Facile Preparation of Purified Sinapate Ethyl Ester from Rapeseed Meal Extracts Using Cation-exchange Resin in Dual Role as Adsorber and Catalyst

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Abstract: In this study, cation-exchange resin was used to prepare an esterified antioxidant, sinapate ethyl ester (SE), using ethanolic extracts from rapeseed. A concentration of sinapic acid using the cation-exchange resin in 80% ethanol (aq) and subsequent interesterification of the extract in ethanol using the same resin afforded a product with a purity of 64 wt% and 100% of SE yield. Moreover, after purification using preparative thin-layer chromatography, almost 100 wt% purity was obtained. In an auto-oxidation test, purified SE conferred a much higher antioxidative effect on the bulk oil, emphasising the effectiveness of the protocol using cation-exchange resin for the purification.

Key words: sinapate ester, interesterification, rapeseed oil, ion-exchange resin

1 Introduction

Among numerous naturally occurring bioactive molecules, phenolic compounds, which exhibit antioxidant, anti-UV and antimicrobial activities, are highly desirable resources. While numerous phenolic acids can be used directly in various applications, the challenge of enhancing their function via chemical modification without producing toxic waste has been eagerly examined.

Rapeseed meal is a by-product of the respective oil processing¹⁾. Simple phenolic compounds in rapeseed are mainly sinapine (SP), the choline ester of sinapic acid (SA) and a small portion of SA in the free form. The free form can be readily obtained by hydrolysis of SP and microwave irradiation affords 4-vinylsyringol via decarboxylation ($-\text{CO}_2$) of SA¹⁻⁴⁾. Since chemical modifications, including these reactions and others, frequently occur in the engineering process, the exploration of value-added product has been performed as a part or an extension of the study¹⁻¹⁰⁾.

Among them, sinapic alkyl ester has been identified as one of the value-added phenolic compounds^{1, 3, 5-10)}. The methyl ester of SA was confirmed as a major constituent of methanolic extract from radish sprout (*Raphanus sativus* L.) and brown mustard (*Brassica nigra*) and as a minor constituent in rapeseed oil¹⁾. Therefore, a series of sinapic alkyl esters, regarded as naturally related compounds, have

been investigated regarding their physio-chemical and bio-active properties^{1, 5-10)}. Concerning the antioxidative effects, their performances were compared with other SA derivatives in versatile situations. The sinapic alkyl ester has been known to exhibit superior antioxidative effects compared to the free form, as exemplified in the oxidised low-density lipoprotein⁵⁾, membrane protection *in vivo*⁶⁾ and oil-based products^{7, 9, 10)}. These effects have been further recognised as these alkyl esters form to be intermediates for the further derivations^{11, 12)}.

Meanwhile, the sinapic alkyl esters without natural occurrence were prepared by conventional chemical synthesis using Fisher acids, such as sulfuric acid^{6, 7, 9, 10, 12)} and hydrochloric acid¹¹⁾, as catalysts for esterification. Here the SA materials were highly purified commercial product^{5-7, 9-11)} or had been synthetically prepared¹²⁾, meaning that the purification protocols of the SA and its derivative were separately conducted. Considering the versatile potential of sinapic alkyl ester as a value-added antioxidant and unique intermediate for the value-extended material, the development of cheap and facile modification methodology for the sinapic alkyl esters must be significant.

Ion-exchange resin has been used as a recyclable adsorbent, in addition to zeolite and activated carbon^{13, 14)}. By appropriately selecting the resin type, such as cation and

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anion resins, the extracts can be effectively separated (i.e. cation-exchange resin can trap cationic species)^{15–17}. Moreover, the resin is known to be used as a catalyst for the esterification of phenolic acids^{18,19}. Recently, a cation-exchange resin was used as a catalyst for the esterification of chlorogenic acid¹⁹. Thus, cation-exchange resin can be dually utilised as both adsorbent for cation species and catalysis for esterification, but as far as we know, dually use of these functions have been rarely reported.

