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Physicochemical properties and *in vitro* digestibility of potato starch after inclusion with vanillic acid

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ABSTRACT

While research regarding phytochemicals has been topical due to their many positive health effects, modification of starch with phytochemical fractions has been limited. In the current study, 50 or 100 mg/g of vanillic acid (VA), which possesses antioxidant activity and positive health effects, was added to potato starch on a dry weight basis at various pH levels (3, 5, 7, 9, & 11). Both total phenolic content and antioxidant activity generally increased over the control. X-ray diffraction detected no V-amylose peaks indicating a lack of amylose-VA inclusion complexes. In addition, a decrease in the ratio of 1045/1015 cm^{-1} was measured by FTIR denoting a loss of crystalline order. Enthalpy of gelatinization was unaffected in most samples, however, $T_c - T_o$ was significantly ($p < 0.05$) reduced in all modified starches. Trough viscosity, final viscosity, and pasting time all significantly increased with increasing pH ($p < 0.05$), and peak viscosity reached a maximum of 11360 mPa·s. The resistant starch (RS) content of modified starches ranged from 51.71 to 77.16 g/100 g; in cooked samples, RS was not significantly different from the control, and ranged from 11.66 to 15.10 g/100 g.

1. Introduction

Starch, a major agricultural product that is globally consumed and/or used industrially, is comprised of two gluco-homopolysaccharides, amylose and amylopectin. In terms of digestibility, starch is typically divided into three fractions: rapidly digesting starch (RDS), slowly digesting starch (SDS), and resistant starch (RS), with RDS and SDS being the fractions which are hydrolyzed to dextrins by α-amylase within 20 and 120 min after ingestion, respectively (Englyst, Kingman, & Cummings, 1992). RS is the portion which is not hydrolyzed after 120 min and continues its passage from the small to the large intestine (Englyst et al., 1992) where it can then be fermented by the intestinal microflora to produce beneficial short-chain fatty acids, namely acetic, propionic, and butyric (Nugent, 2005). RS can be further divided into five classes: physically unavailable, resistant native granules, retrograded, chemically modified, and amylose-inclusion complexes (Dupuis, Liu, & Yada, 2014; Fuentes-Zaragoza et al., 2011).

Recently, phytochemicals have been at the forefront of research due to their many positive health benefits (Shahidi, 2004). Currently there has been limited research on the modification of starch with phytochemicals, with most focusing on the thermal, retrogradation, and pasting properties of starches after the direct addition of the phytochemical, with little attention given to the interaction between the phytochemical and starch (Zhu, Cai, Sun, & Corke, 2009). Research in which ferulic acid was incubated with debranched cassava starch for 24 h found that the RS level was unaffected (Hung, Phat, & Phi, 2013). Vanillic acid (VA, Fig. 1) is a commonly found phenolic acid in cereal grains and their related food products (Tomás-Barberán & Clifford, 2000) with daily intake as high as 4.09 mg/day (Radtke, Linseisen, & Wolfram, 1998). The inclusion of VA in foodstuffs may lead to a lowering of inflammation; in bioprocessed bread, lower cytokine production, specifically of IL-6 and IL-1β, in simulated blood was shown to be associated with higher bioavailable phenolics, including VA (Anson et al., 2011). The bioprocessing was accomplished by subjecting bran to a combination of yeast fermentation and cell wall-degrading enzymes. Prior to ingestion, VA may be dehydroxylated and/or decarboxylated by colonic microflora to simpler benzoic acids, or benzoic acid itself (Anson et al., 2011; Laparra & Sanz, 2010; Selma,

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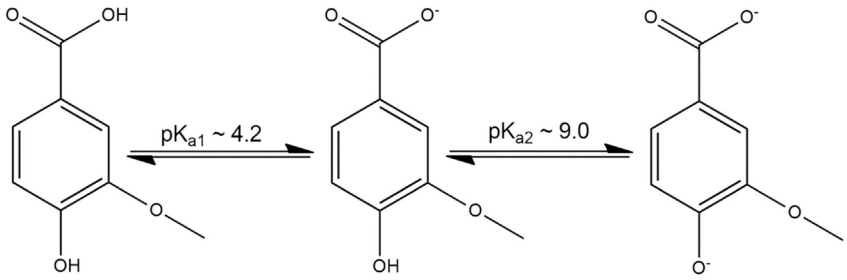

Fig. 1. Skeleton formulae for vanillic acid (left) and its deprotonated forms (center and right).

Espín, & Tomás-Barberán, 2009). Following colonic absorption, the degraded microflora metabolites circulate *in vivo* via the vascular system, and are ultimately excreted renally (Laparra & Sanz, 2010), either as is, or after hepatic condensation with glycine to hippuric acid derivatives (Anson et al., 2011).

