2017

Extraction and Quantification of Sinapinic Acid from Irish Rapeseed Meal and Assessment of Angiotensin-I Converting Enzyme (ACE-I) Inhibitory Activity

Leah Quinn
Teagasc Food Research Centre

Steven Gray
St James's Hospital, Dublin

Steve Meaney
Technological University Dublin, steve.meaney@tudublin.ie

See next page for additional authors

Follow this and additional works at: <https://arrow.tudublin.ie/scschbioart>

Part of the [Biology Commons](#), [Chemistry Commons](#), and the [Food Chemistry Commons](#)

Recommended Citation

Quinn L, Gray SG, Meaney S, Finn S, McLoughlin P, Hayes M. Extraction and Quantification of Sinapinic Acid from Irish Rapeseed Meal and Assessment of Angiotensin-I Converting Enzyme (ACE-I) Inhibitory Activity. *J Agric Food Chem*. 2017 Aug 16;65(32):6886-6892. doi: 10.1021/acs.jafc.7b02670. Epub 2017 Aug 8. PMID: 28748695.

This Article is brought to you for free and open access by the School of Biological Sciences at ARROW@TU Dublin. It has been accepted for inclusion in Articles by an authorized administrator of ARROW@TU Dublin. For more information, please contact arrow.admin@tudublin.ie, aisling.coyne@tudublin.ie, gerard.connolly@tudublin.ie.

Authors

Leah Quinn, Steven Gray, Steve Meaney, Stephen Finn, Padraig McLoughlin, and Maria Hayes

Extraction and Quantification of Sinapinic Acid from Irish Rapeseed Meal and Assessment of Angiotensin-I Converting Enzyme (ACE-I) Inhibitory Activity

Leah Quinn,^{†,‡} Steven G. Gray,[‡] Steve Meaney,[§] Stephen Finn,^{||} Padraig McLoughlin,[†] and Maria Hayes^{*,†,§}

[†]Food Biosciences Department, Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland

[‡]Department of Clinical Medicine, Trinity Centre for Health Sciences, Institute of Molecular Medicine, St. James's Hospital, Dublin 8, Ireland

[§]School of Biological Sciences, College of Sciences and Health and Environment, Sustainability and Health Institute, Dublin Institute of Technology, Dublin 8, Ireland

^{||}Department of Histopathology, Central Pathology Department, St. James's Hospital, Dublin 8, Ireland

ABSTRACT: Phenolic compounds, including phenolic acids, are known to play a protective role against the development of cardiovascular disease. The aim of this work was to generate a phenolic acid extract from Irish rapeseed meal, to determine the quantity of sinapinic acid (SA) in this fraction and to assess the ability of this fraction to inhibit the enzyme angiotensin-I converting enzyme (ACE-I; EC 3.4.15.1). A crude phenolic extract (fraction 1), free phenolic acid containing extract (fraction 2), and an extract containing phenolic acids liberated from esters (fraction 3) were generated from Irish rapeseed meal using a methanol:acetone:water solvent mixture (7:7:6). The total phenolic content (TPC) of each extract was determined and proximate analysis performed to determine the fat, moisture, and protein content of these extracts. Nuclear magnetic resonance (¹H NMR) spectroscopy was used to quantify the level of SA in extract 3, which inhibited ACE-I by 91% ± 0.08 when assayed at a concentration of 1 mg/mL, compared to the control, captopril, which inhibited ACE by 97% ± 0.01 when assayed at a concentration of 1 mg/mL.

KEYWORDS: ACE-I, rapeseed meal, sinapinic acid, solvent extraction, total phenolic content

