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In situ silylation for the multicomponent analysis of canola oil by-products by gas chromatography–mass spectrometry

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Abstract

In this study, a simple method for the determination of free fatty acids, phytosterols, tocopherols, mono and diglycerides present in canola oil deodorizer distillate (DD) and soapstock samples was developed. Analytes were derivatized “in situ” using a mixture of hexamethyldisilazane (HMDS), pyridine and trifluoroacetic acid; separated by gas chromatography (GC) with mass spectrometry (MS) for final detection. Two drying procedures were evaluated for drying deodorizer distillate samples before derivatization: freeze drying and drying at 100 °C for 24 h. The use of high temperatures caused the degradation of tocopherols and phytosterols, while lyophilization did not affect the substances negatively. The chromatographic conditions used in this work allow for the separation and quantification of oleic, linoleic and linolenic acids, monoolein and monolinolein in both samples, and brassicasterol and α-tocopherol in deodorizer distillate samples. MS provided an accurate identification for the compounds which were at very low concentrations (>0.09%). Oleic acid was the most abundant compound in both samples. Deodorizer distillate was an important source of tocopherols which were not detected in the soapstock samples.

Keywords: Canola oil; Soapstock; Deodorizer distillate; Silylation; GC–MS

1. Introduction

Canola, an important economic cultivar in Canada has become the world’s third leading source of vegetable oil [1]. Canola oil produced in Canada is obtained from the seeds of *Brassica napus* and *Brassica rapa* containing low erucic acid and glucosinolates cultivars, which possesses chemical, physical and nutritional properties that are very different from high erucic acid rapeseed oil [2]. The nutritional advantages of canola oil as compared with other common oils are well documented (see for example Ackman’s review [3]). The crude canola oil is refined to remove the nontriglyceride components from the oil which include the fatty acids, mono and diglycerides, phosphatide, glycerol, sterols, tocopherol, hydrocarbons, pigments, vitamins, glycolipides, proteins fragments, and traces of other materials such as metals, pesticides and resinous and mucilaginous materials. The chemical refining process is the most used process. It consists of hydration and degumming, neutralization, bleaching, and deodorization steps [4]. The hydration and degumming steps remove the phospholipids and gums by addition of phosphoric acid and/or water. The neutralization step removes the free fatty acids, metals and the residual phospholipids and leads to the formation of soap. The residual soap, pigments, and phospholipids are removed in the bleaching step. The waste stream from these steps is a hydrophilic material commonly called soapstock. Finally, the volatiles, oxidation products, and the contaminants which could influence the taste and smell of the oil as well as its appearance (and thus reduce consumer acceptance and marketability) are removed by a distillation process known as deodorization. The side-product of this step is the deodorizer distillate. The soapstock composition changes according to the class of crude oil from which it is obtained. Generally soapstock samples contain fatty acids, glycerides, and phytosterols [5,6]. Deodorizer distillate contains fatty acids, tocopherols, phytosterols, sterol esters, waxy hydrocarbons, odoriferous compounds, pigments, mono and diglycerides [7,8].

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Both side-products, the soapstock and deodorizer distillates, are wastes that contaminate the environment but some of their components are very useful nutriments and valuable molecules. The value of deodorizer distillate is strongly dependent on its tocopherol content, a natural antioxidant present in oilseeds. Its dextrorotatory (D)-isomers occurring in plant seeds have shown higher biological activity than those obtained by chemical synthesis [9,10]. α-Tocopherols are used as pharmaceuticals and in cosmetics, whereas α-, δ- and γ-tocopherols are used as additives in various foods, such as fats and oils. Phytosterols are very interesting compounds. They are used in the pharmaceutical industry as reagent for the synthesis of steroids, pesticides and emulsifiers and are expected to add value in functional foods. It has been suggested that they have cholesterol-lowering properties by limiting the intestinal solubility of cholesterol through cholesterol displacement from the micelles, or by limiting cholesterol esters hydrolysis in the gut [11,12]. Phytosterols imparts to this by-product a high aggregated value [13,14].

Depending on the crude oil and the physical or chemical processes used during refining, fatty acids can account for 25–75% of the deodorizer distillate. These may be used as starting material for the synthesis of hypocholesterolaemic phytosteryl esters added to functional foods as shown with the efficient in situ esterification of canola oil deodorizer distillate fatty acids with phytosterols [15].

