Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2024, **15**, 9880

β-Glucan content and *in vitro* bile-acid binding capacity of *Agaricus bisporus* and *Pleurotus* spp.†

Damien Paul Belobrajdic, *[a,b] Henri Brook,[a] Genevieve James-Martin[a] and Welma Stonehouse [a]

The cholesterol lowering properties of oats and barley, attributed to their high β-glucan content, are well established, but it remains unclear whether mushrooms, also rich in β-glucan, exhibit a similar functionality. We aimed to quantify the β-glucan content of commonly consumed Australian mushrooms and evaluated their bile acid binding capacity, the primary cholesterol lowering mechanism of β-glucan. Raw, boiled and fried Australian grown *Agaricus bisporus* (button, cup, flat and brown mushrooms) and *Pleurotus* spp. (shimeji and oyster) along with oats were freeze-dried and the β-glucan content was determined. The bile acid binding capacity of these samples was assessed using an *in vitro* digestion assay. The β-glucan content of freeze-dried raw *A. bisporus* mushrooms (4.5–8.1 g per 100 g) was similar to that of oats (7.6 g per 100 g, all $p > 0.05$), whereas *Pleurotus* mushrooms contained ~5 times more β-glucan (32.5–37.4 g, $p < 0.05$). Boiling increased the β-glucan content of oyster, button, flat and brown mushrooms by 3–7% ($p < 0.05$), but did not affect the β-glucan content of shimeji or cup mushrooms. Frying had no effect on any mushroom type. The bile acid binding capacity of *A. bisporus* mushrooms (29–36%) was equivalent to that of raw oats (36%, $p > 0.05$), whereas the bile acid binding capacity of oyster mushrooms (22%) was lower than that of oats ($p < 0.05$). Both boiling and frying increased the bile acid binding capacity. The cholesterol lowering effects of *A. bisporus* mushrooms and the acceptability of consumption at the required levels need to be confirmed by clinical trials.

Received 22nd May 2024,
Accepted 27th August 2024

DOI: 10.1039/d4fo02416h

rsc.li/food-function

Introduction

Reducing blood cholesterol is one of the key ways to reduce cardiovascular disease risk,[1] the leading cause of mortality worldwide.[2] Diet and lifestyle interventions are the first-line therapy for the treatment of hypercholesterolaemia before pharmacological therapies, such as statin medications, are introduced.[3] The cholesterol lowering effects of soluble fibre β-glucan from oats and barley products are well established.[4,5] The primary mechanism proposed whereby β-glucan lowers circulating cholesterol concentrations is through interfering with bile acid enterohepatic circulation.[6,7] Beta-glucan is thought to be a bile acid sequestrant, resulting in decreased bile acid reabsorption in the small intestine, which in turn results in the up-regulation of bile acid synthesis in the liver using cholesterol as a substrate, leading to reduced circulating cholesterol concentrations.[6,8,9] In addition, health claims pertaining to β-glucan from oats and barley and cholesterol lowering have been authorised by several regulatory bodies in the USA, Europe and Australia.[10–12]

Although, the cholesterol lowering properties of oats and barley are well established, there is less evidence for other dietary sources of β-glucan such as fungi, yeast, and algae. The β-glucan in these foods is structurally different to oat and barley β-glucan, with fungi and yeast containing β-glucan that is made from straight β (1 → 3) glucan with short-branched chains connected through β (1 → 6), whereas cereal β-glucan is predominantly composed of mixtures of β (1 → 3) and β (1 → 4) glycosidic linkages without any β (1 → 6) bonds.[13] It is unclear whether these structural differences in β-glucan may affect the bile acid binding and cholesterol lowering properties of mushrooms. A small number of clinical trials have reported that consumption of *A. bisporus* and *P. ostreatus* (oyster mushroom) as dried or cooked products lowered fasting total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C).[14–18] Furthermore, it is not known whether the commonly consumed *A. bisporus* has effects similar to those of *P. ostreatus* on binding bile acids, especially given that β-glucan content varies between mushroom varieties, cooking methods and mushroom fractions (*e.g.* cap *vs.* stalk).[19–22]

[a]*Commonwealth Scientific Industrial Research Organisation (CSIRO) Health and Biosecurity, Adelaide, South Australia, Australia.*
E-mail: damien.belobrajdic@csiro.au
[b]*College of Medicine and Public Health, Health Flinders University, Bedford Park, SA 5042, Australia*

† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4fo02416h

In this study, we aimed to report the β-glucan content of a range of mushroom varieties, *A. bisporus* (button, cup, flat and brown mushrooms) and *Pleurotus* spp. (shimeji and oyster), that had been prepared in the most common ways they are consumed (raw, boiled and fried). An additional aim was to compare the cholesterol lowering mode of action of these mushrooms with that of oats by assessing their bile acid binding capacity using *in vitro* digestion methods. A subsequent investigation compared the β-glucan content and bile acid binding capacity of stalk and cap fractions of *A. bisporus* button and cup mushrooms.

