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Supercritical fluid extraction for quality control in beer industry

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Abstract

The knowledge of lipid composition in beer ingredients (malt and corn grits) and wort enables the quality control for final product. Since supercritical fluid extraction (SFE) is an efficient technique for preparing samples for analysis without the use of solvents, in this research Supercritical CO_2 (SC–CO_2) extraction was compared with the traditional Soxhlet one for a gravimetric determination of total lipids on malt and corn grits. The obtained extracts were then analyzed by HPLC-ELSD after TLC separation of triacylglycerols (TAGs) for lipids fingerprint. The extraction of total fats achieved by a 60-min run with pure CO_2 at 65 MPa and 100 °C was 43% higher than that produced by Soxhlet performed for 9 h for malt. The extraction was intermediate for SFE at 60 and 80 °C. The recovery of the TAG obtained with SC–CO_2 at 100 °C was statistically comparable with results from Soxhlet extraction.

Keywords: Lipids; Malt; Corn grits; Supercritical carbon dioxide; Extraction

1. Introduction

One of the most important issues in modern brewing is how to produce beer having adequate flavor and foam stability, and these quality characteristics can be adversely affected by the presence of lipids in the product.

With ageing the flavor may definitely change, and in particular the musty aroma of stale beer is related to the moieties originating from the oxidation of the unsaturated fatty acids found in beer [1,2]. The enzymatic- and/or auto-oxidation of lipids results in the formation of their hydroperoxides, which are unstable and degrade into low molecular weight and flavor active compounds [2]. Trans-2-nonenal is produced via lipoxygenase lipid oxidation during wort production and causes an unpleasant papery, cardboard off-flavor, characteristic of staleness, in beer during storage [3].

The damaging effect of lipids on beer foam is widely documented and easily demonstrated [4]. During foam formation, amphipathic proteins may surround the bubbles forming a stable bridge between the hydrophobic gas bubbles and the aqueous phase of beer. Lipids are able interfere with this hydrophobic interaction, causing instability of the foam and its subsequent rapid collapse. Fatty acids with either six or ten carbons have been shown to have no impact on foam stability, but longer chain fatty acids destabilized beer foam through a mechanical film-bridging mechanism (collapses rapidly), similar to that used in antifoam systems [5].

Due to the negative effects of lipids on beer flavor and foam stability, the control of their presence is of the utmost importance for the improvement of beer quality, taking also into account that lipids can positively affect the yeast metabolism during fermentation, playing an important role in the formation of the cell wall of the microorganisms [6].

The aim of this research was to develop a rapid extraction method for analysis of fatty acids in brewing raw materials (malt and corn grits). In this paper, two different extraction methods (Soxhlet with petroleum ether, and Supercritical CO_2–SC–CO_2) were compared as useful tools for brewing industries in process quality control.

A reverse-phase HPLC method with an evaporative light scattering detector (ELSD) was used for fatty acids determination in lipid extracts separated by TLC and transesterified with methanol.

2. Experimental

2.1. Materials

Malt and corn grits were supplied by "Associazione degli Industriali della Birra e del Malto" (ASSOBIRRA, Rome, Italy).

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HPLC grade water was purchased from Panreac (Spain); HPLC grade methanol, analytical grade petroleum ether, *n*-hexane, chloroform and HPLC grade diethyl ether were purchased from Carlo Erba (Milan, Italy). Analytical grade nitrogen and 99.92% CO_2 were purchased from Linde Gas Tecnici (Perugia, Italy). HPLC standards were purchased from Sigma–Aldrich SRL (Milan, Italy). Silica gel plates were purchased from Merck (Darmstadt, Germany).

2.2. Lipid extraction

Malt and corn grits samples were finely milled in a Bühler MLI-204 (Bühler S.p.A. Milan, Italy) grain laboratory mill. Lipid extraction was performed with two different techniques: Soxhlet extraction with petroleum ether at 60 °C for 9 h [7,8], and extraction with supercritical CO_2 at 65 MPa and three different temperatures (60, 80 and 100 °C); the SC–CO_2 densities were therefore 0.99, 0.93 and 0.88 g/mL. The SC–CO_2 tests were conducted using a Speed SFE^{TM} extraction unit (Applied Separation, Lehigh, PA, USA) and for all SC–CO_2 fat determinations approximately 40 g of sample, exactly weighted, were loaded into a 50-mL extractor vessel. Total extraction time was 70 min, with an initial static extraction period of 10 min followed by a dynamic extraction lasting 60 min, with the SC–CO_2 flow-rate set at 2 g/min. Collection of the extract was achieved at room temperature and atmospheric pressure. An "HR 200" analytical balance (AeD Instrument Ltd., Oxon, UK) was used for the gravimetric determinations.