1. INTRODUCTION

Rapeseed is one of the world's major oilseeds and a globally valuable crop, with Europe being the largest producer.¹ In 2013/2014, the EU produced 9–93 million metric tons of rapeseed oil.² Rapeseed meal is a byproduct of rapeseed oil production, and this is traditionally used as animal feed. Rapeseed meal is unsuitable for human consumption due to the presence of antinutritional compounds including phytates and glucosinolates, which result in poor digestibility, unpleasant color, and bitter taste.³ Glucosinolates are hydrolyzed to produce harmful products including oxazolidinethione and isothiocyanate, both of which impair the absorbance of iodine.⁴ Erucic acid, or *cis*-13-docosenoic acid, is another antinutritional compound found in high concentrations in rapeseed. The toxic effects of erucic acid are mainly targeted toward the heart, impairing cardiac muscle and altering the fat content of the heart.⁴ However, rapeseed is reported to contain more phenolic compounds than any other oilseed plant, with the majority of phenolics remaining in the meal after oil production.⁵ These phenolic acids, including sinapinic acid (SA), have gained attention due to their bioactive properties such as antioxidant and antidiabetic activities. SA has also been shown to possess histone deacetylase (HDAC) inhibitory activity, with histone deacetylase inhibitors (HDACi) known to play a protective role against diabetes development.⁶ Furthermore, phenolic acids are also thought to play a protective role in heart health and may help to prevent cardiovascular disease (CVD).^{7–9}

Clinical studies have shown that the amount of dietary phenolics required to exert their beneficial effects exceeds what is obtainable from dietary intake alone.¹⁰ In this work, phenolic acids containing SA were generated from Irish rapeseed meal using a methanol:acetone:water (7:7:6) solvent system. Cardiovascular disease remains the most common cause of death globally. In 2013 the Global Burden of Disease (GBD) study found that CVD accounted for 17.3 million deaths worldwide.¹¹ In Europe alone, CVD results in 4 million deaths each year and 45% of all deaths.¹¹ In terms of CVD, hypertension is a critical factor. ACE-I is a zinc-dependent peptidase which cleaves angiotensin I (a vasodilatory peptide) to angiotensin II, which is a vasoconstrictor peptide involved in regulating blood pressure.¹² Inhibition of ACE-I reduces vasoconstriction and reduces hypertension, therefore improving heart health (Figure 1). The aim of this paper was to generate extracts from Irish rapeseed meal and to assess the impact of these extracts containing SA on the enzyme ACE-I. Given that current ACE-I inhibitors such as captopril and enalapril have various side effects including cough, rash, and vomiting, identifying ACE-I inhibitors from a natural food source is an

Received: June 8, 2017

Revised: July 26, 2017

Accepted: July 27, 2017

Published: July 27, 2017

Figure 1. Total phenolic content of Irish rapeseed meal phenolic fractions. All samples were tested at 1 mg/mL. Extract 1 had a TPC of 572 mg SAE/g $\pm$ 0.05, with 1832 mg SAE/g $\pm$ 0.02 for extract 2 and 1726 mg SAE/g $\pm$ 0.08 for extract 3. Data is representative of three biological replicates ($n = 3$), performed in triplicate. Data is expressed as mean $\pm$ SEM, carried out using Excel 2013.

area of interest.¹³ This is the first paper to report the ACE-I inhibitory activity of a rapeseed meal phenolic extract containing SA, with potential use as a functional food ingredient.

2. MATERIALS AND METHODS

2.1. Chemicals. Methanol, acetone, captopril, Folin–Ciocalteu phenol reagent, sinapinic acid, and DMSO- d_6 (with 1% TMS v/v) were supplied by Sigma-Aldrich (Dublin, Ireland). The ACE-I kit-WST was supplied by Dojindo Molecular Technologies Inc. Japan.

2.2. Sample Preparation. Rapeseed meal was kindly supplied by Donegal Rapeseed Company, Co. Donegal, Ireland. The rapeseed meal was prepared from seed harvested in the summer of 2014. Rapeseed meal was defatted overnight with hexane using a meal:solvent ratio of 90 g:5 L (w/v). Defatting was carried out at room temperature on a JENWAY 1002 stirrer (Wolfabs, U.K.) and repeated three times. Defatted meal was subsequently air-dried.