Materials and methods

Sample processing

The fruiting bodies of four *A. bisporus* (common names: button, cup, flat and brown mushrooms) and two *Pleurotus* spp. (common names: shimeji and oyster) were assessed. Mushroom varieties (1.5 kg of each) were sourced from The Mushroom Man (Adelaide Central Market, South Australia), except for shimeji, which was sourced from White Prince Mushrooms (Vineyard, New South Wales). For stalk *vs.* cap analyses, *A. bisporus* button and cup mushrooms were sourced from SA Mushrooms (Waterloo Corner, South Australia). The mushrooms were harvested with the whole stalk intact and delivered to the CSIRO laboratory on the day they were picked.

The mushrooms were stored at 4 °C for no longer than 2–5 days from the time that they were harvested to minimise any variation that storage time may have on β-glucan content.

Fresh mushroom fruits were cleaned of soil and substrate (using a soft brush and forceps), and then cut along their vertical axes into slices 4–5 mm thick. Each mushroom variety was randomly divided into three groups (500 g each). One group remained raw mushrooms, and the rest were cooked by two different methods (boiling and frying). The culinary conditions included boiling, where mushroom slices (100 g per batch) were boiled in a pot containing 1 L of filtered water for 6 min. This method resembles the addition of mushrooms to dishes such as risotto or stews. For frying, mushroom slices (100 g per batch) were fried in a non-stick pan with 20 mL of olive oil (160 °C) for 6 min. After cooking, all samples were placed on filter paper to drain water or excess oil.

The stalk and cap of fresh button and cup mushroom fruits were separated manually by lightly twisting and applying pressure to the stalk until it snapped out from the mushroom cap. The stalks were then trimmed at the lowest possible point to remove the substrate. Any residual substrate on the cap or stalk was removed using a paper towel.

All mushroom samples were freeze-dried using a freeze-drier (Martin Christ Alpha 1–2 LD Plus, Osterode am Harz, Germany) until a constant weight was obtained and then powdered using a Kitchen Wizz (Cuisinart Mini-prep, Stamford, CT, USA). Weights were obtained of the raw and freeze-dried mushrooms to calculate the moisture content of the samples by difference.

Uncle Toby's Quick Oats (purchased from Coles supermarket), a commonly consumed, commercially available whole oat product, was used as the oat control. The oat flakes were analysed raw and cooked as porridge. Oat porridge was prepared by following the on-pack instructions. Half a cup (125 ml) of oat flakes and $\frac{3}{4}$ of a cup (187.5 ml) of filtered water were cooked in an 800 watt Panasonic microwave for $1\frac{1}{2}$ min on high, stirred and then cooked for an additional 1 min on high. The raw oat flakes and porridge were freeze-dried (until a constant weight was obtained), the moisture content was determined, and the samples were then ground in a cyclone mill (Foss Analytical Cyclotec 1093, Hillerød, Denmark).

β-Glucan content analysis

The β-glucan content of the mushrooms was determined by the method of McCleary and Draga (2016)[23] in duplicate or triplicate using reagents in the kit supplied by Megazyme (K-YBGL, Megazyme, Wicklow, Ireland). This method involved two separate analyses of the food sample. The first involved the hydrolysis of total glucans by treating the sample with sulfuric acid to hydrolyse α-glucans (starch and simple polysaccharides) and β-glucans, followed by treatment with *exo*-1,3-β-glucanase to fully hydrolyse the β-glucan. Glucose was measured in the sample to quantify the total glucan content. The second analysis involved hydrolysis of the samples using amyloglucosidase and the glucose content of the digested sample was measured at 510 nm using a spectrophotometer (Agilent Technologies Cary 100, Santa Clara, CA, USA) to determine the α-glucan content. The β-glucan was then calculated as the difference between the values obtained in the two assays. β-Glucan from oats was analysed in the raw form using AOAC Method 995.16. The β-glucan contents of mushrooms and oats are reported as fresh and dry weights (g per 100 g), as well as the serving size in grams and cups and, where possible, the number of whole raw mushrooms[24] needed to provide 1 g of β-glucan.

