Food Research International 103 (2018) 182–191

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Slow digestible colored rice flour as wall material for microencapsulation: Its impacts on gut bacterial population and metabolic activities

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ARTICLE INFO

Chemical compounds:
1,1-Diphenyl-2-picrylhydrazyl (PubChem CID: 74358)
2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (PubChem CID: 16240279)
Trolox (PubChem CID: 40634)

Keywords:
Black waxy rice
Pullulanase
Heat moisture treatment
Synbiotic
Fluorescence *in situ* hybridization

ABSTRACT

Black waxy and red jasmine rice flour (6.5% and 18.7% amylose content, respectively) were modified using pullulanase followed by heat-moisture treatment (Hydrolyzed-HMT) to produce microcapsules that entrapped *L. plantarum* TISTR 1465. Hydrolyzed-HMT of colored rice flours showed restricted pasting properties, lower breakdown and higher thermal properties than native flour ($p < 0.05$). Hydrolysis treatment was able to promote a low molecular weight starch that easily formed a crystalline structure after HMT. As a consequence, a significant increase in slowly digestible starch was observed (from 23.7% to 37.0% in waxy type and 22.2% to 34.6% non-waxy type). The survival of *L. plantarum* TISTR 1465 after simulated gastric fluid (90 min) and simulated intestinal fluid (120 min) of the Hydrolyzed 36 h-HMT black waxy rice was higher (8.07 ± 0.13 log CFU/g and 7.48 ± 0.12 log CFU/g) than gum arabic (6.12 ± 0.11 and 4.72 ± 0.28 log CFU/g) and no carrier (3.34 ± 0.23 and 0.43 ± 0.75 log CFU/g) respectively. Moreover, these microcapsules also obtained the highest survival (8.67 ± 0.20 log CFU/g) after storage for 90 days at 4 °C. Under scanning electron microscopy, starch granules of the hydrolyzed 36 h-HMT were seen as polyhedral shapes in the spherical aggregates that carried the microorganisms and reduced their injury and mortality. Short-chain fatty acids of the hydrolyzed 36 h-HMT were much higher than positive control at every fermentation time ($p < 0.01$). The fluorescence *in situ* hybridization result showed that the prebiotic property of hydrolyzed 36 h-HMT black waxy rice can better aid the beneficial probiotic *Lactobacillus* spp. growth after 24 h fermentation than the negative control (from 8.40 ± 0.48 to 7.03 ± 0.21 log CFU/g, $p < 0.05$) and commercial prebiotic Orafti®Synergy1 (8.40 ± 0.48 to 7.47 ± 0.08 log CFU/g, $p < 0.01$). Microencapsulation of hydrolyzed black waxy rice flour followed by HMT is proposed as a synbiotic ingredient to apply in synbiotic foods.

1. Introduction

Slowly digestible starch (SDS) and resistant starch (RS) are defined as the slow- to non- digestible portion of starch that cannot be digested after ingestion for 20–120 min and longer than 120 min, respectively. Indigestible carbohydrates, which can pass through the upper part of the gastrointestinal tract into the large intestine, are subsequently fermented by gut microbes (Casterline, Oles, & Ku, 1997; Wang et al., 2002; Zhang & Hamaker, 2009). These prebiotic carbohydrates should be able to provide an absorption site for probiotic adherence and, at the same time, a carbon source for probiotic bacteria. This results in favorable metabolites such as short-chain fatty acids (SCFAs) in the human colon (Brouns, Kettlitz, & Arrigoni, 2002; Crittenden et al., 2001; Johnson & Gee, 1996). SDS and RS are known to aid human health benefits, such as stable glucose metabolism, reduced risk of diabetes, obesity, cardiovascular disease and colonic cancer (He, Liu, & Zhang, 2008; Lehmann & Robin, 2007). RS content of native colored rice from indigenous Thai rice varieties is about 5.3–5.7% (Pongjanta, Chomsri, & Meechoui, 2016). Factors affecting SDS and RS formation are crystallinity, chain length distribution, amylose and amylopectin ratios, and retrogradation (Miao, Jiang, & Zhang, 2009; Shi & Gao, 2011). SDS and RS can be produced by physical, chemical and enzymatic modifications. Physical modification is considered safe for human consumption.

