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In vitro gastric digestion antioxidant and cellular radical scavenging activities of wheat-shiitake noodles

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ABSTRACT

A high glycaemic index diet causes a rapid increase in blood sugar level and may lead to chronic metabolic disorders such as obesity and type 2 diabetes. Shiitake is rich in bioactive compounds. Wheat flour noodles were enriched with shiitake (*Lentinus edodes*) powder (cap, stem, whole) at different levels to investigate the effects of shiitake addition on the nutritional composition, physical and textural properties. *In vitro* digestion was conducted to determine the glycaemic glucose equivalents and bioaccessibility of antioxidants in digesta. The addition of 15% shiitake stem powder in the noodles resulted in a significant ($p < 0.05$) decrease in reducing sugars released after *in vitro* digestion. Digesta also exhibited cellular antioxidant ability on IEC-6 cells after H_2O_2-induced oxidative stress. These results show the potential beneficial use of shiitake, especially the stem, as a high-value ingredient to improve the nutritional profile and reduce the glycaemic index of foods.

1. Introduction

Obesity is rising around the world as a result of many factors, including an imbalanced food intake, a high-calorie diet and lack of physical exercise. Obesity not only leads to an increased risk of type 2 diabetes, cardiovascular disease and cancer, but also negatively affects longevity and quality of life (Martel et al., 2017). It also increases the risk of incidence of Alzheimer's disease, whereas a diet rich in phytochemicals can manipulate gene expression related to the control of metabolic and other diseases (Hossain, Brennan, Guo, Zeng, & Brennan, 2020).

Mushrooms have multiple health-promoting properties, including anti-inflammation, anti-tumour and anti-bacterial actions, as well as being involved in immuno-modulating therapies (Palacios et al., 2011; Adebayo et al., 2018; Li, Guo, Zhuang, Qin, & Sun, 2018). Shiitake mushroom contains numerous biologically active compounds, such as dietary fibre (DF). Compared to other conventional sources of DF, such as cereals, legumes, fruits and vegetables, the DF of mushrooms or fungi are underutilized (Cheung, 2013; Radzki et al., 2019). The total dietary fibre (TDF) of shiitake has been reported to be 41.97 ± 1.08 g/100 g, which is much higher than white button mushroom and porcini mushrooms (Lu, Brennan, Serventi et al., 2018). The consumption of edible mushrooms as part of the daily diet can provide 25% of the recommended dietary intake of DF (Cheung, 2013). Particularly, beta-glucan is a typical kind of DF; and the beta-glucan from shiitake lentinan has the immunostimulatory activity (Muszyńska, Grzywacz-Kisielewska, Kała, & Gdula-Argasińska, 2018). In addition, higher glucan contents have been shown to be present in the stem of mushrooms compared to the mushroom caps, which provides support for the potential use of mushroom by-products (Sari, Prange, Lelley, & Hambitzer, 2017).

Shiitake is also an excellent source of vitamin D since it has a high ergosterol (vitamin D precursor) content of 84.9 mg/100 g dry weight (DW) (Phillips et al., 2011). Although shiitake is not a choice of lipids due to low fat concentration, it has higher polyunsaturated fatty acids content (82.0 ± 0.4% of total fatty acids) than other plant sources (Sande et al., 2019). Some other bioactive compounds identified in shiitake include lectin, eritadenine, indoles, all of which have great potential for therapeutic applications of metabolic diseases (Wang, Brennan, & Brennan, 2020).

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Shiitake mushrooms, therefore, serve as a model for investigating functional fungi properties and isolating pure compounds for pharmaceutical use of severe symptoms (Finimundy, Dillon, Henriques, & Ely, 2014). Whole functional foods have the advantages of wide availability, ease of preparation and low adverse effects (Martel et al., 2017). Studies of mushroom food products have been carried out, and the consumption of foods rich in mushroom bioactive compounds appears to be beneficial to health (Jin et al., 2018; Lu, Brennan, Serventi et al., 2018; Gaglio et al., 2019). However, the complex binding of starch, protein, fat and all other bioactive compounds present in the food matrix and which they interact with physically and chemically influences the release and digestibility of many food compounds (Aguilera, 2018; Wang, Pan et al., 2018, Wang, Wu et al., 2018). Thus, to claim that a functionalised food will bring beneficial effects needs more in-depth studies.

A model food system was devised in order to illustrate the connection between shiitake bioactive ingredients and human health. This work also investigated the effect that the plant and food matrix plays on its bioavailability, since plant material matrix may alter absorption and bioavailability of phytochemicals. In this study, noodles had the cap, stem or both cap and stem of the shiitake mushroom incorporated into them on a replacement basis. The physical characteristics, cooking properties, potential glycaemic response, digestibility and antioxidant bioavailability were evaluated.

2. Materials and methods

2.1. Materials

Wheat flour (Champion Flour Milling, Auckland, New Zealand) and iodised table salt (Cerebos, Auckland, New Zealand) were obtained locally. Fresh shiitake mushrooms was harvested from a local edible fungus cultivation centre (Tianjin, China). Folin-Ciocalteau reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), pepsin, pancreatin, amyloglucosidase and trypsin were purchased from Sigma-Aldrich (St. Louis, MO, USA). IEC-6 cells were obtained from the Tianjin University of Commerce, Tianjin, China.