A yeast sample (K-YBGL, Megazyme, Wicklow, Ireland) was included as a quality control and shown to contain 56.0 ± 0.6% total glucan, 1.2 ± 0.0% α-glucan and 54.8 ± 0.6% β-glucan. For subsequent cap *vs.* stalk analyses, the yeast control contained 48.1% total glucan (% CV = 4.4), 0.54% α-glucan (% CV = 4.1) and 47.5% β-glucan. These levels were considered to be in the acceptable range for the assay as the control sample was reported to contain 49% β-glucan by the kit manufacturer (Megazyme).

In vitro bile acid binding

Bile acid binding of samples was determined by a modification of the method of Kim and White.[25] Dried samples (500 mg) underwent simulated digestion in triplicate using a method described previously.[26] In brief, during the gastric phase, a 0.02 M HCl solution (pH 2) was added and a sodium acetate buffer (pH 6) was then added in the intestinal phase, prior to the addition of 2.5 mg of a 50/50 mixture of cholic acid and deoxycholic acid (B8756, Sigma-Aldrich, Castle Hill, NSW, Australia). All digestion stages were conducted at 37 °C. At completion of the ileal phase in the small intestine, the samples were centrifuged (2000*g*). The amount of bile acids

remaining in the supernatant was determined by high-performance liquid chromatography (Agilent Technologies 1260 Series, Santa Clara, CA, USA). Cholestyramine and α-cellulose were included in the assay as positive and negative controls, respectively. Data ware reported as the % of bile acids bound per 500 mg of food (dry weight).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8. Differences between the raw mushroom varieties and oats were analysed using one-way Analysis of Variance (ANOVA) and Tukey's *post-hoc* tests. For oats and each mushroom type, the effect of cooking was determined by comparing cooking methods using one-way ANOVA. A two-way ANOVA was used to compare the effect of mushroom type and cooking on bile acid binding. A two-tailed unpaired Student's *t*-test was conducted to compare the β-glucan content and bile acid binding capacity between raw and cooked oats.

Cap *vs.* stalk statistical analyses were performed using IBM SPSS Statistics, version 29. Differences between cap and stalk fractions were analysed using an independent samples Student's *t*-test. ANOVA was used to compare the effects of mushroom variety (cup *vs.* button) on cap *vs.* stalk differences by including an interaction term in the model, mushroom variety × mushroom fraction. *Post-hoc* analyses were performed when significant mushroom variety × mushroom fraction interactions were observed using Tukey's *post-hoc* test with Bonferroni adjustments.

Values are expressed as mean ± SEM. A P value < 0.05 was considered as the level of statistical significance in all analyses.

Results and discussion

Beta-glucan content of mushrooms and oats

Across all mushroom varieties evaluated, a majority of the total glucan was from β-glucan, with α-glucan only making up a small proportion (ESI Table 1†). In fresh raw mushrooms, the β-glucan content was the highest in shimeji mushrooms (3.7 g per 100 g) and oyster mushrooms (3.2 g per 100 g), followed by button (0.7 g per 100 g), brown (0.7 g per 100 g), and cup mushrooms (0.5 g per 100 g), with flat mushrooms containing the lowest amounts (0.3 g per 100 g) (Table 1).

Raw freeze-dried *Pleurotus* spp. mushrooms had ~5 times more β-glucan than *A. bisporus* per 100 g dry weight. The β-glucan content was the highest in oyster (37.4 ± 1.3 g per 100 g dry weight) and shimeji mushrooms (32.5 ± 0.6 g per 100 g dry weight), followed by button (8.1 ± 0.0 g per 100 g dry weight), brown (7.6 ± 0.7 g per 100 g dry weight), and cup mushrooms (6.4 ± 0.4 g per 100 g dry weight), with flat mushrooms containing the lowest amounts (4.5 ± 0.1 g per 100 g dry weight). Freeze dried raw oats contained 7.6 ± 0.1 g per 100 g dry weight, similar to *A. bisporus* on a weight-for-weight basis for freeze dried samples (ESI Table 1†).

