Food Chemistry 153 (2014) 393–397

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Various concentrations of erucic acid in mustard oil and mustard

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ARTICLE INFO

Article history:
Received 25 July 2013
Received in revised form 11 December 2013
Accepted 19 December 2013
Available online 28 December 2013

Keywords:
Fatty acid
Erucic acid
Mustard oil
Prepared mustard
Tolerable daily intake

ABSTRACT

Erucic acid is a typical constituent of mustard or rape. Foodstuff with a high content of erucic acid is considered undesirable for human consumption because it has been linked to myocardial lipidosis and heart lesions in laboratory rats. As a result, several countries have restricted its presence in oils and fats. In this study, the erucic acid content in several mustard oils and prepared mustard samples from Germany and Australia was determined. Seven of nine mustard oil samples exceeded the permitted maximum levels established for erucic acid (range: 0.3–50.8%, limit: 5%). The erucic acid content in mustard samples ($n = 15$) varied from 14% to 33% in the lipids. Two servings (i.e. 20 g) of the mustards with the highest erucic acid content already surpassed the tolerable daily intake established by Food Standards Australia New Zealand. However, a careful selection of mustard cultivars could lower the nutritional intake of erucic acid.

1. Introduction

Oilseeds of *Brassicaceae* such as rapeseed and mustard are major sources of vegetable oil for nutritional purposes on a global scale (United States Department of Agriculture, 2013). In the past, rapeseed oil was characterised by a high content of erucic acid (22:1*n*-9). Since erucic acid consumption has been associated with myocardial lipidosis and heart lesions in test animals, oils high in erucic acid are considered undesirable for human nutrition (Charlton et al., 1975; Food Standards Australia New Zealand., 2003; Kako & Vasdev, 1979). Although none of the studies with test animals enabled to establish a tolerable level for humans (Food Standards Australia New Zealand, 2003), these oils (erucic acid >40%) were mainly restricted for use in industrial purposes (Nieschlag & Wolff, 1971) including biofuel production (Ciubota-Rosie et al., 2013). This situation has changed for rapeseed when new breeds were introduced, including Canola from Canada (Abbadi & Leckband, 2011; Mag, 1983). Canola oil is characterised by low erucic acid and low glucosinolate content and is thus considered safe for human consumption (Abbadi & Leckband, 2011).

To avoid potential public health risks the erucic acid content in vegetable oils was regulated in several countries. For instance, the European Union has set the maximum level of erucic acid to 5% of the total fatty acids. This regulation applies to oils or fats and foodstuff with a fat content of 5% or more to which oils or fats have been added (Council Directive 76/621/EEC, 1976). Likewise, the joint Food Standards Code of Australia and New Zealand has classified erucic acid as natural toxicant and maximum levels in edible oils were set to 20 g/kg (2%) (Abbott et al., 2003). Furthermore, the tolerable daily intake of erucic acid was set at 7.5 mg/kg bodyweight (Food Standards Australia New Zealand, 2003). The dietary exposure assessment was calculated on the basis of the Canola oil consumption (Food Standards Australia New Zealand, 2003).

While a lot of attention has been put on the absence of erucic acid in rapeseed oil, the presence of this undesired fatty acid in other members of *Brassicaceae* has been largely overlooked. For instance, mustard oil is widely consumed in the Asian region owing to its nutty and pungent flavour (Alim, Iqbal, & Dutta, 2012; Mortuza, Dutta, & Das, 2006). Likewise, mustard oil is the most commonly used oil in cooking in North India (Rastogi et al., 2004). Due to the consumers' interest in new types of foodstuff, mustard oil is now increasingly marketed worldwide as salad and cooking oil. However, several studies have pointed out that mustard seed oil may contain high levels of erucic acid and consumers were advised against its intake as food (Abul-Fadl, El-Badry, & Ammar, 2011; Genet, Labuschagne, & Hugo, 2004; Mortuza et al., 2006). For this reason, mustard oil has been suggested for the production of biodiesel (Ciubota-Rosie et al., 2013). Likewise, efforts were made on breeding cultivars with low or zero erucic acid content (Getinet, Rakow, Raney, & Downey, 1994; Males, Rakow, Potts, & Raney, 2000). However, despite the maximum levels of erucic acid in oils, little is known about the actual situation for mustard oil from the German market.

