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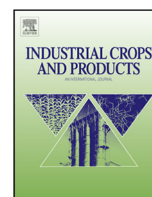

Influence of rapeseed meal treatments on its total phenolic content and composition in sinapine, sinapic acid and canolol

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Rapeseed meal is the co-product of the pressing and de-oiling process of rapeseed seeds and is used as animal feed. Most of phenolic compounds remain in the meal after processing the seeds. While sinapine (sinapoyl choline), sinapoyl glucose and sinapic acid are naturally present in the seed, canolol (4-vinylsyringol) is formed during processes of pressing, oil extraction and roasting treatments via decarboxylation of sinapic acid. Canolol was recently described as a free-radical scavenger with various biological activities. One of the objectives of this work was the valorization of rapeseed meal as a source of canolol, this latter being produced through the transformation of sinapine and sinapic acid under hydration and roasting processes applicable at industrial scale. The parameters studied for the rapeseed meal processing were: (i) time of incubation after hydration: 0, 2 and 18 h and, (ii) thermal treatment: high-temperature steam (105°–160 °C) or microwave roasting (160°–180 °C). It was concluded that temperature, and exposure time in case of microwaves, were the most important factors in increasing concentrations of canolol in rapeseed meal. Incubation time after hydration did not influence the total phenolic compounds content suggesting the absence of endogenous enzymatic hydrolysis. However, it showed a particular contribution in sinapine, sinapic acid and canolol transformation during the microwave treatment. Finally, whatever the treatment, only a part of the sinapic acid initially present or generated during the processes, was converted into canolol.

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1. Introduction

Rapeseed (*Brassica napus* L.) is a plant from the oleaginous cruciferous species grown mainly for its oil (Harbaum-Piayda et al., 2010; Kuwahara et al., 2004). The products obtained after de-oiling the seeds are crude oil (43.6% of the seed) and meal (53.8% of the seed). Around 23.6 million tons of rapeseed are expected to be produced in the European Union in 2015, up to 17.5% more compared to 2013 (CETIOM, 2013, 2014; Fine et al., 2015).

The industrial processes of oil extraction from rapeseed generally employ mechanical or combines mechanical and solvent extraction methods. Before the oil extraction step, the seeds are normally submitted to different unit processes of preparation such as cleaning, drying, grinding, hull removing, cooking, flaking and extruding. Except for cold pressing, seeds are generally roasted and sometimes dried prior to their extraction. When roasting time is longer than 10–15 mins, this step is referred as ‘cooking’. Conditioning the seeds facilitated the heat transfer from saturated steam to the oilseed without allowing the steam to enter into contact with the material being conditioned. The preparation of the seeds is a critical step in terms of temperature, time of roasting and moisture content. These parameters could influence malleability and porosity of the cake as oil viscosity. When no solvent is applied to oil extraction (cold pressing), a double pressing separated by a cooking step is required. This kind of treatment affords rapeseed cakes with a 9–10% oil residue. A single full press gives slightly more fatty cakes

Abbreviations: TLC, thin layer chromatography; R_t, retention time; R^2, determination coefficient; UPLC, ultra performance liquid chromatography; TPC, total phenolic compounds; SAE, sinapic acid equivalents; DM, dry matter; H0, without hydration and incubation; H2, hydration followed by two hours incubation; H18, hydration followed by eighteen hours incubation; SR, simple roasting; SRSH, simple roasting with superheated steam; MWR, microwave roasting; MWRSH, microwave roasting with superheated steam.

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(10–12%). Rapeseed cakes originated from cold pressing could have from 12 to 25% oil content, depending on the capacity and quality of the press. The solvent extraction is beneficial in terms of yields of oil extraction. The most common solvent used in industrial processes is *n*-hexane. The removal of this solvent (desolventization) from the meal is primordial to enable its application as animal feed.

Rapeseed contains more phenolic compounds than other oleaginous plants (Kozlowska et al., 1990; Lomascolo et al., 2012). When the oil is pressed from the seeds, most of them remain in the meal (Liu et al., 2012). Rapeseed meal is fully used to feed livestock, either in compound feed, either directly in manufacturing farm. The total content of phenolic compounds ranges between 6400 and 18,400 $\mu g\ g^{-1}$ of dry matter, depending on the variety of plant and the method of processing the oil (Kozlowska et al., 1990). Furthermore, growth conditions and the degree of maturity of the plant affect its phenolic composition. Thermal and hydrolysis treatments of the seeds can lead to the release of these phenolic molecules (Niu et al., 2013; Spielmeyer et al., 2009; Vuorela, 2005). The most abundant phenolic compound present in rapeseed is sinapine, the choline ester of sinapic acid which account for up to 80% of the total phenolic compounds (Kozlowska et al., 1990). During rapeseed germination, some sinapine can be released as free sinapic acid, namely about 70% of the total free phenolic acids in rapeseed meal (Koski et al., 2003; Kuwahara et al., 2004; Terpinc et al., 2011).