The stalk of cup mushrooms contained significantly higher levels of β-glucan compared to the cap. However, button mushroom stalk and cap β-glucan levels did not differ significantly (Table 2). The higher β-glucan levels in the stalk *vs.* the cap in cup mushrooms agree with the findings from Sari *et al.*[22] who showed higher levels of β-glucan in the stalk *vs.* the cap of *A. bisporus* white, *A. bisporus* brown and several other mushroom varieties. Sari *et al.*[22] unfortunately did not differentiate

Table 1 Moisture and β-glucan content in fresh raw and cooked mushrooms and oats and serving sizes to provide 1 g of β-glucan

Samples	Cooking methods	Moisture, g per 100 g	β-Glucan content, g per 100 g	Serving size of food to provide 1 g of β-glucan		Freeze dried powder amount (g) to provide 1 g of β-glucan
				g	Number of cups[a] (250 ml per cup)	
Oats	Raw	8.9	6.9	14	0.2	13.2
	Cooked	80.2	1.0	100	0.4	20.4
Pleurotus, shimeji	Raw	88.4	3.7	30	0.4	3.1
	Boiling	91.8	2.6	40	0.2	3.2
	Frying	66.3	5.1	20	0.1	3.3
Pleurotus, oyster	Raw	91.4	3.2	30	0.4	2.7
	Boiling	92.2	3.7	30	0.2	2.1
	Frying	79.6	5.5	20	0.1	2.5
A. bisporus, button	Raw	91.5	0.7	150	1.8	12.4
	Boiling	92.2	1.0	100	0.6	7.5
	Frying	65.0	1.2	80	0.5	13.0
A. bisporus, cup	Raw	92.0	0.5	200	2.4	15.7
	Boiling	91.8	0.7	150	0.9	12.4
	Frying	72.7	0.8	120	0.7	14.4
A. bisporus, flat	Raw	92.6	0.3	300	3.5	22.1
	Boiling	93.0	0.5	200	1.2	13.9
	Frying	69.4	0.6	170	1.0	21.9
A. bisporus, brown	Raw	91.3	0.7	150	1.8	13.2
	Boiling	90.4	1.1	90	0.5	8.8
	Frying	64.3	1.2	90	0.5	13.8

[a] Cup size for raw mushroom is approximately 85 g for raw and 167 g for cooked mushroom.[27] Cup size for raw oats is approximately 82.5 g and 250 g for porridge oats.[27.]

between types of white mushroom. The differences in results between cap *vs*. stalk seen between cup and button mushrooms were significant ($P < 0.01$) and driven by both higher levels of β-glucan in the stalk and lower levels of β-glucan in the cap of cup mushrooms compared to button mushrooms (Table 2).

The main difference between button and cup mushrooms is related to when they are harvested and the resulting shape and openness of their caps/veils. As button mushrooms are harvested first, their caps are tightly closed and they are often smaller than cup mushrooms that are harvested second with a longer development time, resulting in a more open cap shape and often a larger size.[28] Based on these differences, it is hypothesised that β-glucan levels in the stalk may increase as the stalk matures, but as the cap matures and becomes more open, the β-glucan levels may either decrease or the concentration of β-glucan may be lower relative to the increased size of the cap. Nevertheless, repurposing the stalk of commercially cultivated mushrooms, that is currently a production waste product, as a source of β-glucan may offer value for use in human food applications.[22]

Boiling and frying had no effect on the β-glucan content of shimeji or cup mushrooms (Fig. 1). Boiling increased the β-glucan content of oyster by 7%, button by 5.2%, flat by 2.7% and brown mushrooms by 3.8% compared to raw mushrooms, whereas frying had no effect (Fig. 1). In contrast, oat porridge contained 2.7% less β-glucan than raw oats (ESI Table 1†).

The β-glucan levels presented in the current study are at the lower end of the range of β-glucan concentrations reported for *A. bisporus* (8–12 g of β-glucan per 100 g dry weight).[19] Another study showed higher levels of β-glucan for *A. bisporus* (white buttons) (12.9 g of β-glucan per 100 g dry weight) and that β-glucan levels increased by 4.1% after boiling and decreased by 2.3% when fried.[21] For *Pleurotus ostreatus* (oyster mushroom), the authors reported a β-glucan content of 40.9 g per 100 g (dry matter) in raw mushrooms and that β-glucan levels increased by 2.4% when boiled and decreased by 20.4% when fried.[21] It has been suggested that the small increase in β-glucan following boiling could be due to the leaching of soluble substances during boiling, which could result in a concentration effect of the fraction of insoluble carbohydrates[29] and could be due to matrix effects caused by structural differences between the mushroom varieties and oats.

