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Leah Quinn
Teagasc, Ireland

Steven G. Gray
Trinity College Dublin, James' Hospital, Ireland

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

See next page for additional authors

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Authors

Leah Quinn, Steven G. Gray, Steve Meaney, Steven Fiinn, Owen Kenny, and Maria Hayes

Sinapinic and protocatechuic acids found in rapeseed: isolation, characterisation and potential benefits for human health as functional food ingredients

Leah Quinn^{1,2}, Steven G. Gray², Steven Meaney³, Stephen Finn⁴, Owen Kenny⁵, Maria Hayes^{1*}

¹Food BioSciences Department, Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland

²Trinity Centre for Health Sciences, Institute of Molecular Medicine, Department of Clinical Medicine, St. James' Hospital, Dublin 8, Ireland

³School of Biological Sciences, College of Sciences and Health and Environment, Sustainability and Health Institute, Dublin Institute of Technology, Dublin 8, Ireland

⁴Department of Histopathology, Central Pathology Department, St. James's Hospital, Dublin 8, Ireland

⁵Institute for Global Food Security, Queen's University Belfast, Northern Ireland

Abstract

Rapeseed is one of the world's major oilseeds, and rapeseed oil is produced by pressing of the seeds. This process results in the production of a low-economic-value by-product, rapeseed meal, which is commonly used as animal feed. Rapeseed meal is rich in bioactive phenolic compounds, including sinapinic acid (SA) and protocatechuic acid (PCA). Isolation of these bioactive compounds 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. In this review, current information concerning traditional and novel methods to isolate phenolic compounds – including SA and PCA – from rapeseed meal, along with in vitro and in vivo studies concerning the bioactivity of SA and PCA and their associated health effects, is collated. These health effects include anti-inflammatory, anti-cancer, anti-diabetes activities, along with histone deacetylase inhibition and protective cardiovascular, neurological and hepatic effects. The traditional extraction methods include use of solvents and/or enzymes. However, a need for simpler, more efficient methodologies has led to the development of novel extraction processes, including microwave-assisted, ultrasound-assisted, pulsed electric field and high-voltage electrical discharge extraction processes.

Keywords

cardiovascular health • diabetes • protocatechuic and sinapinic acid

Introduction

Rapeseed is a globally valuable crop, and its production has steadily grown over the past 20 yr. Rapeseed is cultivated in China, India, Canada and Europe; however, in terms of rapeseed production, Europe is the largest producer (Carre, 2014). In the year 2013/2014, the European Union (EU) produced 9.93 million t of rapeseed oil, while China produced 6.58 million t (List, 2015). A by-product of the rapeseed oil industry, rapeseed meal contains bioactive constituents, such as the phenolics sinapinic acid (SA) and protocatechuic acid (PCA), which have potential uses as functional food ingredients and for use in cosmetic and pharmaceutical applications. Improving and developing methods to isolate phenolic compounds from rapeseed meal could enhance the economic value of this resource. The isolation of phenolics from rapeseed meal encompasses the idea of a “circular economy”, whereby resources that would otherwise be considered waste are recycled into products (Velis, 2015). Traditional methods of isolating phenolics from rapeseed meal include organic solvent and enzymatic extraction;

however, this can require large volumes of solvent, while the associated toxicity of certain organic solvents such as methanol makes them unsuitable for use in final food applications. This has encouraged the development of novel isolation methods such as ultrasound-assisted (UAE), microwave-assisted (MAE), high-voltage electrical discharge (HVED) and pulsed electric field (PEF) extraction processes (Teh and Birch, 2014; Barba *et al.*, 2015; Teh *et al.*, 2015). In the first part of this review, existing and novel methods of isolating these phenolic compounds from rapeseed meal are examined. The second part of the review focusses on the diverse bioactive properties of these phenolics, which could make them potentially useful in improving human health.

Poor diet plays a role in the development of major diseases such as heart disease, diabetes and cancer. Consumption of a diet rich in vegetables is strongly implicated as a protective factor against such diseases, due to the presence of polyphenols or phenolic compounds. Clinical trials regarding the use of dietary phenolic compounds for the prevention of colorectal cancer

*Corresponding author: Maria Hayes

E-mail: Maria.Hayes@teagasc.ie

revealed that the amount required to exert their effects exceeds what is obtainable from dietary intake alone (Nunez-Sanchez *et al.*, 2015). In this review, diverse and potentially important physiological roles of SA and PCA in terms of human health and disease are discussed.

Rapeseed meal and oil production

Rapeseed meal is a by-product of the rapeseed de-oiling process, with the majority of phenolic compounds remaining in the meal after extraction of the oil (Figure 1). Traditionally, the production of rapeseed oil involves mechanical extraction by pre-pressing and extrusion of rapeseeds, followed by extraction with solvents such as food grade hexane. The processing of oil and meal involves various steps, including seed pre-conditioning and flaking, seed cooking, pressing and solvent extraction, followed by the removal of solvent and toasting. Some small-scale facilities do not use solvents to extract rapeseed oil, using only mechanical extraction (Booth, 2004). Although food grade solvents are used during extraction of food grade oils, further refinement of the rapeseed oil is necessary to make it more palatable and suitable for human consumption. Refinement steps include the removal of unwanted components, including phospholipids and free fatty acids, which can be achieved by either physical or chemical means. Chemical methods include either water or acid degumming and alkali refining, while the physical process involves “steam distillation”.

Phenolic compounds in rapeseed

Rapeseed is reported to contain more phenolic compounds than any other oilseed plant (Lomascolo *et al.*, 2012). Reports suggest that rapeseed contains 240–590 $\mu\text{g/g}$ of SA, while rapeseed cake (an initial by-product produced by crushing the rapeseeds to remove the oil) contains 170–454 $\mu\text{g/g}$ of SA (Nićiforović and Abramović, 2014). PCA is also found in rapeseed, with concentrations ranging from 28 $\mu\text{g/g}$ to 612 $\mu\text{g/g}$ in rapeseed hulls (Liu *et al.*, 2012). Phenolic compounds present in rapeseed can be categorised into various subgroups: phenolic acids, lignans, stilbenes, flavonoids, isoflavonoids and complex phenolic polymers such as tannins (Vuorela, 2005). Phenolic acids tend to be found in a soluble form, conjugated with sugars or organic acids, and are usually constituents of more complex structures such as lignans and hydrolysable tannins (Cheynier *et al.*, 2013). In rapeseed, the main phenolic compounds are derivatives of hydroxycinnamic acid (Cvjetko. *et al.*, 2009). The biosynthetic origin of both benzoic and cinnamic acid derivatives is the aromatic amino acid L-phenylalanine, which itself is the end product of the shikimate pathway. The preceding conversion of L-phenylalanine to various hydroxycinnamic acids involves a three-step sequence, referred to as the “general phenylpropanoid metabolism” (Robbins, 2003). Phenolic compounds found in rapeseed mainly exist in the esterified form. Sinapine, which is the choline ester of SA, is the main phenolic ester in rapeseed.

