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Rapid gas-chromatographic method for the determination of diacetyl in milk, fermented milk and butter

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Abstract

In this work, a simple and fast method for the determination of diacetyl by gas-chromatographic technique coupled with flame ionisation detector (GLC-FID) was developed. Diacetyl is the typical butter flavour, but it is also commonly present in others fermented dairy products. Recently, diacetyl determination has also attracted interest because it is one of the parameters on which lactic acid bacteria (L.A.B.) are characterized and valued. Only acetone and 2,3-pentanedione were used as chemicals. After centrifugation of acetone–milk mixture, supernatant was filtered and directly injected into gas-chromatographic apparatus, without a further purification procedure step.

This method was accurate and precise; diacetyl recovery on milk was 97% and the detection limit was 1 mg L^{-1}. Finally, by using this method, diacetyl was easily determined in fresh and high-temperature treated milk, commercial butter, yoghurt and also in a series of L.A.B. performance tests.

Keywords: Diacetyl; Milk flavour; Butter flavour; Gas-chromatography

1. Introduction

In all the world, the production and consumption of fermented milk products are increasing (Baron, Roy, & Vuillemard, 2000). Simultaneously, the commercial production and use of lactic acid bacteria (L.A.B.), in the dairy-industry, are also increasing. L.A.B.'s quality are principally evaluated on their ability to metabolise with some milk components, during specific biochemical processes such as glycolysis, proteolysis, lipolysis and diacetyl production.

Diacetyl or 2,3 butanedione is the typical butter flavour/aroma, but it is also commonly found in other fermented dairy-products such as sour cream, yoghurt and others. Recently, mutant strains have been constructed by genetic engineering to increase the production of diacetyl in cottage cheese or other soft cheese (Law, 2001).

The precursor of diacetyl is citric acid; cow's milk contains approximately 1750 mg citrate per litre (Fox, Law, McSweeney, & Wallace, 1993). At pH 5.0–5.2 lactic acid displaces citric acid from its salts; free citric acid in the presence of lactose is converted into CO_2, diacetyl, acetoin and 2-acetolactate by citrate-utilising (Cit^+) strains of *Lactococcus lactis* subsp. *Lactis* (Fox, Lucey, & Cogan, 1990; McSweeney & Fox, 2004).

The biochemical pathway is: citrate → oxalacetate → pyruvate → acetolactate → diacetyl/acetoin/2,3-butandiol (Palles, Beresford, Condon, & Cogan, 1998).

Recently, diacetyl has also attracted interest since it is one of the parameters on which L.A.B. are characterized and valued (Beshkova, Simova, Frengova, Simov, & Dimitrov, 2003). Indeed, the quantification of diacetyl in fermented milk products is highly affected by analytical

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methods used to determine it (Escamilla, Valdés, Soriano, & Tomasini, 2000). At present, a specific and internationally proven method to determine diacetyl in milk and milk products has been not validated. Some time ago, the common analytical method carried out a steam distillation step to separate diacetyl from the sample, followed by a colorimetric reaction (Pack, Sandine, Elliker, Day, & Lindsay, 1964; Prill & Hammer, 1938; Walsh & Cogan, 1974; Westerfeld, 1945). This procedure was laborious, poorly selective and inaccurate. In fact, colorimetric determination is not specific and the diacetyl measured was in reality the sum of diacetyl and acetoin. Furthermore, during the steam distillation step, 2-acetolactate contained in the samples could be converted by chemical decarboxylation to diacetyl and acetoin causing an overestimation of both compounds (Cronin & Rispin, 1996; Veringa, Verburg, & Stadhouders, 1984).

Direct analysis of diacetyl by gas–liquid chromatography (GLC) was performed and validated in bacterial culture supernatant (Lee & Drucker, 1975). The gas-chromatographic analysis is more selective, but direct analysis in milk products is very difficult owing to interferences with other components (Thomhill & Cogan, 1984).