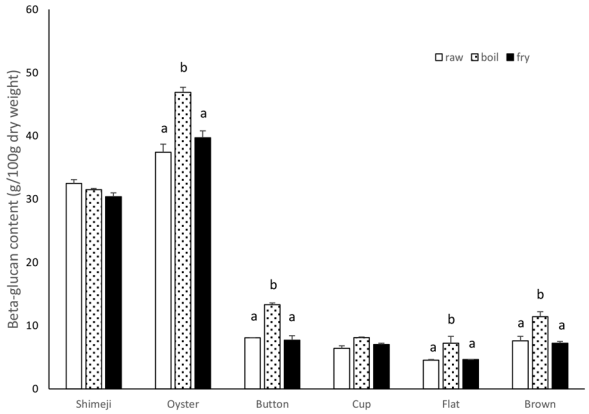

Fig. 1 Effect of cooking on the β-glucan content (g per 100 g dry weight) of mushrooms. Different letters between groups denote a significant difference between cooking methods for each mushroom type ($P < 0.05$) by one-way ANOVA and Tukey's *post-hoc* test. Values are mean ± SEM, $n = 2$.

As raw mushrooms contain a high quantity of moisture (88–93% in the varieties tested), it is important to consider the amount of β-glucan in the context in which it will be consumed, namely per fresh/wet weight and serving size (Table 1). Subsequently, to obtain 1 g of β-glucan (the amount needed in a serving of oats and barley products to allow a cholesterol-lowering health claim in Australia[11] and the EU[10]) from raw mushrooms, the smallest serving size was approximately 30 g (0.4 of a cup) for shimeji and oyster mushrooms, 150 g (1.8 cups, ~6.5 raw mushrooms) for button and brown mushrooms, 200 g (2.4 cups, 5.2 raw mushrooms) for cup mushrooms and 300 g (3.5 cups, 4.2 raw mushrooms) for flat mushrooms (Table 1). In comparison, the serving size of freeze-dried mushrooms to provide 1 g of β-glucan was only 12–22 g for the *A. bisporus* varieties and 3 g for the *Pleurotus* spp. varieties (Table 1). 1 g of β-glucan can be obtained from 6.9 g of raw oats.

When mushrooms were boiled or fried, the serving size of cooked mushrooms was generally smaller than that of raw mushrooms (Table 1). For shimeji and oyster mushrooms, 20–40 g (0.1–0.2 of a cup) of cooked mushrooms provided 1 g of β-glucan. For common mushrooms, the lowest serving sizes of cooked mushrooms that provided 1 g of β-glucan were for

Table 2 β-Glucan content of cap compared to stalk fractions from *A. bisporus* button and cup mushrooms (g per 100 g)

	Fresh mushrooms (g per 100 g)			Freeze-dried mushrooms (g per 100 g)		
	Cap	Stalk	*P*-value (cap *vs*. stalk)	Cap	Stalk	*P*-value (cap *vs*. stalk)
Cup	0.60 ± 0.02[a]	0.83 ± 0.03	0.002	8.35 ± 0.23[a]	10.9 ± 0.36[a]	0.004[a]
Button	0.73 ± 0.02[b]	0.79 ± 0.02	0.08	9.42 ± 0.22[b]	9.68 ± 0.30[b]	0.52[b]

Values are mean ± SEM, $n = 3$. Differences between cap and stalk fractions were analysed using an independent samples Student's *t*-test. The interaction between mushroom variety × mushroom fraction was significant (fresh mushrooms, $P = 0.007$; freeze-dried mushrooms, $P = 0.004$) as determined by two-way ANOVA. Different letters (a and b) indicate a difference between cup *vs*. button mushrooms for the respective mushroom fraction (fresh mushrooms caps, $P = 0.004$; freeze-dried mushroom caps, $P < 0.001$; freeze-dried mushroom stalk, $P = 0.02$) as determined by Tukey's *post-hoc* test with Bonferroni adjustments. *P*-values < 0.05 denote statistically significant differences.

button and brown mushrooms (80–100 g or 0.5–0.6 cup). The largest serving sizes to achieve 1 g of β-glucan were for cup and flat mushroom varieties and ranged from 120 to 200 g (0.7–1.2 cups) of cooked mushrooms, while 100 g of cooked oats provided 1 g of β-glucan.

Bile acid binding capacity of mushrooms and oats

The bile acid binding capacity of dried raw *A. bisporus* mushroom varieties ranged from 29% to 36%, which was similar to that of raw oats which bound 36% of the bile acids (Fig. 2). Both cooking methods increased the bile acid binding capacity of the *A. bisporus* varieties evaluated in this report (Table 3).

