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Generation of sinapinic acid-containing extracts from Irish rapeseed meal and characterisation of their bioactive properties for potential human health applications

Key external stakeholders:

Cereal growers and processors specifically Rapeseed oil growers
Donegal Rapeseed Oil Company Ltd.

Practical implications for stakeholders:

Rapeseed meal is a low-economic value by-product from the rapeseed de-oiling process and is ordinarily used as animal feed. Rapeseed is rich in bioactive compounds including the phenolic acid – sinapinic acid (SA), the majority of which remain in the meal after processing of the oil. This project developed methods to generate sinapinic acid rich extracts from rapeseed meal and identified the potential health benefits of these ingredients – i.e. anti-inflammatory and heart health benefits in human clinically relevant cell lines. The work to isolate and characterise these phenolics from rapeseed meal enhances the economic value of this by-product, but also encompasses the idea of a 'circular economy' and provides information for new market development.

Main results:

The first aim of this work was to generate extracts containing the phenolic sinapinic acid from Irish rapeseed meal. An initial methodology was chosen based on the phenolic acid composition of rapeseed meal, in terms of both its free and esterified forms. This resulted in the generation of an extract containing 0.0531 mg/g of SA (Extract I). Further modifications to the initial methodology were performed, and this resulted in the generation of an extract with an SA content of 0.569 mg/g (57 %) (Extract II).Previous studies have reported SA in rapeseed to be in the ranges of 170-454 µg/g and 0.11-.0.59 mg/g. The modified methodology developed here is therefore an efficient way to ensure that maximum levels of SA are retrieved from rapeseed meal in one extraction.

The SA containing extracts inhibited the heart-health relevant enzyme Angiotensin-I-converting enzyme (ACE-I), which plays a role in hypertension. ACE-I inhibitors work by competitive inhibition of the ACE-I enzyme, blocking the formation of angiotensin II. This results in dilation of veins and arteries. The level of inhibition of the extracts was on a par with the known ACE-I inhibitor, Captopril when assayed at at 1 mg/mL, Captopril displayed 96 % ± 0.5 ACE-I inhibition, compared to SA containing extracts I and II at 97 % ± 4.1 and 91 % ± 3.6, respectively. Inhibition of ACE-1 was previously shown to play a role in the development of diabetes and kidney disease.

Chronic inflammation is well-established in the underlying pathology of numerous diseases, including cardiovascular disease, diabetes and obesity. Both of the SA-containing extracts, along with commercial SA, were found to significantly reduce the expression of pro-inflammatory cytokines including TNF-alpha, IL-6 and IL-12. Recent studies have made significant progress in elucidating the mechanisms linking inflammation with hypertension. Studies have shown that inhibition of TNF-alpha leads to decreased blood pressure and inflammation in animal models of metabolic syndrome and pre-eclampsia. The levels of IL-6 have also been shown to be elevated in hypertensive conditions, including pulmonary hypertension. Anti-IL-6 therapy has been successfully used in a one-patient trial with severe pulmonary arterial hypertension

Opportunity / Benefit:

The nutraceutical market in Europe is predicted to register a Compound Annual Growth Rate (CAGR) of 7.5 % from 2019-2024. The reason for this increase is due to an increase in lifestyle-related diseases, aging and increased consumer awareness of the impact dietary factors can play in health and disease. Current pharmacological agents used to treat inflammation and hypertension are associated with various side effects, and have undesirable methods of administration i.e. I.V or subcutaneous injection. Natural extracts

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possessing anti-inflammatory and hypertensive properties could eliminate these effects. Extracts could be consumed pro-actively to prevent the onset of inflammation and to maintain normal blood pressure.

Collaborating Institutions:

Trinity College Dublin (TCD); St. James Hospital, Dublin.

Teagasc project team: Dr. Maria Hayes (PI)
Dr. Leah Quinn

External collaborators: Prof. Stephen Gray; Prof. Stephen Finn (St. James Hospital); Dr Steve Meaney (TUD).