The dual modification proposed in this study was a combination of two safe processes: heat-moisture treatment (HMT) and enzymatic modification. Significant increase in SDS (40.8%) was found after HMT of native brown rice (Chung, Cho, Park, Kweon, & Lim, 2012). Enzymes

Abbreviations: Hydrolyzed-HMT, hydrolyzed heat-moisture-treated rice flour; Hydrolyzed 36 h-HMT, hydrolyzed for 36 h and heat-moisture-treated rice flour; FISH, fluorescence *in situ* hybridization; SCFAs, short-chain fatty acids; RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch

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http://dx.doi.org/10.1016/j.foodres.2017.10.027
Received 25 July 2017; Received in revised form 11 October 2017; Accepted 12 October 2017
Available online 14 October 2017

such as α amylase, β amylase, amylo-sucrase, pullulanase and iso-amylase were often used to cleave the outer chain of amylopectin, hence increasing starch chain mobility which led to a highly crystalline structure. Shorter amylose chains were usually required, accounting for its ability to be readily re-associated into a more orderly crystalline structure (Shi & Gao, 2011). For SDS and RS production, amylo-sucrase was reported to be able to hydrolyze starch chains in waxy/normal rice and potato starch into DP 25–36 that aided crystalline perfection (Shin, Choi, Park, & Moon, 2010). Pullulanase treatment followed by repeated retrogradation was reported to increase SDS and RS content (25.4% and 50.1% respectively) in waxy maize starch (Miao et al., 2009). The most SDS was reported to be produced by debranching waxy starch with pullulanase for 4 h and subsequent storage at 1 °C. Waxy starch is reported to be more suitable to produce SDS (Guraya, James, & Champagne, 2001). Starch that was hydrolyzed and allowed to crystallize showed higher RS than those only debranching (Cai & Shi, 2010). The co-process of hydrolysis followed by HMT was reported to increase the ratio of linear glucan α-D-(1, 4), which supported crystalline structure and resulted in a more enzymatic-resistant starch (Lin, Wang, & Chang, 2009; Mutungi et al., 2010).

Various polysaccharides that have been used as encapsulating materials include gum arabic, inulin, oligosaccharides, maltodextrin and resistant starch (Desmond, Ross, O'Callaghan, Fitzgerald, & Stanton, 2002; Fahimdanesh et al., 2012; Fritzen-Freire, Prudêncio, Pinto, Muñoz, & Amboni, 2013; Perdana, Fox, Siwei, Boom, & Schutyser, 2014; Soukoulis, Behboudi-Jobbehdar, Yonekura, Parmenter, & Fisk, 2014). These polysaccharides have different degrees of prebiotic effects, depending on its structure and composition that favor probiotic growth. The carrier matrix of gum arabic and sodium caseinate in low-melting-point fat microparticles was reported to enhance probiotic survival after spray drying, storage and *in vitro* digestion (from 1.20 log CFU/g to 2.55 log CFU/g) (Liu et al., 2016). Prebiotic edible films made from native rice and corn starch, mixed with gelatine, sodium caseinate and soy protein concentrate were used to encapsulate *L. rhamnosus* GG in a bread coating. The viability of *L. rhamnosus* GG is increased by 3 to 7-fold under simulated gastro-intestinal conditions (Soukoulis, Yonekura, et al., 2014; Soukoulis, Singh, Macnaughtan, Parmenter, & Fisk, 2016). Glucose-oligosaccharides and polydextrose were also reported to enhance *L. rhamnosus* GG viability in prebiotic edible films during air drying. Inulin was the most effective material to maintain sub-lethal amounts of *L. rhamnosus* GG during storage (Soukoulis, Yonekura, et al., 2014).

The nutritious colored rice of interest in this study accounted for high antioxidant activity. The anthocyanin content in black/purple, red and wild rice were 3276.0, 93.5 and 27.2 μg/g, respectively (Abdel-Aal, Young, & Rabalski, 2006). The synergistic effect of malvidin-3-glucoside mixed with other anthocyanins was reported to improve the growth of the good bacteria (Hidalgo et al., 2012). The colored rice flour was modified by the dual process of using HMT and enzymatic modification to obtain appropriate degrees of slow to indigestible prebiotic starch. Highly indigestible starch aids probiotic survival but lowers its utilization as a carbon source. On the contrary, rapidly digestible starch lowers probiotic protection in the gut system but serves as a good carbon source. Therefore, this study aimed to evaluate this trade-off effect using *in vitro* human faecal batch fermentation that closely mimics the real human gut system.