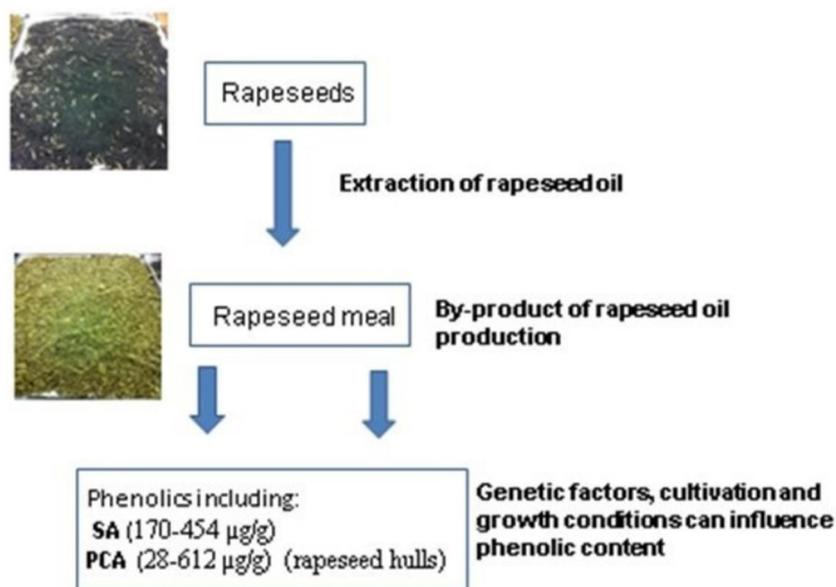


Figure 1. Rapeseed de-oiling process and the phenolics remaining in the meal after extraction.

However, SA can also be esterified to other phenolic acids, sugars or kaempferols (Vuorela, 2005). SA comprises 70%–96% of the esterified phenolic acids in rapeseed meal, while PCA is present in small quantities in rapeseed flours (Shahidi and Naczki, 1992). Sinapine plays an important role in rapeseed, acting as storage for both SA and choline in young plants (Kozłowska *et al.*, 1990). As the seeds mature, some of the sinapine is enzymatically hydrolysed by sinapine esterase to choline and free SA (Tzagoloff, 1963). Apart from the esterified forms, free phenolic acids can also be found in rapeseed, comprising up to 15% of the total phenolics in rapeseed meal. SA comprises 65%–85% of free phenolic acids in defatted rapeseed meals, with PCA also present in small quantities (Naczki *et al.*, 1992; Shahidi and Naczki, 1992; Vuorela, 2005).

Sources and derivatives of SA

SA is found in varying amounts primarily among citrus fruits. For example, lemon and Murcott orange are known to contain the highest quantities of SA in comparison to other natural sources. Additionally, cereal grains such as rye and rice are also known to contain substantial concentrations of SA, while it has also been shown to be present in various herbs such as sage and thyme. SA and its derivatives are particularly abundant among the different varieties of *Brassica* vegetables, including rapeseed, kale, white cabbage, turnip and broccoli (Table 1). In terms of its structure, SA is 3, 5-dimethoxy-4-hydroxycinnamic acid, which is found either in free or esterified form. The most common glycoside of SA is 1-O- β -D-glucopyranosyl sinapate, which can be found in numerous Brassicaceae species. SA can also form dimers with itself and ferulic acid in the walls of cereal cells, with C8–C8-coupled SA dehydrodimers and three sinapate-ferulate heterodimers discovered in various insoluble and soluble cereal grain dietary fibres. SA in rapeseed also exists as the glucosidic ester, glucopyranosyl sinapate. SA does not usually exist in free form, and <16% of SA is present as free SA. Moreover, 4-vinylsyringol, which is found in crude rapeseed oil, is a decarboxylation product of SA. During rapeseed oil extraction, elevated temperatures and pressures cause SA to undergo structural changes, resulting in the formation of 4-vinylsyringol, a decarboxylation product of SA, along with syringaldehyde. The amount of SA derivatives (which include sinapoyl glucose, methyl sinapate, sinapoyl malate and 1-sinapoyl-2-feruloylgentiobiose) found in rapeseed ranges between 6,390 and 18,370 $\mu\text{g/g}$, but this is dependent on the method used to process the oil (Lomascolo *et al.*, 2012; Nićiforović and Abramović, 2014).

Sources and structure of PCA

PCA is chemically known as 3,4-dihydroxybenzoic acid. This compound is purported to have various pharmacological activities, including anti-inflammatory, anti-cancer and cardiac beneficial activity. It is frequently detected in edible plants used in folk medicine and is one of the suggested biologically active components in medicinal plants such as Sudan Mallow (*Hibiscus sabdariffa* L.) and St. John's wort (*Hypericum perforatum* L.) (Kakkar and Bais, 2014). It is also found in common fruits and vegetables, such as blackberries, blackcurrant, cauliflower and lentils (Masella *et al.*, 2012). PCA is a major metabolite of other complex polyphenols, such as the anthocyanins. Anthocyanins are mainly found in fruits such as those of the berry family, pomegranate and pigmented varieties of oranges (e.g. Tarocco, Moro and Sanguinello). In humans and animals, anthocyanins are absorbed as intact glycosides and metabolites. While they can undergo methylation and glucuronidation, they primarily undergo degradation, followed by considerable phase II conjugation, particularly methylation and sulphation. De Ferrars *et al.* (2014) studied the metabolism of the anthocyanin cyanidin-3-glucoside and found that it spontaneously degrades to PCA in the small intestine and the circulation (De Ferrars *et al.*, 2014).

Traditional extraction processes for recovering phenolics from rapeseed meal

Traditionally, extraction of phenolic acids involves a defatting step to remove excess oil. This is usually performed with hexane in a Soxhlet apparatus, with the use of food grade hexane important if the product is to be utilised in food applications. Phenolics found in rapeseed can be divided into three categories: (1) free phenolic acids, (2) soluble esters and (3) insoluble-bound phenolics (Naczki *et al.*, 1992; Szydłowska-Czerniak *et al.*, 2010). Solvents used to extract these phenolics include methanol, ethanol, acetone or a combination of these (e.g. methanol: acetone: water 7:7:6) (Naczki *et al.*, 1992; Cai and Arntfield, 2001; Khattab *et al.*, 2010; Liu *et al.*, 2012). Free phenolic acids can be extracted using methanol: acetone: water (7:7:6, v:v:v) solvent system, and the soluble esters can be obtained through hydrolysis with sodium hydroxide or enzymes and subsequently extracted with diethyl ether: ethyl acetate (1:1, v:v) (Naczki *et al.*, 1992). Extraction and hydrolysis can also be performed with enzymes, such as Ultraflo L and ferulic acid esterase (Vuorela *et al.*, 2003). The main compound released using enzymatic extraction is SA, with a study by

Vuorela (2005) revealing that enzymatic extractions yielded a higher total phenolic content compared to solvent-based extractions. Enzymes are also useful agents for isolation in that they are considered food-friendly, which makes the isolated compounds suitable for food applications (Vuorela, 2005).

Limitations of solvent extraction

Given the shift towards “green and sustainable” methods to extract bioactive compounds, reducing or eliminating solvent use is a key factor when developing new methodologies. This is due to the harmful properties of organic solvents; they are flammable, volatile and toxic, while they may also cause environmental pollution (Chemat *et al.*, 2012). The use of organic solvents also poses problems in terms of using the extracted compounds in food applications. In order for SA and PCA to be used as food components, the solvents used to extract them must be food grade and have safe status. If solvents with toxic properties are used in the extraction process, they must be removed to a specified residual level in order to be eligible for use in food applications, e.g. 5–30 mg of hexane/kg of product (such as edible oils) (Starmans, 1996).

Novel extraction processes for recovering phenolics from rapeseed meal

Developing novel methods to isolate phenolic compounds from rapeseed meal not only overcomes the issues associated with the use of solvents but also closely links with the biorefinery concept. Efficient, simpler and more economic methods of isolation allow for the utilisation of rapeseed by-products and ensure total utilisation of the crop, with no or limited waste generation (Chemat *et al.*, 2012). Various novel methods to extract phenolic acids from various natural product sources have been carried out to date, including UAE, MAE, HVED and PEF extractions (Teh and Birch, 2014; Barba *et al.*, 2015; Teh *et al.*, 2015).