2.2. Mushroom and noodle preparation

The three parts (cap, stem and whole) of shiitake mushrooms were cleaned and cut into slices. After drying at 40 °C, the mushroom slices were milled into a powder, sealed in polythene bags and stored at −20 °C. The noodle formula consisted of flour, 38% (v/w, flour weight basis); water, 2% (w/w); sodium chloride; and shiitake powder at concentrations of 5%, 10% and 15%, to replace the flour composition in noodles. Control samples were prepared using wheat flour. Noodles were produced using a pasta machine fitted with a 2.25 mm spaghetti die (MPF15N235M, Fimar, Villa Verucchion, Italy) as previously described (Lu, Brennan, & Serventi et al., 2018, Lu, Brennan & Narciso et al., 2018). Dough blends were mixed for 20 min in the pasta machine and then extruded.

2.3. Nutritional composition analysis

Total starch was determined by using the Megazyme starch analysis kit (Megazyme International Ireland Ltd, Wicklow, Ireland). Briefly, sample (0.1 g of dried noodles powder) and 5.0 mL of 80% EtOH) was incubated at 80–85 °C for 5 min. The contents were mixed on a vortex stirrer and 5.0 mL of 80% EtOH was added. The tube was centrifuged (ROTINA 380, Hettich LAB TECHNOLOGY, Tuttlingen, Germany) for 10 min at 1800 × g and then the supernatant was discarded. Thermostable α-amylase and amyloglucosidase were added and incubated at 50 °C for 30 min. Glucose oxidase–peroxidase (GOPOD) reagent was added to each tube and incubated. The absorbance was read at 510 nm against the reagent blank.

The protein content was determined using a Rapid Max N exceed (Elementar, Langenselbold, Germany) with the conversion factors of 5.7 for shiitake mushroom noodles. The total fat content was determined by Soxhlet extraction method (De Castro & Garcıa-Ayuso, 1998).

Total dietary fibre (TDF) content was determined in duplicate by using the Total Dietary Fibre assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland) with determinations for soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) fractions.

2.4. Physical characteristics

The optimal cooking time (OCT), cooking loss (CL), swelling index (SI), and water absorption index (WAI) were determined according to methods approved by AACC 66–50 (AACC, 2002). Noodle strands were cut into lengths of 40 mm and cooked in 300 mL of boiling water. A noodle strand was taken every 30 s and squeezed between two transparent glass side. OCT was recorded when the core of the noodle disappeared. Noodles (10 g) were cooked in 600 mL of boiling water for their OCT, then rinsed with 100 mL of cold water and strained for 30 s. The calculation of CL, SI and WAI were performed as previously reported (Lu, Brennan, Serventi, Mason, & Brennan, 2016).

2.5. Texture analysis

Firmness and elasticity of the cooked noodles were determined according to the method previously described (Desai, Brennan, & Brennan, 2018). Texture Analyser (TA.XT2; Stable Micro Systems, Godalming, UK) was equipped with a 5 kg load cell. Prior to the testing, noodles samples were cooked for the OCT and kept at room temperature for 10 min.

2.6. Colour

The colour was measured using a colourimeter CR-210 (Minolta Camera Co., Osaka, Japan) using the L*(lightness), a*(redness) and b*(yellowness) parameters, and colour differential index (△E) was calculated as the following equation (Foschia, Peressini, Sensidoni, Brennan, & Brennan, 2015b),

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2}.$$

2.7. In vitro digestion

An *in vitro* digestion was conducted according to the previous study (Gao, Brennan, Mason, & Brennan, 2016). Noodles (2.5 g) were mixed with 30 mL of distilled water in plastic biopsy pots and placed on a preheated magnetic stirring block (IKAAG RT 15, IKA-Werke GmbH & Co., Staufen, Germany). After constant stirring at 37 °C for 10 min, stomach digestion was mimicked by addition of 0.8 mL 1 M HCL and 1 mL of 10% pepsin solution in 0.05 M HCl for 30 min and then stopped by the addition of 2 mL of 1 M $NaHCO_3$. Small intestine digestion was initiated by the adding 5 mL 0.1 M sodium maleate buffer (pH 6) and 5 mL 2.5% pancreatin solution in 0.1 M sodium maleate buffer (pH 6) and with constant stirring for 120 min. Aliquots of 1 mL were taken before adding pancreatin, then at 20, 60 and 120 min and added to 4 mL ethanol. The samples were stored at 4 °C until analysis of reducing sugar content using the 3,5-dinitrosalicylic acid (DNS) method. The area under the curve (AUC) was calculated by dividing the graph into trapezoids. All the supernatants of the digesta were freeze-dried and stored at −20 °C.

2.8. Antioxidant abilities

All noodles were cooked for optimum cooking time before extraction occurred.

2.8.1. Total phenolic content (TPC)

Total phenolic content was determined as reported previously (Rana, Gupta, Rana, & Bhushan, 2015). Samples were extracted with 70% methanol and stirred overnight on a magnetic stirrer. A 0.25 mL aliquot of the sample (extract or supernatant of digesta), 2.5 mL of 0.2 N Folin-Ciocalteu reagent, 2 mL of 7.5% sodium carbonate were mixed and incubated for 2 h at room temperature. The absorbance was measured at 725 nm on a spectrophotometer (V-1200, Global Science, VWR, Radnor, PA, USA). Results were expressed as gallic acid equivalents mg/g of dry weight (DW).