Compared to raw mushrooms, boiling increased the bile acid binding capacity by 23% for flat, 19% for brown, 17% for button and 11% for cup, and frying increased the bile acid binding capacity by 16% for flat, 9% for brown, 11% for button and 8% for cup (Table 3). As the bile acid binding assay was conducted using 500 mg of dried mushrooms, the quantity of β-glucan in each sample of *A. bisporus* tested ranged from 23 to 66 mg, which is comparable to the amount of β-glucan in raw oats (38 g) and cooked oats (25 g). Thus, we can conclude that on a per gram basis, the bile acid binding capacities of β-glucan of oats and *A. bisporus* are similar.

The bile acid binding capacity of dried raw *Pleurotus* spp. ranged between 22 and 25% (Fig. 2 and Table 3). Although shimeji mushrooms' bile acid binding capacity did not differ significantly from that of raw oats (25% shimeji and 36% oats), the bile acid binding capacity was significantly lower for oyster mushrooms (22%) compared to oats and brown mushrooms (both 36%). Both cooking methods increased the bile acid binding capacity of shimeji and oyster mushrooms to levels about 2-fold greater than that seen with oats (Table 3). When boiled, the bile acid binding capacity of shimeji and oyster mushrooms was increased by 31% and 27%, respectively, and frying shimeji and oyster mushrooms increased the bile acid binding capacity of these mushrooms by 17 and 18%, respectively. However, it is important to consider the amount of β-glucan present in the bile acid binding assay. For the

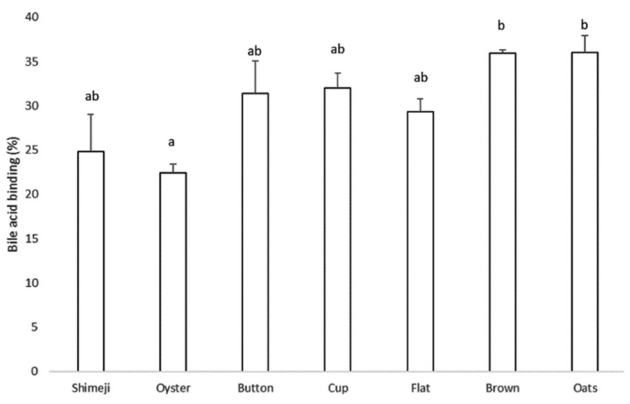

Fig. 2 *In vitro* bile acid binding of raw mushrooms and oats (per 500 mg dry weight). Different letters between groups denote a significant difference ($P < 0.05$) by one way ANOVA and Tukey's *post-hoc* test.

Table 3 *In vitro* bile acid binding of raw and cooked freeze dried mushrooms and oats (per 500 mg dry weight)

Samples	Cooking methods	Bile acid binding (%)	mg of β-glucan in 500 mg of sample
Oats	Raw	36.0 ± 1.9^{b}	38
	Cooked	25.3 ± 1.7^{a}	25
Pleurotus, shimeji	Raw	24.8 ± 4.2^{a}	163
	Boiling	56.5 ± 1.7^{c}	158
	Frying	41.7 ± 2.6^{b}	152
Pleurotus, oyster	Raw	22.4 ± 1.0^{a}	187
	Boiling	49.2 ± 2.1^{c}	235
	Frying	39.8 ± 1.5^{b}	199
A. bisporus, button	Raw	31.4 ± 3.7^{a}	40
	Boiling	48.4 ± 1.9^{b}	66
	Frying	42.3 ± 2.0^{b}	38
A. bisporus, cup	Raw	32.0 ± 1.7^{a}	32
	Boiling	42.7 ± 0.7^{b}	40
	Frying	40.2 ± 0.3^{b}	35
A. bisporus, flat	Raw	29.3 ± 1.5^{a}	23
	Boiling	52.7 ± 1.0^{c}	36
	Frying	45.0 ± 1.6^{b}	23
A. bisporus, brown	Raw	35.9 ± 0.4^{a}	38
	Boiling	55.1 ± 2.7^{c}	57
	Frying	44.7 ± 0.8^{b}	36

Values are mean ± SEM, $n = 3$. For each mushroom type, different letters denote a significant difference between cooking methods ($P < 0.05$) as determined by a two-way ANOVA and Tukey's *post-hoc* test. For oats, different letters denote a significant difference between cooking methods ($P < 0.0001$) as determined by a two-tailed Student's *t*-test.

