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Application of a microwave-assisted extraction method for the extraction of organic acids from Greek cheeses and sheep milk yoghurt and subsequent analysis by ion-exclusion liquid chromatography

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Abstract

A method for the determination of 13 organic acids (OA) in Greek cheeses and sheep milk yoghurt was proposed and assessed. The method was based on microwave-assisted extraction (MAE) of OA by an aqueous solution (10 mM) of sulphuric acid; extracts were subsequently analysed by ion-exclusion liquid chromatography associated with PhotoDiode Array detection (IE-LC–PDA). Chromatographic conditions (column temperature and mobile-phase composition) were screened to optimise separation. MAE operational parameters (extraction temperature, duration, and extractant molarity) were also evaluated. The effect of extractant molarity was not significant; however, the extraction temperature and duration exerted a significant effect ($P<0.05$) on the overall recovery of the majority of the target compounds. Recoveries >92% were found for most target analytes in cheese but recoveries of tartaric, succinic, valeric, and propionic acids were lower (67–78%). In the case of yoghurt, recoveries ranged from 78% to 125% but that of succinic acid was quite low (53%). The overall performance of the proposed MAE-based method, compared to the commonly used extraction method by stirring, was found to be superior in terms of accuracy and repeatability.

Keywords: Organic acids; Microwave-assisted extraction (MAE); Ion-exclusion chromatography; Greek cheeses; Sheep milk yoghurt

1. Introduction

Organic acids (OA) play an important role in the flavour of dairy products and they occur as a result of animal metabolism, fat hydrolysis, and bacterial growth (Marsili, Ostapenko, Simmons, & Green, 1981). Many authors have tried to correlate the age/ripeness of a cheese to the level of OA (Marsili, 1985; Bevilacqua & Califano, 1992; Fedio, Ozimek, & Wolfe, 1994; Careri, Spagnoli, Panari, Zannoni, & Barbieri, 1996; Hough, Califano, Bertola, & Bevilacqua, 1996; Akalin, Gönç, & Akbaşt, 2002; Lues & Bekker, 2002; Lues, Botha, & Smit, 1998a; Bouzas, Kantt, Bodyfelt & Torres, 1991). The OA profile was found to differ among cheese types. Also, the level of individual OA was found to vary according to the processing procedure, the ripening temperature and duration, bacterial counts, etc. The effect of the starter and non-starter lactic acid bacteria on the distribution of OA in cheese was investigated as well (de Llano, Rodríguez, & Cuesta, 1996; Skeie, Londberg, & Narvhus, 2001).

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Several methods have been applied so far for the determination of OA in food. Among them, high performance liquid chromatography (HPLC) found many applications as it allows the fast, sensitive, and nearly specific determination and involves uncomplicated sample treatment (Gomis & Alonso, 1996). In most methods applied in dairy products, an ion-exchange or ion-exclusion column was used (Marsili et al., 1981; Lues et al., 1998a; Skeie et al., 2001; Lues & Botha, 1998; Bouzas et al., 1991; Panari, 1986; Güzel-Seydim, Seydim, Greene, & Bodine, 2000), but the use of reverse-phase columns has also been reported (Akalin et al., 2002; Bevilacqua & Califano, 1989). Lues, Botha, and Smit (1998b) compared reverse-phase to ion-exclusion HPLC and concluded in favour of the latter.

Due to their high water solubility, the OA can be extracted from solid and semisolid samples by stirring the ground material with water, diluted acids, 80% acetonitrile, or 80% ethanol. Sulphuric acid solutions (0.4–92.5 mM) were used in most cases (Gomis & Alonso, 1996). A barium hydroxide/zinc sulphate extractant (Marsili, 1985) and a phosphate buffer/acetonitrile mixture (Bevilacqua & Califano, 1989) are also reported. Blending time applied varied from 1 min (Marsili, 1985) to 60 min (Bevilacqua & Califano, 1989) depending on the homogenisation speed. Lues et al. (1998a) compared the extraction efficiency of sulphuric acid (4.5 mM), barium hydroxide/zinc sulphate, and phosphate buffer/acetonitrile and found that the method using 4.5 mM sulphuric acid gave best recoveries for most of the OA.

Microwave ovens, initially used for sample digestion, have been also used for extraction, offering advantages like improved efficiency, reduced extraction time, low solvent consumption, and high level of automation compared to conventional extraction techniques (Eskilsson & Björklund, 2000; Buldini, Ricci, & Sharma, 2002). Microwave-assisted extraction (MAE) has been mainly used for extracting persistent organic pollutants and pesticides from a variety of matrices (Vryzas, Papadakis, & Papadopoulou-Mourkidou, 2002; Papadakis & Papadopoulou-Mourkidou, 2002) but there are also applications to the extraction of natural tissue constituents (Carro, Garcia, & Cela, 1997; Kiss et al., 2000). A focused MAE method has been compared to a conventional one for fat extraction; it was reported that the former was superior in terms of speed, solvent consumption, and efficiency (Garcia-Ayuso, Velasco, Dobarganes, & Castro, 1999).

The aim of this study was to test the efficiency and repeatability of the MAE method for extracting OA from Greek cheeses and sheep milk yoghurt, and to select the conditions for an adequate ion-exclusion HPLC method for the separation and quantification of as many OA as possible per run of this extract.

2. Materials and methods

2.1. Samples

Samples of various Greek cheeses and of traditional sheep milk yoghurt were purchased from the local market. Each sample originated from a different manufacturer. Analyses were performed immediately after analytical sample preparation. However, when testing the repeatability of the method, portions of shredded cheese were stored at −18 °C until analysed.

2.2. Reagents

Analytical grade OA standards were used: lactic, hippuric, orotic, pyruvic, butyric, and fumaric (Sigma, St. Louis, MO, USA), propionic, citric, and tartaric (Merck, Darmstadt, Germany), valeric and isovaleric (Fluka, Buchs, Switzerland), succinic (Riedel-de Haën, Seelze, Germany), and acetic acids (Panreac, Barcelona, Spain). Analytical grade sulphuric acid (Riedel-de Haën) was used for the preparation of the extractants and the chromatographic mobile phase. Water used throughout the experiment was distilled and filtered through 0.2 μm membrane filters (Millipore, Bedford, MA, USA).

Analytical standards of the OA were prepared in water except for orotic acid, which was prepared in 0.01 M NaOH. These solutions were stored at +4 °C for a period of not more than 2 months. Calibration solutions prepared by mixing appropriate volumes of the aforementioned analytical standard solutions were also stored at +4 °C and were renewed at biweekly intervals.

2.3. Instrumentation

The Mars5 Microwave System (CEM, Matthews, NC, USA) equipped with a 14-vessel carousel operated in the closed vessel mode was used for the extraction. The HP-500 Plus vessels were used and during operation both temperature and pressure were monitored in one vessel. When not otherwise stated, the operational parameters of the MAE apparatus were those shown in Table 1.

The liquid chromatographic (LC) analysis was carried out on a Spectra System, thermo separation products (TSP, Austin, TX, USA) consisting of an on-line TSP degasser, a P4000 tertiary solvent pump, an AS3000 autosampler equipped with a 100 μL loop, incorporating a column oven, and a UV6000LP diode array detector equipped with 50 mm path length flow cell. Chromatographic data were monitored and processed by ChromQuest (TSP).