2.8.2. ABTS (2,2′-amino-di(2-ethyl-benzothiazoline sulphonic acid-6) ammonium salt) assay

The ABTS radical scavenging assay was based on the method of Pérez-Jiménez (Pérez-Jiménez & Saura-Calixto, 2008). $ABTS^{\cdot+}$ was prepared by reacting colourless $ABTS^{\cdot+}$ stock solution (7 mM in deionised water) with 2.45 mM potassium persulfate and allowing the reaction to stand for 16 h in the dark at room temperature. Then the $ABTS^{\cdot+}$ solution was diluted with PBS (pH 7.4) to an absorbance of 0.70 (± 0.02) at 734 nm and 3 mL transferred to a cuvette. Trolox or a sample extract (300 μL), was added and mixed well. The cuvette was allowed to stand for 6 min, and the absorbance was read at 734 nm. The results were expressed as Trolox equivalents (TE) (μM)/g DW.

2.8.3. Ferric-reducing antioxidant power (FRAP) assay

The FRAP reagent was prepared by combining tripyridyltriazine solution (10 mM), $FeCl_3$ (20 mM), and acetate buffer (300 mM, pH 3.5) as the ratio of 1:1:10 (v/v/v) (Bolanos de la Torre, Henderson, Nigam, & Owusu-Apenten, 2015). A 30 μL aliquot of shiitake extract or digesta was mixed with 90 μL FRAP solution using a 96 well plate and incubated at 37 °C for 30 min. Absorbance was read at 593 nm. The results were expressed as TE (μM)/g DW.

2.9. Cell work

2.9.1. Cell culture

The IEC-6 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) containing 10% of the foetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 100 units/mL penicillin and 100 units/mL streptomycin (Solarbio, Beijing, China) at 37 °C, with 5% CO_2 in a humidified incubator (Thermo Fisher Scientific, Waltham, MA, USA) (Wang, Pan et al., 2018, Wang, Wu et al., 2018).

2.9.2. Cell viability assay

Cell viability was analysed by using 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO, USA). Five different doses of each digesta were chosen to examine the cells viability: physiological (1x), five (5x), ten (10x), fifty (50x), hundred (100x) times higher. The 1 × dose was calculated according to a 75 g/day adult recommended serving size of dry pasta or noodles, which will then be distributed on 200 m2 intestine surface (Hidalgo et al., 2018). Cells were grown in 96-well plates at a density of 5×10^3 cells/well for 24 h. The cells were then washed with fresh medium, followed by treatment with *in vitro* digesta (digestion buffer was added at the same volume to act as a control) and incubated for 24 h. Then the cells were washed, and 100 μL of MTT (1 mg/mL) was added, before incubating for 4 h. DMSO (150 μL) was then added to solubilise the formazan salt. The amount of formazan salt was determined by measuring the optical density at 540 nm using a microplate reader (Synergy HT; BioTek Instruments, Winooski, VT, USA).

2.9.3. Intracellular reactive oxygen species (ROS) assay

The production of intracellular ROS was measured by dichlorofluorescin (DCFH) oxidation (Wang et al., 2016). IEC-6 cells were seeded into 96-well cell culture plate at a density of $8*10^3$ cells/well and cultured overnight. Followed by pre-treatment of cells with shiitake digesta with selected concentrations for 24 h, 10 μL H_2O_2 (300 μg/mL) was added to the cells and incubated for 6 h to generate ROS. After treatment, the cells were treated with 10 μM/L 2′,7′-dichlorofluorescin diacetate (DCFH-DA) at 37 °C for 20 min and then washed with PBS. Fluorescence was measured at 485 nm excitation and 530 nm emission.

2.10. Statistical analysis

All experiments were carried out in triplicate except where stated otherwise. All data are expressed as mean ± standard deviations (SD). One-way analysis of variance, with the multiple range significant difference (Turkey's) test ($p < 0.05$) was carried out using Minitab Express 1.5.0 (Minitab Pty, Sydney, Australia). Figures were created in Graphpad Prism 8.0 (GraphPad, San Diego, CA, USA). Pearson's correlations were conducted by R 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

3.1. Nutritional composition

The shiitake noodles contained less starch (Table 1) than the control noodles, and this decreased incrementally with the increasing addition of the shiitake powder. The shiitake stem powder noodles had less total starch compared to the cap powder and whole mushroom powder noodles at each level of addition, however there were no significant differences except at 15% addition ($p < 0.05$).

The protein content of noodles increased with increased shiitake powder. Protein content in the noodles containing shiitake cap powder was higher than noodles containing stem and whole shiitake powder (Table 1). There were no significant differences in fat content, however, the noodles containing stem powder had slightly higher values than those containing cap powder (Table 1).

No statistical difference was observed in the SDF values of the cap, stem and whole powder containing noodles, except for the whole 15%

Table 1
Nutritional compositions of shiitake noodles (g/100 g dry matter basis).