2.3. Extraction of Phenolic Acids. Extraction of free phenolic acids and phenolic acids liberated from esters was performed according to the method of Nacz et al. (1992), with modifications as described.¹⁴

Briefly, phenolic acids were extracted five times from Irish rapeseed using a total meal:solvent ratio of 1:20 (w/v) of methanol:acetone:water (7:7:6 v:v:v). The extractions were carried out separately with 2 g of meal and 40 mL of methanol:acetone:water (7:7:6 v:v:v) in six separate 50 mL tubes. Extracts were vortexed for 15 s, followed by centrifugation for 15 min at 5000 rpm (2655g) at 20 °C. The supernatants from each extraction were combined and reduced to approximately 240 mL using rotary evaporation at 30 °C. From this, 40 mL was kept, before being dried under nitrogen at 30 °C and labeled as extract 1 (total phenolics). In order to extract the soluble esters, 30 mL of this initial extract was also kept for hydrolysis. The remaining extract was extracted six times with diethyl ether:ethyl acetate (1:1 v:v), evaporated at 30 °C using rotary evaporation, and then was dried under nitrogen at 30 °C. This was labeled as extract 2 (free phenolic acids). To release the esterified phenolic acids, 30 mL of the initial extract was hydrolyzed with 30 mL of 4 M NaOH under nitrogen for 4 h at room temperature, before being acidified to pH 2 with 6 M HCl. The acidified solution was then extracted six times with diethyl ether:ethyl acetate (1:1 v:v) evaporated at 30 °C using rotary evaporation (BUCHI, Switzerland), before being dried under nitrogen at 30 °C. This was labeled as extract 3 (soluble esters).

Multiple extractions were carried out and pooled. From 144 g of defatted rapeseed meal, the yield of extract 1 was 1.003 g (0.7% yield),

extract 2 was 0.70 g (0.5% yield), and extract 3 was 2.11 g (1.5% yield). All samples were freeze-dried (Cuddon Engineering, New Zealand) overnight and stored at -20 °C.

2.4. Total Phenolic Content. The total phenolic content was determined using the Folin–Ciocalteu (F–C) assay, according to the method described previously by Khattab et al. (2010).¹⁵ Briefly, the phenolic extracts (1–3) were prepared to a final concentration of 1 mg/mL with methanol, in triplicate (Sigma-Aldrich, Dublin, Ireland). Aliquots (0.2 mL) of each sample were diluted to 0.5 mL with water, and 0.5 mL of Folin–Ciocalteu phenol reagent (Sigma-Aldrich, Dublin, Ireland) was added. After 3 min, 1 mL of 19% sodium carbonate was added to each sample. After 60 min, absorption was measured at 750 nm using a spectrophotometer (Hitachi U-2900). Sinapinic acid (Sigma, Dublin, Ireland) was used as the standard (1–10 mg/mL), and results were expressed as mg of sinapinic acid equivalents per gram (mg SAE/g).

2.5. Protein Analysis. Analysis of protein content was performed on each phenolic extract (1–3) using the Dumas method in accordance with the AOAC Method 968.06 (15th ed.) using a Leco FP629 protein analyzer. A conversion factor of 5.70 was used in order to determine the total protein content from the nitrogen concentration.

2.6. Fat and Moisture Analysis. Total fat was determined for the three phenolic extracts, along with rapeseed meal, defatted rapeseed meal, and rapeseeds. This was carried out using the acid hydrolysis filter bag technique using an automated Ankom Fat Analyzer XT15 based on the method ISO 6492.¹⁶

2.7. Ash Analysis. The ash content of samples was determined by furnace overnight at 600 °C.^{17,18}

2.8. ACE-I Inhibition. Measurement of ACE-I inhibition was performed using the ACE-I kit-WST following the manufacturer's instructions (Dojindo Molecular Technologies Inc., Japan). Briefly, the phenolic extracts (1–3) and positive control (captopril) were assayed in triplicate at a concentration of 1 mg/mL. The concentration of each extract which inhibited ACE-I by 50% (the IC_{50} value) was determined for extract 3 and captopril. Each extract was dissolved in deionized water for the initial assay or 1% DMSO for the IC_{50} determination. Absorbance was measured using an Omega microplate reader at 450 nm, and % inhibition was calculated using the following:

$$\frac{[(\text{absorbance blank 1} - \text{absorbance inhibitor}) / (\text{absorbance blank 1} - \text{absorbance blank 2})] \times 100}{}$$

where blank 1 is the control without the addition of any inhibitor, blank 2 is the reagent blank, and the inhibitor is the positive control or test sample (extracts).

IC_{50} was determined by plotting % ACE-I inhibition against concentration (mg/mL) of extract or captopril, and solving the equation of the line, substituting 50 for the Y value and solving for X.