UAE process

UAE provides a unique advantage over traditional methods of extraction in that it can overcome physical barriers, such as poor solubility of phenolics in rapeseed cake, which can make extraction difficult. These physical barriers arise as phenolics in the cake are encompassed in lignin, hulls and cell walls, which are insoluble structures. This, along with the fact that phenolic acids, including SA, occur as esters, glycosides and insoluble-bound forms, has hindered their extraction from rapeseed cake. Ultrasound generates sound waves that create “cavitation bubbles” close to the sample tissue; this leads to the breakdown and disruption of cell

walls and release of the bioactive contents (Khoddami *et al.*, 2013). Teh and Birch (2014) previously determined the optimal parameters to extract phenolics from de-fatted canola seed cakes to comprise a 200 W ultrasound extraction with a solvent volume of 50 mL, extraction time of 20 min, and temperature of 70°C. This method was found to be advantageous over traditional methods in that it involves low cost, has low solvent consumption and increases the antioxidant activity of the extracts (Teh and Birch, 2014). Other protocols have demonstrated UAE to be a suitable technique to extract phenolics from rapeseed and related species. An optimised protocol for the UAE of antioxidant compounds, including phenolics, from rapeseed cultivars was previously described by Szydłowska-Czerniak and Tułodziecka (2014). They concluded that the optimal conditions to perform UAE were an extraction time of 13.8 min, a solvent-to-material ratio of 26.2 mL/g and a solvent concentration of 50.3% (Szydłowska-Czerniak and Tułodziecka, 2014). Another study investigated the optimal UAE conditions to extract phenolics from kale, which is a member of the Brassicaceae family, along with rapeseed. They determined that the optimal conditions were 80% ethanol, with an extraction time of 60 min, ultrasound frequency of 20 kHz and power of 100 W (Oniszczyk and Olech, 2016). Dubie *et al.* (2013) also extracted phenolic antioxidant compounds from mustard (*Brassica juncea*) seed meal, another member of the Brassicaceae family, using UAE. They determined that the optimal conditions were 75°C for 20 min with 70% ethanol and a solvent-to-meal ratio of 40:1, with 20 kHz and 0.5 W/mL ultrasound treatments using a Branson Sonifier probe with input power of 400 W (Dubie *et al.*, 2013).

MAE process

MAE of phenolics from rapeseed meal offers advantages, including shorter extraction times and greater yields, over traditional methods such as solvent extraction. Teh *et al.* (2015) investigated MAE of phenolics from rapeseed cakes. The optimal parameters for MAE of rapeseed cakes were found to be 5 min, with a liquid: solvent ratio of 6 mL/g and power of 633.33 W. Microwave pre-treatment using the optimal parameters described maximised the phenolic yield from rapeseed cake when subsequently extracted with ultrasound and methanol: acetone: water mixture (7:7:6, v:v:v). This method was economically viable due to the shorter extraction time, moderate microwave power and low electroporation voltage and frequency (Teh *et al.*, 2015). MAE has also been used in the extraction of phenolic compounds from broccoli, which is a member of the Brassicaceae (or mustard) family along with rapeseed. The optimal conditions were determined to be 71.11°C, microwave power of 167.03 W, solvent concentration (methanol/water) of 75.95% and an extraction time of 16.34 min (Jokić *et al.*, 2012). Other

studies have demonstrated the efficacy of this technique in the extraction of phenolics, although from different sources, including rice grains and almond skin by-products (Setyaningsih *et al.*, 2015; Valdés *et al.*, 2015). The success of this technique in extracting phenolic compounds means it could be a useful method to isolate phenolic compounds from rapeseed meal, but further optimisation and validation are necessary.

PEF extraction

PEF extraction is a process that does not require heat but generates an electric field, which results in the electroporation of the cellular membrane and the release of the bioactive constituents from the “protoplasm”. The efficacy of this technique depends on various factors, including the strength of the electric field as well as the duration and number of pulses. A recent study (Teh *et al.*, 2015) optimised the extraction of polyphenols using PEF from rapeseed cake. In order to recover the maximum yield of polyphenols, the optimal parameters for PEF extraction were found to be as follows: voltage of 30 V, frequency of 30 Hz, 10% ethanol and an exposure time of 10 s. This method also proved advantageous over traditional methods due to the shorter extraction time and low solvent use (Teh *et al.*, 2015). Yu *et al.* (2016) evaluated PEF-assisted extraction of polyphenols in order to valorise a by-product of rapeseed oil production, rapeseed stem or green biomass. They determined that the number of pulses that yielded higher total polyphenol content of juices pressed at 10 b was 200, with a strength of 8 kv/cm. The efficacy of PEF pre-treatment was demonstrated by the increase in total polyphenols with PEF pre-treatment and subsequent pressing. Without PEF pre-treatment, after 30 min of pressing, the result was 24% polyphenol yield from rapeseed. PEF pre-treatment with 200 pulses and an increase in strength to 8 kv/cm resulted in maximum polyphenol yield for pressing (Yu *et al.*, 2016). These initial studies have had success in isolating phenolics from rapeseed by-products, and it could be a promising novel method to isolate phenolic compounds; however, further studies are necessary to fully validate this method for use with rapeseed meal.

HVED approach

HVED is based on “electrical breakdown in water”, which is accompanied by various phenomena including high-amplitude pressure shock waves and cavitation bubbles. This subsequently leads to the breakdown of cellular structures and the release of the cell contents. The use of HVED to isolate polyphenols from rapeseed cake was also investigated previously (Barba *et al.*, 2015). The study found that when isolating polyphenols from rapeseed cake, the liquid-to-solid ratio was a key parameter. The highest

polyphenol content was observed using a liquid-to-solvent ratio of 20, while a liquid-to-solvent ratio of either 5 or 20, combined with HVED treatment at 80 kJ/kg also resulted in increased polyphenol content. However, it was noted that higher energy inputs >80 kJ/kg in rapeseed cake led to a decrease in polyphenol content. The authors suggest that high-energy inputs result in the creation of radicals, which in turn decrease the polyphenol content (Barba *et al.*, 2015). Crucially, it was found that the extracts from rapeseed cake had a significant increase in their antioxidant activity following HVED treatments. Other studies have shown the efficacy of this technique to extract phenolics from different oilseed sources, including sesame cake and flaxseed cake (Boussetta *et al.*, 2013; Sarkis *et al.*, 2015). The study by Barba *et al.* was an initial optimisation, in addition to being the first step in the search for more efficient methods to isolate phenolic compounds from rapeseed by-products. Further optimisation and validation are necessary in order to ensure the efficacy of this novel technique; however, it is worthy of mention due to its proven ability to extract phenolic compounds and its apparent efficiency over traditional methods, such as lower solvent consumption.

Characterisation strategies for rapeseed meal phenolics

As SA and PCA found in rapeseed possess various bioactive properties, including anti-inflammatory, anti-diabetes and anti-cancer activities, they are of interest as functional food components (Cherng *et al.*, 2013; Del Corno *et al.*, 2014; Tsao *et al.*, 2014). Therefore, further separation and purification are necessary to isolate these phenolic compounds from the initial fractions. Initially, the total phenolic content is often quantified using the Folin-Ciocalteu assay (Vuorela, 2005). Chemical characterisation and quantification are commonly performed using high-performance liquid chromatography (HPLC) or gas-liquid chromatography (GLC)/gas chromatography-mass spectrometry (GC-MS) (Cai and Arntfield, 2001; Canini *et al.*, 2007; Khattab *et al.*, 2010). HPLC is the preferred method for separation and quantification of phenolic compounds due to the fact that there is a wide range of commercially available columns, as well as the potential to combine two or more columns with differing column chemistries (Stalikas, 2007). However, despite the fact that HPLC is a commonly used technique, it has been suggested that GC-MS is more efficient, providing improved resolution and baseline separation with minimum co-elution. It must also be noted that when quantifying phenolic compounds with GC, there is sometimes a requirement for chemical modification, such as transformation to more volatile derivatives (Khoddami *et al.*, 2013).