Different analytical strategies have been utilized for identifying and quantifying fatty acids, sterols and tocopherols in deodorizer distillates and soapstocks. The American Oil Chemist's Society (AOAC) has recommended the Standard Official Method Ce 3–74 [16] to analyze sterols and tocopherols. In this method, the deodorizer distillate is saponified and the unsaponifiable compounds are extracted and determined by packed-column GC. The main inconvenience of the method is that it is time consuming. Moreover, it has been reported that it is not applicable to deodorizer distillates from rapeseed oils [17]. The AOAC, has also recommended a methodology (Ce 7–87) for analyzing total tocopherols in deodorizer distillates, in which the chromatographic separation is performed by using a non-polar column [16]. Total tocopherols in soybean oil deodorizer distillate have been analyzed using capillary GC after silylation of the sample [16,17]. Verleyen et al. [18] separated different compounds contained in deodorizer distillates. After derivatization, the sample was introduced into a cold on column injector GC and the different compounds (including tocopherols, sterols, acids and glycerides) were separated. However, they did not separate and identify the different free fatty acids present in the sample.

Trimethylsilylation (TMS) involves the introduction of a trimethylsilyl group into a molecule. The formation of trimethylsilyl esters or ethers is one of the most popular and versatile derivatization techniques available for GC because it improves chromatographic properties such as volatility and thermal stability, of the compounds. These same properties have made the technique appropriate for MS analysis [19]. The derivatives are prepared by the reaction of the trimethylsilylating agent and the compounds containing one, or more, active hydrogen. TMS has enabled the analysis of different lipidic compounds, such as sterols, fatty acids and glycerides [20].

The TMS technique provided a good approach to overcome the analytical challenges of the characterization of soapstocks from vegetable oils. Dowd used it for the quantitative analysis of corn, cottonseed, and peanut soapstocks. After derivatization the samples were analyzed by GC using a non-polar capillary column [5,6].

The purpose of this study was: (1) the use of a new silylation approach for derivatization of canola oil deodorizer distillate samples; (2) to present a GC–MS analytical method, in full scan mode, that allows for the simultaneous determination of free fatty acids, phytosterols, tocopherols, and glycerides present in the samples and (3) to characterize the composition of canola oil soapstocks.

2. Experimental

2.1. Chemicals and materials

Canola deodorizer distillate and soapstock samples were supplied by Canbra Foods Ltd. (Lethbridge, AB, Canada) and were stored at −10 °C until use. Lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), oleic acid (18:1, $n-9$), linoleic acid (18:2, $n-6$), linolenic acid (18:3, $n-3$), arachidic acid (20:0), behenic acid (22:0), lignoceric acid (24:0), monoolein, monolinolein, diolein, α- and δ-tocopherol campesterol, stigmasterol and sitosterol and cholesterol (IS) standards were obtained from Sigma–Aldrich (St. Louis, MO, USA). β- and γ-Tocopherol standards were supplied by Matreya LLC (PA, USA). Hexamethyldisilazane (HMDS), anhydrous pyridine 99% and trifluoroacetic acid (TFA) 98% were purchased from Sigma–Aldrich (USA). Analytical grade chloroform was obtained from Caledon (Georgetown, Ont., Canada).

2.2. Standard solutions

Standard solutions were prepared at a 10 mg/ml concentration in chloroform for each fatty acid, sterols, tocopherols and glycerides and were kept at −20 °C. Because cholesterol was not detected in canola oil deodorizer distillate or soapstock samples, it was used as internal standard instead of dihydrocholesterol, a much more expensive chemical. A mixture at a concentration of 10 mg/ml of these standards solutions, including the internal standard, was silylated and injected into the GC, following the chromatographic conditions described below (Section 2.4). The separated compounds were used for determining the elution of the compounds, and for MS identification.

2.3. Silylation experiments

2.3.1. Deodorizer distillate samples

Derivatization experiments were performed by freeze drying, or by drying the samples (1 g) at 100 °C for 24 h. Subsequently, 30 mg of the dried sample were weighed in a screw-capped derivatization vial. An amount of 50 μl of the internal standard (IS) (10 mg/ml) were added. Finally, 2 ml of pyridine, 2 ml of hexamethyldisilazane and 0.175 ml of trifluoroacetic acid were added, and the reaction was performed at 60 °C for 1 h.