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# Effect of chestnut extract and chestnut fiber on viability of potential probiotic *Lactobacillus* strains under gastrointestinal tract conditions

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ABSTRACT

The main challenge to probiotics, during their passage through the gastrointestinal tract, are the acidic gastric secretions of the stomach, and the bile salts released into the duodenum. The survival of the strains, in this phase, is strongly influenced by the food used for their delivery.

This work is part of a project studying the development of novel food processes, based on the use of chestnuts from cultivar "Castagna di Montella". In detail, the effect of indigestible chestnut fiber and of chestnut extract on the viability of selected lactic acid bacteria strains was evaluated. Among 28 cultures, twelve strains were selected, on the basis of tolerance to low pH values and bile salts, and submitted to exposition to simulated gastric or bile juice in presence of chestnut extract with or without immobilization in chestnut fiber. The presence of chestnut extract proved to play a significant role on the gastric tolerance improvement of lactobacilli. The recorded protective effect could not be simply related to the starch or reducing sugars content. RP-HPLC demonstrated that in the chestnut flour, there are one or more hydrophobic peptides or oligopeptides, which specifically offer a marked resistance to simulated gastric juice, albeit present at low concentration. These beneficial effects proved to be dependent by the cultivar used to produce the flour.



## 1. Introduction

Due to the tremendous increase in public concern over health issues and the diversification of food products, the functional food industry needs to explore novel processes. In accordance with the worldwide accepted definition, functional food is coined to describe foods or nutrients whose ingestion leads to important physiological changes in the body that are separate and distinct from those associated with their role as nutrients (FDA, 2004). Fermented foods containing probiotic microorganisms are generally considered as functional foods. Probiotics are defined by the FAO/WHO as live microorganisms, which when administered in adequate amounts, confer a health benefit on the host and are widely used as a live microbial food supplement that can improve the intestinal microbial balance (FAO/WHO, 2001). Many nutritional and therapeutic benefits have been attributed to probiotic microorganisms, including metabolism of lactose, control of gastrointestinal infection, suppression of cancer, reduction of serum cholesterol, immune stimulation, and increased digestibility of foods (Collins et al., 1998). The therapeutic potential of probiotics is dependent upon a number of factors, but most important are their survival during manufacture, storage, transit through the gastrointestinal tract, and the ability to proliferate in the large intestine (Tannock, 1998). Selection of suitable strains must account for these factors (Saarela et al., 2000).

The main challenges presented to probiotics, during their passage through the gastrointestinal tract (TGI), are the acidic gastric secretions of the stomach, and bile salts released into the duodenum (Bezkorovainy, 2001). Several researchers have studied the survival of microorganisms in the presence of bile salts and at low pH, and it has become clear that the survival of probiotics, both *in vitro* and *in vivo*, is strongly influenced by the food used for their delivery (Charteris et al., 1998; Dunne et al., 2001). Buffering capacity, pH, as well as physical and chemical characteristics of the food carrier have all been demonstrated to be significant factors. Foods used for the delivery of probiotics are usually dairy fermented foods (Gomes and Malcata, 1999), however probiotics are

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also delivered in fruit drinks, sweetened milks, infant formula and confectionary (Patel et al., 2004).