2.9. ^1H NMR Analysis. ^1H NMR spectroscopy was used to quantify the level of sinapinic acid in extracts 1–3. It was performed using the method of Tan and Shahidi (2013), using Topspin software 2.1.¹⁹ A standard curve was prepared using SA (Sigma-Aldrich, Dublin, Ireland), which dissolved in dimethyl sulfoxide- d_6 with 1% (v/v) tetramethylsilane (DMSO- d_6 with 1% TMS (v/v) (Sigma-Aldrich, Dublin, Ireland). TMS served as an internal standard for the analysis. Phenolic extract 3 was analyzed in triplicate by dissolving 0.1 g of sample in 1 mL of DMSO- d_6 containing 1% (v/v) TMS. In order to calculate the quantity of SA in the extract, the average values for each standard were plotted in Excel and a linear curve was generated. The equation of the line was solved, substituting the value obtained for extract 3 for the Y value and then solving for X.

2.10. Statistical Analysis. Data was derived from samples obtained in biological triplicate, and each experiment was performed in triplicate. The exception was ACE-I IC_{50} determination, which was performed using two biological replicates in triplicate. Data was subsequently analyzed for standard deviation (SD) and standard error of mean (SEM), which was calculated and plotted using Microsoft Excel 2013.

3. RESULTS

3.1. Protein, Fat, Moisture, and Ash Analysis. The moisture, protein, and fat content of phenolic extracts 1–3, along with defatted rapeseed meal and seeds, are shown in Table 1.

Table 1. Protein, Fat, Ash, and Moisture Content of Irish Rapeseed Meal Phenolic Extracts along with Oilseed Rape and Oilseed Rape Meal

sample	protein (N $\times$ 5.70)	fat	ash	moisture
extract 3	1.6	4.1	not tested	4.8
oilseed rape	3.1	47.3	4.0	7.3
oilseed rape meal	3.9	25.7	5.3	10.5

3.2. Total Phenolic Content of Phenolic Extracts. The Folin–Ciocalteu assay measures the total phenolic content of samples based on the transfer of electrons in an alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes, forming a blue complex which can be measured using a spectrophotometer.²⁰ The total phenolic content for each phenolic extract is shown in Figure 2. The total phenolic content ranged from 572 mg SAE/g $\pm$ 0.05 for extract 1 to 1832 mg SAE/g $\pm$ 0.02 for extract 2 and 1726 mg SAE/g $\pm$ 0.08 for extract 3.

3.3. Rapeseed Phenolic Extracts Inhibit ACE-I Activity. Extract 3 was examined for its potential to inhibit the enzyme ACE-I, with ACE-I known to play a role in heart health by ameliorating hypertension. The ACE-I inhibitory values obtained for the phenolic extracts 1–3 are shown in Figure 3. ACE-I inhibitory activity ranged from 89% $\pm$ 0.04 for extract 1 to 90% $\pm$ 0.01 for extract 2 and 91% $\pm$ 0.08 for extract 3. The ACE-I inhibitory activity values of the three extracts were comparable to those of the positive control, captopril (97% $\pm$ 0.01), at a concentration of 1 mg/mL.

3.3.1. ACE-I IC_{50} Values. The concentration of extract 3 that inhibited ACE-I by 50% (the IC_{50} value) was determined and compared to the positive control, captopril. Extract 3 was

chosen for further analysis as it inhibited ACE-I by 91% $\pm$ 0.08. Extract 3 had an IC_{50} value of 0.25 mg/mL $\pm$ 0.05, compared to the positive control, captopril, which had an IC_{50} value of 0.05 mg/mL $\pm$ 0.01, which is lower than reported in the literature, at approximately 0.004 mg/mL.²¹

3.4. ^1H NMR Analysis. ^1H NMR spectroscopy was performed in order to quantify the level of SA in extract 3, given that it displayed the highest total phenolic content and ACE-I inhibitory activity (Figures 4 and 5). Using the equation of the line generated from the standard curve of SA, extract 3 was found to contain 5.31 mg of SA (5.3%) per 100 mg of extract.