SA and PCA in human health and disease

Properties of SA

Antioxidant activity of SA

SA can modulate oxidative stress pathways in the cell and may have potential in treating diabetes and high blood pressure (Roy and Prince, 2012; Silambarasan *et al.*, 2015). Oxidative stress can be defined as an imbalance in the “redox” status of a cell, which occurs when the levels of reactive oxygen species (ROS) and antioxidants are unbalanced (Nordberg and Arner, 2001). ROS can be found extrinsically in the form of pollutants, tobacco smoke and radiation; however, ROS may also be generated intrinsically through various pathways in the mitochondria (Trachootham *et al.*, 2009). The single electron reduction of molecular oxygen leads to the formation of a superoxide anion, a molecule that acts as a precursor for the formation of most other ROS (Turrens, 2003). In order to maintain homeostatic levels of reactive superoxide anions, the mitochondrial matrix contains a family of metalloenzymes known as superoxide dismutases (SODs). Compounds such as ascorbic acid, tocopherols and glutathione (GSH) also provide antioxidant activity (Jalaludeen and Pari, 2011). High levels of ROS are known to be carcinogenic, damaging vital cellular components including lipids, proteins and DNA. Given the destructive potential of ROS, it is generally thought that the use of antioxidants could reduce intracellular ROS levels and thus alleviate ROS-mediated damage (Brieger *et al.*, 2012). Recent studies indicate the antioxidant activity of SA and SA's superoxide radical and hydroxyl radical, as well as its $-O_2^-$ -scavenging activity (Zou *et al.*, 2002; Jalaludeen and Pari, 2011). Using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, it was found that SA had higher free radical-scavenging activity than its alkyl ester methyl sinapate or the control Trolox, a vitamin E analogue. Ferric reducing antioxidant power (FRAP) assays also revealed the higher antioxidant scavenging activity of SA compared to its alkyl ester methyl sinapate or the control Trolox (Gaspar *et al.*, 2010). The mechanism and kinetics of SA's antioxidant activity were measured using its hydroperoxyl radical ($HOO\cdot$)-scavenging activity in lipidic and aqueous solutions, which occurs through the transfer of a hydrogen atom from its $-OH$ group. The anionic form of SA at physiological pH was also more reactive than its neutral form towards oxygenated radicals (Galano *et al.*, 2011).

Histone deacetylase inhibition

Histone deacetylases (HDACs) remove acetyl groups from histones, resulting in a “closed” chromatin conformation that blocks gene transcription and therefore gene expression. Aberrant HDAC activity is common in various cancer types, driving carcinogenesis through the repression of genes involved in the cell cycle regulation, cellular differentiation

and apoptosis (Tortorella *et al.*, 2015). Thus, HDAC inhibitors are emerging as potential therapeutic agents. There has been success in developing clinical therapeutics using HDAC inhibitors from both natural and synthetic sources. For example, suberoylanilide hydroxamic acid (SAHA) (vorinostat) and the natural product, romidepsin, were both approved for the treatment of cutaneous T-cell lymphoma (Mair *et al.*, 2014). SA was previously shown to have HDAC inhibitory activity. Senawong *et al.* (2013) extracted phenolic compounds from the rhizome of *Hydnophytum formicarum* Jack, and SA was found to be one of the major components. SA was found to block HDAC enzyme activity *in vitro* at a similar concentration as the established HDAC inhibitor sodium butyrate, with the authors suggesting that SA partly accounted for the HDAC inhibitory activity of the phenolic extract. The phenolic extract possessing HDAC inhibitory activity was found to inhibit the growth of HeLa cells in a dose- and time-dependent manner, while SA was also found to significantly inhibit the growth of HT-29 cells. The phenolic extract and SA were also found to have a significant effect on the induction of apoptosis, up to 78.9% and 8.4%, respectively.

Anti-cancer and antibacterial activities

SA was found to be cytotoxic against various cancer cell lines, including the human colon cancer cell lines HT-29 and SW480 and the human laryngeal carcinoma Hep-2 cells, when assayed previously (Balaji *et al.*, 2014; Janakiraman *et al.*, 2014). The cytotoxicity is probably due to the anti-proliferative activity of SA, which is supported by a study demonstrating SA's anti-proliferative activity against the breast cancer cell line T47D (Kampa *et al.*, 2004). The antibacterial effect of SA was demonstrated to be anti-carcinogenic, brought about by decreasing mucinase activity, an enzyme secreted by gut microflora. This inhibited mucin degradation and enhanced contact between carcinogens and colon epithelial cells. SA also inhibited carcinogenesis by increasing the activities of enzymatic and non-enzymatic antioxidants, including SOD, catalase (CAT) and GSH, with ROS known to play a role in early carcinogenesis. SA could potentially prevent the onset of carcinogenesis, as demonstrated in a study by Balaji *et al.* (2015). Colon cancer is the third most common malignancy worldwide, with a high-fat diet being a primary risk factor for developing colon cancer. Consumption of a high-fat diet has been reported to increase the development of pre-neoplastic lesions. Aberrant crypt foci (ACFs) are pre-neoplastic lesions, which are formed during an early event in carcinogenesis in humans and rodents. Rats fed a high-fat diet consisting of peanut oil had increased crypt numbers and “multiplicity”. SA supplementation in 1,2-dimethylhydrazine (DMH)-induced colon cancer rats decreased the development and number of ACFs. SA also decreased β -glucuronidase activity in DMH-induced rats. Increased β -glucuronidase activity is

considered a primary causative factor in colon cancer, via the formation of methylazoxymethanol, a metabolite that induces the formation of DNA adducts. SA was effective in reducing the development of pre-cancerous lesions when studied previously (Balaji *et al.*, 2015).

Protective role in cardiovascular disease

Cardiovascular disease is the number one cause of death globally and the leading cause of death among Europeans (Townsend *et al.*, 2015). The available figures from 2013 revealed that 30.8% of deaths in the US were due to cardiovascular disease (Mozaffarian *et al.*, 2016). Lifestyle modifications, including a healthy diet, can play a protective role. Although no human studies have been performed, SA was shown to have a protective effect on isoproterenol (ISO)-induced myocardial infarction (MI) in rats. MI induction with ISO is a well-established animal model used to evaluate the effects of various cardioprotective agents because the pathophysiological changes are analogous to those taking place in humans with MI. Pre- or co-treatment with SA normalised the activity of serum creatine kinase (CK-MB), suggesting that SA protects the heart and reduces the damage leading to leakage of CK-MB. SA normalised the levels of lipid peroxidation products, such as cardiac malondialdehyde, and as such, apparently inhibited oxidative stress and stabilised lysosomal membranes. In accordance with this finding, SA normalised lysosomal enzymes including β -glucuronidase and β -galactosidase, as well as cathepsins B and D. Co-treatment with SA also reduced the size of the infarct, protecting the heart from ISO-induced MI (Roy and Prince, 2012).