This work is part of a project, focused on the development of novel food processes, based on the use of chestnuts (*Castanea sativa* Mill.), produced in the Irpinia district (Campania Region, Italy) and termed with the Protected Geographical Indication (PGI) "Castagna di Montella" (Blaiotta et al., 2012). The good adaptation of lactic acid bacteria in chestnut-base media suggests that the utilization of potentially probiotic strains, as starter culture in this substrate, would produce a fermented food with defined and consistent characteristics and possibly health-promoting properties (Blaiotta et al., 2012). The chemical composition of chestnut fruits have been recently reviewed (De Vasconcelos et al., 2010a) revealing the presence of various nutrients that are important for human health. Chestnut fruits are mainly composed of carbohydrates: primarily starch. The free sugar sucrose can be up to one-third of the total sugars, but several studies revealed the presence of several mono- and disaccharides (glucose, fructose, sucrose and maltose) as well as of fiber (De Vasconcelos et al., 2010a). On the other hand, chestnuts contain very small amounts of crude fat content that is low in saturated fatty acids and high in monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), which are known for their anticancer effects and for decreasing the risk of cardiovascular diseases and neurological function disorders (Whelan and Rust, 2006). Chestnut fruits also contain significant amounts of γ-aminobutyric acid (Tixier and Desmaison, 1980) and are a good dietary source of vitamins E, C, B1, B2, B3, pantothenic acid, pyridoxine, folate and of important mineral macro-(Ca, P, K, Mg and S), and micro (Fe, Cu, Zn and Mn) – elements (De Vasconcelos et al., 2010a). Moreover, the chestnut fruit content of phenolics (gallic and ellagic acid) have been linked with various positive health effects such as antioxidant effects, decreases in the risk of cardiovascular diseases, anticancer mechanisms and anti-inflammatory properties (De Vasconcelos et al., 2010b).

Actually, chestnut appears as a functional fruit and, moreover, due to the presence of non-digestible components of the matrix, chestnut might also serve as prebiotics. However, due to the complexity of chestnut composition, a systematic approach is needed in order to identify the factors which may enhance the growth and survival of probiotic strains, both *in vitro* and *in vivo*.

The overall aim of the present study was to evaluate chestnut-based substrates as probiotic carriers by examining the effect of indigestible chestnut fiber and of chestnut extract on the viability of selected LAB strains.

## 2. Materials and methods

### 2.1. Microorganisms and culture conditions

LAB strains used in this study were as follows: *Lactobacillus* (*Lb.*) *rhamnosus* VT1, RBM526, and RBT739 isolated from Parmigiano reggiano cheese and kindly provided by the Departmentof Agricultural, Environmental and Food Sciences (DIAAA, Campobasso, Italy), two *Lb. casei* (Lbg491, Lbg496), ten *Streptococcus* (*St.*) *thermophilus* (309, 43, 69, 82a, 80, 48, 381, 247, 75, 246) and ten *St. macedonicus* (67, 393, 72, 282, 71, 109, 335, 51, 395, 240) isolated from Provolone del Monaco cheese (Aponte et al., 2008); five *Lb. paracasei* subsp. *tolerans*, isolated from water kefir grains (1A, 4AM, 1S), commercial probiotic drink (1DM) and commercial probiotic tablet (4E); one *Enterococcus durans* strain from water kefir grains (2E); two *Lb. casei* coming from probiotic drink (1B) and probiotic tablet (5 EG), and, finally, one probiotic strain – *Lb. rhamnosus* GG from ATCC (ATCC 53103) (Table 1). Before experimental use, cultures were propagated twice in M17 (Oxoid, Basingstoke, United Kingdom) for streptococci or in MRS (Oxoid) for lactobacilli and enterococci.