Project background: Rapeseed is one of the world's major oilseeds, with Europe being the largest producer with 20 million metric tonnes per year. The process of pressing the seeds to produce rapeseed oil results in a low-economic value by-product, rapeseed meal. Rapeseed meal is commonly used as animal feed, however it is rich in bioactive phenolic compounds, including sinapinic acid. Isolation of bioactive phenolics from a by-product of rapeseed oil production is largely in agreement with the current concept of the circular economy and total utilisation of crop harvest using a biorefinery approach. Sinapinic acid has been shown to possess various bioactive properties including anti-diabetic, anti-inflammatory and histone deacetylase inhibitory activities. However, the amount obtained from our diet is insufficient to produce beneficial effects on health. Therefore, isolating these bioactive phenolics from a natural source, such as rapeseed meal, could generate extracts containing concentrated amounts of phenolic acids which could be consumed to prevent health-related disease.

Questions addressed by the project:

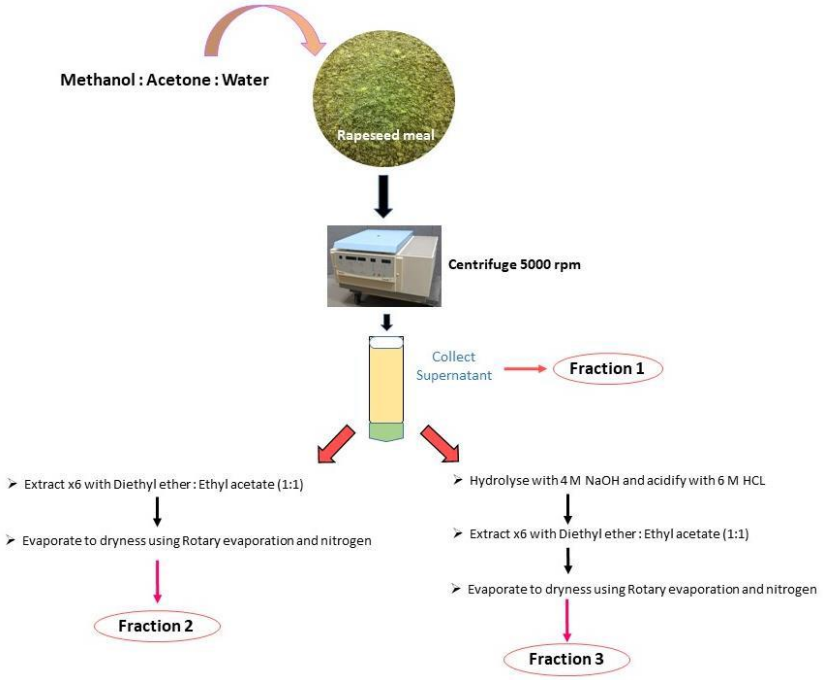
- How can sinapinic acid be extracted from rapeseed meal, a by-product of rapeseed oil production?
- What health benefits does rapeseed derived sinapinic acid have on inflammation and heart health?

The experimental studies:

The aim of this project was to generate and characterise extracts containing sinapinic acid (SA) from Irish rapeseed meal and to assess the potential anti-diabetic, anti-obesogenic, heart health and anti-inflammatory benefits of SA containing extracts using cell culture methods with human derived cell lines and pro-inflammatory markers.

SA containing extracts were generated using the method outlined in Figure 1.

Figure 1: Extraction of SA containing extracts from rapeseed meal



1. The level of SA in extracts I and II was determined using a combination of ^{1}H-NMR and MS. The

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effect of time for extraction and temperature on the amount of SA extractable from rapeseed meal was also determined. Extracts were stabilised using rotary evaporation and freeze-drying. Proximate analysis (protein, ash, lipid content) of derived extracts was also determined. The bioactivities of the SA containing extracts I & II (enriched extract) were then determined using different bioassays.

