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Bioavailability of Rapeseed Oil Fortified with Ethyl Sinapate †

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Abstract: In recent years, phenolic acid esters have been proposed as valuable additives in the food and cosmetic industries. Therefore, ethyl sinapate (ESA) was synthesized using an enzymatic approach. Hence, the bioaccessibility of antioxidant compounds after the in vitro digestion process of rapeseed oil fortified with ESA was evaluated. The antioxidant activity (AA) of the oil samples before and after digestion was analyzed using three spectrophotometric methods. The addition of synthesized ESA at concentrations of 0.02 and 0.5% caused a two- to ten-fold increase in the AA of the rapeseed oils. Therefore, the obtained phenolipid can be used as an efficient antioxidant in the oil industry.

Keywords: bioavailability; rapeseed oil; ethyl sinapate; antioxidant activity

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1. Introduction

Rapeseed oil is the most popular vegetable oil in Poland. It is a valuable source of bioactive compounds such as polyphenols, phospholipids, and sterols. Additionally, it contains a high amount of essential fatty acids. Unfortunately, oils containing a high amount of unsaturated acids are easily oxidized. Therefore, it is desirable to protect the oil from the oxidation process [1]. However, recent studies have demonstrated the toxic effect of commercially available, artificial phenolic antioxidants, e.g., butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), and especially their metabolites [2]. This fact prompts the search for new compounds dedicated to the fat industry. Unfortunately, naturally phenolic antioxidants have hydrophilic properties. Hence, phenolic acids are esterified to obtain their amphiphilic derivatives. The research focused on phenolic acids because they are widespread in the plant world and have many health beneficial properties such as antioxidant, chelating, free radical scavenging, antiallergic, anti-inflammatory, antimicrobial, antiviral, anticarcinogenic properties [3–5]. Lipophilization of phenolic acids has been carried out in chemical, enzymatic, and chemo-enzymatic ways [2]. However, enzymatic synthesis offers milder reaction conditions. Additionally, it is known that phenolic acid alkyl esters possess antioxidant properties and enhance the AA of food products [3–5].

To the best of our knowledge, there are no data on the influence of ESA on the AA of enriched refined rapeseed oil after the in vitro gastrointestinal digestion process. In general, antioxidants should be bioavailable and bioaccessible to be biologically active. At the same time, their bioavailability depends on the release of molecules from the food matrix during the digestive process [6,7].

Given the above, this study focused on the enzymatic synthesis of ESA. Moreover, in order to increase the nutritional value of rapeseed oil and to valorize ESA, in the present study, the digestive stability of phenolic compounds from rapeseed oils enriched with ESA at two concentration levels was investigated and changes in their AA using the standardized static in vitro digestion model was monitored.

2. Materials and Methods

2.1. Reagents

All of the reagents, reactants, and solvents were purchased from Merck (Warszawa, Poland). Silica gel (pore size 60Å, 230–400 mesh, Kieselgel, Macherey-Nagel, Dueren, Germany) was purchased from Alchem, (Toruń, Poland). The biocatalyst used in this work, Novozym 435 (*Candida Antarctica*) lipase B immobilized on a macroporous acrylic resin, was a generous gift from Novozymes (Madrid, Spain).

2.2. Samples

The refined rapeseed oil in its original packaging (polyethylene terephthalate (PET) bottle) was kindly provided by a local vegetable oil factory.

2.3. NMR Analysis

The structure of the ESA was confirmed by nuclear magnetic resonance (NMR) spectroscopy. The ^{1}H NMR spectrum was recorded with a Bruker Avance III 400 MHz spectrometer (Bruker Corporation, Karlsruhe, Germany) at 298 $\pm$ 1 K. The sample was dissolved in $CDCl_3$ containing TMS as an internal standard. Chemical shifts were recorded in δ values in parts per million (ppm), and coupling constants (J) were reported in hertz (Hz).