DISCUSSION

Rapeseed meal is a low economic value byproduct of rapeseed oil production and is ordinarily used as animal feed. Rapeseed contains more phenolic compounds than any other oilseed plant, and the majority of these phenolics remain in the meal after oil production. Phenolic acids including SA are also found in fruits, vegetables, and members of the Brassicaceae family including broccoli, cabbage, and kale. However, isolating these potentially valuable phenolic acids from rapeseed meal could enhance the economic value of this resource and make use of a byproduct which is unfit for human consumption. It also fits in with the concept of a “circular economy”, whereby resources which would otherwise be considered waste are recycled back into the economy.²²

A review of the literature regarding the phenolic composition of rapeseed meal identified that SA comprises between 73 and 85.4% of free phenolic acids.^{23,24} Up to 80% of phenolic acids in rapeseed meal are esterified, and hydrolysis with sodium hydroxide releases the esterified phenolic acids, including sinapine, yielding SA as the main phenolic acid.²³ According to the literature, SA constitutes between 70.9 and 96.7% of the soluble esters in rapeseed; therefore extracts were successfully generated containing free phenolics and soluble esters using the method of Naczki et al.^{14,23} This method was chosen as it is a published method which allows the extraction of phenolic acids in their different forms, i.e., free or esterified, using methanol:acetone:water (7:7:6). During the extraction process, all solvents are completely evaporated, leaving our extract of interest for use in downstream studies. Extract 3, containing the soluble esters, was found to have the highest yield at 2.11 g (1.5% yield). It has previously been shown that esterified phenolics appear to be extracted more effectively than free phenolic acids, as is the case in this work.¹⁴ However, no explanation has been proposed for this observation. Analysis of the total phenolic content found that extract 3 contained the highest level of total phenolics at 1726 mg SAE/g. Given that extract 3 had the highest total phenolic content, ^1H NMR spectroscopy was used to determine the quantity of SA in this extract. Quantitative analysis of extract 3 using ^1H NMR analysis found that extract 3 contained 5.31 mg/100 mg of extract. This is higher than the values previously reported, which ranged from 0.17–1.7 mg/g of SA in rapeseed meal phenolic isolates.²⁵ The high level of SA in extract 3 is likely a result of multiple extractions which were carried out to generate a sufficient quantity of the extract. Efforts will be made in order to upscale the extraction of extract 3 from rapeseed meal, using techniques such as accelerated solvent extraction (ASE).

All three extracts generated in this work were found to inhibit ACE-I, with extract 3 found to inhibit ACE-I by 91% $\pm$ 0.08. ACE-I inhibition has also been reported for other phenolic

Figure 2. Angiotensin I-converting enzyme inhibition by Irish rapeseed meal phenolic fractions and positive control captopril. All samples were tested at 1 mg/mL. ACE-I inhibitory activity ranged from $89\% \pm 0.04$ for extract 1 to $90\% \pm 0.01$ for extract 2 and $91\% \pm 0.08$ for extract 3. Data is representative of three biological replicates ($n = 3$), performed in triplicate. Data is expressed as mean $\pm$ SEM, carried out using Excel 2013.

Figure 3. Angiotensin-I converting enzyme IC₅₀ inhibition values for positive control, captopril, and phenolic fraction 3. Extract 3 had an IC₅₀ value of $0.25 \text{ mg/mL} \pm 0.05$, compared to the positive control, captopril, which had an IC₅₀ value of $0.05 \text{ mg/mL} \pm 0.01$. Data is representative of 2 independent experiments ($n = 2$), performed in triplicate. Data is expressed as mean $\pm$ SEM, carried out using Excel 2103.

extracts generated from fruits and vegetables. For example, phenolic extracts from black and red raspberries were found to inhibit ACE-I by over 70% when assayed previously.²⁶ ACE-I is a zinc-dependent peptidase which cleaves angiotensin I to angiotensin II, which is a vasoconstrictor involved in regulating blood pressure.¹² Recent evidence suggests that various processes including oxidative stress, inflammation, and hypertension are interconnected, with each influencing the other in terms of the pathology of “hypertension-related cardiovascular events”.²⁷ Therefore, developing inhibitors which can target ACE-I and which also possess antioxidant activity is of great interest.²⁷ The antioxidant activity of phenolic acids including sinapinic acid is well-known.^{7,28} The inflammatory process is also known to contribute to cardiovascular events related to hypertension, and it has been shown that ACE-I inhibitors can alter inflammatory molecules.²⁹ Such molecules include

