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Analytical Methods

Determination of free fatty acids in beer wort

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ABSTRACT

The importance of free fatty acids (FFAs) in wort has been known for a long time because of their influence on beer quality and yeast metabolism. Lipids have a beneficial effect on yeast growth during fermentation as well as negative effects on beer quality. Lipids content of beer affects the ability to form a stable head of foam and plays an important role in beer staling. Moreover, the ratio of unsaturated and saturated fatty acids seems to be related to gushing problems. A novel, simple, and reliable procedure for quantitative analysis of FFAs in wort was developed and validated. The determination of FFAs in wort was achieved via liquid–liquid cartridge extraction, purification of FFA fraction by solid phase extraction, boron trifluoride in methanol methylation, and injection into GC-FID system. The proposed method has high accuracy (<0.3%, expressed as the bias), high precision (<1.2%, RSD), and recoveries ranging from 74% to 98%. The method was tested on two different wort samples (9° and 12° Plato).

1. Introduction

Lipids in beer wort and beer are important because they affect yeast metabolism and beer quality. The presence of lipids has long been considered to have adverse effects on beer quality. Some long- chain fatty acids have a high flavor potential (Garbe, Barbosa De Almeida, Nagel, Wackerbauer, & Tressl, 2006; Segawa, Yamashita, Mitani, & Masachika, 2002; Yano, Morikawa, Yasui, Ogawa, & Ohkochi, 2004). In particular linoleic and linolenic acids have received great attention because of their oxidative degradation leading to the formation of a characteristic ageing flavor. The most commonly recognised of these products, trans-2-nonenal, possesses a flavor threshold of only 0.11 μg/L (DeVries, 1990). Although the levels of long-chain fatty acids are normally very low in beer, an increase in their concentration, coupled with harsh storage conditions, can result in concentrations of stale-flavored breakdown products that exceed threshold levels (Vanderhaegen, Neven, Verachtert, & Derdelinckx, 2006). Moreover, some authors (Gallardo et al., 2008) reported on the contribution of lipids to the Strecker degradation of amino acids. This contribution is not only due to lipid oxidation products but also to unoxidized lipids. The damaging effect of lipids on beer foam has bee n widely documented (Dickie, Cann, Norman, Bamforth, & Muller, 2001; Roberts, Keeney, & Wainwright, 1978; Van Nierop, Evans, Axcell, Cantrell, & Rautenbach, 2004). Furthermore, the ratio of unsaturated (gushing-suppressors) and saturated fatty acids (gushing-promoters) seems to be related to gushing problems (Kobayashi, Kaneda, Kano, & Koshino, 1993; Muller, Schmid, Becker, & Gastl, 2010). On the other hand, in fermenting wort, lipids, including long-chain unsaturated fatty acids, are necessary for activation of yeast cell growth under anaerobic conditions and significantly affect the fermentation process, leading to a more intensive and faster fermentation (Bravi, Perretti, Buzzini, Della Sera, & Fantozzi, 2009; Briggs, Hough, Stevens, & Young, 1995; Gallardo et al., 2008). Lipids involved in brewing process are present in different forms, such as (i) simple lipids (triacylglycerols, diacylglycerols, monoacylglycerols, and free fatty acids), (ii) complex lipids, and (iii) bound lipids, and the most of them derives from malt and from lauter turbidity (Gallardo et al., 2008; Kuhbeck, Back, & Krottenthaler, 2006). Not all of the lipids adversely affect the beer quality and brewing process. In fact, despite the widely preference for a lauter wort as clear as possible, some authors reported that an improvement of fermentations performance was observed and that none of the quality parameters of the final beers were significantly compromised by employing worts with higher content in linoleic acid (Kuhbeck et al., 2006).

To our knowledge a few methods, employing complex liquid–liquid extraction technique, that require large volumes of toxic organic solvents and that are time-consuming and labour-intensive multi-step procedures prone to analyte loss, have been reported in the literature for the determination of free fatty acids in wort. DeVries (1990) reported on the extraction of fatty acids from the acidified wort on a separatory funnel using a chloroform/methanol mixture. The FFAs were then extracted from the solvent, as their water-soluble potassium salts, with 10% KOH (w/v). The method addresses the simultaneous extraction of the short chain fatty acid, C8:0 through the longer C18:3, the precision of the method ranged from 2.5% to 18.8%, and the recovery, for longer chain fatty acids, was about 31%. Kobayashi et al. (1993) and Kuroda, Kobayashi,

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Kaneda, Takashio, and Shinotsuka (2001) reported on the determination of hydroperoxides of fatty acids during mashing using liquid–liquid extraction methods.

More modern procedures, based on the use of solid-phase extraction (SPE), were developed, these procedures lead to good recovery, allow to minimizing solvent consuming, are quite fast, and more reproducible (Vaghela & Kilara, 1995).

Battistutta, Buiatti, Zenarola, and Zironi (1994) proposed a procedure for the determination of free medium chain fatty acids (caproic, caprylic, capric, and lauric) and related ethyl esters in beer by SPE, derivatization with diazomethane, and GC-FID analysis. The recoveries ranging from 68% to 121%, the analytical repeatability (RDS), evaluated analysing eight time the same beer sample, is quite high (<10.3%). However, no validation procedure has been reported for the mentioned method. Schutz and Back (2005) reported on the development of a method for the determination of free long chain fatty acids (myristic, palmitic, stearic, oleic, linoleic, and linolenic) in centrifuged beer wort and beer by SPE, methylation by diazomethane, and GC–MS analysis; no validation procedure has been reported. Horak, Culìk, Jurkovà, Cejka, and Kellner (2008) reported on the extraction of free FFAs in beer with a new technique known as Stir Bar Sorptive Extraction (SBSE). The authors applied this novel method in order to determine the amounts of four free medium fatty acids (caproic, caprylic, capric, and lauric) in beer, thus excluding the long chain fatty acids present in wort. The repeatability (RSD) of the method is quite good (<6.7%), and recoveries ranging from 57% to 89%. The method is advantageous for the use of a small sample volume, the simple extraction, and the small volume of solvent consumption. However, the procedure showed a very low recovery of short fatty acids that tend to stay in the aqueous phase; this behaviour seems to be related to the lower molecular weight of these fatty acids. Moreover, as the concentration of ethanol in beer influence the performance of the method, for quantitation it is important to calibrate the instrument using calibrating solutions with a content of ethanol similar to the measured sample. This aspect could reduce the versatility of the method.