Reperfusion injury occurs due to ischaemia followed by reintroduction of blood flow to a previously ischaemic tissue, causing additional myocardial damage and cardiac contractile dysfunction (Silambarasan *et al.*, 2015). As oxidative stress is an important contributor to ischaemia/reperfusion (I/R) injury, antioxidants have been considered as a potential therapeutic option (Yang *et al.*, 2013). Oral pre-treatment with SA significantly improved the percent rate–pressure product and percent coronary flow when compared to untreated I/R hearts. Mitochondrial dysfunction results in cardiac contractile dysfunction and hypertrophy; moreover, under conditions of oxidative stress, the enzymes of the tricarboxylic acid (TCA) cycle are disrupted. Decreases in the activity of several TCA enzymes were blunted after pre-treatment with SA. SA was also found to reduce H9c2 cardiomyoblast cell injury caused by H_2O_2 , probably due to its scavenging abilities (Silambarasan *et al.*, 2015).

In N^w -nitro-L-arginine methyl ester hydrochloride (L-NAME)-treated rats, SA decreased the mean arterial pressure, along with kidney and liver weights. SA increased the activities of the antioxidant enzymes SOD and CAT, along with the levels

of non-enzymatic antioxidants such as the vitamins C and E. Ventricular hypertrophy was observed due to L-NAME treatment, causing a reduction in contractile function. SA treatment restored this ventricular function, which the authors attributed to its antioxidant abilities, considering that previous studies have shown that oxidative stress plays a role in fibrosis during hypertension. Damaged cells leak hepatic enzymes such as aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT), causing an increase in their activities. Treatment with SA decreased their activities and controlled liver damage, which was validated by histopathological examination of the liver. SA was also found to decrease renal function markers, including urea, uric acid and creatinine, towards normal levels. Epidemiological studies have linked hypercholesterolaemia to hypertension. Furthermore, SA treatment was found to lower total cholesterol levels in the liver and kidney of L-NAME spontaneously hypertensive rats. Docking studies revealed that this effect was due to the repression of endogenous cholesterol biosynthesis by inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (Silambarasan *et al.*, 2016).

Anti-diabetes activity

Diabetes is a group of “heterogeneous, hormonal and metabolic disorders”, with hyperglycaemia being one of its physiological characteristics. Type 2 diabetes (T2D), in particular, is one of the most prevalent “metabolic pathologies” worldwide, with >220 million people affected. Chronic hyperglycaemia has detrimental cellular effects, as oxidation of glucose as well as protein glycation and glycoxidation all enhance the formation of ROS. The biochemical and physiological effects of SA treatment on streptozotocin (STZ)-induced diabetic rats revealed that oral administration of SA was found to cause a significant improvement in body weight, attributed to the anti-hyperglycaemic effect of SA (Stumvoll *et al.*, 2005). SA also influenced other physiological parameters, such as decreasing kidney weight to near-normal values. SA significantly decreased plasma glucose when compared to diabetic control rats, which the authors suggest could be due to the regeneration of existing pancreatic β -cells by SA and enhanced transport of glucose to peripheral tissues. SA significantly increased the levels of both C-peptide and insulin in STZ-induced diabetic rats, while decreasing the level of glycosylated haemoglobin (HbA1c) and increasing the level of total haemoglobin. The authors speculate that these changes could be due to the reduction of blood glucose level. Increased activities of enzymes involved in gluconeogenesis, i.e. glucose-6-phosphatase and fructose-1, 6-biphosphatase, were observed in the liver and kidney of diabetic rats. SA restored their level of activity to near normal, resulting in improved glycaemic control (Kanchana *et al.*, 2011).

SA can directly influence glucose utilisation, by significantly lowering postprandial glucose without altering plasma insulin levels. Glucose transporter type 4 (GLUT4) expression was significantly increased in the soleus muscle of STZ-induced rats, with SA causing a concentration-dependent increase in glucose uptake in the soleus muscle. This was confirmed in L6 myoblast cells, indicating that SA may directly stimulate glucose uptake. *In vivo*, SA increased insulin sensitivity, probably due to the enhanced glucose uptake in skeletal muscle (Cherng *et al.*, 2013). Taken together, these results indicate that SA may be clinically useful in the treatment of diabetes.

Anti-inflammatory activity

Nuclear transcription factor kappa-B (NF- κ B) plays a key role in the pathogenesis of inflammation, and as such, there are numerous drugs designed to treat inflammatory disease, which are based on the inhibition of NF- κ B, e.g. non-steroidal anti-inflammatory drugs. In response to lipopolysaccharide (LPS), SA inhibited the expression levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2, which were markedly upregulated (Yun *et al.*, 2008). The mRNA levels of both iNOS and COX-2 were also reduced, indicating transcriptional inhibition. SA reduced the production of tumour necrosis factor (TNF)- α and interleukin (IL)-1 β , as well as their mRNA expression levels. RAW 264.7 cells treated with SA had a reduction in the DNA-binding activity of p65, preventing transcription initiation by NF- κ B and subsequent expression of genes involved in the immune response (Schmitz and Baeuerle, 1991; Pahl, 1999). The nuclear translocation of both p65 and p50 was also inhibited. Another key finding was SA's ability to inhibit the LPS-induced degradation of I kappa B-alpha (I κ B- α), preventing the release of NF- κ B. These results demonstrate that SA inhibits iNOS, TNF- α , COX-2 and IL-1 β expression, and this is at least partly mediated via an NF- κ B mechanism. This anti-inflammatory activity was also demonstrated *in vivo*, with SA inhibiting oedema caused by acute inflammation in serotonin- and carrageenan-induced rat paw oedema models (Yun *et al.*, 2008).

Properties of PCA

Antioxidant activity of PCA

The physiological effect of PCA is believed to be linked to its antioxidant activities, which were recently investigated *in vitro*. DPPH and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assays demonstrated that PCA had better scavenging activity than the positive controls Trolox and butylated hydroxytoluene (BHT). When compared to Trolox and BHT in terms of reducing power percentage (%-Fe³⁺ and %-Cu²⁺), PCA was found to be much more efficient. PCA also had lower half-maximal inhibitory concentration (IC₅₀) values for both Fe³⁺ and Cu²⁺ assays, in comparison to

Trolox and BHT. It was found that PCA generally exhibited higher inhibition levels than Trolox, but not BHT, in terms of scavenging of superoxide anion and hydroxyl radical. The generally lower IC₅₀ values obtained for PCA in comparison to the positive controls Trolox and BHT indicate that PCA is a better antioxidant, as a lower IC₅₀ value indicates higher antioxidant activity. The DPPH and ABTS assays were conducted in organic solutions, while the reducing power (Fe³⁺ and Cu²⁺) and superoxide anion- and hydroxyl radical-scavenging assays were carried out in aqueous solutions. Therefore, PCA exhibited significant antioxidant abilities in both lipidic and aqueous media and is thus considered an ideal natural antioxidant in both media (Li *et al.*, 2011). The potent antioxidant activity of PCA was also shown in ageing rats. Treatment with PCA caused a significant rise in the enzymatic activities of CAT and GSH peroxidase (GSH-PX), both of which are endogenous enzymatic antioxidants (Zhang *et al.*, 2011). This highlights the therapeutic potential for PCA in the treatment of age-associated disorders, such as cancer, through decreasing the levels of harmful ROS.