intercellular adhesion-molecule-1, tumor necrosis factor- α , and C-reactive protein.²⁹ In line with this, SA has been shown to exert its anti-inflammatory activity by inhibiting the expression of iNOS, COX-2, TNF- α , and IL-1 β .³⁰ SA could potentially play a role in the treatment of not only hypertension but also disease states caused by hypertension; these include vascular hypertrophy, retinopathy, and stroke.³¹

Inhibition of ACE-I is just one potential mechanism by which SA could potentially improve heart health. SA has been shown to restore ventricular function and improve both percent rate pressure product and percent coronary flow.^{7,9} Therefore, further studies are necessary to examine the effects of SA through confocal analysis of stress fibers and focal adhesions.

A potential mechanism has been proposed by which phenolic acids, including hydroxycinnamic acids like SA, inhibit ACE-I.³² The above study found that hydroxycinnamic acids had

Figure 4. ^1H NMR spectra of extract 3. The peak for extract 3 is highlighted by the red circle. Extract 3 was analyzed in triplicate.

Standard curve of SA

Figure 5. Standard curve of SA. The data generated from ^1H NMR analysis of SA at various concentrations was used to generate a standard curve. The equation of the line and R -squared value are displayed, which were used to calculate the mg of SA in extract 3.

increasing ACE-I inhibitory activity with increasing numbers of hydroxyl groups. They also found that the potency of ACE-I inhibition depends upon the presence of particular functional groups, including hydroxyl, carboxyl, and acrylic groups, which can act as hydrogen-bond acceptors or donors.³² Gallic acid, which possesses five hydrogen-bond acceptor groups just as SA does, was found to have higher activity when compared to syringic acid, which has a low number of hydrogen-bond acceptor groups.³²

The ACE-I inhibitory properties of SA mean that it could also prove potentially useful in the treatment of other diseases including cancer, with a recent study demonstrating improved

survival in adenocarcinoma patients following treatment with ACE-I inhibitors.³³

The fat content determined for the oilseed rape sample was higher than that obtained for the oilseed meal. This would be expected, as during the pressing of the oilseed rape to produce rapeseed oil, much of the oil and lipid content is expelled. Oilseed rape meal is a byproduct of this deoiling process, and therefore is expected to contain less fat than the oilseed rape itself (Table 1). The ash content of rapeseed has been reported to be approximately 4%, with our oilseed rape found to have an ash content of 4.01%. Values reported for rapeseed meal have been reported to be slightly higher at around 8%, with similar

values obtained with our samples, as the oilseed meal had a slightly higher ash content at 5.3%.⁴

However, there are limitations to this work. The phenolic extract isolated as part of this work is not purified and, therefore, contains other phenolic acids besides SA. Some of these include protocatechuic, caffeic, and syringic acid.¹⁴ PCA was found to be a poor inhibitor of ACE-I activity, with less than 50% inhibition observed at a concentration of 0.5 mM.³⁴ Another study found that, at a similar concentration of 0.8 mM, PCA only displayed 9% ACE inhibition.³² When the concentration was increased to 10 mM, the ACE-I inhibitory activity increased to 91%.³² Syringic acid has also been reported to be a weak inhibitor of ACE-I, with 54% inhibition at a concentration of 10 mM.³² Similarly, caffeic acid has been shown to have poor ACE-I inhibitory activity, displaying just 34% ACE-I inhibition at a concentration of 0.93 mM. In the same study, caffeic acid was unable to be studied at a higher molarity due to its poor solubility.³² Based on the existing evidence, it appears unlikely that these phenolics contribute to the ACE-I inhibitory effects of our generated extract. Given the high concentration of SA in our generated extract, 5.3 mg or 23 mM, and that a previous study has shown that SA can lower plasma ACE activity, it would appear that SA does contribute to the observed ACE-I inhibitory activity of our extract.⁹

The yield of extract generated was low, and as such multiple extractions were required. Given that the extract displayed good ACE-I inhibitory activity, it is a promising candidate to move forward into animal studies, to determine its efficacy as an antihypertensive agent in vivo, but also for use in toxicology studies. As such, it is planned to upscale the extraction process and move forward to in vivo studies as mentioned. Another limitation of this work is the stability of the extract, which is yet to be determined. This information would be pertinent regarding the potential use of this extract as a functional food ingredient, which is ultimately what the project aims to achieve.

