Journal of Food Engineering 110 (2012) 289–293

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

No invasive methodology to produce a probiotic low humid apple snack with potential effect against *Helicobacter pylori*

E. Betoret [a], N. Betoret [a], A. Arilla [a], M. Bennár [a], C. Barrera [a], P. Codoñer [b], P. Fito [a,*]

[a] Institute of Food Engineering for Development, Department of Food Technology, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain
[b] Department of Pediatrics, Obstetrics, and Gynecology, Facultad de Medicina y Odontología, Universidad de Valencia, Avenida Blasco Ibáñez, 15, 46010 Valencia, Spain

ARTICLE INFO

Article history:
Available online 28 April 2011

Keywords:
Probiotic
Lactobacillus salivarius spp. *salivarius*
Mandarin juice
Pineapple/grape juice
Vacuum impregnation
Helicobacter pylori

ABSTRACT

A probiotic low humid apple snack with potential effect against the infection caused by *Helicobacter pylori* has been developed from apple (cv. Granny Smith) and mandarin juice with a high microbial content of *Lactobacillus salivarius* spp. salivarius, by vacuum impregnation and hot air drying techniques. The moisture content reached in the final product ($0.144 \pm 0.012\ g_{water} \cdot g^{-1}_{sample}$) ensured stability, and although the drying process affected the microbial content, the concentration in the final product (9.486 ± 0.013) $\times\ 10^7$ CFU $g^{-1}_{dry\ sample}$) was sufficient to confirm that with this procedure it is possible to obtain a stable probiotic fruit with a low moisture content. Additionally, a preliminary *in vivo* test with five dyspeptic children was undertaken that suggested the possible effect of this new product on *H. pylori* as measured by a standard infection indicator.

1. Introduction

Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health (Salminen et al., 1999) or as live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance (Fuller, 1989). Scientific evidence suggests that probiotic bacteria, consumed at high levels (10^9–10^{11} CFU day^{-1}) can decrease the incidence, duration, and severity of some intestinal illnesses (Sanders, 1999). For example, it has been shown that *Lactobacillus salivarius* suppress *Helicobacter pylori* and reduces the inflammatory responses in probiotic mice better than *Lactobacillus casei* (Aiba et al., 1998) and *L. acidophilus* has been demonstrated to inhibit *H. pylori* better than other strains (Mrda et al., 1998; Canducci et al., 2000; Sheu et al., 2002). *H. pylori* is an etiologic agent of chronic gastritis and gastroduodenal ulcers and its inclusion by the International Agency for Research on Cancer in 1994 as a carcinogenic agent type 1, has turned it into one of the most interesting microorganisms in human pathology. In developing countries *H. pylori* can affect up to 90% of the population and its treatment is based on antibiotic therapy, but this has the disadvantages of being expensive, risks poor compliance, causes side effects and in particular, encouraging the emergence of resistance (Gold et al., 2000).

Currently, industrial probiotic foods are mainly dairy products, which have a microbial content in excess of 10^6 CFU cm^{-3} at the end of their shelf life (Ouwehand et al., 1999). However, lactose intolerance and the cholesterol content are two drawbacks related to their consumption. Techniques such as vacuum impregnation though, make it possible to obtain fruit and vegetables enriched with probiotic microorganisms or minerals (Betoret et al., 2003). Vacuum impregnation allows, by means of pressure gradients, the incorporation of components into the structural matrix of foodstuffs without substantially modifying its organoleptic properties (Patent P99 02730-5 titled "Procedimiento de impregnación de alimentos naturales con productos nutracéuticos y dispositivo para su puesta en práctica"). The pressure gradients created in this system and the capillary pressure at the entrance of the pores produces an important transference of gas and liquid between the solid and the impregnating liquid (Fito et al., 2001; Betoret et al., 2003; Cháfer et al., 2003; Alzamora et al., 2005). Following vacuum impregnation, fruit and vegetables are highly unstable due to their high water content, and therefore it is necessary to apply a preservation method to increase their shelf life.

The development of a functional food from fresh fruit or vegetables with high microorganism content is extremely interesting not only because it represents an opportunity within the functional food industry but also because it can be used to reduce the disadvantages and side effects of traditionally used treatments. In order

* Corresponding author. Address: Institute of Food Engineering for Development, Department of Food Technology, Polytechnic University of Valencia, Camino de Vera s/n, 46022 Valencia, Spain. Tel.: +34 96 387 7367; fax: +34 96 387 7056.
E-mail address: pfito@tal.upv.es (P. Fito).

0260-8774/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jfoodeng.2011.04.027

to commercialise a functional product it is necessary to prove scientifically, using both *in vitro* and *in vivo* studies its beneficial effects (Regulation CE N° 1924/2006). In the case of *H. pylori* infections, these studies should be directed towards reducing infection (Hamilton-Miller, 2003). To determine levels of infection, the breath test using labelled urea has been used extensively in studies (Michetti et al., 1999; De Vrese and Schrezenmeir, 2000; Sakamoto et al., 2001).

The aim of this research is to determine both adequate material and process conditions to develop a probiotic low humid apple snack with potential effect against infection caused by *H. pylori*.

2. Materials and methods

2.1. Bacterial cultures

L. salivarius spp. *salivarius* CECT 4063 and *Lactobacillus acidophilus* CECT 903 were obtained from the Spanish Type Culture Collection (CECT).