Due to the presence of phenol rings diversely substituted, phenolic compounds exhibit more or less marked antioxidant properties. One of the most active antioxidant components found in the polar fraction of the rapeseed extracts is canolol (4-vinylsyringol or 2,6-dimethoxy-4-vinylphenol). Canolol is produced by decarboxylation of sinapic acid during the process of roasting of rapeseed (Koski et al., 2003; Kuwahara et al., 2004; Spielmeyer et al., 2009; Wakamatsu et al., 2005). The increased oxidative stability of rapeseed crude oil after roasting treatment is mainly attributed to the formation of canolol (Koski et al., 2003; Siger et al., 2013; Wijesundera et al., 2008). Recently, canolol has been reported as a powerful radical scavenger and is also known for its anticarcinogenic and antimutagenic properties (Khattab et al., 2014; Terpinc et al., 2011). To enhance canolol content in rapeseed, pretreatments of the seeds by infrared, microwave roasting and/or high-temperatures were proposed (Cai and Arntfield, 2001; Spielmeyer et al., 2009; Wijesundera et al., 2008). The present work was conducted in order to study and characterize the phenolic extracts of different treated rapeseed meals in terms of concentration in sinapine, sinapic acid, canolol and total phenolic compounds. The objective of this study was to identify possible treatments of rapeseed meal after pressing and solvent extraction promoting the formation of canolol.

2. Materials and methods

2.1. Chemicals

Sinapine thiocyanate (99.9%) was from Wilshire Technologies Inc. (Princeton, USA), Sinapic acid (98%) was from Alfa Aesar (Karlsruhe, Germany). Syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde, ≥98%), malonic acid (99%), piperidine (≥99.5%), toluene (99.8%), Folin–Ciocalteu phenol's reagent, sodium carbonate (≥99.5%), acetic acid (>99.7%), *n*-hexane (95%), ethyl acetate (99.8%), methanol and water (for HPLC, >99.9%) were purchased from Sigma–Aldrich (Saint Quentin, France).

2.2. Raw materials

A commercial lot of 150 kg rapeseed (glucosinolates content = 18.1 $\mu mol\ g^{-1}$) were purchased from Arterris (Castelnaudary, Aude—France). The processing of the material (Fig. 1) was carried out at the oil-mill pilot plant CREOL (Pessac—France) with a capacity for 200 $kg\ h^{-1}$. Sample 1 referred to seeds, sample 2 (cake) corresponds to the seeds after a mechanical cold pressing de-oiling process on a MBU 20 press. Sample 3 (rapeseed meal) was the product of a process of de-oiling, with *n*-hexane, of the sample 2. The desolventization process was carried out under vacuum at 50 °C to avoid denaturation of the endogenous enzymes in raw materials. Sample 3 was subjected to different hydration/incubation and roasting treatments, as described in Table 1. These processes led to samples 4–15 (Fig. 1) which treatments are described below.

2.2.1. Hydration step

The meal produced in the third step of the process (sample 3, water content 10.3% w/w) was divided into three equal parts (Fig. 1). The first portion was set aside without any treatment (H0) while the other two were first hydrated to a water content of 21.4 ± 0.1% (w/w) and then mixed at 32 ± 2 °C for 2 (H2) and 18 h (H18). After incubation, H2 and H18 fractions were dried in a warm air cabinet at 70 °C for 1 h 15. This step was done to verify the possibility of an endogenous enzymatic hydrolysis of the sinapine present in the meal, with a consequent release of sinapic acid whose could be decarboxylated into canolol by the thermal treatments.

2.2.2. Roasting treatments

All meal samples (H0, H2 and H18) were exposed to various industrial thermal treatments as follows (Table 1):

- SR: Simple roasting—This treatment was intended to reproduce the temperature and moisture conditions occurring during regular desolventization. The samples were pre-heated at 80 °C in a stirred bench cooker then exposed to an injection of steam to reach 105 °C and kept at this temperature for 50–60 min.
- SRSH: Simple roasting with superheated steam—The meal was first pre-heated at 80–90 °C. Then, a 180–200 °C steam was applied to reach a temperature of 160 °C which was maintained for 47–51 min.
- MWR: Microwave roasting (2.45 GHz and 10 kW)—The microwave oven tunnel was conditioned to reach the desired temperature of 160 °C as follows: the tunnel was first filled by rapeseed flakes to avoid an empty oven run. The feeding rate of the tunnel was about 50 $kg\ h^{-1}$. The transportation belt on which the meals were transported in the cavity of the tunnel has a linear speed of 4 $mm\ s^{-1}$. The power was set at the highest possibility when the temperature measured at the exit of the oven was under 160 °C. Otherwise, the power was adjusted to obtain a temperature of 160 °C in the core of the tunnel. The typical residence time in the active part of the tunnel was 375 s (6 min 15), except for sample MWR-H0 (with no hydration treatment) that rested in the tunnel for 333 s (5 min 33). After the treatment, the material was cooled by spreading.
- MWRSH: Microwave roasting (2.45 GHz and 10 kW) with a superheated steam treatment—To the material obtained at the previous step (MWR) was applied an injection of superheated steam at 180 °C (2 $kg\ h^{-1}$) in the middle of the active part of the tunnel and about 10 mm above the bottom of the tunnel.

The temperatures of the treatments were chosen taking into account the results obtained by various authors (Pudel et al., 2014; Spielmeyer et al., 2009; Wakamatsu et al., 2005) where roasting temperature of 160 °C and 165 °C provided the highest canolol contents.

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