2.4. Enzymatic Synthesis

Lipase-catalyzed esterification of sinapic acid with ethanol was conducted in a 20 mL vial equipped with a tight plastic cap. Sinapic acid (5 mM) with 10 mL of ethanol (dried over 3 Å molecular sieves) was mixed. After dissolving the sinapic acid, the reaction was started by the addition of 60 mg/mL of the biocatalyst. Molecular sieves (3 Å, 40 mg/mL) were also used to remove water formed as a by-product. The reaction was carried out in an orbital shaker at 120 rpm at 65 °C for five days (Incu-Shaker™ Mini Shaking Incubator-Benchmark Scientific; New York, NY, USA). The biocatalyst and molecular sieves were filtered and washed on a Buchner funnel. The ethanol was evaporated under vacuum using a rotary evaporator (Laborota 4003, Heidolph Instruments, Schwabach, Germany). The ESA was purified on silica gel using dichloromethane:ethyl acetate (90:10) as an eluent. The synthesized ESA [ethyl (E)-3-(4-hydroxy-3,5-dimethoxyphenyl)-propenoate] had the following characteristics: light yellow solid, ^{1}H NMR ($CDCl_3$) δ: 0.90 (t, J = 7.0 Hz, 3H), 3.95 (s, 6H), 4.19 (t, J = 6.8 Hz, 2H), 6.31 (d, J = 15.9 Hz, 1H), 6.82 (s, 2H), 7.69 (d, J = 15.9 Hz, 1H).

2.5. Addition of the ESA to Refined Rapeseed Oil

The refined rapeseed oil was fortified with the ESA at two different concentrations: 0.02% and 0.5%. The ESA was weighed (at 0.01 and 0.25 g, respectively), added to 50.0 g of oils, and transferred into Erlenmeyer flasks. The samples were placed in an ultrasonic cleaner bath (Sono Swiss, SW 6H, Labo Plus, Warszawa, Poland) with an ultrasound input power of 180 kW for 5 min to completely dissolve the ESA.

2.6. In Vitro Digestion

The in vitro digestion process was carried out according to the procedure described by Seiquer et al. [6]. Before the digestion process, 1 g of the oil sample and 9 mL of distilled water were placed in an ultrasonic cleaner bath (Sono Swiss, SW 6H, Labo Plus, Warszawa, Poland) with an ultrasound input power of 180 kW for 5 min. The first step was a digestive process in the stomach (gastric digestion). An initial pH was adjusted to 2 with 1M HCl, then pepsin/HCl solution was added to each oil sample and put on an orbital shaker (Incu-Shaker™ Mini Shaking Incubator-Benchmark Scientific; New York, NY, USA) at 37 °C at 110 rpm for 2 h. After this time, the pH was adjusted to 6 by a dropwise addition of 1 M $NaHCO_3$. Then, 2.5 mL of pancreatic enzyme solution was added to simulate the next step, duodenal digestion. The pH was adjusted to 7.5 with 1 M $NaHCO_3$. The solutions were again incubated for 2 h at 37 °C at rpm 110. After the digestion process, the enzymes were inactivated by heating for 4 min at 100 °C. The solutions were frozen for 15 min and then

centrifuged for 15 min to separate fractions. For each test, the top oil layer formed was weighed and processed for methanol extracts.

2.7. Antioxidant Activity

2.7.1. Sample Preparation

The methanolic extracts of the studied oils were prepared according to the procedure described in our previous work [8]. Briefly, 2.00 g of oil was weighed into test tubes and extracted with 5 mL of methanol for 30 min using an orbital shaker (SHKA25081 CE, Labo Plus, Warszawa, Poland). Then, the extracts were frozen to separate the oil from the methanol (−20 °C, 30 min) and transferred quantitatively into glass bottles. Each oil sample was extracted in triplicate. The extracts were stored in a refrigerator until the AA analyses were carried out.

2.7.2. Analytical Methods

The AA of the refined rapeseed oil, with and without ESA and before and after each step of the digestion process, was determined by three spectrophotometric methods: 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and Folin–Ciocalteu (FC), as described in our previous work [8]. The UV–Vis spectra of the obtained solutions were recorded using a Hitachi U-2900 spectrophotometer (Tokyo, Japan) in a 1 cm quartz cell.

The obtained results were expressed in μmoL of Trolox equivalents per 100 g of sample.

2.8. Statistical Analysis

The AA of the oil samples was determined five times within one day by the modified ABTS, DPPH, and FC methods. The obtained results were presented as mean (c) ± standard deviation (SD).

3. Results

The enzymatic synthesis was carried out in milder conditions without organic solvents, while the yield of this reaction was 21%.