Pleurotus spp. varieties tested in the current study, the β-glucan amount was 3-fold higher than the amount of β-glucan in oats (Table 3). Thus, it is estimated that approximately 3 times more β-glucan from shimeji or oyster mushrooms is needed to be consumed, to have the equivalent bile acid binding capacity when compared to oats (Table 3). For instance, a serving size of 27 g or 0.3 cup of raw shimeji provides 1 g of β-glucan, but we estimated that a serving size of approximately 90 g or 1 cup would be needed to have an equivalent bile acid binding capacity to that seen for 1 g of β-glucan provided by oats. It is not clear why bile acid binding changes following cooking or between different mushroom varieties, but it is likely to be due to interactive effects between other mushroom components such as chitin and deserves further investigation.

In the current study, when oats were cooked, the bile acid binding capacity was reduced from 36% to 25% ($P < 0.05$). This level of bile acid binding was similar to that previously reported by Kim and White[25] for different molecular weight fractions of raw oats which ranged from 21% to 27%.[25] Subsequently, this study suggests that the structural differences in β-glucan between cereals and fungi/yeast may change the response to cooking, with the bile acid binding properties of β (1 → 3) and β (1 → 4) glycosidic linkages present in cereals negatively impacted by cooking, whereas cooking may enhance bile acid binding properties when β (1 → 6) glucan is present in fungi and yeast.

Despite higher β-glucan levels in cup mushroom stalk compared to the cap, the bile-acid binding capacity of the two fractions did not differ significantly (Table 4). In fact, the bile

Table 4 *In vitro* bile acid binding (%) of cap compared to stalk fractions from *A. bisporus* button and cup mushrooms (per 500 mg dry weight)

	Cap	Stalk	*P*-value
A. bisporus, cup			
Bile acid binding (%)	40.0 ± 1.12	36.4 ± 0.87	0.06
β-Glucan (mg per 500 mg sample)	41.8 ± 1.15	54.4 ± 1.81	0.004
A. bisporus, button			
Bile acid binding (%)	38.8 ± 1.40	35.0 ± 0.41	0.06
β-Glucan (mg per 500 mg sample)	47.1 ± 1.09	48.4 ± 1.49	0.52

Values are mean ± SEM, n = 3. Differences between cap and stalk fractions were analysed using an independent samples Student's t-test. No interaction between mushroom variety × mushroom fraction was observed (P = 0.90). P-values < 0.05 denote statistically significant differences.

acid binding capacity tended (although not significantly) to be lower in the stalk compared to the cap fraction. The bile acid binding capacity also did not differ in button cap compared to stalk fractions (Table 4). These results appear to be slightly higher than those reported in Table 3. This may be due to the variability between experiments performed at different times that is typical in this type of biological assay, as a result of different batches of enzymes and buffer solutions being used.

The capacity of *A. bisporus* mushrooms to bind bile acids similarly to oats demonstrates the potential to lower cholesterol. However, the clinical evidence is limited and of low quality. Three studies showed that daily consumption of ~150–200 g per day fresh *A. bisporus* or *P. ostreatus* lowered TC and/or LDL-cholesterol.[16–18] Other studies provided study participants with 3–30 g per day of dried *P. ostreatus* powder,[14,15,30,31] equating to a β-glucan dose of 1–10 g per day, and also showed improvements in cholesterol levels. However, these studies all have significant weaknesses in study design that include lack of a control group or randomisation,[14–18] short intervention periods (7 days[32]), and/or poorly control, *e.g.* olive oil was added to the mushroom intervention,[18] limiting the ability to identify the independent lipid lowering potential of the mushrooms. Thus, high-quality clinical trials are needed to confirm these preliminary findings, especially for the more commonly consumed *A. bisporus*, for which only one clinical trial on fresh (*i.e.*, not powdered) mushrooms has been conducted to date.[18] The proposed level of mushroom intake needed to provide 1 g of β-glucan is between 150 and 300 g of mushrooms for *A. bisporus* varieties. This is consistent with a daily serving of ~150–200 g per day fresh *A. bisporus* or *P. ostreatus* that was shown to have cholesterol lowering effects.[16–18] While this is higher than the amount of mushrooms commonly consumed by most populations, it could be achievable, particularly for a health-motivated consumer. For instance, Australian mushroom consumers are estimated to consume 60 g of mushrooms each day,[33] whereas per capita intakes in the Netherlands and China suggest event higher rates of intake.[28] Furthermore, the target population may be willing to make certain dietary choices to improve or maintain their cholesterol levels, especially when higher mushroom intake aligns with Australian dietary recommendations and priorities to increase vegetable consumption for health.[34]

Conclusions

Conventional *A. bisporus* (button, cup, flat and brown) mushrooms had similar bile acid binding properties to oats, but the levels of β-glucan were relatively low. On the other hand, although *Pleurotus* spp. mushrooms had higher β-glucan levels compared to *A. bisporus*, their bile acid binding capacity was significantly lower.