Considering that FFAs in beer wort affect the fermentation process and are related to the flavor and foam stability of final beer, the aim of this research was to develop and validate a simple and reliable procedure for quantification of FFAs in beer wort in order to assist the brewing industry to control brewing process for the quality improvement of final product. In order to determine FFAs in a complex aqueous matrix as the beer wort, is required the development of an accurate extraction method which avoids emulsion formation and allows the analysis of not centrifuged wort, since most part of the lipid fraction is removed together with the trub during centrifugation (Kuhbeck et al., 2006). The high porosity, the high dispersing capacities and the high capacity for aqueous adsorption of the inert support of Chem elut cartridges, used in this research for sorbent assisted liquid–liquid extraction, ensure immiscibility of organic solvent and aqueous phase, avoiding emulsion formation and facilitating efficient interaction between the sample and the organic solvent. Moreover, in this procedure the methylation step avoids the use of diazomethane, which is potentially explosive, stable for very short periods, and needs to be prepared with carcinogenous reagents.

2. Experimental methods

2.1. Materials

Wort samples (9° and 12° Plato pitching wort) were produced in a pilot plant and supplied by CERB (Italian Brewing Research Centre). The 9° Plato wort was obtained using a mixture of different malts: Pilsner (13.12 kg, Durst, Heidelsheim, Germany), Carapils (1.28 kg, Weyermann, Bamberg, Germany), Special B (640 g, Castle Malting, Beloeil, Belgium), Carahell (800 g, Weyermann, Bamberg, Germany), and Chocolate malt (160 g, Crisp Malting, Ditchingham, UK); the 12° Plato wort was obtained using 100% Pilsner malt (23 kg, Durst, Heidelsheim, Germany). HPLC grade n-hexane, diethyl ether, methanol, chloroform, 2-propanol, analytical grade glacial acetic acid, boron trifluoride (BF_3) methanol complex solution (13–15%), Supelco® 37-component FAME MIX, heptadecanoic acid (⩾98%), linoleic acid (⩾99%), oleic acid (⩾99%), methyl nonadecanoate (⩾98%), and glyceryl trioleate (⩾99%), were purchased from Sigma–Aldrich (Milan, Italy).

An Internal Standard (IS) solution was prepared by diluting 10 mg of methyl nonadecanoate to 200 ml with n-hexane. The stock standard solution was prepared diluting 100 mg of standard mix to 5 ml of the IS solution. The stock solution was stored at 4 °C protected by light. Diatomaceous earth cartridges (ChemElut) and solid phase extraction amino cartridges (aminopropyl SampliQ) were obtained from Agilent Technologies (Milan, Italy).

In order to obtain an homogenous purification procedure, a VAC Elut 20 Tall Glass Manifold (Agilent Technologies, Milan, Italy) was used.

2.2. Equipment and chromatographic conditions

An Agilent Model 6850 gas chromatograph (Agilent Technologies, Milan, Italy) equipped with a flame ionisation detector (FID), a capillary inlet system, a DB-23 (60 m × 0.25 mm × 0.25 μm) column, and a Model Maestro MPS 2XL multipurpose sampler with a 10 μL syringe (Gerstel Inc., Baltimore, MD) was employed. The programmed oven temperature was: 130 °C for 1 min, 130–170 °C at 6.5 °C/min, 170–215 °C at 2.75 °C/min, 215 °C for 12 min, 215–230 °C at 40 °C/min, 230 °C for 3 min. The carrier gas (H_2) flow rate was 1.7 mL/min; constant pressure mode was set up. The split ratio was set at 50:1. The temperatures of injector and detector were 270 and 280 °C, respectively. Peak areas were measured by using an Agilent MSD Chemstation (version E.01.00.237) for HRGC-FID.

2.3. Calibration

An IS (methyl nonadecanoate, 0.05 mg/ml) method was used for the calibration curves construction. Table 1 reports the six concentrations of FAMEs used for calibration curves, the retention times for the individual FAME, and correlation coefficients of the calibration plots. According to the Eurachem Guide (1998) each point of calibration curve was obtained by six replicates.

2.4. Lipid extraction

2.4.1. Liquid–liquid cartridge extraction procedure

The extraction was carried out using porous diatomaceous earth (Chem Elut cartridges). The samples of tempered (20 °C) wort (20 ml) were loaded on the top of a dry Chem Elut cartridge. The column was eluted with 20 ml of diethyl ether; the effluent was collected in a 50 ml bottom flask, and evaporated to dryness under vacuum. The residue was then recovered with 3 portions of 2 ml of diethyl ether, collected in a test tube, and evaporated to dryness under nitrogen flow.

2.4.2. Free fatty acids purification and esterification procedures

A comprehensive survey of the literature on the FFAs purification in food products allowed us to identify a reference method, developed by Kaluzny, Duncan, Merritt, and Epps (1985), utilising aminopropyl bonded phase for lipid mixture separation into individual classes in high yield and purity. This method, together with the FA methylation, allowed us to obtain a accurate procedure for the measurement of FFAs in beer wort.