Sample	Total starch	Protein	Fat	IDF	SDF	TDF
control	71.14 ± 0.98^{a}	13.35 ± 0.06^{g}	0.33 ± 0.02^{a}	2.55 ± 0.01^{c}	3.89 ± 0.35^{b}	6.44 ± 0.34^{c}
cap 5%	63.25 ± 1.40^{b}	13.66 ± 0.09^{f}	n.a	n.a	n.a	n.a
cap 10%	63.70 ± 2.56^{b}	14.79 ± 0.08^{c}	n.a	n.a	n.a	n.a
cap 15%	59.46 ± 2.15^{bc}	15.64 ± 0.12^{a}	0.31 ± 0.01^{a}	7.55 ± 0.03^{b}	4.42 ± 0.11^{ab}	11.97 ± 0.09^{b}
stem 5%	65.05 ± 2.64^{ab}	13.63 ± 0.02^{f}	n.a	n.a	n.a	n.a
stem 10%	61.70 ± 0.30^{bc}	14.24 ± 0.03^{e}	n.a	n.a	n.a	n.a
stem 15%	50.30 ± 2.07^{d}	14.44 ± 0.02^{d}	0.34 ± 0.03^{a}	8.98 ± 0.04^{a}	4.43 ± 0.12^{ab}	13.42 ± 0.10^{a}
whole 5%	65.56 ± 1.15^{ab}	13.70 ± 0.08^{f}	n.a	n.a	n.a	n.a
whole 10%	60.97 ± 0.91^{bc}	14.70 ± 0.03^{c}	n.a	n.a	n.a	n.a
whole 15%	55.65 ± 4.39^{cd}	15.23 ± 0.06^{b}	0.31 ± 0.03^{a}	9.09 ± 0.19^{a}	4.77 ± 0.07^{a}	13.81 ± 0.12^{a}

Values represent means ± SD of analysis; within columns, values with different letters are significantly different ($p < 0.05$). n.a: not available.

Table 2
Cooking qualities and texture properties of shiitake mushroom noodles.

Samples	Optimal Cooking Time (min)	Cooking Loss (g/100 g)	Swelling Index (g H_2O/100 g)	Water Absorption Index (g/100 g)	Moisture content (%)	Firmness (force (g))	Elasticity (tensile strength force (g))
control	4	7.40 ± 0.28^{abc}	2.97 ± 0.17^{abc}	74.22 ± 3.45^{a}	41.26 ± 2.29^{c}	177.99 ± 1.05^{e}	20.97 ± 0.67^{e}
cap 5%	4	8.09 ± 0.02^{ab}	2.95 ± 0.06^{ab}	71.39 ± 2.52^{ab}	41.82 ± 0.31^{bc}	229.47 ± 3.18^{a}	18.89 ± 0.68^{f}
cap 10%	4	8.02 ± 1.20^{ab}	3.01 ± 0.19^{ab}	65.99 ± 3.73^{abc}	44.84 ± 2.45^{ab}	198.26 ± 3.14^{c}	23.59 ± 1.08^{bc}
cap 15%	3.5	9.02 ± 0.72^{a}	2.80 ± 0.03^{bcde}	59.04 ± 2.28^{cd}	43.25 ± 0.38^{abc}	176.37 ± 0.78^{e}	26.09 ± 1.11^{a}
stem 5%	4	6.02 ± 0.43^{c}	2.63 ± 0.03^{e}	55.01 ± 1.59^{de}	41.12 ± 0.12^{c}	205.26 ± 4.24^{c}	21.11 ± 0.77^{de}
stem 10%	3.5	6.92 ± 0.45^{bc}	2.65 ± 0.02^{de}	54.43 ± 1.70^{de}	41.66 ± 0.74^{c}	185.52 ± 1.71^{d}	22.71 ± 0.73^{cd}
stem 15%	3	7.94 ± 0.58^{ab}	2.69 ± 0.03^{cde}	50.67 ± 1.70^{e}	43.96 ± 0.08^{abc}	182.45 ± 5.14^{de}	23.25 ± 1.13^{c}
whole 5%	4	7.67 ± 0.47^{abc}	2.89 ± 0.07^{abcd}	64.91 ± 3.66^{bc}	42.92 ± 0.07^{abc}	214.75 ± 7.99^{b}	18.76 ± 0.60^{f}
whole 10%	4	7.26 ± 0.67^{abc}	3.07 ± 0.10^{a}	68.43 ± 2.07^{ab}	45.06 ± 1.04^{a}	165.31 ± 2.73^{f}	21.06 ± 1.05^{e}
whole 15%	3	6.03 ± 0.27^{c}	2.93 ± 0.04^{abcde}	59.08 ± 1.90^{cd}	43.77 ± 0.52^{abc}	180.27 ± 4.07^{de}	24.90 ± 0.45^{ab}

Values represent means ± SD of analysis; within columns, values with different letters are significantly different ($p < 0.05$).

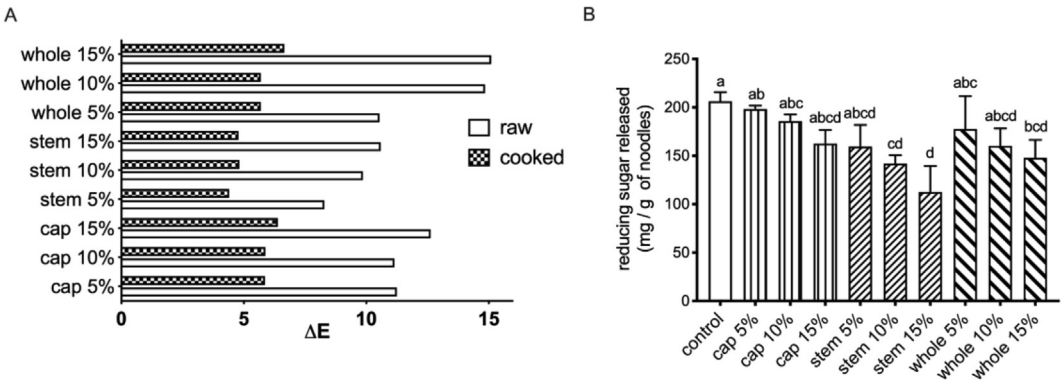

Fig. 1. Colour change (△E) and AUC (area under curve) of shiitake fortified noodles. A. △E of raw (□) and cooked (▩) shiitake noodles. B. The AUC of shiitake fortified noodles at level of 5%, 10%, and 15% after *in vitro* digestion. Error bars represent standard deviation (SD) of replicates ($n \geq 3$). Values with different letters are significantly different ($p < 0.05$).

shiitake noodles which contained more SDF than the control (Table 1). The IDF of shiitake stem noodles was significantly higher than the shiitake cap group and the control.