Bioaccessibility is the amount of a food constituent present in the gut due to its release from the solid food matrix, which affects the organism through the intestinal barrier [6,7]. Therefore, the AA of the oil samples was determined before and after each simulated digestion step, and the data are presented in Table 1. The AA of the non-supplemented oil was similar to the results reported in our previous work [9]. As was observed, the antioxidant potential of the rapeseed oils analyzed by the three methods differed, probably due to the different mechanisms of the analytical methods used. The refined rapeseed oil enriched with ESA had higher ABTS (1939.12–4036.24 μmol TE/100 g), DPPH (580.74–5183.95 μmol TE/100 g), and FC (292.28–581.98 μmol TE/100 g) results than those of the oils without the new antioxidant (ABTS = 1277.62 μmol TE/100 g, DPPH = 520.32 μmol TE/100 g, and FC = 120.01 μmol TE/100 g). Furthermore, an increase in the radical scavenging activity determined by the ABTS (2.08-fold) and DPPH (8.9-fold) assays and total phenolics analyzed by the FC method (1.99-fold) for the rapeseed oils spiked with increasing concentrations of ESA (0.02 and 0.5%) was found. Differences in antioxidant properties were observed during the digestion process. However, after the two-stage digestion process, no significant changes in AA were noted for the non-supplemented oil (Table 1). On the contrary, Seiquer et al. observed a 3–20-fold increase in AA measured by the ABTS, DPPH, FRAP, and FC methods for extra virgin argan oil. This can be explained by the compounds in argan oil transforming into different forms with higher antioxidant potential during digestion [6].

Table 1. Antioxidant activity before and after the two-step in vitro model of the digestion of refined rapeseed oil fortified with ESA.

Step of Digestion	Sample	ABTS ± SD	DPPH ± SD	FC ± SD
		(μmol TE/100 g)		
before digestion	RO	1277.62 ± 189.47	520.32 ± 5.69	120.01 ± 15.53
	RO + 0.02% ESA	1939.12 ± 22.98	580.74 ± 40.86	292.28 ± 6.99
	RO + 0.5% ESA	4036.24 ± 688.55	5183.95 ± 493.69	581.98 ± 15.71
after first step	RO	1631.97 ± 82.83	616.44 ± 32.69	231.31 ± 12.44
	RO + 0.02% ESA	1148.05 ± 80.40	533.83 ± 39.35	261.41 ± 19.00
	RO + 0.5% ESA	10,507.47 ± 626.17	3570.44 ± 333.11	422.40 ± 11.97
after second step	RO	761.37 ± 60.04	356.70 ± 44.66	61.46 ± 8.82
	RO + 0.02% ESA	1703.82 ± 20.42	447.67 ± 45.72	504.66 ± 21.23
	RO + 0.5% ESA	11,597.04 ± 646.61	4177.28 ± 374.25	486.23 ± 27.08
after first and second steps	RO	1489.95 ± 106.01	574.55 ± 42.96	127.29 ± 8.75
	RO + 0.02% ESA	1518.43 ± 77.76	525.71 ± 16.94	352.13 ± 15.03
	RO + 0.5% ESA	10,485.70 ± 1000.22	4684.67 ± 253.84	768.01 ± 7.47

The first step of digestion caused a 1.28–1.93-fold increase in the AA of the enriched rapeseed oils. In contrast, the second step showed a 0.51–0.68-fold decrease in the AA of these oils compared to the non-supplemented oil before digestion. The fortification of rapeseed oil with 0.02% ESA resulted in an AA decrease in each digestion step, except for the total phenolic contents determined after the second step by the FC method. Moreover, the rapeseed oil fortified with 0.5% ESA had lower amounts of total phenolics and other compounds capable of scavenging DPPH radicals. Surprisingly, the ABTS radical scavenging activity measured both hydrophilic and hydrophobic antioxidants and demonstrated an increase of 160% in gastric digestion and 187% in duodenal digestion of the oil with 0.5% ESA. On the other hand, the oil enriched with 0.5% ESA was characterized by significantly higher antioxidant properties than the non-supplemented oil. The AA values determined by the ABTS, DPPH, and FC methods were about 7.04, 8.15, and 6.03-fold higher, respectively, than the antioxidant potential of the refined rapeseed oil without the synthetic antioxidant after the digestion process. For comparison, a significant decrease in the ABTS values of extra virgin olive oil after in vitro gastric and small intestinal digestion was reported by other authors [10].

4. Conclusions

ESA was successfully synthesized in an enzymatic way without organic solvents. The two-step in vitro digestion process slightly affected the antioxidant potential of the oil sample without ESA. The antioxidant properties of the fortified oil strongly depend on the concentration of the added phenolic acid ester. The fortification of rapeseed oil with 0.02% ESA did not improve its radical scavenging activity as measured by the ABTS and DPPH methods, while the total phenolic content, measured by the FC method, increased by about two times.

Nevertheless, the addition of ESA by up to 0.5% significantly increased the AA of the refined rapeseed oil. Additionally, the presence of ESA in a higher amount in the oil probably caused the release of antioxidant compounds that reacted with the ABTS radicals and consequently improved the antioxidant properties as compared to the oil samples before the digestion process. Therefore, ESA could be used as an efficient additive to enhance the antioxidant potential of vegetable oils.

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