Herein, we introduce the preparative procedure of the sinapic ethyl ester (SE) from the extract of rapeseed meal using cation-exchange resin (Scheme 1). One is the direct interesterification of the extracts. The other dually use the resin as the adsorbent of cationic SP from the extract of rapeseed meal, and as the catalyst for the subsequent interesterification reaction. Then, the antioxidative properties were evaluated using the SE-containing products with different purities in terms of the radical scavenging effect and the preservation of oil substance. Throughout the experiment, we only used ethanol and water as solvent to avoid highly toxic solvent.

2 Experimental Procedures

2.1 Chemicals

Sinapic acid source: Rapeseed meal was provided from Showa Sangyo Co., Ltd. (Tokyo, Japan). Sinapic acid (SA) used as a standard was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan).

Cation-exchange resin: Amberlite XH 2071H was purchased from Organo Corp. (Tokyo, Japan) and DOWEX™ 50Wx2 50-100 Mesh (H) was obtained from Fujifilm Wako Pure Chemical Industries (Osaka, Japan), respectively. They were sufficiently washed with water prior to use.

Free radical: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was

purchased from Fujifilm Wako Pure Chemical Industries (Osaka, Japan).

Oil substance. High linoleic safflower oil used for the auto-oxidation test was obtained from The Nisshin Oil Group, Ltd. (Tokyo, Japan). First, the oil was purified with activated carbon (Powder, Product code 037-02115; FUJIFILM Wako Pure Chemical Industries, Ltd., Osaka, Japan) to remove the contained antioxidant in commercial oil. Then, oil substance was purified using a silica gel column chromatography purification with a mixed eluent of *n*-hexane/diethyl ether (85:15, v/v). The purified oil substance was used immediately after the purification.

Commercial antioxidant: Vitamin E mixture (α -form: 14.3%, β -form: 1.6%, γ -form: 69.0% and δ -form: 15.1%) used for the auto-oxidation test was obtained from The Nisshin Oil Group, Ltd. (Tokyo, Japan).

2.2 Preparation of sinapate ethyl ester (Fig. 1)

2.2.1 Solvent and hot water extraction of rapeseed phenolics from rapeseed meal

Defatted meal (10 g) was extracted three times with 50 mL extraction solvent (80% ethanol) by heating under reflux for 1 h with magnetic stirring. Each extract was obtained from insoluble fractions by filtration and the filtrate was concentrated by evaporating under reduced pressure to give $1.36 \text{ g} \pm 0.179$ ($n=3$) extract including 156.4 mg of SP.

2.2.2 Direct interesterification of extracts

The extract (0.2 g) and cation-exchange resin, Amberlite XH 2071H or DOWEX™ 50Wx2 50-100 Mesh (H), (0.5 g) were added to ethanol (10 mL) and the interesterification reaction was initiated by heating at 80°C with magnetic stirring. After the 48-hour reaction in ethanol, the resin was removed by filtration. The filtrate was evaporated under reduced pressure to afford the sinapate ethyl ester (SE)-containing product (Product-A). The yield was 85%

Scheme 1 (i) Direct interesterification of extracts with ethanol using cation-exchange resin as a catalyst. (ii) Separation and interesterification of extracts with ethanol and water using cation-exchange resin as adsorbent and catalyst. SE, SP and SA in the Scheme are the abbreviations of sinapate ethyl ester (SE), sinapine (SP) and sinapic acid (SA), respectively.

Fig. 1 Preparation of sinapate ethyl ester (SE) products (Product-A, -B and -C) from rapeseed meal.

based on initial SE molar and the mass percentage (purity) of SE fraction was 18 wt% against total mass fraction.

2.2.3 Interesterification of loaded extracts on cation-exchange resin

The extract (0.2 g) and cation-exchange resin, Amberlite XH 2071H, (0.5 g) were added to ethanol (10 mL) and the mixture was stirred for 0.5 h to load SP onto the cation-exchange resin. The resin was obtained by filtration and dried with filter paper. To ethanol, the SP-loaded resin was added and interesterification reaction initiated by heating at 80°C with magnetic stirring. After the 48-hour reaction in ethanol, the resin was removed by filtration. Subsequently, the filtrate was concentrated to dryness to afford the SE-containing product (Product-B). The yield and purity for the SE product were 98% against total mass and 64.0 wt% of SE fraction, respectively.