In conclusion, a phenolic extract isolated from Irish rapeseed meal has potential utility as an antihypertensive agent to improve heart health, through inhibition of ACE-I and decreased hypertension. Therefore, this phenolic extract containing SA is a promising candidate to move forward to in vivo studies for the treatment of hypertension-related cardiovascular events.

AUTHOR INFORMATION

Corresponding Author

*E-mail: maria.hayes@teagasc.ie. Phone: + 353 (0) 18059957. Fax: +353 1 8059950.

ORCID

Maria Hayes: [0000-0002-2805-8007](https://orcid.org/0000-0002-2805-8007)

Funding

This work forms part of a Teagasc Walsh Fellowship (Grant No. 2015015).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ACE-I, angiotensin I-converting enzyme; ASE, accelerated solvent extraction; CVD, cardiovascular disease; F-C, Folin-Ciocalteu; GBD, global burden of disease; NMR, nuclear magnetic resonance; SA, sinapinic acid; TMS, tetramethylsilane; TPC, total phenolic content

REFERENCES

- (1) Carre, P.; Pouzet, A. *OCL: Oilseeds Fats, Crops Lipids* **2014**, *21*, 1–12.
- (2) List, G. *Lipid Technol.* **2015**, *27*, 120–120.
- (3) Tan, S. H.; Mailer, R. J.; Blanchard, C. L.; Agboola, S. O. *J. Food Sci.* **2011**, *76*, R16–28.
- (4) Dingyuan, F.; Jianju, Z. *Rapeseed Conference, 12th IRC* (Wuhan, China); 2007; pp 265–270.
- (5) Vuorela, S. *Analysis, isolation, and bioactivities of rapeseed phenolics*, PhD Thesis, University of Helsinki, 2005; pp 1–75.
- (6) Senawong, T.; Misuna, S.; Khaopha, S.; Nuchadomrong, S.; Sawatsitang, P.; Phaosiri, C.; Surapaitoon, A.; Sripan, B. *BMC Complementary Altern. Med.* **2013**, *13*, 232. Raichur, S.; Teh, S. H.; Ohwaki, K.; Gaur, V.; Long, Y. C.; Hargreaves, M.; McGee, S. L.; Kusunoki, J. *J. Mol. Endocrinol.* **2012**, *49*, 203–211.
- (7) Silambarasan, T.; Manivannan, J.; Priya, M. K.; Suganya, N.; Chatterjee, S.; Raja, B. *Biochem. Biophys. Res. Commun.* **2015**, *456*, 853–859.
- (8) Cherng, Y. G.; Tsai, C. C.; Chung, H. H.; Lai, Y. W.; Kuo, S. C.; Cheng, J. T. *J. Agric. Food Chem.* **2013**, *61*, 12053–12059.
- (9) Silambarasan, T.; Manivannan, J.; Raja, B.; Chatterjee, S. *Eur. J. Pharmacol.* **2016**, *777*, 113–123.
- (10) Nunez-Sanchez, M. A.; Gonzalez-Sarrias, A.; Romo-Vaquero, M.; Garcia-Villalba, R.; Selma, M. V.; Tomas-Barberan, F. A.; Garcia-Conesa, M. T.; Espin, J. C. *Mol. Nutr. Food Res.* **2015**, *59*, 1274–1291.
- (11) Townsend, N.; Wilson, L.; Bhatnagar, P.; Wickramasinghe, K.; Rayner, M.; Nichols, M. *Eur. Heart J.* **2016**.
- (12) Bernstein, K. E.; Ong, F. S.; Blackwell, W. L.; Shah, K. H.; Giani, J. F.; Gonzalez-Villalobos, R. A.; Shen, X. Z.; Fuchs, S. *Pharmacol. Rev.* **2013**, *65*, 1–46.
