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Polyphenols from Australian-grown pigmented red and purple rice inhibit adipocyte differentiation

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ABSTRACT

Coloured rice is rich in polyphenols and has been shown to have significant antioxidant and anti-adipogenic potential. The study aimed to investigate the anti-adipogenic properties of polyphenol extract (PE) derived from Australian-grown rice varieties. Eight wholegrain pigmented rice varieties were screened for their polyphenol content and antioxidant activity, of which, Yunlu29 (red), Purple (purple) and Reiziq (brown) had the highest values. The selected varieties were then subjected to *in-vitro* investigation to determine the effect of rice-derived PE on adipocyte differentiation. Lipid accumulation and peroxisome proliferator-activated receptor gamma (PPARγ) expression were quantified by oil red O staining and RT-PCR respectively. PE from Yunlu29 (red) and Purple rice varieties significantly reduced ($p < 0.001$) lipid accumulation by 53% and 56% respectively and PPARγ expression in adipocytes by 61.2% and 35.6% respectively. PE from Reiziq (brown), did not inhibit lipid accumulation in adipocytes however, did reduce PPARγ expression ($p < 0.001$).

1. Introduction

It has been universally established that obesity is the central component facilitating the development of metabolic syndrome (Huang, 2009). Obesity is defined as an excessive accumulation of adipose tissue (AT) resultant of a high-energy intake that exceeds total body expenditure (Visioli, 2016). AT is a fundamental homeostatic regulatory endocrine organ with lymphatic and blood circulatory systems. Increased adiposity as a consequence of obesity can lead to the disruption of the homeostatic balance in AT leading to AT dysfunction (Spiegelman et al., 1997). As a result, increased levels of oxidative stress and inflammation arise due to abnormal free fatty acid release and dysregulated adipokine production (Bastard and Feve, 2013).

Comprehension of lipid accumulation pathways in adipocytes may be key in developing anti-obesity therapeutics. Adipocyte differentiation is an intricate sequential process involving morphological transformation of fibroblastic mesenchymal stem cells (MSC's) into spherical adipocytes (Westerink and Visseren, 2011). At the molecular level, peroxisome proliferator-activated receptor gamma (PPARγ) and CAAT/enhancer-binding protein-α (C/EBPα) are the master regulatory transcription factors involved in adipogenesis (P Cornelius et al., 1994). In MSC's, adipogenesis is initiated via the expression of C/EBPϑ to promote proadipocyte formation. As the proadipocyte develops, the expression of C/EBPϑ

Abbreviations: ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid; ACO, acyl-CoA oxidase; CYP4A10, cytochrome P450; AMPK, AMP-activated protein kinase; AT, adipose tissue; C/EBPϑ, CAAT/enhancer-binding protein-ϑ; C/EBPα, CAAT/enhancer-binding protein-α; C3G, cyanidin-3-glucoside; CE, catechin equivalents; CPT1A, carnitine palmitoyltransferase; DMSO, dimethyl sulfoxide; FRAP, ferric reducing ability of plasma; GAE, gallic acid equivalents; IBMX, isomethylbutylxanthine; MSC's, mesenchymal stem cells; P3G, peonidin-3-glucoside; PE, polyphenol extract; PPAR-α, peroxisome proliferator activated receptor; PPAR-γ, peroxisome proliferator activated receptor - γ; SREBP-1, sterol regulatory element-binding protein-1; TAC, total anthocyanin content; TE, trolox equivalents; TPAC, total proanthocyanidin content; TPC, total phenolic content.

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decreases while PPARγ simultaneously increases (Wu et al., 1999). PPARγ activates the promoter of the gene encoding C/EBPα and vice versa creating a positive feedback loop to promote the development of mature adipocytes. In addition, PPARγ and C/EBPα induce the expression of genes that are involved in insulin sensitivity, lipogenesis and lipolysis (Lowe et al., 2011). Therefore, it is important that the anti-adipogenic effects of potential therapeutics are explored.

Whilst effective, many anti-obesity medications have been associated with undesirable side effects, resulting in a demand for therapeutic alternatives. In particular, there is increasing consumer demand for functional foods that not only meet the consumer's culinary requirements but are also enriched with bioactive compounds that can assist consumers to overcome their individual medical ailments. Coloured wholegrain rice is of particular interest to consumers due to its potential health benefits which have been primarily attributed to the presence of polyphenols. In particular, rice-derived polyphenols have been demonstrated to have antioxidant (Sompong et al., 2011) and anti-inflammatory (Candiracci et al., 2014) potential. Furthermore, human dietary intervention trials have shown wholegrain rice consumption to reduce serum low density lipoprotein, total cholesterol and total triglyceride levels (Cheng et al., 2010).

The health properties of rice-derived polyphenols have been broadly studied on international rice varieties (Jang et al., 2012; Shimoda et al., 2015). However, mechanistic studies on the health properties of Australian-grown rice varieties are yet to be explored. Investigating the anti-adipogenic properties of Australian-grown whole-grain pigmented rice varieties may elucidate their potential as a functional food ingredient and promote the consumption of Australian-grown pigmented rice.