Product-B was purified through preparative thin-layer chromatography (PTLC) using PLC Silica gel 60 (Merck KGaA, Darmstadt, Germany) with a mixed solvent of benzene/ethyl acetate (3: 7, v/v). SE-containing product (Product-C) was obtained after PTLC with 91% recovery rate and 100 wt% purity of SE fraction. The purity of Product-C was confirmed by ¹H-NMR analysis.

2.3 Measurement

The general experimental procedure was described in ESI.

2.3.1 Evaluation of radical scavenging ability

A free radical scavenging activity test of compounds was measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as a stable radical²¹. Each substrate was dissolved in methanol (2 mL) and 0.1 M acetic acid buffer (pH 5.5) (2 mL) was added to the solution. Subsequently, 1 mL of DPPH in methanol (0.5 mM) was mixed with the test solutions at the indicated concentrations or their diluent (similar solution). Each mixture was magnetically stirred and held in the dark for 30 min at room temperature. The absorbance of DPPH at 517 nm was then measured. The radical scavenging activity was estimated as (%) inhibition of DPPH absorbance. The radical scavenging ability in mol was expressed using that of the vitamin E mixture as a standard (unity). The value was averaged of three times experiments.

2.3.2 Evaluation of antioxidation activity on auto-oxidation test

The antioxidant capacity of prepared SE was evaluated by the comparison with that of the commercial antioxidant, the vitamin E mixture. Oxidation stability at the primary step was evaluated by an auto-oxidation test^{22, 23}. Briefly, each of the substances containing 500 ppm of antioxidant were added to a petri dish (90 mm × 15 mm) and kept at 30°C in the dark during several days. According to the standard method for the analysis of fats, oils and related materials as prescribed by the Japan Oil Chemists' Society, the peroxide value (PV) (meq/kg), which is one of the representative evidence of rancidity in unsaturated oils, was evaluated in a time-course manner by a potentiometric titration method using a GT-200 General-Purpose Automated Potentiometric Titrator (Mitsubishi Chemical Analytech Co., Ltd, Kanagawa, Japan).

3 Results and Discussion

3.1 Preparation of SE

3.1.1 Direct interesterification of extracts

Extraction from rapeseed meal using 80% ethanol afforded phenolic extracts containing 156 mg of SP and 19 mg of SA, respectively. These extracts consisted of 12.9 wt% of total extract. These values were included in the ranges reported by previous works¹. To the extracts, we applied the esterification reaction in ethanol solvent under reflux condition using the cation-exchange resin as a catalyst. By using Amberlite XH 2071 or DOWEXTM 50Wx2 resins (Fig. S1, ESI), the interesterification reaction successfully proceeded. After a 24-hour reaction time, more than 90% of conversion was confirmed, respectively.

After the removal of resin, Amberlite XH 2071H, and the concentration of the solvent under reduced pressure, the

Product-A was obtained with the purity of 18.0 wt% of SE fraction in total mass and 85% of SE yield. Since the SE yield prepared from purified SA using Fisher esterification was reported around 80%⁶⁾, such high yield of 85% indicates the usefulness of the direct interesterification reaction from SP using Amberlite XH 2071 resin. For the product, the main fraction containing SE was separated by preparative TLC, resulting in a product with 26.8 wt% of purity of SE fraction. Owing to the strong affinity of SE and other components contained in the product, the separation was not a practical protocol.

3.1.2 Esterification of SP loaded on cation-exchange resin

3.1.2.1 Separation of SP-rich extract

Figures 2a and 2b show the HPLC chromatograms obtained from the extracts before and after the addition of cation-exchange resin. While no change in the peak attributed to SA was recognised (Fig. 2a), the peak attributed to SP vanished corresponding to the filtrate after the addition of resin (Fig. 2b). This result clearly indicates the separation of SP via adsorption on the resin through the ionic interactions between cationic SP and anionic sulfonate substituent in the resin^{16, 17)}. The addition of 10% HCl(aq) to the resin ended the interaction by exchanging the ionic species trapped on resin and redissolving the SP in solution. The presence of SP was confirmed by HPLC in the washed solution (Fig. S2, ESI). The numerous peaks indicate that not only SP but also other cationic species in the extract were adsorbed on the resin. Thus, the purity of recovered SP was not high. However, a large amount of impurity could be removed by the selective loading of cationic extract. The resin, where the large amount of SP was loaded, was used for the esterification.

