LWT - Food Science and Technology 73 (2016) 397–405

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Identification and quantification of phenolic compounds in rapeseed originated lecithin and antioxidant activity evaluation

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ARTICLE INFO

Article history:
Received 29 April 2016
Received in revised form
2 June 2016
Accepted 15 June 2016
Available online 18 June 2016

Keywords:
Rapeseed gum
Phenolics
Phospholipids
Antioxidant activity
Identification

Chemical compounds studied in this article:
Sinapic acid (PubChem CID: 637775)
Sinapine (PubChem CID: 5280385)
Canolol (PubChem CID: 35960)

ABSTRACT

Phenolic compounds may exist in the gum generated from rapeseed oil refining as rapeseed is rich in phenolics. Therefore, this work identified the main phenolic compounds, followed by tracking their flow according to typical lecithin production procedures. Antioxidant activities of the extracts were also investigated to get a clue of the potential oxidative stability of the final products. Column chromatography, UV spectra, ^{1}H NMR, and HPLC were used to separate and identify the phenolic compounds. Sinapic acid was found to be the dominant phenolic while relatively low contents of sinapine and canolol were also identified. Nonpolar solvent aid extraction was better for extracting more sinapine and sinapic acid while direct ethanol extraction gave more canolol. Almost half of the phenolics in dried gum were removed by acetone during deoiling process, leaving only a small amount in deoiled lecithin. Extracts from deoiled lecithin showed the weakest antioxidant activity while the ethanol solubles showed the strongest. This work demonstrated the occurrences of bioactive phenolic compounds in rapeseed originated gum, which may be an advantage of rapeseed lecithin compared with other types of lecithin. This work could also be a reference for rapeseed lecithin production with different contents of bioactive compounds in industry.

1. Introduction

Soapstock/acid oil, deodorized distillates, and spent bleaching clay are main side-streams from vegetable oil refining (Echim, Verhé, De Greyt, & Stevens, 2009). Valorization of these side-stream products has been investigated either for high value products such as lecithin (Ceci, Constenla, & Crapiste, 2008), vitamin E (Isso & Ryan, 2012), and antioxidants (Chen, Thiyam-Hollander, Barthet, & Aachary, 2014) or for biofuel production (Echim et al., 2009).

Phospholipids are either present in a hydratable or a non-hydratable form. The non-hydratable form occurs when the phospholipids are combined with calcium, magnesium or iron cations. Acid treatment must be performed in order to convert them into hydrated gums. Then water is introduced to precipitate the gums (Dumont & Narine, 2007). The best known and the most widely used method to remove phospholipids, free fatty acid, and excess of phosphoric acid is the caustic soda process. The soapstock accompanied with gum is then continuously separated from crude oil by centrifugation (Dumont & Narine, 2007). Wet gum from degumming process mainly contains phospholipids and soapstock.

Phenolics, which are considered as major plant secondary metabolites, are much richer in rapeseed than in the other oilseeds (Yang et al., 2014). Sinapic acid and its derivatives especially sinapine are reported as the main phenolic compounds in rapeseed (Chen et al., 2014; Yang et al., 2014). Consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of their antioxidant activity. Additionally, natural antioxidants are attracting more attention due to safety concerns of synthetic antioxidants (Shahidi & Ambigaipalan, 2015). Some of the phenolics are accompanying with oil when the oil is pressed from rapeseeds (Naczk, Amarowicz, Sullivan, & Shahidi, 1998). Previously, many studies focused on phenolic compounds produced from rapeseed meal (Amarowicz, Naczk, & Shahidi, 2000; Khattab, Eskin, & Thiyam-Hollander, 2013; Szydłowska-Czerniak & Tułodziecka, 2014; Vermorel, Hocouemiller, & Evrard, 1987; Vuorela, Meyer, & Heinonen, 2004) since a large proportion of phenolic compounds are still in rapeseed meal after oil press. Amongst, Chen et al. (2014) investigated sinapic acid derivatives and tocopherols from canola oil refining

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http://dx.doi.org/10.1016/j.lwt.2016.06.039
0023-6438/

byproducts; where the content and DPPH radicals scavenging equivalency of sinapic acid derivatives in bleaching clay, wash water, soapstock, and deodorization distillate were quantified. However, no relevant data with regards to the phenolics in the gum were reported.

Soybean lecithin is now the dominant product on market. However, the concerns on its genetically modified organism have restricted its wide acceptance (Paparini & Romano-Spica, 2004). Rapeseed, on the contrary, does not have this problem, making rapeseed oil as one of the most promising feedstock for lecithin production. As discussed above, a large amount of phenolics existed in rapeseed than the other oilseeds. The phenolics, on one hand, are potent antioxidants (Hassasroudsari, Chang, Pegg, & Tyler, 2009). On the other hand display bitter taste (Clandinin, 1961), leading to an urgent investigation of the phenolics distribution in gums from different processes. This could benefit further commercialization of rapeseed lecithin production. Meanwhile, consumers could make their decision based on the composition of alternative lecithin product.

To the best of our knowledge, no systemic investigation about the phenolic compounds in rapeseed gum and the potential antioxidant activity of different products from the gum has been carried out. In the present work, several methods including ^{1}H NMR, UV spectra, and HPLC were employed to identify and elucidate the molecular structures of the phenolics presented in wet gum. Thereafter, direct ethanol extraction (DEE) and nonpolar solvent aid extraction (NPSAE) were compared for determination of phenolic compounds in dried gum. Subsequently, the content of phenolic compounds was tracked following the typical lecithin production procedures. The antioxidant activities of the extracts were also evaluated in order to find out the potential oxidative stability of the resultant products. This work is believed to establish new knowledge basis for advanced utilization of rapeseed originated lecithin.

