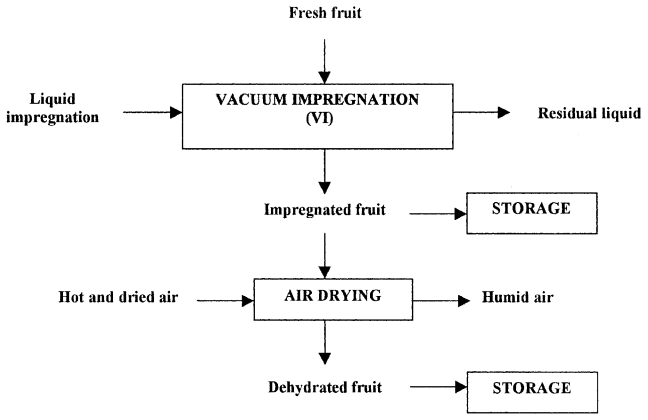

Fig. 1. Flow chart to produce probiotic-enriched dried fruit products.

fruit matrices, aiming to develop a procedure to produce dehydrated fruit products enriched with probiotics.

2. Materials and methods

Fig. 1 shows the flow chart to prepare apple products enriched with probiotics.

2.1. Cultures

S. cerevisiae, strain CECT 1347 and *L. casei* (spp. *rhamnosus*), strain CECT 245 were obtained from the Spanish Collection of Type Cultures (CECT).

2.2. Impregnation liquid preparation

Lyophilized cultures were grown on Saboureau (*S. cerevisiae*) or MRS (*L. casei* spp. *rhamnosus*) broth, respectively. Then, they were transferred to commercial apple juice (*S. cerevisiae*) and to either whole milk or apple juice (*L. casei*). When apple juice was used as culture media for *L. casei*, a pH value between 5.8–6.0 was maintained by adding 5 g/l of sodium bicarbonate. In all cases cultures were incubated at 26 °C for 48 h (*S. cerevisiae*) and at 37 °C for 72 h (*L. casei*).

2.3. Sample impregnation

Apples (var G. Smith) with adequate ripening from local market were used as raw material. Apples were peeled and cut into cylindrically-shaped samples (40 mm length and 18 mm diameter) following their vertical axis. Three samples were obtained from each apple and submitted to a VI experiment with the impregnation liquid obtained as described above. A vacuum pressure of 50 mbar was applied for 10 min, and then atmospheric pressure was restored leaving samples under the liquid for an additional 10 min period. Sample weight was monitored during the process to calculate VI parameters (the amount of liquid that has been incorporated to the sample or volumetric impregnation parameter (X), volumetric deformation of the sample (γ) and effective porosity (ε)) according to the following equation (Salvatori, Andrés, Chiralt, & Fito, 1998):

$$X - \gamma = \varepsilon_e \left(1 - \frac{1}{r}\right) - \frac{\gamma}{r}$$

2.4. Air drying and rehydration

Impregnated apple samples were dried for 48 h in a pilot scale air dryer at 40 °C under a flow rate of 4 m/s. Dried samples were rehydrated using milk or apple juice (pH = 5.8) in a ratio of 50 mL liquid/g sample at 25 °C for 24 h.

2.5. Microscopic observations

Cryo-SEM technique was used to verify introduction of impregnation liquid into intercellular spaces of apple tissue after VI experiments. A transversal section from a slice taken from the middle section of a cylinder was excised, mounted in stainless steel stubs, immediately frozen in liquid nitrogen slush, gold coated and observed by SEM using a JEOL microscope, model JSM-5410.

2.6. Probiotic-enriched dried fruit characterization

Moisture content, a_w and pH of the dried products were measured. Moisture content was determined by AOAC 20.013 method. Water activity was measured by a dewpoint hygrometer (Aqualab CX-2, 0.003 a_w).

3. Results and discussion

3.1. Microorganism location in the impregnated samples. Microscopic observations

Figs. 2–5 correspond to Cryo-SEM microscopic observations of apple parenchymatic tissue after the following impregnation treatments: control (no treatment) (Fig. 2), apple juice (Fig. 3), apple juice inoculated with *S. cerevisiae* (Fig. 4) and milk inoculated with *L. casei* spp. *rhamnosus* (Fig. 5). Dentritic structures observed in the intercellular spaces (IS) of impregnated apple (Figs. 2–5) show that gas has been replaced by impregnation liquid. The size of the IS (210–350 μm) (Lapsley, Escher, & Hoehn, 1992) allows microbial cells present in the impregnation liquid to enter these spaces. Accordingly, Fig. 4 shows yeast cells (*S. cerevisiae*) imbibed in dentritic structures in the IS. Accumulation of yeast cells can be observed in the narrowest zones of the IS, probably due to the impregnation liquid flow originated by pressure gradients created during the VI operation. In the