**Table 1**
Tolerance to low pH and bile salts, expressed as percent of survival (%±sd), of strains used in this study.

| Taxon | Source | Strain | Survival percentage (% ± sd) | |
|---|---|---|---|---|
| | | | pH 2.50 | 0.30% oxgall |
| *St. macedonicus* | Provolone del Monaco cheese | 67 | 6.8 ± 1.6 | 30.8 ± 1.4 |
| | | **393**[a] | 78.8 ± 0.4 | 60.6 ± 1.1 |
| | | 72 | 48.8 ± 1.7 | 41.8 ± 1.4 |
| | | 282 | 40.0 ± 1.1 | 11.2 ± 0.6 |
| | | 71 | 8.7 ± 2.4 | 1.2 ± 0.9 |
| | | 109 | 9.1 ± 1.0 | 46.1 ± 0.8 |
| | | **335** | 95.0 ± 0.5 | 100.0 ± 0.5 |
| | | 51 | 17.7 ± 0.4 | 0.9 ± 0.8 |
| | | 395 | 45.9 ± 1.1 | 22.2 ± 1.1 |
| | | 240 | 5.8 ± 1.2 | 1.0 ± 1.3 |
| *St. thermophilus* | | 309 | 7.4 ± 0.8 | 28.7 ± 0.6 |
| | | 43 | 19.1 ± 0.2 | 0.4 ± 0.9 |
| | | **69** | 54.5 ± 0.8 | 49.1 ± 1.1 |
| | | 82a | 3.8 ± 0.5 | 13.9 ± 0.7 |
| | | **80** | 64.1 ± 1.8 | 12.8 ± 2.5 |
| | | 48 | 19.2 ± 4.0 | 15.4 ± 4.9 |
| | | 381 | 10.3 ± 0.8 | 0.1 ± 0.7 |
| | | 247 | 100.7 ± 0.4 | 3.2 ± 0.2 |
| | | 75 | 15.7 ± 1.3 | 4.7 ± 0.8 |
| | | 246 | 22.2 ± 1.7 | 3.5 ± 0.7 |
| *Lb. paracasei* subsp. *tolerans* | Water kefir grains | 1A | 54.8 ± 0.5 | 33.9 ± 0.8 |
| | | 4AM | 16.9 ± 1.4 | 54.2 ± 1.4 |
| | | 1S | 99.9 ± 2.2 | 19.5 ± 2.2 |
| *Enterococcus durans* | | 2E | 0.3 ± 1.1 | 10.2 ± 0.6 |
| *Lb. casei* | Probiotic drinks | 1B | 21.4 ± 1.1 | 60.9 ± 1.3 |
| *Lb. paracasei* subsp. *tolerans* | | **1DM** | 100.0 ± 0.7 | 100.9 ± 0.8 |
| *Lb. paracasei* subsp. *tolerans* | Probiotic tablets | **4E** | 64.6 ± 0.4 | 100.4 ± 0.6 |
| *Lb. casei* | | 5 EG | 42.5 ± 0.6 | 1.1 ± 1.3 |

[a] Strains in bold were selected for further analysis.

### 2.2. LAB strains survival under conditions simulating the human GI tract

The effect of low pH was examined by the method of Kimoto et al. (1999). Cells of LAB strains were harvested from MRS or M17 overnight cultures (5 mL) by centrifugation (1840 *g* for 10 min) and resuspended in 0.85% NaCl solution at pH 2.50 adjusted with 1 N HCl, and then held at 37 °C for 30 min. The number of surviving cells was determined by a direct plate counting on MRS or M17 agar. Saline solution at pH 6·00 was used as control. The sensitivity of strains to bile salts was tested on MRS or M17 agar plates containing 0.30% of Oxgall (Sigma, Milan, Italy), and on the unmodified media as control. In all trials, agar plates were incubated at 30 or 37 °C for 24–48 h, and the number of colonies counted. Each experiment was performed in triplicate.

### 2.3. Preparation of chestnut media and chestnut extracts

Chestnut flour from cv "Castagna di Montella" was used for the preparation of culture media. 5 g were mixed with 95 mL of distilled water under heating at 80 °C for 20 min and sterilized at 121 °C for 20 min before use as chestnut media (CM). The chestnut extract (CE) was prepared by centrifuging (5000 rpm, 10 min) CM to separate solids. Chestnut flour (protein: 4.61%; lipid: 3.8%; carbohydrate: 69.3) was kindly provided by Ipafood srl (Avellino, Italy).

### 2.4. Preparation of free cells and cells immobilized within chestnut fiber

Strains were grown overnight in MRS or M17, medium, for lactobacilli (incubation at 30 °C) and streptococci (incubation at 37 °C),