2. The phenolic fractions generated (total, free and esterified) were tested for their ability to inhibit Angiotensin-converting enzyme-I (ACE-I), compared to a positive control Captopril. All three fractions were found to inhibit ACE-I, with 91 ± 3.6, 89 ± 2.2 and 90 ± 0.3 % inhibition for esterified, total and free phenolics, respectively.
3. The effects of the generated extracts I and II on various cell lines in order to determine a non-toxic dose was also determined. Once this was determined, the extracts, along with commercial SA (control), were tested to determine their potential to ameliorate T2DM and obesity using histone deacetylase expression in the H9c2 cell line.
4. The non-toxic dose of SA extracts I and II, along with commercial SA in SGBS, H9c2, HuH7, HepG2, Hep3B and Caco-2 cell lines was determined. The effects of phenolic extracts I, II and commercial SA on GLUT4 expression (relevant to type 2 diabetes) in the H9c2, HuH7 and SGBS cell lines was determined. The anti-obesogenic effects of commercial SA on adipogenesis in the SGBS cell line was also determined.
5. The anti-inflammatory effects of extract I and II generated from Irish rapeseed meal, along with commercial SA were also determined using THP-1 cells and in human peripheral blood mononuclear cells (PBMCs).

Main results:

Rapeseed meal is a low-economic value by-product from the rapeseed de-oiling process, and is ordinarily used as animal feed. Rapeseed is rich in bioactive compounds including phenolic acids, the majority of which remain in the meal after processing of the oil. Developing and improving methods to extract these phenolics from rapeseed meal could not only enhance the economic value of this resource, but also encompasses the idea of a 'circular economy'. The first aim of this work was to generate extracts containing the phenolic sinapinic acid from Irish rapeseed meal. An initial methodology was chosen based on the phenolic acid composition of rapeseed meal, in terms of both its free and esterified forms. This resulted in the generation of an extract containing 0.0531 mg/g of SA. Further modifications to the initial methodology were performed, and this resulted in the generation of an extract with an SA content of 0.569 mg/g (57 %). Previous studies have reported SA in rapeseed to be in the ranges of 170-454 μg/g and 0.11-.0.59 mg/g. The modified methodology used in this study is therefore an efficient way to ensure that maximum levels of SA are retrieved from rapeseed meal in one extraction. The generation of these extracts was an important and novel aspect of the project moving forward, as previous studies examining the bioactivities of SA have used a chemically manufactured version.

The extracts and commercial SA were found to inhibit the ACE-I enzyme, which plays a role in hypertension. ACE-I inhibitors work by competitive inhibition of the ACE-I enzyme, blocking the formation of angiotensin II. This results in dilation of veins and arteries. The level of inhibition of the extracts was on a par with the known ACE-I inhibitor, Captopril. When assayed at 1 mg/mL, Captopril displayed 96 % ± 0.5 ACE-I inhibition, compared to extracts I and II at 97 % ± 4.1 and 91 % ± 3.6, respectively. Inhibition of ACE-1 was previously shown to play a role in the development of diabetes and kidney disease. Hypertensive patients with T2DM who received ACE-I inhibitors had a significant reduction in cardiovascular events; while evidence is also emerging to suggest that ACE-I inhibition plays a role in kidney disease. One possible mechanism by which the extracts could inhibit the ACE-I enzyme is through the carboxylic acid functional group present in SA. This acts as the ligand which binds to the zinc ion in the active site of the ACE-I enzyme. This is the same mechanism of action as Enalapril, a drug used in the clinical management of hypertension.

Chronic inflammation is well-established in the underlying pathology of numerous diseases, including cardiovascular disease, diabetes and obesity. Both of the SA-containing extracts, along with commercial SA, were found to significantly reduce the expression of pro-inflammatory cytokines including TNF-alpha, IL-6 and IL-12 (Figure 2). Recent studies have made significant progress in elucidating the mechanisms linking inflammation with hypertension. Studies have shown that inhibition of TNF-alpha leads to decreased blood pressure and inflammation in animal models of metabolic syndrome and pre-eclampsia. The levels of IL-6 have also been shown to be elevated in hypertensive conditions, including