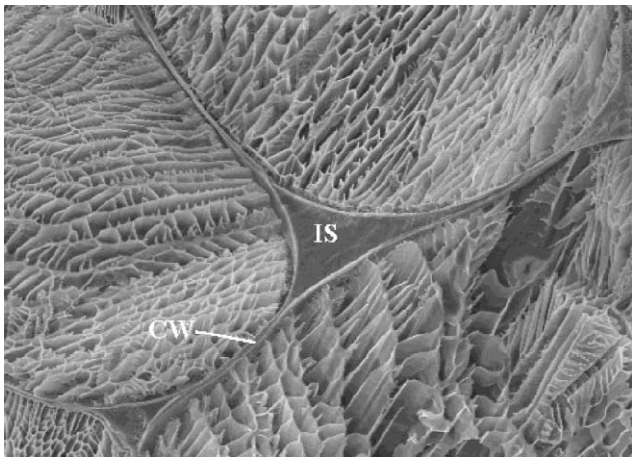

Fig. 2. Fresh apple microstructure (magnification: 750) (CW: cell wall; IS: intercellular space).

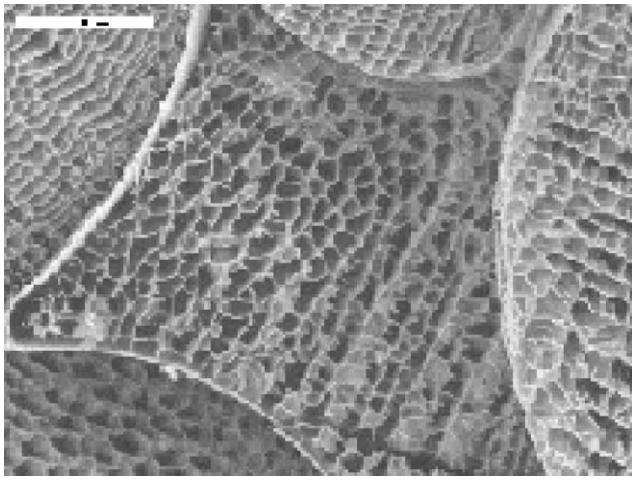

Fig. 3. Intercellular space in impregnated apple tissue (magnification: 1500) (CW: cell wall; IS: intercellular space full of impregnation liquid).

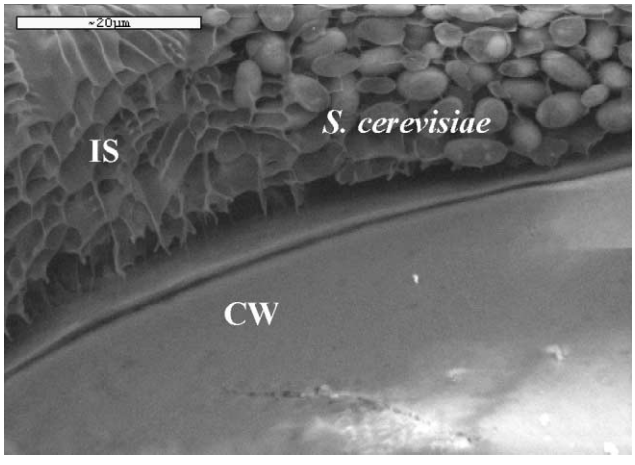

Fig. 4. *S. cerevisiae* in intercellular space of impregnated apple tissue (magnification: 2000) (CW: cell wall; IS: intercellular space full of impregnation liquid with *S. cerevisiae* cells).

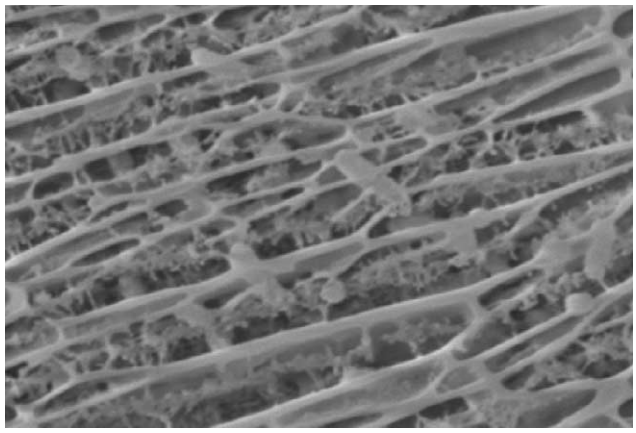

Fig. 5. *L. casei* in intercellular space of impregnated apple (magnification: 7500) (IS: intercellular space full of impregnation liquid with *L. casei* cells).

same way bacterial cells of *L. casei* may be observed in the dentritic structures of IS in Fig. 5.