Mushrooms are a good source of dietary fibre, with 100 g of fresh mushrooms providing between 10 and 40 % of the recommended dietary intake of fibre (Cheung, 2013), and higher glucan contents are present in the mushroom stipe compared to the mushroom cap, indicating a potential use of mushroom by-products (Sari et al., 2017). Although a previous study used stalks and basal hyphae clumps of chestnut mushrooms as fibre-rich ingredient in the production of ready-to-eat extruded cereal snack (Brennan, Derbyshire, Tiwari, & Brennan, 2012), there is little or no research into the SDF and IDF contents of the stem and cap of shiitake mushroom. Mushrooms generally have high IDF contents (chitin and other polysaccharides) which present nutritional advantages, which coupled with low lipid and glycogen contents results in low overall energy values (Sari et al., 2017).

3.2. Cooking properties of shiitake noodles

The cooking quality of the noodles is indicated by OCT, CL, WAI and SI (Table 2). The OCT gradually decreased with increasing shiitake powder addition. OCT of noodles with 15% whole shiitake powder was 1 min shorter than the control. The decreased OCT could be due to the reduction of gluten content by the substitution of wheat flour for fibre (Aravind, Sissons, Egan, & Fellows, 2012).

The CL of the shiitake noodles ranged from 6/100 g to 9/100 g and no significant differences were found. However, it was noted that the CL of noodles with shiitake stem powder addition was lower than the CL of noodles with cap and whole powder addition. A possible explanation for this might be that starch and protein contents in the stem were lower than that in cap and whole mushroom (Table 1). It has been reported that the increase of CL in pasta or noodles might the result of the weakening of the gluten network (Foschia, Peressini, Sensidoni, Brennan, & Brennan, 2015a).

There was a continual decrease of SI in noodles with increasing shiitake powder. The SI of noodles containing the stem powder was lower than the SI of noodles containing cap and whole powder. This same trend was also observed in WAI and moisture. In foods containing bran there is typically less absorption of water by the starch (Brennan, Merts, Monro, Woolnough, & Brennan, 2008). Avarind et al. found that SI and WAI decreased when spaghetti was fortified with pollard (Aravind et al., 2012). A possible explanation for these results may be that the IDF content of noodles containing stem powder is higher than the IDF of noodles containing cap or whole shiitake powder, and the IDF competes more highly for water with the starch. Dietary fibre has a faster hydration dynamic than starch; however, the water is held more loosely by fibre than by starch (Brennan et al., 2008).

3.3. Colour change

Uncooked noodles with shiitake powder addition showed an increase in redness (a*), while lightness (L*) and yellowness (b*) were lower than the control (Table S1). Similarly, the cooked noodles also had a decrease, albeit smaller, in lightness and yellowness (Table S1), this might be due to the occurrence of Maillard's reaction during cooking which made the colour of the control group darker. The cooked and uncooked noodles containing stem powder exhibited a smaller colour change (△E) than noodles containing cap or whole shiitake powder (Fig. 1A).