Recently, diacetyl in milk products has often been determined by headspace technique coupled with gas–liquid chromatography, using flame-ionization or mass spectrometer detectors. Diacetyl measured by headspace technique is conventionally considered as the true value of total diacetyl, but actually, only its volatile part is detected (Monnet, Schmitt, & Divies, 1994). Repartition of diacetyl between liquid and air phases is affected by several factors, such as temperature, vapour pressure and sample matrix composition. In particular, protein and fat influence the diacetyl's coefficient of partition (Haahr, Bredie, Stahnke, Jensen, & Refsgaard, 2000; Lee, Lo, Richter, & Dill, 1995). Among other techniques, HPLC, spectrophotomeric and fluorometric methods have been used to determine diacetyl in milk products (Guerra Hernández, Garcıa Estepa, & Rodriguez Rivas, 1995; Matsuura, Fujiyama, Minagawa, & Sawa, 1990; Zeppa, Conterno, & Gerbi, 2001).

In this research, a new method to easily and rapidly determine the diacetyl content of milk products was developed. The gas-chromatographic technique coupled with a flame ionisation detector has been used. The method was first validated and then, used to determine diacetyl on several milk products and for a series of L.A.B. performance tests.

2. Materials and methods

2.1. Materials

Only acetone of reagent grade quality (C.Erba, Rodano, MI, Italy) was used as a solvent. The standard compounds, 2,3-pentanedione and diacetyl, were from Sigma Chemical Co. (St. Louis, MO, USA). The milk products analysed were: (1) several samples of fresh raw cow, sheep or goat's milk, drawn directly from a local breeding farm; (2) two samples of UHT commercial milk; (3) four samples of commercial butter; and (4) three samples of commercial yoghurt. For the L.A.B. performance test, fermented milk samples were prepared by Mediterranea Biotecnologie s.r.l. (Termoli, CB, Italy), a specialised company that produces L.A.B. starters. Different mixtures of *Lactococcus* cultures from Mediterranea Biotecnologie collections were cultured in sterilized milk at the following experimental growing conditions: sample no. 1 was incubated at 30 °C for 6 h, sample no. 2 at 20 °C for 16 h and finally, sample no. 3 at 10 °C for 17 h. Sterilized milk was analysed as a control. All samples were stored chilled until required for analysis.

2.2. Sample preparation for GLC analysis

Extraction of diacetyl was performed using acetone. The butter was melted for two minutes at 40 °C. About 2 g of sample (milk, fermented milk or butter) were accurately weighted, and shaken vigorously for 30 s with 2 mL of acetone and with 50 μg mL^{-1} of 2,3-pentanedione as internal standard. After centrifugation at 4000*g* for 5 min, the supernatant was filtered through a 0.20 μm disposable syringe membrane filter (Sartorius AG, Göttingen, Germany) and subsequently injected directly to the gas-chromatographic apparatus. Final concentration of diacetyl (expressed as μg of diacetyl per g of sample) was calculated using the following formula:

$$\text{Diacetyl } (\mu g\ g^{-1}) = \frac{w_{IS} \cdot A_D}{A_{IS} \cdot W_s}$$

w_{IS} is μg of internal standard (2,3-pentanedione); A_D is the area GLC-FID peak of diacetyl; A_{IS} is the area GLC-FID peak of internal standard; and W_s is the g of sample used.

2.3. Gas-chromatographic analysis

Gas-chromatographic analysis of diacetyl was performed using a gas-chromatograph Model 8000 Finnigan (Milan, Italy) equipped with a flame ionisation detector (FID). One 30 m ZB-Wax (Phenomenex, Torrance, CA, USA) capillary column, with 0.32 mm i.d., 0.5 μm film and 100% polyethylene glycol phase, was used. Gas-chromatographic parameters were: carrier gas helium at 50 kPa flow; injected amount 1 μL; split mode injection at 1:15 splitting ratio; injector and detector temperatures were 250 °C and 260 °C, respectively; oven temperature was running from 50 °C to 240 °C at 7 °C min^{-1}.

2.4. Validation of method

Linearity was determined with solutions of acetone:water 50:50 (v/v) containing diacetyl standard at concentrations between 0.30 and 0.67 mg L^{-1}. Each point was repeated six times. Accuracy was estimated from the slope of regression line between diacetyl added and detected. Repeatability or precision of the method was determined