3.2. Volumetric impregnation parameters and microbial content at each stage of the process

Table 1 shows results obtained in a typical experiment. Although yeast growth was not influenced by culture media, *Lactobacillus* strain showed a significant reduction in biomass production when milk or commercial apple juice were employed. As it can be observed, in all cases, the volumetric impregnation parameter value (X) was around 0.2 (20%). This value is similar to that obtained using sucrose isotonic solutions in a VI experiment by Betoret, Martínez-Monzó, Gras, and Fito (2001a,b). Microbial content, evaluated by plate count after sample homogenization, at each stage of the process are also shown in the same table. Values have been ordered to illustrate the effect of each treatment on microbial content. X-values and microbial plate count data were used to calculate the reduction in viable cell concentration of impregnation liquid entrapped into the different samples. VI operation appears to reduce microbial content of the incorporated liquid by one logarithmic cycle. Afterwards, air drying operation reduces microbial content by three additional logarithmic cycles. In spite of the observed reduction in microbial concentration of probiotic-enriched dried fruits with respect to the impregnation liquid, the viable cell content of the final products are high enough to consider the proposed process as adequate to develop dehydrated fruits with probiotic effect.

3.3. Storage of vacuum impregnated and air dried samples

VI samples were stored at 8 °C for six days. Microbial content was evaluated after three and six days of

Table 1
Impregnation parameters, number of microorganisms and some physico-chemical properties of impregnated apple products

Microorganism	Culture medium/product	pH	X[a]	γ[b]	ε[c]	X_w[d]	a_w	Number of micro-organisms
S. cerevisiae	Saboureau	5.1						2.1E+10 cfu/ml
	Apple juice	3.8						3.2E+10 cfu/ml
	Apple impregnated in apple juice	3.9	0.186	−0.01	0.204	84.14	0.984	2.8E+9 cfu/g
	Apple impregnated in apple juice, dried and rehydrated	4.0				3.74	0.32	4.3E+6 cfu/g
L. casei (spp. *rhamnosus*)	MRS	5.7						1.5E+11 cfu/ml
	Milk	4.3						2.6E+8 cfu/ml
	Apple juice	5.8						1.9E+8 cfu/ml
	Apple impregnated in milk	4.1	0.207	0.041	0.175	86.55	0.991	1.7E+7 cfu/g
	Apple impregnated in milk, dried[e]	4.1				4.40	0.373	8.3E+8 cfu/g[e]
	Apple impregnated in milk, dried and rehydrated	5.8				82.51	0.996	1.1E+5 cfu/g
	Apple impregnated in apple juice	4.1	0.194	0.004	0.199	86.98	0.984	4.5E+5 cfu/g
	Apple impregnated in apple juice, dried[e]	4.1				3.74	0.321	1.8E+8 cfu/g[e]
	Apple impregnated in apple juice, dried and rehydrated	4.1				82.3	0.982	6.28E+5 cfu/g

[a] Volumetric impregnation parameter.
[b] Volumetric deformation of sample.
[c] Effective porosity.
[d] Moisture content (%).
[e] Calculated by means of mass balance.

Table 2
Growth of microorganisms in apple impregnated with *L. casei* (spp. *rhamnosus*)

Apple product	Time (d)	T (°C)	Nunber of microorganisms (cfu/g)
Impregnated milk	0	20	1.71×10^8
	3	8	6×10^7
	7	8	1.65×10^7
Impregnated milk, dried	0	20	3.42×10^8
	15	20	8.21×10^9
Impregnated milk, dried, rehydrated	0	20	6.9×10^7
	15	20	1.2×10^6
Impregnated apple juice	0	20	4.5×10^6
	3	8	8.85×10^5
	7	8	2.43×10^6
Impregnated apple juice, dried	0	20	1.5×10^7
	15	20	3.1×10^9
Impregnated apple juice, dried, rehydrated	0	20	3.75×10^6
	15	20	2×10^5

storage. Dried samples were stored at 20 °C for 15 days. Microbial content of these samples was evaluated at the end of storage. Results are shown in Table 2. In all cases storage caused a reduction of microbial content of less than one logarithmic cycle, indicating that dried products kept an adequate microbial content to accomplish the required probiotic effect.

4. Conclusions

VI experiments using impregnation liquid inoculated with *S. cerevesiae* and *L. casei* spp. *rhamnosus* showed that it is possible to introduce microbial cells into structural matrix of fresh apple tissue by means of this technique. A process combining VI with air drying, is proposed for developing dried fruit products with probiotic effect. This process has successfully produced dehydrated apple with microbial content ranging 10^6–10^7 cfu/g. This level corresponds to average values usually found in commercial probiotic dairy products.

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