The effects of VA may be experienced prior to absorption; increased levels of phenolic acids have been shown to modulate the colonic microflora landscape by exhibiting bacteriostatic and/or antimicrobial effects, depending on the population in question (Laparra & Sanz, 2010). VA in particular has been shown to inhibit the growth of specific *Escherichia coli* strains, as well as methicillin resistant *Staphylococcus aureus* (Cueva et al., 2010). Additionally, phenolic acids may modulate beneficial bacteria as well, particularly by decreasing the ratio of *Firmicutes* to *Bacteroides*, which is lower in non-obese individuals, and has been associated with weight loss in obese subjects (Parkar, Trower, & Stevenson, 2013). Caffeic acid, a highly related structural homologue to VA, has been shown to increase short-chain fatty acid synthesis by colonic microflora, particularly of butyric acid, which is an energy source for colonocytes and aids in protection from colonic diseases such as colon cancer (Parkar et al., 2013). *In vitro*, VA has been demonstrated to have potent cardio (Prince, Dhanasekar, & Rajakumar, 2015) and nephroprotective (Sindhu, Nishanthi, & Sharmila, 2015) properties, and inhibits DNA oxidation greater than other common phenolic acids, such as gallic acid (Sevgi, Tepe, & Sarikurkcu, 2015). Additionally, VA has also been shown to reduce the action of α-amylase, the primary human carbohydrase enzyme, by 71.9 percent at a level of 20 μg/g starch (Chethan, Sreerama, & Malleshi, 2008). Due to the benefits of phenolic acids together with those of RS, it would be prudent to include a VA fraction in RS-rich products for consumption, such as a bread or fortified grain product (Igoumenidis & Karathanos, 2016), to form a functional food. The RS will ensure colonic delivery, and the VA may be readily taken up by the colonic microflora. VA has shown to be reasonably thermally stable at typical food processing temperatures and times (Volf, Ignat, Neamtu, & Popa, 2014), and food matrices may provide further thermal stability (Shafi, Baba, Masoodi, & Bazaz, 2016).

The inner cavity of an amylose helix is hydrophobic in nature, and will preferentially attract a hydrophobic moiety. Using molecular dynamics simulations, β-cyclodextrins have been shown to form energetically favourable complexes with caffeic acid, a phenolic acid structurally similar to VA (Górnas, Neunert, Baczyński, & Polewski, 2009). Vanillic acid has two protonatable groups (Fig. 1); a carboxylic acid (pK_a ~4.2) and a hydroxyl group (pK_a ~9.0) (Ozkorucuklu et al., 2009). Similarly, starch has several pK_a's; the starch-phosphate monoesters present on starch have pK_a's of approximately 0.9 and 5.9 (Lim & Seib, 1993), and potato starch has a pK_a of approximately 13.0 at 40 °C (Lammers, Stamhuis, & Beenackers, 1993). Due to the presence of ionizable groups, and to avoid excessive acidic hydrolysis or base-induced gelatinization, the modification process in the present study was done from pH 3 to 11, in increments of 2 pH units, to examine the effect of altered ionization states on the modification treatment of the potato starch. Therefore, the purpose of this work was to create novel VA-amylose complexes, and subsequently determine the physicochemical properties of these modified starches. In addition, *in vitro* digestibility was determined on both native and cooked starches to evaluate the effect of modification on digestibility. VA-starch complexes may result in a functional food with the ability to both lower carbohydrate digestibility while having antioxidant ability.

2. Materials and methods

2.1. Materials

Potato starch (S4251) and vanillic acid (H36001) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were of analytical grade.

2.2. Methods

2.2.1. Starch modification

Starch (on a dry weight basis, dwb) was mixed with water to create a 1:2 starch:water ratio slurry, followed by the addition of 50 or 100 mg VA/g dry starch to the slurry, and pH was adjusted to 3, 5, 7, 9, or 11 by drop-wise addition of 2 moL/L HCl or NaOH. Following pH adjustment the slurry was placed in a water bath at 40 °C for 24 h with constant stirring at 250 rpm using a magnetic stirring hotplate. After 24 h, either 1 moL/L HCl or NaOH was added to neutralize the slurry to pH 7, followed by vacuum filtration. Starches were washed twice with 150 mL distilled water and once with 150 mL ethanol solution (950 mL ethanol/L), after which the samples were allowed to air dry at room temperature for 24 h, ground with a mortar and pestle to pass through a 125 μm sieve, and stored at room temperature in air-tight containers until further use.

2.2.2. Phenolic extraction

Approximately 0.25 g of modified starch (dwb) was placed in a 50 mL centrifuge tube along with 15 mL methanol solution (700 mL methanol/L) and allowed to shake for 1 h on a roller mixer, followed by centrifugation (800×*g*, 10 min) and removal of the supernatant. This procedure was repeated twice to remove free vanillic acid from the samples. NaOH (2 moL/L, 15 mL) was then added to each sample pellet to liberate any bound phenolic acids. The samples were allowed to shake for 16 h, and then adjusted to pH 1.5 using 12 moL/L HCl followed by the addition of 15 mL of 1:1 diethyl ether/ethyl acetate (DE/EA) solution to each tube of hydrolysate. The tubes were allowed to shake for 15 min to partition the freed phenolics into the nonpolar DE/EA phase. Centrifugation (800×*g*, 5 min) separated the two phases with the non-polar phase being pipetted