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Short communication

Determination of phenolic acid decarboxylase produced by lactic acid bacteria isolated from shalgam (şalgam) juice using green analytical chemistry method

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ABSTRACT

Lactic acid bacteria were isolated from Shalgam (Şalgam) juice samples and phenolic acid decarboxylase activity of the strains were examined by HPLC method. The isolates were identified with biochemical tests and according to the results a significant number of the strains were determined as *Lactobacillus plantarum*. The abilities of 21 selected isolates to decarboxylate p-coumaric, caffeic, ferulic, o-coumaric, gallic and sinapic acids were determined. Six isolates were detected to have phenolic acid decarboxylase activity. The strains GK1, GK3, GK5, GK11 and GK13 reduced both p-coumaric and caffeic acid, and all the strains except GK3 and GK12 could metabolize gallic acid to p-coumaric acid. Our results demonstrate that some important phenolics were produced by different lactic strains during Şalgam fermentation process and these strains could be declared as potential starter cultures. In this study phenolic acid decarboxylase (PAD) activity of lactic acid bacteria were also determined by HPLC method in which ethanol was used as a component of the mobile phase to avoid deleterious environmental side effects associated with acetonitrile. Accordingly, ethanol can be used as a component of the mobile phase instead of acetonitrile in the HPLC method for the determination of phenolics.

1. Introduction

Lactic acid bacteria (LAB) are industrially important organisms used in the fermentation of food products of plant origin due to their GRAS (Generally Recognized as Safe) status. These bacteria not only produce lactic acid but also preserve nutrients, vitamins of fruits and vegetables and are used as starter cultures to convert sugars into lactic acid and other end products which give the typical flavour to fermented products (Carr, Chill, & Maida, 2002; Leroy & De Vuyst, 2004; Madigan, Martinko, & Parker, 2003, Chap. 12; Ray & Panda, 2007, Chap. 5).

Phenolic compounds, important constituents of fermented vegetable products, are directly related to sensory characteristics of foods. The most important phenolic acids produced by several *Lactobacillus brevis* strains isolated from different origins were p-coumaric, ferrulic and cafeic acids (Curiel, Rodriguez, Landete, De las Rivas, & Munoz, 2010). These compounds are also beneficial to health due to their chemopreventive activities. Shalgam juice (Şalgam/turnip) is a fermented drink with health benefits attributed to its high antioxidant capacity and rich mineral and vitamin content. Şalgam juice is made with black carrot, wheat flour (setik), yeast, sourdough, turnip, rock salt (unrefined salt), spices and flavoured with çelem obtained by lactic acid fermentation. It is a red coloured, cloudy and sour soft drink mainly consumed in southern Turkey (Canbaş & Fenercioğlu, 1984; Erten, Tangüler, & Canbaş, 2008; Kabak & Dobson, 2011; Tanguler & Erten, 2012).

In recent years, several studies have been conducted on phenolics, which are known as beneficial compounds to health due to their antioxidant activities (Curiel et al., 2010; Öztan, 2006; Rodriguez et al., 2009; Erginkaya & Hammes, 1992). Phenolics are also known to play an important role in the prevention of cancer,

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Table 1
The retention times of phenolic compounds at 280 nm.

Phenolic compound	Retention time (minute)
Gallic acid	8,92
Caffeic acid	29
p-Coumaric acid	33,5
Sinapic acid	41
Ferulic acid	43,5
o-Coumaric acid	61

cardiovascular and some other degenerative diseases (Tangüler, 2010). However, there is a lack of knowledge pertaining to the efficacy of phenolic compounds on the growth of *Lactobacillus plantarum* and the other lactic acid bacteria.

The techniques used in the determination of phenolic compounds or the phenolic acid decarboxylase mostly depend on chromatographic methods. Among them HPLC is the most preferred method. In recent years, the field of energy has been heavily impacted by the use of green technology (De la Guardia & Garrigues, 2012, Chap. 1). The novel branch of analytical chemistry known as Green Analytical Chemistry (GAC) is also in demand recently as it provides inherent safety, non-toxicity, and environmental protection (Vaher & Kaljurand, 2012). Thus, there is an increased interest on the green methods in HPLC as well as to find alternative solvents and reagents which do not have persistent biocumulative and toxic effects (De la Guardia & Armenta, 2012). In analytical processes like HPLC assays, solvents are of vital importance because of their unique characteristics. Many studies have been focused on green solvents and their contribution to provide environmental safety. When selecting a green solvent, solute solubility, viscosity, compatibility and also cost are some of the factors that should be considered (De la Guardia & Armenta, 2012; De la Guardia & Garrigues, 2012; Vaher & Kaljurand, 2012; Young & Raynie, 2011). To date while we know the beneficial health effect of Şalgam, we do not have any commercial LAB starter cultures. While Şalgam is a traditional Turkish beverage, other fermented vegetable juice samples exists in the world. The studies conducted on this topic showed that *Lb. plantarum* is a significant potential starter culture for fermented juices. This strain also contributes some nutritional properties to end product during fermentation process [3, 18]. The aims of this study were: 1) to isolate LAB from Şalgam juice samples 2) to identify the strains isolated from Şalgam juice samples with biochemical and API 50 CHL test 3) to analyse the phenolic acids decarboxylase activity of isolated strains by HPLC. Additionally, ethanol as a component of the mobile phase was used in order to provide a green analytical alternative, as a replacement for acetonitrile. In this respect, this is one of the first studies conducted on using ethanol as a green solvent in HPLC analysis.