- (13) Sharma, S. *Int. J. Sci. Res.* **2015**, *4*, 65–66.
- (14) Nacz, M.; Wanasundara, P. K. J. P. D.; Shahidi, F. *J. Agric. Food Chem.* **1992**, *40*, 444–448.
- (15) Khattab, R.; Eskin, M.; Aliani, M.; Thiyam, U. *J. Am. Oil Chem. Soc.* **2010**, *87*, 147–155.
- (16) Seenger, J.; Nuernberg, G.; Hartung, M.; Szucs, E.; Ender, K.; Nuernberg, K. *Arch. Tierz., Dummerstorf* **2008**, *51*, 449–457.
- (17) Arif, M.; Nasiruddin; Masood, T.; Shah, S. S.; Faayaz, H. *ARNP J. Agric. Biol. Sci.* **2012**, *7* (9).
- (18) AOAC (Association of Official Analytical Chemists). Helrich, K., Ed.; AOAC: Arlington, VA, 1990.
- (19) Tan, Z.; Shahidi, F. *Food Chem.* **2013**, *138*, 1438–1447.
- (20) Ainsworth, E. A.; Gillespie, K. M. *Nat. Protoc.* **2007**, *2*, 875–877.
- (21) Ben Henda, Y.; Labidi, A.; Arnaud, L.; Bridiau, N.; Delatouche, R.; Maugard, T.; Piot, J. M.; Sannier, F.; Thiery, V.; Bordenave-Juchereau, S. *J. Agric. Food Chem.* **2013**, *61*, 10685–10690.
- (22) Velis, C. A. *Waste Manage. Res.* **2015**, *33*, 389–391.
- (23) Shahidi, F.; Nacz, M. *J. Am. Oil Chem. Soc.* **1992**, *69*, 917–924.
- (24) Thiyam, U.; Stöckmann, H.; Zum Felde, T.; Schwarz, K. *Eur. J. Lipid Sci. Technol.* **2006**, *108*, 239–248.
- (25) Vuorela, S.; Meyer, A. S.; Heinonen, M. *J. Agric. Food Chem.* **2004**, *52*, 8202–8207. Nićiforović, N.; Abramović, H. *Compr. Rev. Food Sci. Food Saf.* **2014**, *13*, 34–51.
- (26) Cheplick, S.; Kwon, Y.-I.; Bhowmik, P.; Shetty, K. *J. Food Biochem.* **2007**, *31*, 656–679.
- (27) Pacurari, M.; Kafoury, R.; Tchounwou, P. B.; Ndebele, K. *Int. J. Inflammation* **2014**, *2014*, 1–13.
- (28) Gaspar, A.; Martins, M.; Silva, P.; Garrido, E. M.; Garrido, J.; Firuzi, O.; Miri, R.; Saso, L.; Borges, F. *J. Agric. Food Chem.* **2010**, *58*, 11273–11280.
- (29) Ferrario, C. M.; Strawn, W. B. *Am. J. Cardiol.* **2006**, *98*, 121–128.
- (30) Yun, K. J.; Koh, D. J.; Kim, S. H.; Park, S. J.; Ryu, J. H.; Kim, D. G.; Lee, J. Y.; Lee, K. T. *J. Agric. Food Chem.* **2008**, *56*, 10265–10272.
- (31) Harlan, S. M.; Ostroski, R. A.; Coskun, T.; Yantis, L. D.; Breyer, M. D.; Heuer, J. G. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2015**, *309*, R467–474.

(32) Al Shukor, N.; Van Camp, J.; Gonzales, G. B.; Staljanssens, D.; Struijs, K.; Zotti, M. J.; Raes, K.; Smagghe, G. *J. Agric. Food Chem.* **2013**, *61*, 11832–11839.

(33) Al-Shamsi, H. O.; Shalaby, A.; Dar, A. Y.; Hassan, M.; Wolff, R. A.; Li, D. *J. Clin. Oncol.* **2016**, *34*, 420.

(34) Hidalgo, M.; Martin-Santamaria, S.; Recio, I.; Sanchez-Moreno, C.; de Pascual-Teresa, B.; Rimbach, G.; de Pascual-Teresa, S. *Genes Nutr.* **2012**, *7*, 295–306.