2. Materials and methods

2.1. Samples and chemicals

Rapeseed gum and rapeseed meal were provided by DLG food oil (Alborg, Denmark). The gum is a mixture of phospholipids etc. polar lipids and soapstock and therefore the pH value is around 10.5. HCl (6.0 mol/L) was used to neutralize the gum to pH value of 6.8 and then the neutralized gum was stored at 4 °C until use. The neutralized gum was dried under vacuum to obtain dried gum. Hexane was used to dissolve the dried gum and obtain the hexane-solubles. Acetone was then used to deoil the hexane-solubles to obtain deoiled lecithin and recovered oil. The deoiled lecithin was further fractionated by absolute ethanol to obtain both ethanol-solubles. All chemicals used in this study were purchased from Sigma-Aldrich and of HPLC grade.

2.2. Preparation of extracts for column chromatography

Neutralized gum (20 g) was extracted with ethyl acetate in a ratio of 1:10 (w/v) for 20 min. The extracts were dried in a rotary evaporator at 50 °C and then re-dissolved in 5 mL of 70% ethanol. Hexane was used in a ratio of 1:1 to extract hydrophobic compounds thereafter. After separation, the lower phase was collected and condensed for column separation.

2.3. Column chromatography

Silica gel (high-purity grade, pore size 60 Å, 220–440 mesh particle size, 35–75 μm particle size) was used as stationary phase and diethyl ether:petroleum ether:acetic acid (80:20:1) and methanol were used as mobile phase A and B, respectively. Mobile phase B was used to elute the last fraction. Eluates (6 mL) were collected manually and their absorbance was recorded at 300 nm after 10 times dilution.

2.4. Isolation of sinapine and structure determination

Sinapine was prepared following a previously reported method (Clandinin, 1961). Briefly, trichloroethylene (TCE) was employed to remove the fat and resinous material from rapeseed meal. The TCE extracted meal was further extracted for 4 h in a Soxhlet apparatus with ethanol. The resultant ethanolic extract was concentrated and 100 mL of 20% KSCN solution was added. The mixture was stored at 4 °C for 48 h and the crystals of sinapine SCN were recovered by centrifugation at 3095*g*. Hot ethanol was used to re-dissolve the recovered sinapine SCN and the solution was stored at 4 °C for 48 h again. Crystals were collected by filtration and then dried. The dried sample was dissolved in CD_3OD and analyzed with a Bruker Ascend 400 spectrometer for structural confirmation purpose.

2.5. Preparation of canolol and structure determination

The synthesis of canolol was performed following the method (Khattab et al., 2013). Sinapic acid solution was put in a 100 mL conical glass flask and 100 μL of 1,8-diazabicy- clo [5.4.0]undec-7-ene (DBU), 5 mg hydroquinone, and 2.0 g aluminum oxide were added. The mixture was vortexed and the solvent was completely evaporated under N_2. The flask was then treated in a microwave oven for 5 min. Neutralization with 1 mol/L HCl was performed when the flask was cooled down to room temperature. Diethyl ether/ethyl acetate mixture (1:1) was then used to extract the phenolics. Canolol was further isolated from the mixture of phenolic by using TLC plate (TLC silica gel 60, 5 cm × 10 cm, Merck, Germany) with diethyl ether: petroleum ether: acetic acid (80:20:1) as the developing solvent. The compounds were visualized by using the DPPH radical solution in pure ethanol based on their DPPH radical scavenging ability. The purified sample was dissolved in CD_3OD and analyzed with a Bruker Ascend 400 spectrometer for the purpose of structural confirmation.

2.6. Extraction of sinapic acid derivatives

Hexane, heptane, and cyclohexane were selected as typical nonpolar solvents. Briefly, 1 g of sample was dissolved by 5 mL of a nonpolar solvent and mixed by a vortex. Subsequently, 2.5 mL of 70% ethanol (v/v) was employed to extract polar compounds twice. In DEE, 2.5 mL of 70% ethanol was mixed with 1 g of sample and extract for twice. The ethanolic extracts were collected for further analyses.

2.7. HPLC analysis of sinapic acid derivatives

Sinapic acid derivatives were quantified using a reversed-phase HPLC-PDA, with a 25 cm × 4.6 mm, 5 μm, SUPELCOSIL LC-18 column (Sigma-Aldrich Co., St Louis, MO). Gradient elution with two mobile phases, namely A (0.08 mol/L KH_2PO_4:CH_3CN:85% H_3PO_4 (85:15:0.25)), and B (60% MeOH: 85%H_3PO_4 (100:0.05)), was adopted for the separation with the following strategy: t_0-t_{18} 100% A, t_{23}-t_{32}0%A, and t_{37}-t_{42}100%A (t_0 … t_{42} means elution at 0 … 42 min). Standards of sinapine, sinapic acid, and canolol were used to authenticate the retention time and UV adsorption spectra. The contents of all sinapic acid derivatives were expressed as sinapic acid equivalents (SAE), wherein sinapine and sinapic acid were detected at 330 nm; while canolol was detected at 270 nm.