2. Material and methods

2.1. Isolation and identification of LAB strains

Nine commercial Şalgam samples were purchased from local markets and a total of 38 samples were taken from fermentation processes at two different Şalgam juice plants located in Mersin region. For this purpose, Şalgam juice samples were carried aseptically from factories to laboratory and 2.5 mL of the Şalgam samples were inoculated into 5 mL MRS broth (de Man Rogosa Sharpe Medium, Merck, Germany) and incubated 24 h at 30 °C. The proportion of 0.1 mL from growth cultures was transferred into MRS agar plates and incubated at the same conditions. (Harrigan & McCane, 1996). Typical colonies were randomly picked from MRS agar plates and transferred into MRS broth (Merck, Germany) medium for phenotypic characterization. These strains were

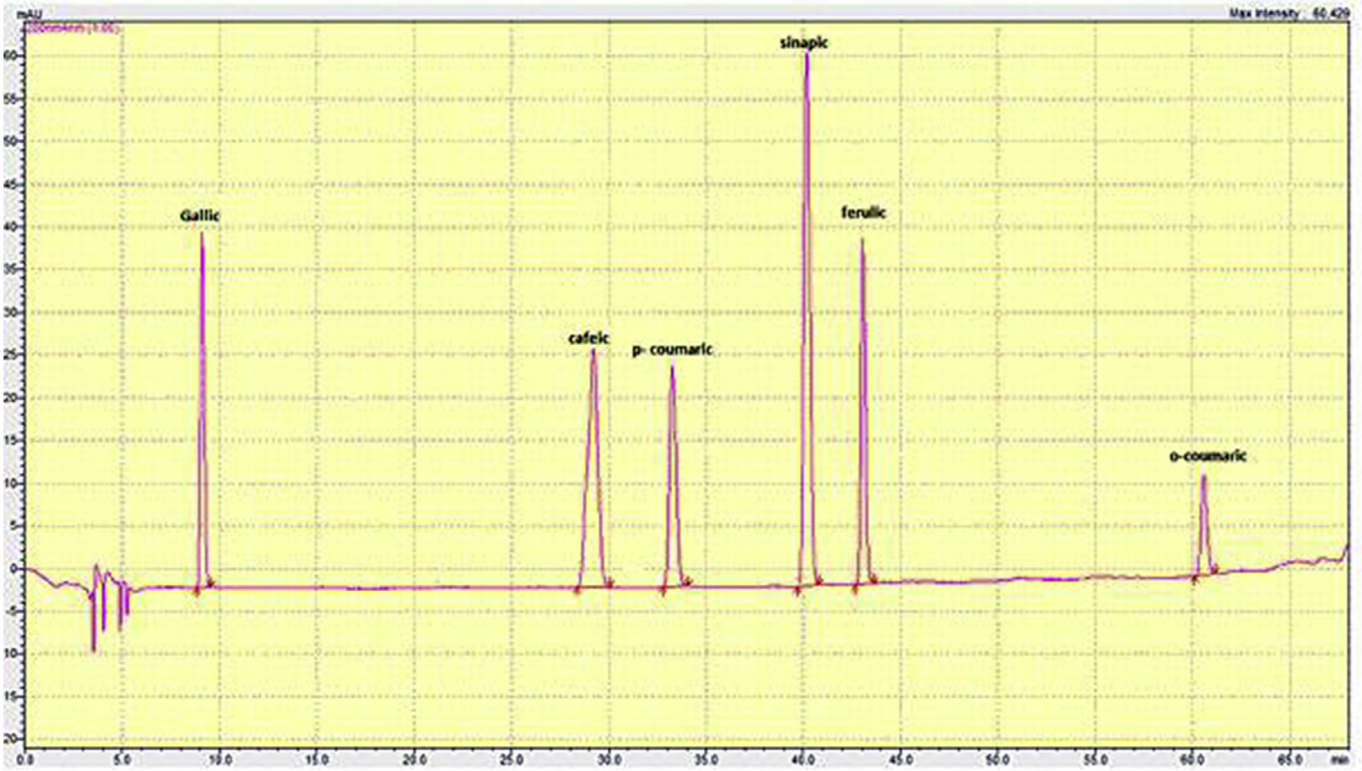

Fig. 1. The phenolic acid standard chromatogram at 280 nm and the retention times of the phenolic acids used in this study.