In this present study, eight whole-grain Australian-grown rice varieties of various pigmented pericarps were explored. This study aimed to develop a preliminary polyphenol profile of the eight rice varieties using analytical chemistry techniques. Preliminary analytical screening resulted in the selection of the best performing varieties from the red, purple and brown pericarps. The anti-adipogenic properties of the selected rice varieties were further explored using polyphenol extracts (PE) which were assessed for their ability to inhibit lipid accumulation and expression of PPARγ in mesenchymal stem cells differentiated into adipocytes.

2. Materials and methods

All chemicals and reagents were purchased from Sigma-Aldrich (St Louis, Missouri, USA) unless otherwise stated. Acetone, acetic acid, acetonitrile, anhydrous sodium acetate, ethanol, hexane, hydrochloric acid, iron (III) chloride, methanol, potassium chloride, potassium persulfate sodium carbonate and sulphuric acid were sourced from Chem Supply Pty Ltd (Port Adelaide, South Australia, Australia).

2.1. Rice samples

Rice samples were obtained from field trials conducted by the New South Wales Department of Primary Industries and Rice Research Australia Pty Ltd. The varieties consisted of non-pigmented: brown (Reiziq, Sherpa, Doongara and Koshihikari); pigmented: red (Yunlu29, IR45427 and Black Gora) and purple rice (Purple). All rice samples were subjected to the same post-harvest handling and storage (4 °C). Analysis of all the biological replicates was conducted in triplicate.

2.2. Polyphenol extraction

Wholegrain rice samples were finely ground in a Perten Laboratory Mill 3000 (Hägersten, Sweden) with a 0.5 mm sieve. The extraction procedure was a slight modification from our previous work by Zhou et al. (2014). Defatted rice flour samples of 1 g were extracted with acetone, water and acetic acid solution (70:29.5:0.5 v/v/v) at a ratio of 20:1 (v/w). The acetone was removed via vacuum evaporation (Rotavapor R-210 BUCHI Labortechnik, Flawil, Switzerland). The samples were freeze-dried to powder using a Christ-Alpha 2–4 LD Plus freeze dryer (Martin Christ, Osterode am Hartz, Germany). The lyophilised PE was re-constituted in 50% methanol to 1 mg mL^{-1} for chemical analysis or 50% dimethyl sulfoxide (DMSO) for cell-based analysis. The PE was stored at −20 °C until required.

2.3. Total phenolic content (TPC)

The total free phenolic content was determined using methods described by Qiu et al. (2010) with minor modifications. Briefly, 125 μL of the PE was combined with 125 μL of Folin-Ciocalteu reagent and 500 μL of deionized water and incubated in the dark for 6 min. The mixture was neutralised by adding 1.5 mL of 7% sodium carbonate solution and 1 mL of deionized water. The absorbance was measured at 725 nm on a microplate reader (BMG Labtech FLUOstar Omega, Offenburg, Germany) against a methanol blank after a 90-min incubation in the dark. Total phenolic content of the rice samples was expressed as mg 100 g^{-1} gallic acid equivalents (GAE).

2.4. Total anthocyanin content (TAC)

Monomeric TAC was determined using the pH differential method by Wrolstad (1993) and Shao et al. (2014). Briefly, two solutions were prepared at pH 1 (1.49% potassium chloride acidified with hydrochloric acid) and pH 4.5 (1.64% sodium acetate acidified with hydrochloric acid). The PE were diluted accordingly to be within a measurable absorbance range. The absorbance was read at dual wavelengths of 520 nm and 700 nm on a microplate reader (BMG Labtech FLUOstar Omega, Offenburg, Germany). The TAC was expressed as mg 100 g^{-1} cyanidin-3-glucoside (C3G) equivalents.

2.5. Total proanthocyanidins content (TPAC)

TPAC was quantified by conducting a vanillin assay with methods adapted from Sun et al. (1998) and Min et al. (2012). Briefly, 0.2 mL PE was added to 0.5 mL of 1% (w/v) vanillin in methanol and 0.5 mL of 25% sulphuric acid in methanol. The mixture was vortexed and placed in a 37 °C water bath for 15 min. The absorbance was measured at a wavelength of 500 nm at room temperature using a microplate reader (FLUOstar Omega microplate reader, BMG Labtech, Offenburg, Germany). Total proanthocyanidin content was determined as mg 100 g^{-1} (+)-catechin equivalents (CE).

2.6. Ferric reducing ability of plasma assay (FRAP)

The ferric reducing ability of the rice extracts was determined using methods described by Sompong et al. (2011). To perform the assay, 1.8 mL of FRAP reagent, 180 μL deionized water and 60 μL of PE or standard was added to an assay tube and incubated at 37 °C for 40 min. The absorbance was measured at 593 nm using a microplate reader (FLUOstar Omega microplate reader, BMG Labtech, Offenburg, Germany). The FRAP reagent working solution was