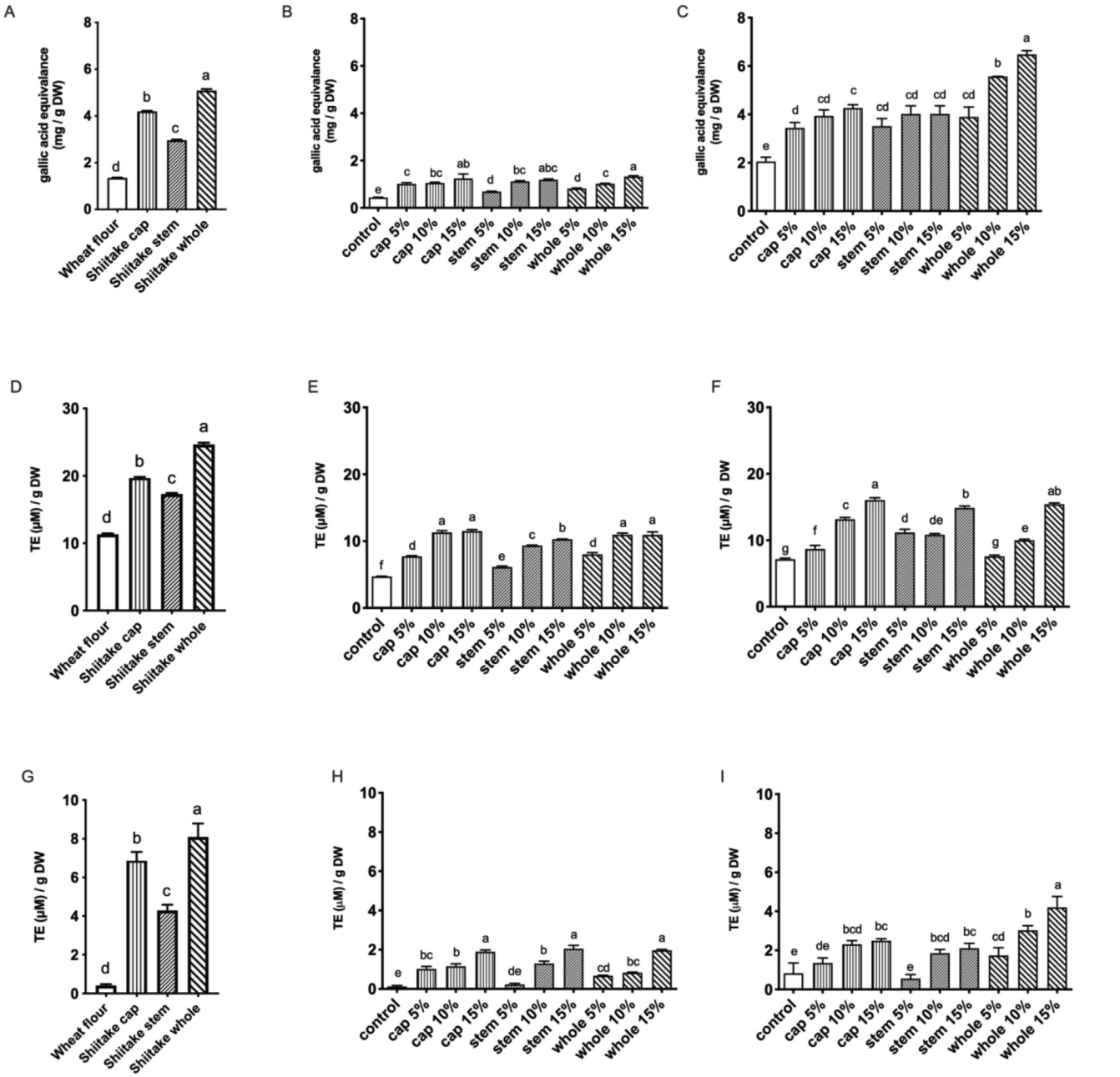

Fig. 2. Antioxidant abilities of shiitake powder and noodles containing shiitake. A-C: TPC of shiitake cap, stem and whole powder (A), noodles containing shiitake (B), and noodles digesta (C). D-F: ABTS values of shiitake cap, stem and whole powder (D), noodles containing shiitake (E), and noodles digesta (F). G-I: FRAP values of shiitake powder (G), noodles containing shiitake (H), and noodles digesta (I). Error bars represent standard deviation (SD) of replicates ($n \geq 3$). Values with different letters are significantly different ($p < 0.05$). TE: Trolox equivalent; DW: dry weight;

3.4. Texture properties

A significant increase in firmness was observed in noodles with shiitake powder (Table 2). Within each group of shiitake addition, firmness was highest at 5% addition and then decreased, but all values remained higher than the control.

The increase of firmness might be attributed to the interaction of nonpolar lipids and starch. The shiitake mushroom lipids may reduce starch granule disruption when they bind to the granules (Lu et al., 2016), thus forming the firmer a starch gel in noodles leading to a firmer product.

The elasticity of the noodles was reduced with the addition of 5% powder in all three groups, then increased as the shiitake powder increased. The tensile strength value ranged from 18.76 to 26.09 g. As the shiitake noodles have a higher protein content, this protein may form an insoluble network within the food matrix which then entraps the swollen and gelatinised starch granules, preventing the noodles from disruption (Desai et al., 2018).

3.5. In vitro digestion

A significant reduction in the amount of reducing sugars released during the digestion process occurred in the noodles containing shiitake stem at 10% and 15% and whole shiitake powder at 15% (Fig. 1B). Shiitake stem noodles released a smaller amount of reducing sugars during digestion than the shiitake cap or whole shiitake noodles at the same inclusion levels; however, the differences were not significant. The 5% stem noodles had a reduction of 22%; this reduction is greater

Table 3
Antioxidant activities standardised with respect to the Trolox measurement (μM/g dry matter basis).

		ABTS	FRAP	Weighted average
Noodles	control	4.72	0.12	0.27
	cap 5%	7.73	1.02	0.70
	cap 10%	11.31	1.16	0.91
	cap 15%	11.52	1.90	1.16
	stem 5%	6.14	0.23	0.37
	stem 10%	9.32	1.30	0.86
	stem 15%	10.29	2.04	1.14
	whole 5%	8.04	0.68	0.60
	whole 10%	10.98	0.83	0.79
	whole 15%	10.96	1.97	1.15
Intestinal Digesta	control	7.16	0.82	0.61
	cap 5%	8.70	1.36	0.85
	cap 10%	13.17	2.31	1.37
	cap 15%	16.06	2.50	1.57
	stem 5%	11.18	0.56	0.72
	stem 10%	10.84	1.86	1.11
	stem 15%	14.88	2.11	1.39
	whole 5%	7.59	1.74	0.92
	whole 10%	10.02	3.02	1.44
	whole 15%	15.43	4.20	2.07

than would be expected considering that the available carbohydrate was replaced at 5%. The 10% and 15% stem noodles had a 30% and 45% reduction in reducing sugars released respectively, compared to the control group. Noodles containing 15% whole shiitake powder released significantly less reducing sugars (147.88 mg/g) than the control (206.35 mg/g). These results support the idea that the presence of the shiitake powder restricts the digestion of carbohydrate in wheat flour noodles.