HDAC inhibition

Khaopha *et al.* (2015) obtained phenolic-rich testa extracts from peanut testae (skins), which were found to possess HDAC inhibitory activity and anti-cancer activity. PCA and *p*-coumaric acid were found to be the most predominant phenolic acids, with the highest quantity of PCA in the ICG15042 extract, while SA was greater in the KK4 extract (Khaopha *et al.*, 2015). HeLa cells treated with the phenolic-rich testa extracts ICG15042, KS2 and KK4 displayed a significant increase in all four acetylated lysine residues of histone H4, indicating HDAC inhibition. The authors suggested that some of the phenolic compounds in the extracts were functioning as HDAC inhibitors, with extracts ICG15042 and KK4 chosen for further evaluation as they displayed the highest HDAC inhibitory activity. Both extracts significantly inhibited the proliferation of various human cancer cell lines, including HeLa (human cervical adenocarcinoma), HT29 (human colon adenocarcinoma), HCT116 (human colorectal carcinoma) and Jurkat (human T-cell leukaemia) cells, with Jurkat cells being particularly sensitive to this anti-proliferative activity. Both extracts induced apoptosis in the cancer cell lines; however, it appeared that apoptotic induction was most effective in the HeLa and HCT116 cells, a result also observed by Senawong *et al.* (2013). Importantly, when the extracts were tested with a non-cancer cell line, the number of apoptotic cells was similar to that with the solvent control, indicating that the phenolic extracts were not harmful to normal cells (Khaopha *et al.*, 2015). This is crucial given that specificity is a vital attribute to any therapeutic agent, in order to minimise damage to healthy cells and reduce side effects.

*Anti-cancer activity**Cytotoxicity*

PCA is cytotoxic to various cancer cell lines, including human breast MCF-7 cells, lung cancer A549 cell, HepG2 hepatocellular carcinoma, cervix HeLa and prostate cancer LNCaP cells. PCA was able to penetrate the cancer cells, destroying the integrity of the plasma membrane, causing apoptosis and lactate dehydrogenase (LDH) leakage. PCA also decreased Na⁺/K⁺-ATPase activity, thereby disrupting ion homeostasis, resulting in collapse of the mitochondrial membrane. This collapse resulted in the release of pro-apoptotic factors, initiating the intrinsic apoptotic pathway, and was also suggested to cause an increase in the activities of caspase-8 and -3, which are key mediators of apoptosis (Yin *et al.*, 2009). PCA also displayed cytotoxicity against non-small cell lung cancer via similar mechanisms as reported herein. They found that PCA induced mitochondrial dysfunction and apoptosis through increases in Bax and caspase-3 expression and a decrease in Na⁺/K⁺-ATPase activity. PCA also modulated several signalling pathways, including FAK, MAPK and NF- κ B pathways and thus reduced the release of inflammatory cytokines such as IL-6 and IL-8. Production of proteins involved in metastasis (such as basic fibroblast growth factor [bFGF] and the matrix metalloproteinases MMP-2 and MMP-9) were also reduced (Tsao *et al.*, 2014).

Chemoprevention and reduced side effects

Cancer chemoprevention can be defined as the prevention of cancer using non-toxic synthetic chemicals or chemicals from natural substances before malignancy occurs. PCA was previously shown to prevent oral carcinogenesis, reducing incidences of neoplasms in the tongue (squamous cell papilloma and carcinoma), along with significantly decreasing pre-neoplastic lesions (hyperplasia and dysplasia) (Tanaka *et al.*, 1994). Anthocyanins derived from black raspberries, along with the main anthocyanin metabolite PCA, were studied in *N*-nitrosomethylbenzylamine (NMBA)-induced oesophageal tumours in rats. PCA delayed the formation of pre-neoplastic lesions and the progression of these lesions to papillomas, ultimately reducing NMBA-induced oesophageal carcinogenesis. PCA also induced the expression of PTX3, which was shown to possess anti-angiogenic and anti-tumourigenic activity in prostate cancer cells (Peiffer *et al.*, 2014).

Although cancer prevention is crucial, improving existing therapies and reducing harmful side effects is just as vital. Cisplatin is widely used to treat various solid malignancies; however, its use is limited due to its associated nephrotoxicity, which primarily occurs in kidney proximal tubule epithelial cells (Karasawa and Steyger, 2015). PCA was found to prevent induced oxidative damage in kidney proximal tubule

LLC-PK1 cells *in vitro*, while *in vivo* studies showed that PCA prevented cisplatin-induced increase in the renal function marker, creatinine. Cisplatin treatment also resulted in distinct histopathological changes in the rat kidney, such as tubular epithelial cellular necrosis, desquamation, vacuolisation and swelling. PCA treatment caused notable improvement in the histological appearance, with reduced tubular cell damage and swelling, providing almost “complete renoprotection” (Yamabe *et al.*, 2015).

Anti-metastatic activity

Metastasis accounts for almost 90% of cancer-associated mortality and is the biggest problem in the clinical treatment and management of cancer. PCA represents a potentially important agent for those patients who are diagnosed with early cancer lesions, as the anti-metastatic effects of PCA could stop the subsequent dissemination from the primary tumour and prevent metastasis in those patients (Chaffer and Weinberg, 2011). The metastasis process is still poorly understood, and there is an urgent need to develop new therapeutic strategies and targets to prevent or inhibit this phenomenon. The anti-metastatic potential of PCA in the highly metastatic melanoma cell line B16/F10 and in human gastric carcinoma AGS cells was also demonstrated previously. PCA inhibited the secretion of MMP-2 in human gastric carcinoma AGS cells and inhibited the protein kinase B (Akt)/NF- κ B/MMP-2 pathway via the activation of RhoB. Metastasis of the B16/F10 melanoma cells to the liver of C57/BL6 mice was also considerably reduced by PCA treatment (Lin *et al.*, 2011).

Anti-inflammatory activity

Studies have demonstrated the ability of PCA to inhibit the LPS-induced secretion of pro-inflammatory cytokines, such as IL-8, IL-2 and chemokine (C-C motif) ligand-2 (CCL2), from monocyte-derived dendritic cells and human gingival fibroblasts via activation of peroxisome proliferator-activated receptor (PPAR)-g. As cytokines such as IL-8 and CCL2 are involved in recruiting inflammatory cells, a reduction in the IL-8 content in the extracellular environment may limit the recruitment of inflammatory cells and thus prevent the initiation of an inflammatory immune response (Del Corno *et al.*, 2014; Wang *et al.*, 2015b). PCA can also prevent inflammation by inhibiting the oxidation of fatty acids, which are activators of PPAR-g signalling (Nagy *et al.*, 1998). The pathology of neurodegenerative diseases, including Parkinson's and Alzheimer's disease (AD), has been attributed to the overproduction of inflammatory mediators such as TNF- α , IL-6 and IL-1 β . These inflammatory mediators are produced following the activation of microglia, which are immune cells resident in the brain, by LPS, and the subsequent activation of NF- κ B and MAPKs. As such, regulating the activity of

the microglia could be a potential therapeutic strategy. In accordance with this observation, Wang *et al.* (2015) found that PCA could inhibit the inflammatory mediators TNF- α , IL-1 β , IL-6 and prostaglandin-2 (PGE2) but could also inhibit the LPS-induced activation of NF- κ B and MAPKs, both of which are key regulators of inflammatory cytokine production. Another key finding was PCA's ability to inhibit the expression of Toll-like receptor 4 (TLR4), a receptor that activates NF- κ B and MAPKs upon stimulation by LPS. This, therefore, suggests that PCA exerts its anti-inflammatory effects through inhibition of the TLR4 pathway, making it a potential candidate to regulate the activation of microglia in order to treat neurodegenerative diseases (Wang *et al.*, 2015a).

A recently published study has identified PCA as a potential therapeutic agent in the treatment of inflammatory bone disease. Osteoclasts are necessary for bone matrix degradation and to initiate bone re-modelling. However, an imbalance in their activity can lead to bone deterioration, which is a symptom of diseases such as osteoporosis and rheumatoid arthritis. M-CSF and RANK-L are potent inducers of mature osteoclasts, with RANK-L activating transcription factors such as c-Fos, which activate osteoclasts and induce their differentiation. PCA was found to inhibit RANK-L-induced c-Fos protein expression, while also inhibiting the differentiation of osteoclasts in mouse bone marrow macrophages. The bone-resorbing ability of mature osteoclasts was also inhibited. *In vivo*, PCA was shown to have a protective effect against LPS-induced bone loss. PCA was also found to downregulate the expression of osteoclastogenesis-related genes, including DC-STAMP and b3-integrin (Park *et al.*, 2016b).