2. Materials and methods

2.1. Preparation of hydrolyzed-HMT rice flour

Paddy rice of the black waxy "Leum Pua" variety (Phrae Rice Research Center, Phrae, Thailand) and the "Red Jasmine" variety (Khonkaen Rice Research Center, Khonkaen, Thailand) were dehulled to obtain brown rice grains. The brown rice was steeped in water for 3 h and then wet-milled using a double-disk stone mill to produce 10% (w/v) rice flour slurry. The flour slurry was adjusted to pH 4.5 with a 0.1 M sodium acetate buffer. The enzyme pullulanase (OPTIMAX® L-1000, 1000 ASPU/g, Siam Victory Chemicals, Thailand) (0.2 g) was added into the flour suspension (110 g) to obtain a concentration of 20 ASPU/g of flour (dry basis). The solution was incubated at 55 °C for 8, 24 and 36 h in a shaking water bath. The solution was centrifuged (3000*g*) for 10 min, the precipitate was washed twice with distilled water and collected by centrifugation (Miao et al., 2009). The precipitate was oven dried at 40 °C until the target HMT moisture content (25%) was obtained. The rice flour sample was then put in a sealed screw-cap container and equilibrated at room temperature for 24 h. The equilibrated containers were then placed in a hot air oven (100 °C) for 1 h. After that, the treated flour was taken out and dried in a hot air oven (40 °C) until 12% moisture content was obtained. The obtained sample or "hydrolyzed-HMT" rice flour was milled (ultra-centrifugal mill type ZM1, Retsch GmbH, Germany) and sieved to a particle size of 100 mesh, put in sealed plastic bag and kept at 4 °C.

2.2. Physico-chemical properties of hydrolyzed-HMT rice flour

2.2.1. Pasting properties

The pasting property of the hydrolyzed-HMT rice flour was determined by the Rapid Visco Analyzer (model RVA3D; Newport Scientific, NSW, Australia). The hydrolyzed-HMT rice flour (3.00 ± 0.01 g) was mixed with distilled water (25 mL) in a metal RVA canister (AACC Method 61-02, 2000). The sample suspension was heated in the RVA using the heating profile for rice flour. The sample was heated from 50 °C to 95 °C at rate 12 °C/min and held at 95 °C for 2.5 min, cooled down to 50 °C at a similar rate and held at 50 °C for 2 min. The total running time for each sample was 13 min.

2.2.2. Thermal properties

The thermal properties of all samples were determined by a differential scanning calorimeter (DSC Star® System; Mettler Toledo AG, Switzerland). Approximately 12 mg of flour was put directly into an aluminum pan using flour to water ratio of 1:3 (w/w). The pan was sealed and equilibrated 1 h at room temperature before the analysis. The DSC scanning temperature range was set at 25–95 °C using a heating rate of 10 °C/min. The DSC was calibrated using indium as a standard and an empty aluminum pan as reference (Cham & Suwannaporn, 2010). The parameters were analyzed using STARe evaluation software v12.10 (Mettler Toledo AG, Switzerland).

2.3. In vitro digestibility of hydrolyzed-HMT rice flour

The *in vitro* digestibility of hydrolyzed-HMT rice flour was reported as rapid digestible starch (RDS), slow digestible starch (SDS) and resistant starch (RS) content followed the method of Englyst, Kingman, and Cummings (1992). An enzyme solution was freshly prepared by adding porcine pancreatic α-amylase (Sigma A-3176, Sigma-Aldrich, UK; 16 U/mg) (1.5 g) in a sodium acetate buffer (pH 5.2) (10 mL). The mixture was incubated at 37 °C for 10 min and centrifuged (1500*g*) for 10 min. The supernatant was transferred into a beaker and mixed with amylo-glucosidase (Sigma A-7095, Sigma-Aldrich, UK; 300 U/mL) at 8:1 (v/v) (Mutungi et al., 2011). The modified rice flour (100 mg) was suspended in a 0.1 M sodium acetate buffer (21 mL) and incubated at 37 °C with continuous shaking (200 strokes/min) for 15 min. The freshly prepared enzyme solution (1.6 mL) was added to the suspension, mixed for 1 min and incubated at 37 °C in a shaking water bath (200 strokes/min). After incubation for 20 and 120 min, an aliquot (0.2 mL) was taken and added into absolute ethanol (4 mL), mixed well and centrifuged (5000*g*) for 10 min. The supernatant was then collected for RDS and SDS determination. The glucose content was measured by adding a glucose oxidase-peroxidase assay kit (GOPOD, Megazyme International, Ireland) into the aliquot and incubated at 50 °C for 20 and 120 min. The aliquot was then measured in a spectrophotometer at