The goal of our study was to analyse mustard seed oils from the German and the Australian market, including imports, on the content of erucic acid. In Europe, "mustard" is more frequently consumed in form of (prepared) mustard, which is a smooth paste composed of water, vinegar, mustard seed and spices. Mustard is

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0308-8146/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.12.073

commonly consumed with meat products such as sausages or hamburgers, but also as an ingredient in sauces, salad dressings and other foods (Cuhra, Gabrovská, Rysová, Hanák, & Štumr, 2011). Accordingly, mustard samples and other products containing mustard seed were analysed on erucic acid. Based on these results, we estimated the daily intake of erucic acid in Germany.

2. Material and methods

2.1. Samples

The mustard oils (MO) were bought retail in Germany ($n = 6$) and Australia ($n = 3$). According to label, mustard oils from Australia were imports from India or Pakistan. The German mustard oils were produced in Germany in small oil mills but the source of the mustard seeds used for this purpose remained unknown except for one sample produced from mustard seeds grown in Germany. No information was provided on the cultivars used for the individual mustard oil sample. The mustard sauces ($n = 5$) and mustard samples ($n = 15$) were purchased from different supermarkets located in Stuttgart (Germany), including the most popular commercial brands. The samples included different types of mustard, among them were sweet mustard, medium hot and hot ones and mustards of the Dijon type.

2.2. Chemicals and standards

Methanol and *n*-hexane (both HPLC grade, ⩾95%) were from Th. Geyer (Renningen, Germany). Sulphuric acid and ethanol (technical grade), distilled prior to use, were from BASF (Ludwigshafen, Germany). Hydrochloric acid was from Merck (Darmstadt, Germany). NaCl (⩾99.5%) and KOH (85%) were from Carl Roth (Karlsruhe, Germany). A 37 K fatty acid methyl ester (FAME) mix, erucic acid and myristic acid were from Sigma–Aldrich (Steinheim, Germany). The internal standard 10,11-dichloroundecanoic acid (10,11-DC) was prepared according to Thurnhofer, Lehnert, and Vetter (2008). Myristic acid ethyl ester was prepared by heating myristic acid with 1% sulphuric acid in ethanol.

2.3. Sample preparation

2.3.1. Transesterification of mustard seed oils

Mustard seed oils (about ~0.2 mg per sample) and 23.6 μg of the internal standard 10,11-DC (Thurnhofer et al., 2008) were treated with 1.5 mL of 1% sulphuric acid in methanol in a small sealed tube at 80 °C for 1.5 h. The tube was shaken from time to time. Then, 1 mL of saturated NaCl solution and 1 mL of demineralized water was added and the generated FAMEs were extracted once with 2 mL *n*-hexane. An aliquot of the organic layer was mixed with about 3 μg of the second internal standard (myristic acid ethyl ester, 14:0-EE) and the solution was subjected to GC analysis.

2.3.2. Fast screening method for mustard samples and mustard sauces

Mustard samples and mustard sauces (~0.3 g of each sample) were cold extracted with 4 mL *n*-hexane in a small sealed tube. After being shaken for about 1 min, the hexane extract was transferred into a second tube and the solvent was evaporated at 35 °C by means of a gentle flow of nitrogen. Thereafter, the transesterification procedure was performed without using 10,11-DC as mentioned above. Mustards of the Dijon type were heated in a sealed tube with 2 mL of 5 M hydrochloric acid prior to the extraction with *n*-hexane and the transesterification. All samples were analysed in duplicate determinations and the reported values are mean values (duplicate values generally varied less than 10%).