3.6. Antioxidant ability of shiitake noodles

3.6.1. TPC

The wheat flour noodles had the lowest TPC followed by the shiitake stem, then cap and whole shiitake had the highest TPC (Fig. 2A, B, and C). All noodles and digesta containing shiitake had higher TPC values than the control. Digesta containing 10 and 15% whole shiitake powder had a significantly higher amount of TPC than all other samples. Noodles containing 5% stem or whole shiitake powder had a TPC lower than any of the cap noodles. The TPC values after *in vitro* digestion increased by up to four-fold compared to the noodles.

3.6.2. ABTS

The whole shiitake powder showed higher activity than the cap, which had higher activity than the stem which in turn had higher activity than the wheat flour (Fig. 2D). All enriched noodles had a higher $ABTS^{+}$ activity than the control with 10 and 15% cap and stem being the highest (Fig. 2E). After the *in vitro* digestion process, the antioxidant activity of the 15% noodles (stem, cap or whole) was 3-fold higher than the control noodles before digestion (Fig. 2F). These findings provide the evidence that some antioxidants that are bound to cell walls or conjugated with macronutrients in the food matrix are released during the digestion process.

3.6.3. FRAP

The highest antioxidant activity was found in the whole shiitake powder and the same decreasing trend (whole > cap > stem) was exhibited as in the ABTS determination with the stem having a value of 4.30 ± 0.29 μM TE/g (Fig. 2G). The whole shiitake powder had an antioxidant activity which was 19-fold higher than the wheat flour. All shiitake noodles, except stem 5%, had a higher antioxidant activity than control rising to 2 μM TE/g at 15% addition (whole, stem and cap) (Fig. 2H). The antioxidant activity of the digesta was highest in 15% whole, which was double the value of 15% whole shiitake noodles (Fig. 2I). The antioxidant activity values of all digesta, except 5% stem, were higher than the control digesta.

3.6.4. Standardised antioxidant activity based on weighted average

It has been suggested that the use of more than one assay to determine the antioxidant abilities of food extracts is necessary, since different assays may show diverging results. To standardise the results of shiitake samples we used the following equation described by Müller et al. (Müller, Fröhlich, & Böhm, 2011).

$$Weighted\ average = \frac{\frac{ABTS}{\bar{x}(ABTS)all} + \frac{FRAP}{\bar{x}(FRAP)all}}{2}$$

The antioxidant abilities of 15% (cap, stem or whole) noodles show a three-fold increase compared to the control noodles (Table 3). The *in vitro* digesta show higher antioxidant abilities than the noodles. The 15% whole digesta showed the highest antioxidant activity, followed by whole 10% and cap 15%. The bioavailability of antioxidants is determined by multiple factors and the digestion process has influenced some of these factors, leading to an increase in the antioxidant activities of the digesta (Lafarga, Villaró, Bobo, Simó, & Aguiló-Aguayo, 2019). In a food matrix, antioxidants are always bound to other macronutrients, such as fibre, carbohydrate, lipid and protein (Munialo, Naumovski, Sergi, Stewart, & Mellor, 2019). During digestion, the physical and

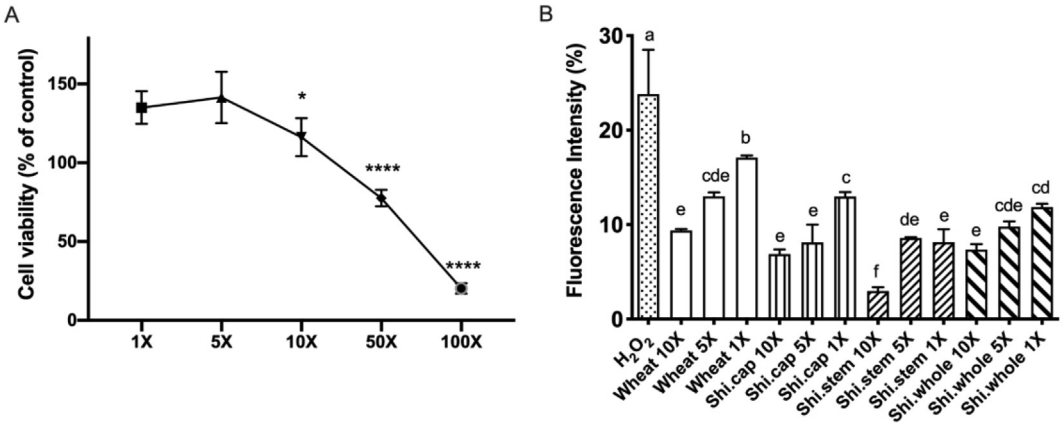

Fig. 3. Shiitake noodles digesta protected intestinal epithelial cells from H_2O_2 induced ROS damage. A. IEC-6 cells viability after shiitake noodles digesta treatment with 1x, 5x, 10x, 50x and 100x doses. Error bars represent standard deviation (SD) of replicates ($n \geq 3$; *, $p < 0.05$; ****, $p < 0.0001$). B. Intracellular ROS of IEC-6 cells after incubation for 24 h with 10x, 5x and 1x doses of wheat flour noodles or shiitake noodles digesta. Graph bars represent mean ± SD of replicates ($n \geq 3$). Values with different letters are significantly different ($p < 0.05$).

chemical bonds are hydrolysed by enzymes and thus releasing nutrients into the gut. However, once in the gut, the antioxidants may interact with dietary fibre and form further chemical complexes or colloidal structures. Dietary fibre can physically entrap the antioxidants in upper intestine. Another role of dietary fibre is to restrict access of the transport enzymes to the substrates by increasing the viscosity of gastric fluids. Since there is more dietary fibre in the shiitake stem, this could possibly explain the decrease in antioxidant activities the digesta containing stem despite the slightly higher antioxidant activity of the shiitake stem noodles.