2.3. Separation of lipid classes

Separation of triacylglycerols (TAG), diacylglycerols (DAG), monoacylglycerols (MAG), free fatty acids (FFA) and polar lipids was achieved by using thin-layer chromatography. The extracts were dissolved in a chloroform–methanol (1:1, v/v) mixture, and an aliquot was then applied on silica gel coated plates (Kieselgel 60, 0.25 mm) (Merck, Darmstadt, Germany). The plates were developed by using a mixture consisting of petroleum ether, diethyl ether and formic acid (70:30:1, v/v/v) and then sprayed with 2′,7′-dichlorofluorescein. The different lipid classes were located under UV light and the spots were then scraped off for subsequent analysis.

2.4. Preparation and analysis of fatty acid methyl esters

The TAG fraction obtained by TLC was analyzed by HPLC-ELSD for lipid fingerprint. From the TAGs fatty acid methyl esters (FAMEs) were prepared according to Christie [9] and separated by using a HPLC system consisting of a Jasco PU-1580 pump (Jasco Co. Tokyo, Japan), a Rheodyne injector (model 7725 with a 50-μL loop) (Rheodyne Inc., Cotati, CA, USA) and an evaporative light scattering detector (ELSD 500, MKIII, Alltech Associates Inc., Deerfield, IL, USA). The ELSD drift tube temperature was set at 75 °C, with nitrogen as a nebulizing gas at a flow rate of 2.75 L/min; the pressure was 0.138 MPa. Two Inertsil ODS-3 (Varian Inc., CA, USA) C18 reversed-phase columns (250 mm × 4.6 mm, 5 μm) in series were used for separations of the analytes, by using a mixture of methanol:water (97:3, v/v) as eluent at the flow rate of 1 mL/min at room temperature [10].

2.5. Statistical analysis

Statistical analysis of the data was performed by using the statistical package SigmaStat (SPSS Science, Chicago, IL, USA). The unpaired *t*-test was used at $P < 0.05$ to determine the statistically significant difference between the two extraction methods, while the Mann–Whitney rank sum test was used to verify if the difference in relative standard deviation (R.S.D.) between the methods could change the *t*-test response.

3. Results and discussion

Total lipid extraction, TAG in lipid extracts and fatty acid profile, are reported for malt and corn grits. The measurement of lipid extraction is reported as weight percent.

The total lipid extraction for the two extraction methods is reported in Table 1 for malt. The lipid extractability was always statistically different, with the lower extractability for Soxhlet and increasing with the increase in temperature. The total lipids extractability obtained with SC–CO_2 at 100 °C was 43% higher than that produced by Soxhlet. The extractability was intermediate for SFE at 60 and 80 °C. Nevertheless, the reproducibility (R.S.D.% reported for each mean) of Soxhlet was generally better in comparison with SC–CO_2. This behavior can be the consequence of the composition in terms of fatty acids and probably the presence of a little amount of polar lipids.

The TAG composition for the different malt lipid extracts is reported in Table 2. The recovery of the TAG obtained with SC–CO_2 at 100 °C was 39.5%, statistically comparable with 37.5% obtained with Soxhlet extraction. Moreover, in these conditions, the extraction with SC–CO_2 is more reproducible. The

Table 1
Total lipid extraction: comparison for Soxhlet and SC–CO_2 extraction for malt

Lipid extract (mg/100 mg malt)	SC–CO_2 extraction conditions (MPa/°C)			Soxhlet
	65/60	65/80	65/100	
Mean	1.65 a	1.92 b	2.06 c	1.44 d
S.D.	0.11	0.03	0.01	0.01
R.S.D.%	6.67	1.56	0.49	0.68

$n = 3$, S.D.: standard deviation, R.S.D.%: relative standard deviation. Values in the same row followed by the same letters are not statistically different ($P < 0.05$).

Table 2
TAG concentration in lipid extracts: comparison for Soxhlet and SC–CO_2 extraction for malt

TAG (mg/100 mg extract)	SC–CO_2 extraction conditions (MPa/°C)			Soxhlet
	65/60	65/80	65/100	
Mean	27.7 a	44.1 b	39.5 bc	37.5 c
S.D.	0.9	4.5	3.9	1.0
R.S.D.%	3.3	10.2	1.0	2.5

$n = 3$, S.D.: standard deviation, R.S.D.%: relative standard deviation, TAG: triacylglycerols. Values in the same row followed by the same letters are not statistically different ($P < 0.05$).