2.4. Gas chromatography coupled to electron ionisation mass spectrometry (GC/EI-MS)

FAME analyses were carried out with a 5890 series II/5971A GC/MS system in combination with a 7673A autosampler (Hewlett-Packard/Agilent, Waldbronn, Germany). Helium (purity 5.0) was used as the carrier gas at a flow rate of 1 mL/min. Two serially coupled 30 m columns (Rtx-2330, internal diameter 0.25 mm, coated with 0.1 μm 10% cyanopropylphenyl, 90% biscyanopropyl polysiloxane, Restek, Bellefonte, PA, USA) were installed in the GC oven. The GC oven program started at 60 °C (held for 1 min), then the temperature was raised at 6 °C/min to 150 °C, at 4 °C/min to 190 °C, and finally at 7 °C/min to 250 °C which was held for 7 min. The solvent delay was set at 6 min. In the full scan mode we recorded m/z 50–500 and in the selected ion monitoring (SIM) mode six ions (m/z 74, m/z 79, m/z 81, m/z 87, m/z 88 and m/z 101) were measured throughout the run. Peak identification was carried out in the full scan mode using the standards mentioned before. The quantification was performed in the SIM mode using 10,11-DC and 14:0-EE as internal standards (Thurnhofer et al., 2008). All preparation steps were performed in duplicate and the recovery rates ranged from 95% to 104%. For each sample, mean values were calculated and the results were expressed as percent (%) of the total fatty acids.

3. Results and discussion

3.1. Fatty acid patterns of mustard oils

Between 15 and 19 fatty acids were detected in the mustard oil samples (Table 1). Major fatty acids were oleic acid (18:1*n*-9), linoleic acid (18:2*n*-6), α-linolenic acid (18:3*n*-3), and, in most samples, erucic acid (22:1*n*-9), which was similar to reports in previous studies (Abul-Fadl et al., 2011; Genet et al., 2004; Mortuza et al., 2006). The main difference in the fatty acid patterns of the different samples was found to be the abundance of erucic acid, which could be the major fatty acid or only low abundant (Fig. 1). Samples low in erucic acid were characterised by a higher content of oleic acid (18:1*n*-9). If not erucic acid, oleic acid was the major fatty acid (Table 1). As a consequence, the contribution of the dominating monoenoic fatty acids to the fatty acid pattern was relatively constant (64.9–73.7%, Table 1). Likewise, the share of saturated (5.2–7.0%) and polyunsaturated fatty acids (PUFA) (20.9–28.6%) varied only with rather low margins (Table 1, Fig. 2). This was valid for both the German (MO 1–5, 9) and the Australian (MO 6–8) samples. Mustard oils from Australian retail (MO 6–8) showed similar fatty acid patterns with high concentrations of erucic acid (~45%) and oleic, linoleic and linolenic acid as further major fatty acids (Table 1). Samples purchased from German retail showed a greater variation including the lowest (0.3%) and highest (50.8%) contribution of erucic acid to the fatty acid pattern found within all samples (Table 1).

Due to the strong variations in the erucic acid content (0.3–50.8%) and its compensation by oleic acid, it was no surprise that oleic acid also shared a wide concentration range (9.2–61.0%) (Fig. 2) (Pearson correlation coefficient: −0.992). This can be explained by the fact that erucic acid is synthesized from oleic acid with elongases (Kanrar, Venkateswari, Dureja, Kirti, & Chopra, 2006; Males et al., 2000; Oram et al., 2005). Accordingly, if the elongation is blocked, the erucic acid content is low, whereas oleic acid content is high.

In addition, the amounts of 20:1*n*-7 and 22:1*n*-7 were found to correlate with erucic acid (Table 1). For instance, the ratio of 22:1*n*-9 to 20:1*n*-7 was ~40 (Pearson correlation coefficient: 0.981). Samples high in erucic acid (MO 6–9) showed 20:1*n*-7 amounts of 1.1–