3.7. Pearson's correlation coefficients

Pearson's correlation coefficient analysis was applied to elucidate the correlation among shiitake noodles nutritional composition, physical characteristics and their antioxidant capacity. In this correlation analysis, cooking properties were influenced negatively by dietary fibre in shiitake. As shown in Fig. S1, the correlation between OCT and IDF was $r = -0.959$ ($p = 0.041$), which demonstrated that the decrease in OCT may be because starch has been substituted for IDF.

Another cooking index, CL, is always considered as an indicator of cooking quality by both consumers and industry. CL was positively correlated with protein ($r = 0.639$, $p = 0.047$), and this is consistent with the previous discussion, that CL was affected by the disruption of the starch-protein network. Petrovska et al. indicated that mushroom proteins have a high proportion of albumins and globulins (Petrovska, 2001). These non-wheat proteins form different covalent bonds with gluten. These bonds, such as more S—H bond or hydrophobic patches, gradually break down and release exudates thus destroy the starch-gluten network during the cooking process (Marlies, Ine, Mieke, & Jan, 2017).

No indices were closely related to firmness. Elasticity was closely positively correlated to the protein content in noodles ($r = 0.856$, $p = 0.002$). This supports the idea that protein helps matrix formation which reduces the rate of starch swelling.

The r value of the AUC and starch content was 0.866 ($p = 0.001$); this correlation would be expected because the AUC is a measure of starch digestion, however, a higher correlation may be expected if there was no interaction by the shiitake mushroom components. IDF is negatively correlated with AUC ($r = -0.955$, $p = 0.045$). Together with previous studies using both *in vitro* and *in vivo* digestion procedures (Brennan, Derbyshire, Brennan, & Tiwari, 2012; Tudorica, Kuri, & Brennan, 2002), these results illustrate that fibre-rich products can significantly reduce starch digestibility and glucose release (Klunklin & Savage, 2018), especially the IDF.

TPC, ABTS and FRAP assays reflect the antioxidant capacities of noodles. There is a strong positive correlation between IDF and TPC ($r = 0.971$, $p = 0.029$), IDF and ABTS ($r = 0.932$, $p = 0.068$), IDF and FRAP ($r = 0.984$, $p = 0.016$). This indicates that the phenolic compounds are bound to the fibre. It has been noted that IDF and SDF may interfere with the macronutrients and biomolecules, thus affect their bioavailability and absorption (Quirós-Sauceda et al., 2014). Also, Guo et al. found that the SDF fraction contained less phenolic acids and lower antioxidant activity than IDF (Guo & Beta, 2013). As shiitake is rich in fibre, more studies are needed to understand the role of dietary fibre in nutrient uptake, especially phenolic compounds and other antioxidants.

3.8. Intracellular ROS

Cells treated with 100x or 50x digesta showed reduced cell viability ($p < 0.01$) (Fig. 3A). This result is in agreement with the previous findings that excess digesta may lead to cell death and detachment (Hidalgo et al., 2018). It also provides the reason and necessity of considering the amount of food that would treat the specific area of cells surface. Thus, 1x, 5x, and 10x doses digesta were chosen to perform the intracellular ROS assay (Fig. 3B).

All digesta showed an antioxidant effect at 1x dose, including wheat flour digesta. A similar effect was observed when the cells were treated with digesta at 5x and 10x doses, except for shiitake stem 10x, which had the lowest fluorescence intensity. Shiitake stem 1x digesta + H_2O_2 significantly decreased ROS formation compared to H_2O_2 alone, which shows a strong *in vitro* antioxidant effect on the IEC-6 small intestine cells.

This provides further evidence that the shiitake noodles can perform ROS scavenging activity on intestinal epithelial cells during digestion, conferring their potential to be considered as an antioxidative food. The shiitake stem digesta displayed the most evident antioxidant scavenging properties at 10x dose. The difference in the distribution of dietary fibre throughout the shiitake mushroom and the fact that IDF and SDF interact with antioxidants in the food matrix differently could be a reason for the results in Fig. 3B.

4. Conclusion

The addition of shiitake to noodles enhanced the protein content, lowered the glycaemic response and increased the antioxidant capacity. Following simulated digestion, the digesta of shiitake noodles also showed antioxidant abilities on cellular level based on the IEC-6 intestinal epithelial cell line, providing evidence that insoluble conjugated phenolic acids are released during digestion. These findings contribute to the understanding of physical characteristics of shiitake fortified noodles and how the nutritional value may be improved while and reducing the potential glycaemic index. They also provide a basis for fully utilising shiitake stems as a functional food source for the potential prevention of chronic diseases.

CRediT authorship contribution statement

Liwen Wang: Writing - original draft. **Hui Zhao:** Supervision. **Margaret Brennan:** Supervision, Writing - review & editing. **Wenqiang Guan:** Supervision. **Jianfu Liu:** Supervision. **Meiyan Wang:** Formal analysis. **Xiang Wen:** Formal analysis. **Jingwen He:** Formal analysis. **Charles Brennan:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.127214.

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