2.2. Food materials

Apples (cv. *Granny Smith*) from a local market were used to be vacuum impregnated. Peeled apples were cut into disc-shaped samples (5 mm thick, with a 65 mm external diameter and 20 mm internal diameter) following their vertical axis. Three samples were obtained from each apple and utilised for vacuum impregnation. Two commercial fruit juices, mandarin Don Simon® and pineapple/grape Don Simon®, were selected as the impregnation liquid in order to minimise the effects on the characteristics of the fresh fruit.

2.3. Impregnation liquid preparation

Lyophilised cultures were recovered following growth on MRS broth for 24 h at 37 °C. The cultures were then transferred into mandarin and pineapple/grape juices where the pH of the juices had been adjusted by the addition of sodium bicarbonate pH 5, 5.5 and 6. MRS broth (4 ml) with microorganism content of 10^9 CFU/ml were added to 1 L of juice. The inoculated juices were grown for 24 and 48 h at 37 °C resulting in 12 different growth media: 6 mandarin and 6 pineapple/grape juices. The mandarin juice inoculated with *L. salivarius* spp. *salivarius* at pH 6 and after 24 h incubation was achieved with maximum microbial content and was selected as impregnation liquid. The values stated are the average of five replicates.

2.4. Physicochemical characterisation

Total soluble solids were measured in Brix units with a refractometer (ABBE ATAGO, NAR T3, Japan) at 20 °C and pH values were determined with a potentiometer (micropH CRISON, 2001). The density of the juices was determined with a liquid picnometer. Water activity was measured using a dewpoint hygrometer (DECAGÓN Aqualab CX-2, ± 0.003) and the water content was quantified by vacuum drying at 60 °C until a constant weight was achieved (method 20.013 AOAC, 1980). The values stated are the average of three replicates.

2.5. Microbial content

The microbial content was determined following growth in MRS agar mandarin juice and pineapple/grape juice with pH values of 5, 5.5, 6 were counted on double layer MRS agar following incubation for 24 h at 37 °C.

To determine the microbial content of the impregnated samples, 5 g of sample were mixed and crushed with 45 ml of buffered peptone water, following 10^{-1}–10^{-8} dilutions the lactobacilli were counted on double layer MRS agar after incubation for 24 h at 37 °C.

To determine the microbial content of the dried samples, these were rehydrated with mandarin juice (pH6) at a ratio of 50 $cm^3_{liquid} \cdot g^{-1}_{sample}$ at 25 °C over 24 h. Following rehydration, the microbial content was determined as for the impregnated samples. For the dried samples, the microbial content was estimated with the mass balance performed in the rehydration operation (Betoret et al., 2003). The values stated are the average of ten replicates.

2.6. Methodology to produce probiotic enriched dried apple

In the Fig. 1 it is shown the flow chart to produce probiotic enriched dried apple.

The vacuum impregnation experiments were performed on a pilot scale using equipment designed in the Institute of Food Engineering for Development of the Polytechnic University in Valencia (Spain) (Fito et al., 2001). A vacuum pressure of 50 mbar was applied for 10 min and then atmospheric pressure was restored. The samples were left submerged in the impregnation liquid for a further 10 min. The values stated are the average of ten replicates.

Impregnated apple samples were dried for 24 h using a pilot scale air dryer (Martin, 2002) at 30 °C under a flow rate of 4 m/s.

2.7. Preliminary in vivo study

A preliminary study was undertaken involving 5 children (3 girls and 2 boys, age 11 years) chronically infected with *H. pylori* and patients of the Gastroenterology Paediatric office in Hospital Universitario Dr. Peset in Valencia. The children's diagnosis was confirmed before commencing treatment by the breath test using labelled ^{13}C urea (commercial test TAU-KIT®) (Michetti et al., 1999; De Vrese and Schrezenmeir, 2000; Sakamoto et al., 2001). TAU-KIT is a breath test suitable for *in vivo* diagnosis of gastroduodenal *H. pylori* infection. Each soluble tablet contains 100 mg of ^{13}C-urea and excipients. During the 28 day study, 30 g of the dried product with a moisture content of 0.144 ± 0.012 $g_{water} \cdot g^{-1}_{sample}$ and microorganism content of (9.5 ± 0.2) × 10^7 CFU $g^{-1}_{dried\ sample}$ was supplied daily to each child. At the end of the study the urea breath test was repeated. The study has been done following rigorous ethical standards. An ethical approval was obtained by the Hospital Universitario Dr. Peset and the Universidad Politécnica de Valencia to carry out the study.

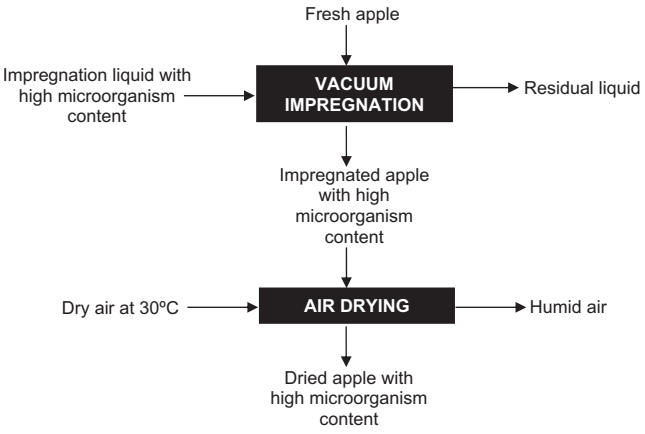

Fig. 1. Flow chart to produce probiotic enriched dried apple.