



Rapeseed for heart health



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Researchers are looking at the use of phenolic-rich rapeseed by-products for development of functional, heart-healthy foods.

Rapeseed (*Brassica napus* L.) is one of the world's major oilseeds with rapeseed oil produced from pressing the seeds. Winter oilseed rape varieties grown commercially in Ireland are classified as *Brassica napus* (Swede Rape) and all have low levels of both erucic acid and glucosinolates. These varieties are known as 'double zero varieties' and their seed is suitable for processing for both food and feed use. The crop is usually grown in rotation with cereals, with usually two or more years between successive crops in the same field (Department of Agriculture, Fisheries and Food, 2009). Rapeseed oil production results in the by-product rapeseed meal. This by-product is rich in polyphenols, including sinapinic acid (SA) and protocatechuic acid (PCA), and these

phenolics are thought to have several beneficial health effects including anti-inflammatory and anti-diabetic applications. SA is a small, natural hydroxycinnamic acid often used in mass spectrometry as a standard. PCA is also found as an antioxidant in green tea and has been studied previously for its effect on cancer cells. Leah Quinn, a PhD candidate at Teagasc Ashdown, is currently researching the beneficial effects of SA and PCA on heart health in conjunction with researchers at Dublin Institute of Technology, Trinity College Dublin and St James's Hospital, Dublin.

Extraction and characterisation of SA and PCA

High blood pressure, or hypertension, is the single largest risk factor attributed to deaths worldwide (World Health Organization [WHO], 2009). Hypertension is responsible for 12.8% of deaths globally, affecting all countries, and all income groups (WHO, 2009). Furthermore, high systolic blood pressure is globally attributable to 51% of stroke, 45%



of ischaemic heart disease and between 37% (South-East Asia region) and 54% (European countries) of cardiovascular deaths (WHO, 2009). Hypertension is, therefore, a considerable problem in our society, not only placing a great burden on our health, but also having substantial impacts on the economy.

An angiotensin-converting-enzyme inhibitor (ACE inhibitor) is a component used primarily for the treatment of hypertension (elevated blood pressure) and congestive heart failure. ACE-I is a zinc-dependent peptidase that cleaves angiotensin I to angiotensin II (ANG II), a vasoconstrictor, involved in regulating blood pressure. Aside from playing a key role in hypertension, ANG II induces cardiovascular damage through its effects on smooth muscle migration and the formation of extracellular matrix, resulting in vascular remodeling and endothelial dysfunction. The use of ACE-I inhibitors suppresses ANG II, thereby reducing high blood pressure, along with reducing the risk of myocardial infarction and mortality in high-risk patients, such as those with diabetes or vascular disease.

The aim of this work was to generate phenolic fractions containing SA and PCA from rapeseed meal, and to test these extracts for their ability to inhibit the enzyme angiotensin-converting enzyme I (ACE-I; EC 3.4.15.1). This enzyme is key to the development of high blood pressure in humans and, if it can be inhibited, can result in reduced blood pressure in persons with high blood pressure.

Scientific approach used

A phenolic acid-rich extract was generated that included SA and PCA using the method of Naczek et al. (1992), which is outlined briefly in Figure 1.

Total phenolics (fraction 1): Defatted meal was extracted and repeated five times with a methanol: acetone: water mix (7:7:6 v.v.v), before being vortexed for 15 sec and centrifuged at 5,000rpm for 15 min. The supernatants were combined and reduced using rotary evaporation at 30°C. This fraction contains total phenolics (fraction 1).

Free phenolic acids (fraction 2): F1 was extracted six times with diethyl ether:ethyl acetate (1:1). The clear layers were pooled together, evaporated to dryness and labelled as free phenolic acids (fraction 2).

Soluble esters (fraction 3): Fraction 1 was hydrolysed with 4M NaOH for 4 hours at room temperature. The hydrolysed solution was then acidified to pH2 using 6M HCl. The acidified solution was then extracted six times with diethyl ether:ethyl acetate (1:1) and the clear layers pooled. The combined supernatants were then evaporated to dryness and labelled as soluble esters (fraction 3).

Figure 1. Method used for extraction of SA and PCA from rapeseed meal.

Measuring ACE-I inhibition

The ACE-I inhibition assay was used to determine the ability of the phenolic fractions isolated from rapeseed meal to inhibit ACE-I. The compound 3-hydroxybutyryl-gly-Gly-Gly (3HB-GGG) was used as an ACE-I substrate and the amount of cleaved 3-Hydroxybutyric acid (3HB) from 3HB-GGG was detected using spectrophotometry.

Results

Figures 2 and 3 show the percentage ACE-I inhibition by each of the rapeseed meal extracts generated and the percentage ACE-I inhibited by the control, Captopril, which is a commercially available antihypertensive drug. IC₅₀ values are shown in Figure 3.

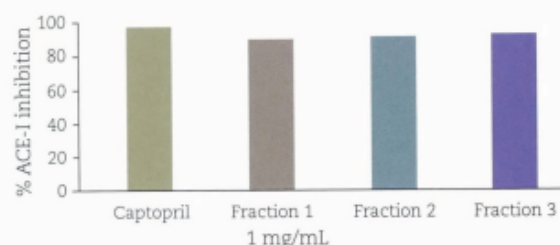


Figure 2. ACE-I inhibitory activity of rapeseed meal phenolic extracts and Captopril, expressed as % ACE-I inhibition. Data expressed as mean ± SEM (n=3).

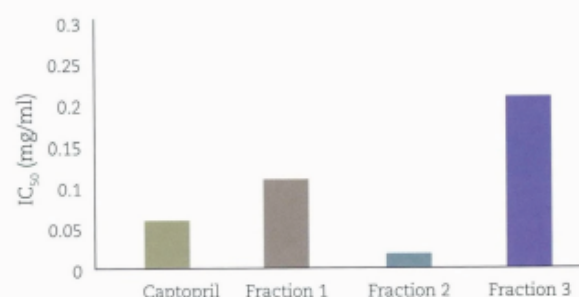


Figure 3. IC₅₀ values of ACE-I inhibition for rapeseed meal phenolic extracts and Captopril, expressed as mg/mL. (n=1).

Industry relevance

Extraction of phenolic compounds, including SA and PCA from rapeseed meal, allows the valorisation of a low-economic-value by-product of the rapeseed de-oiling process through their potential utilisation as functional food ingredients to improve human health.

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