Protective role in cardiovascular disease

The co-morbidities associated with diabetes pose serious health risks, with vascular complications being the primary cause of mortality and morbidity in those suffering from chronic diabetes. The effects of PCA on vascular responses in chronically diabetic rats were also examined. PCA was found to protect blood vessels by increasing nitric oxide production, which prevents the interaction of platelets and leucocytes with the vascular cell wall. Hypertension is a common complication of diabetes. PCA was found to cause significant reductions in the high diastolic, systolic and mean arterial blood pressures observed in chronically diabetic rats. PCA also ameliorated diabetes-induced high blood pressure by restoring the reduced vascular reactivity in the diabetic rats (Semaming *et al.*, 2016). This improved reactivity was exemplified by the restoration of the vasoconstriction and vasodilation responses following PCA treatment. PCA treatment also caused a decrease in the oxidative stress marker malondialdehyde (MDA), while the activities of the antioxidant enzymes SOD and CAT were increased. The authors, therefore, suggested that the improved vascular reactivity observed in the diabetic

rats could be due to the antioxidant activity of PCA, given that it has been postulated that oxidation plays a role in vascular impairment in diabetes. The potential of PCA to prevent or limit cardiac complications in diabetes was demonstrated earlier using type 1 diabetic rats by Semaming *et al.* (2014), who found that PCA reduced MDA levels, along with attenuating mitochondrial depolarisation, decreasing mitochondrial swelling and attenuating mitochondrial ROS production. PCA in combination with insulin could restore echocardiography to a normal status, which could not be achieved by insulin alone. HbA1c levels were also reduced, indicating greater long-term glycaemic control. Cardiac apoptosis is a poor predictor of outcome in those with cardiac disease or heart failure. In line with this, PCA increased the anti-apoptotic protein Bcl2 in the heart tissue of diabetic rats (Semaming *et al.*, 2014).

Currently, there is a need for anticoagulation agents from natural sources in the clinic. In addition, the anti-thrombotic potential of PCA derived from fermented pine needles was evaluated. The processes of coagulation and anticoagulation are tightly regulated, and an imbalance in this process can lead to thrombus formation, which adheres to blood vessel walls. If this thrombus is not broken down, circulation is interrupted, leading to high blood pressure, stroke, ischaemic heart disease and myocardial infarction. PCA was tested for its fibrinolysis activity, by measuring the turbidity in a fibrin clot solution. PCA caused a dramatic decrease in turbidity, indicating that the fibrin clot had dissolved. Further studies aimed to elucidate the mechanism behind the fibrinolytic activity of PCA. A pH range of 2–4 was optimal for PCA to exert its fibrinolytic activity. At this acidic pH, PCA mainly exists in a non-ionised form, therefore allowing it to form greater number of hydrophobic interactions. The authors suggested that this increased tendency towards hydrophobic interactions allows enhanced binding between PCA and polypeptide chains, compromising their stability and causing “loosening of the fibrin network”. The authors concluded that PCA exerted its fibrinolytic activity through a “dissolving effect”, rather than by degradation of fibrin and fibrinogen. *In vivo*, PCA was shown to significantly inhibit thrombosis induced by carrageenan in mouse tail when compared to the control. In FeCl₃-induced carotid arterial thrombus model experiments, rats treated with PCA had no thrombus formation, indicating that PCA could inhibit vascular obstruction (Park *et al.*, 2016a).

Anti-diabetes activity

Functional foods provide a promising alternative therapeutic strategy to drive positive metabolic changes that would impair the T2D process; however, few studies have elucidated the molecular mechanisms of polyphenols on insulin signalling. A recent study (Scazzocchio *et al.*, 2011) has shown that PCA is able to partially counteract the loss of insulin sensitivity in T2DM and activate the insulin receptor substrate-1/

phosphatidylinositol 3-kinase/Akt (IRS-1/PI3K/Akt) signalling pathway, leading to increased glucose uptake. In adipocytes, PCA was found to be dependent on activation of the entire insulin pathway, as inhibition of the major players in the insulin signalling cascade IRS-1, PI3K or Akt prevented glucose uptake and GLUT4 translocation. PCA has also been reported to enhance glucose uptake by increasing translocation of GLUT4 to the cell membrane or, additionally, by increasing adiponectin secretion, a protein hormone capable of modulating glucose and fatty acid metabolism (Scazzocchio *et al.*, 2011).

The effects of PCA on adenosine monophosphate (AMP)-activated protein kinase (AMPK) activation and the role of PCA in glucose homeostasis in mice were examined previously (Talagavadi *et al.*, 2016). AMPK is involved in metabolic regulation and was reported to be central to the control of glucose metabolism. In contrast, mammalian target of rapamycin (mTOR) and S6K1 are known to play a role in insulin resistance. It was found that PCA could significantly activate AMPK, although indirectly, and downregulate S6K in mouse livers. Upon intraperitoneal administration of glucose, there was a significant decrease in the glucose level in PCA-treated mice, with similar results obtained for the positive control metformin, indicating that PCA can induce glucose tolerance. In obese mice, pre-treatment with PCA significantly reduced hyperglycaemia after glucose injection. Furthermore, the insulin tolerance test showed that PCA administration amplified the glucose decrease, indicating that PCA improved insulin sensitivity in obese mice (Talagavadi *et al.*, 2016).

Protective neurological effects

In order to repair injured nerves in adults, proliferation of nerve cells is a vital process. Schwann cells are crucial for the function and regeneration of neurons, and it was recently demonstrated that PCA could stimulate Schwann cell proliferation and survival by phosphorylating the insulin-like growth factor-I (IGF-I)-mediated PI3K/Akt pathway (Ju *et al.*, 2015). PCA enhanced the proliferation of RSC96 cells, inducing cell cycle progression at the G1-to-S phase. An increase in cyclin A was observed, resulting in the promotion of DNA replication and growth of RSC96 cells. A phenomenon that is vital to the repair and regeneration process of nerves is the migration of RSC96 Schwann cells along the growth direction. It was found that PCA enhanced Schwann cell migration, and that the IGF-1/Akt/PI3K pathway may play a key role in this process. The results of that study highlight the potential utility of PCA in the treatment of peripheral nerve injuries caused by trauma, surgery or acute compression. Guan *et al.* (2009) also demonstrated the ability of PCA to stimulate proliferation of rat hippocampal neural stem cells, which are believed to contribute to the regenerative potential of the adult central nervous system.

For patients suffering from neurodegenerative brain diseases, ameliorating symptoms is crucial. PCA was found to improve cognitive defects in a mouse model of AD and attenuate Ab deposition, which is a hallmark of AD (Song *et al.*, 2014). Inflammatory events in AD result from an imbalance in the formation and clearance of Ab, with mounting evidence that Ab accumulation is linked to the secretion of inflammatory cytokines. PCA reduced the levels of TNF- α , IL-1 β , IL-6 and IL-8 in AbPP/PS1 mice. Although the underlying mechanism behind PCA's effect on Ab-induced neurotoxicity was not elucidated, it is attributed to its antioxidant and anti-inflammatory activities. Crucially, PCA displayed similar anti-inflammatory activity as donepezil, a cholinesterase inhibitor used to treat AD.