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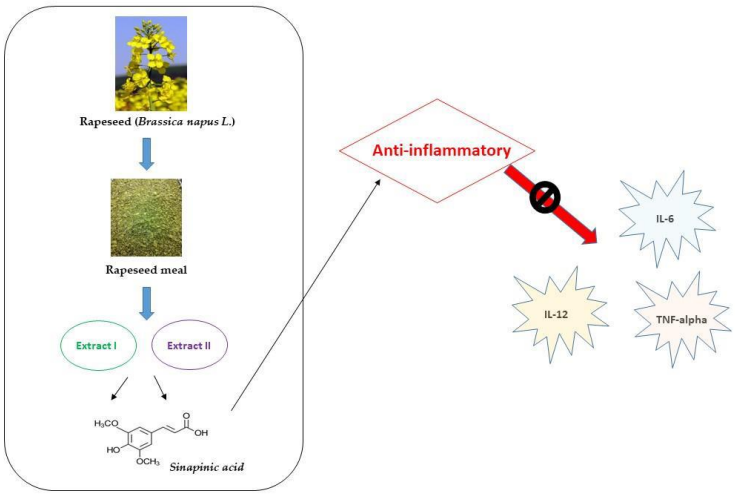
pulmonary hypertension. Anti-IL-6 therapy has been successfully used in a one-patient trial with severe pulmonary arterial hypertension.

Experiments were also carried out to determine if the extracts and commercial SA effect GLUT4 expression. Our results showed no effects on GLUT4 expression in the three cell lines after treatments with the extracts and commercial SA. However, there were limitations to this work in terms of the *in-vitro* models used, and there were no *in-vivo* studies performed.

Figure 2: Summary of the anti-inflammatory activities of Rapeseed meal phenolic SA.



Opportunity/Benefit: Our diet plays a crucial role in the development of diseases such as cardiovascular disease, inflammation, diabetes and obesity. Equally, so too can our diet aide in disease prevention. Consumers today are more aware of the importance of a healthy, balanced diet, and this has resulted in increased sales of functional foods/ nutraceuticals. These are defined as a food or food ingredients which can prevent disease development. Current pharmacological agents used to treat inflammation and hypertension are associated with various side effects, and have undesirable methods of administration. For example, current cytokine-targeting therapeutics, such as the TNF and IL-6 biologics, are administered via I.V or subcutaneous injection. Natural extracts possessing anti-inflammatory and hypertensive properties could eliminate these effects. Supplements, snacks or drinks containing the extracts would provide an amenable method of disease prevention for those of all ages, which are easily consumed and more patient friendly. The extracts could also be consumed pro-actively to prevent the onset of inflammation and to maintain normal blood pressure. There is already a well-established market for these products, including products such as Benecol® which contains plant sterols to lower cholesterol, or Yakult- a probiotic yogurt drinks to maintain healthy digestion. Characterisation of these extracts also provides benefits to animal feed manufacturers as the identified anti-inflammatory activities of rapeseed meal could be used to maintain animal health in pigs and poultry or could be used in aquaculture feed in place of antibiotics.

Dissemination: Main publications

1. Quinn, L., Gray, S. G., Meaney, S., Finn, S., McLoughlin, P., Hayes, M. (2017), Extraction and Quantification of sinapinic acid from Irish Rapeseed Meal and Assessment of Angiotensin-I-converting enzyme inhibitory (ACE-I) activity. *JAFC*, 65, 6886-6892.
2. Quinn, L., Gray, S. G., Meaney, S., Finn, S., Kenny, O., Hayes, M. (2017), Sinapinic and protocatechuic acids found in rapeseed: Isolation, characterisation and potential benefits for human health as functional food ingredients. *Irish Journal of Agricultural and Food Research*, 104-119.
3. Quinn, L., Scott, K. R., Finn, S.P., Hayes, M., Gray, S. G. (2020), An in vitro study determining the anti-inflammatory activities of sinapinic acid-containing extracts generated from Irish Rapeseed Meal. *Medical Research Archives*, 8, 10, 1-15.

Popular publications:

1. Hayes, M., Quinn, L., Meaney, S., Gray, S. (2017) Rapeseed for heart health. Jan 24th 2017, T-Research, 1-2.

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