3.1.2.2 Preparation of SE via esterification of cationic extracts

The esterification was carried out using the SP adsorbing resin in ethanol under reflux condition. After the 48-hour

esterification reaction, **Product-B** was obtained with 98% yield of SE and the purity of 64 wt% of SE fraction, indicating the success of the reaction. In addition, the compound showed a peak around 0.4 in R_f value for TLC analysis using a *n*-hexane/ethyl acetate (1: 9) as eluent, and was separable (Fig. S3a, ESI). After the PTLC purification, the purified product, **Product-C**, was confirmed as nearly 100% of SE for TLC analysis, with ¹H-NMR analysis supporting the result (Fig. S3b, ESI). Compared to the **Product-A**, the impurities that tended to interact with SE and/or showed a similar polarity were reduced and the isolation of SE could be successfully performed, thus achieving the effective concentration of SE fraction.

3.2 Investigation of antioxidative ability

Then, the antioxidative abilities of the SE products, **Product-A** and **-C**, were evaluated using the vitamin E mixture as a standard (Fig. 3). DPPH radical scavenging test showed that the radical scavenging property of the SE-containing products was higher than in the vitamin E mixture and no apparent difference existed despite the difference in the impurity mass (Fig. 3a). Subsequently, the auto-oxidation test was performed using the purified high linoleic safflower oil with and without the addition of antioxidants. Figure 3b showed that the addition of SE prevented the increase of PV value. Noteworthy, for the product with higher purity of SE, the antioxidative effect was more pronounced by two times even though the added SE amount was same. **Product-A** with low purity showed a lower effect than the standard of vitamin E mixture, whereas **Product-C** with high purity showed a higher effect than the standard of vitamin E mixture. The results clearly conveyed that the antioxidant behaviour was prohibited in the presence of impurity due to contaminants. Thiyam *et al.* have mentioned the possibility of the slightly pro-oxidative property of SP⁴⁾. Though SP was mostly consumed during the interesterification reaction in this study, scarce remaining SP or the corresponding decomposed products, such as choline species, may exist and exhibit slightly pro-oxidative properties for the **Product-A**. Specifically, the effective concentration of SE fraction and the accomplishment of SE purification prepared from rapeseed extract was regarded as crucial to exhibit the highly value-added antioxidative effect on oil-based product and the use of cation-exchange resin was critical.

Fig. 2 HPLC profiles for (a) ethanolic extract, and (b) filtrate after adsorbing ingredients on Amberlite resin.

4 Conclusion

The novel and facile preparative method to give highly purified SE from the extracts of rapeseed meal was developed for use of a cation-exchange resin as both adsorber and catalyst. The resulting highly purified SE behaves as a notable value-added antioxidant on the lipid preservation

Fig. 3 Antioxidative performance of sinapate ethyl ester (SE) products (**Product-A** and **-C**) with different purities of 18.0 and 100 wt% of SE fractions, respectively. (a) DPPH radical scavenging ability with SD (standard deviation) and CV (Coefficient of variation) deviations ($n = 3$), and (b) antioxidative effect on the auto-oxidation test. In (a), the DPPH radical scavenging ability of vitamin E mixture (VE-mix) was used as standard (1.00 ± 0.00). SD and CV values were depicted in the figure. In (b), the days requiring for 30 meq/kg were obtained from the day v.s. peroxide value (PV) curves. VE-mix indicates the vitamin E mixture.

compared to the typical SE-containing product with impurity due to contaminants from rapeseed extract.

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Supporting Information

This material is available free of charge via the Internet at <https://dx.doi.org/jos.70.10.5650/jos.ess21036>

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