Protection against nephrotoxicity and hepatotoxicity

Cadmium (Cd) is a heavy metal that is a by-product of zinc production. It is considered to be one of the most toxic environmental and industrial pollutants. It can be ingested from foods, drinking water and contaminated soil or through cigarette smoking. The kidney is the most affected by prolonged low-level exposure to Cd, causing proteinuria, calciuria and tubular necrosis, all of which may result in end-stage renal failure, diabetic complications and osteoporosis. Cd can also lead to nephrotoxicity due to its tendency to settle in the proximal tubule of the nephron, although the underlying mechanism is yet to be fully elucidated. PCA can attenuate the nephrotoxicity and hepatotoxicity induced by Cd in rats. Treatment with Cd led to "severe" hepatic damage, evidenced by the increased activity of the hepatic enzymes alanine transaminase (ALT), AST and ALP in the serum of the Cd-induced hepatotoxicity group compared to the normal control. Co-treatment with PCA was found to ameliorate the activities of these hepatic enzymes. Crucially, PCA at doses of 10 and 20 mg/kg had no adverse effects on the rats (Adefegha *et al.*, 2015).

Potential use of SA and PCA recovered from rape-seed meal

SA and PCA may improve human health, and both compounds are present in traditional Chinese medicines, such as "Yinhuang powder" and *Salvia miltiorrhiza* (also known as Danshen) (Wen *et al.*, 2005; Cao *et al.*, 2014; Su *et al.*, 2015). Research on these phenolic compounds has suggested that they possess diverse bioactive properties, including anti-cancer, anti-diabetes and anti-inflammatory activities, in addition to a role in the prevention and treatment of cardiovascular and neurodegenerative diseases (Yun *et al.*, 2008; Balaji *et al.*, 2015; Ju *et al.*, 2015; Semaming *et al.*, 2016; Talagavadi *et al.*, 2016). Given these potent

bioactivities and the fact that rapeseed is a rich source of phenolic compounds, optimising methods to isolate these compounds from rapeseed meal is pertinent. SA and PCA in dietary sources are present in insufficient quantities to exert a sufficient physiological effect (Nunez-Sanchez *et al.*, 2015). Therefore, optimal extraction of these phenolics from rapeseed meal would allow them to be used as functional food ingredients and exert their physiological effects through dietary intake. The antioxidant properties of these phenolics would make them useful in the cosmetic industry, and indeed, a patent was filed regarding the use of SA as a cosmetic ingredient, such as in a topical agent to protect against the signs of skin ageing (Markert *et al.*, 2011).

Bottlenecks concerning the use of SA and PCA recovered from rapeseed meal

The stability and bioavailability of rapeseed phenolics is an issue in terms of the biorefining process and total utilisation of the rapeseed crop (Nićiforović and Abramović, 2014; Dias *et al.*, 2015). Phenolic compounds are susceptible to spontaneous oxidation and degradation (Tan *et al.*, 2011). Oxidation may occur through enzymatic or non-enzymatic reactions, resulting in oxidation products that are “nutritionally unavailable” (Shahidi and Naczk, 1992). Phenolics can also interact with minerals, such as iron, and inhibit their absorption, and the metabolism of phenolics by phase II enzymes also leads to poor bioavailability (Vuorela, 2005; De Souza *et al.*, 2015). Bioavailability is a key issue, given that little is known about the metabolism of SA and PCA. The epithelium of the small intestine plays a role in the bioavailability and metabolism of SA, involving both phase I and phase II metabolic enzymes; however, compared to other hydroxycinnamic acids such as ferulic acid, little is known about the metabolism of SA (Nićiforović and Abramović, 2014). A recent bioavailability study with rats fed the herb *Polygonum capitatum* found that PCA was primarily distributed in the kidney tissue, followed by the lung tissue; however, >80% was excreted (Ma *et al.*, 2016). PCA was not detected in the brain, indicating its inability to cross the blood–brain barrier; this is in contrast with a study by Guan *et al.* (2009), who detected PCA in rat brain micro-dialysates, indicating that it could cross the blood–brain barrier.

As such, validation of this rapeseed by-product requires stabilisation of the phenolic compounds before application in either food or cosmetics. Indeed, a patent regarding use of SA for cosmetics, including as a topical composition to reduce the signs of ageing, noted the instability of SA and proposed micro-encapsulation as a solution (Markert *et al.*, 2011). Nanoparticle- and microparticle-based delivery systems provide a potential solution to the problem of reduced stability and bioavailability

of phenolics, including SA and PCA. Such systems were found to increase the phytochemical absorption of phenolic compounds in epithelial cells (Dias *et al.*, 2015). The increased bioavailability of resveratrol following micro-encapsulation is proof of the efficacy of this technique (Davidov-Pardo and McClements, 2014). Utilising SA and PCA as functional food ingredients allows validation of a rapeseed by-product and its potent bioactive phenolics. Phenolic compounds have previously been encapsulated using methods such as spray-drying, extrusion and atomisation/coagulation. Spray-drying is a very straightforward and common micro-encapsulation method used in the food industry. Carbohydrate polymers such as starch and cellulose are commonly used as the shell material and are good candidates as they are generally recognised as safe (GRAS) (Dias *et al.*, 2015). A recent *in vitro* study demonstrated the great potential to successfully encapsulate PCA for functional food purposes. Madureira *et al.* (2016) investigated chitosan nanoparticles “loaded” with PCA for potential incorporation into food matrices or as functional food ingredients. The study found that the best nanoparticle for PCA was a low-molecular-weight nanoparticle, and the encapsulation process did not diminish the antioxidant activity of PCA. It was found that the nanoparticles could bypass the acidic environment of the stomach intact and reach the intestine, where the neutral pH results in “coagulation/flocculation” of the chitosan nanoparticle and release of the phenolics. From the intestine, the phenolics can be absorbed into the bloodstream (Madureira *et al.*, 2016). Ultimately, validating this rapeseed by-product and its bioactive compounds for use in food, cosmetic and pharmaceutical industries depends on successful clinical trials. Currently, there are no clinical studies examining the bioavailability or bioactivity of phenolic acids from rapeseed, including SA and PCA. However, a search of Clinicaltrials.gov revealed that there have been trials on phenolic acids from other sources such as black tea, oat and purple wheat products in terms of their pharmacokinetics and bioavailability.

Conclusion

Phenolic compounds such as SA and PCA hold great promise due to their purported bioactive properties, including anti-diabetes, anti-cancer and anti-inflammatory activities, in addition to possessing a protective role in AD and cardiovascular disease. Various traditional methods are used to extract these phenolics from rapeseed meal, based on both solvents and enzymes. However, there are issues associated with these methods, including the requirement for large volumes of solvents and their associated toxicities. The need for simpler, more efficient methodologies has led to the development of novel techniques, such as ultrasound-

assisted, microwave-assisted, HVED and PEF extractions. Development of these novel techniques allows the validation of a rapeseed by-product and total utilisation of the rapeseed crop without generating waste. It must be noted that further optimisation and validation of these techniques are necessary with regard to isolating phenolic compounds from rapeseed meal.

In order to utilise the phenolic compounds isolated from rapeseed meal, there are issues that need to be addressed. The two major issues are stability and bioavailability, which could hinder the use of the phenolics in food and cosmetic industries. A solution is the micro-encapsulation of the phenolics, and initial studies have shown the efficacy of this technique, which would make the phenolics suitable for food applications. Ultimately, maximising the use of rapeseed meal phenolics depends on proven clinical safety and efficacy. To date, there are no clinical trials regarding rapeseed phenolics, including SA and PCA; however, there are clinical trials regarding the use of phenolic compounds from other sources.

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