The daily serving size of fresh or cooked conventional *A. bisporus* mushrooms needed to provide 1 g of β-glucan may be challenging to achieve, considering the levels currently consumed by Australians (an average of 60 g per day for mushroom consumers). The amount may be achieved through consuming a combination of mushroom forms, such as raw, cooked, and freeze-dried forms.

Author contributions

All authors have participated in (a) the conception and design of the experiment, or the analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The project 'Mushrooms and their potential health benefits of lowering blood cholesterol' (MU20001) is a strategic levy investment under the Hort Innovation Mushroom Fund strategic levy investment project.

References

1 F. Mach, C. Baigent, A. L. Catapano, K. C. Koskinas, M. Casula, L. Badimon, M. J. Chapman, G. G. De Backer, V. Delgado, B. A. Ference, I. M. Graham, A. Halliday, U. Landmesser, B. Mihaylova, T. R. Pedersen, G. Riccardi, D. J. Richter, M. S. Sabatine, M. R. Taskinen, L. Tokgozoglu and O. Wiklund, 2019 ESC/EAS Guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk, *Eur. Heart J.*, 2020, **41**, 111–188.

2 World Health Organisation, Cardiovascular diseases, **https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)**.

3 Commonwealth of Australia as represented by the Department of Health and Aged Care, *Australian Guideline for assessing and managing cardiovascular disease risk*, 2023.

4 S. S. AbuMweis, S. Jew and N. P. Ames, β-Glucan from barley and its lipid-lowering capacity: A meta-analysis of randomized, controlled trials, *Eur. J. Clin. Nutr.*, 2010, **64**, 1472–1480.

5 A. Whitehead, E. J. Beck, S. Tosh and T. M. Wolever, Cholesterol-lowering effects of oat β-glucan: A meta-analysis of randomized controlled trials, *Am. J. Clin. Nutr.*, 2014, **100**, 1413–1421.

6 Y. Wang, S. V. Harding, S. J. Thandapilly, S. M. Tosh, P. J. Jones and N. P. Ames, Barley β-glucan reduces blood cholesterol levels via interrupting bile acid metabolism, *Br. J. Nutr.*, 2017, **118**, 822–829.

7 A. Li, J. Gao, Y. Li, S. Qi, T. Meng, S. Yu, Y. Zhang and Q. He, Efficacy of oats in dyslipidemia: A systematic review and meta-analysis, *Food Funct.*, 2024, **15**, 3232–3245.

8 B. Du, M. Meenu, H. Liu and B. Xu, A concise review on the molecular structure and function relationship of β-glucan, *Int. J. Mol. Sci.*, 2019, **20**, 1–18.

9 P. Sima, L. Vannucci and V. Vetvicka, β-Glucans and cholesterol (Review), *Int. J. Mol. Med.*, 2018, **41**, 1799–1808.

10 European Food Safety Authority (EFSA), Scientific opinion on the substantiation of health claims related to beta-glucans and maintenance of normal blood cholesterol concentrations (ID 754, 755, 757, 801, 1465, 2934) and maintenance or achievement of a normal body weight (ID 820, 823) pursuant to Article 13(1) of Regulation (EC) No 1924/2006, *EFSA J.*, 2009, **7**, 1254.

11 Food Standards Australian New Zealand, Reviews of food-health relationships for high level health claims (**https://www.foodstandards.gov.au/consumer/labelling/nutrition/reviews-fhr**).

12 U.S. Food and Drug Administration (FDA), Authorized Health Claims That Meet the Significant Scientific Agreement (SSA) Standard, **https://www.fda.gov/food/food-labeling-nutrition/authorized-health-claims-meet-significant-scientific-agreement-ssa-standard#approved**.

13 A. Singla, O. P. Gupta, V. Sagwal, A. Kumar, N. Patwa, N. Mohan, Ankush, D. Kumar, O. Vir, J. Singh, L. Kumar, C. Lal and G. Singh, Beta-glucan as a soluble dietary fiber source: Origins, biosynthesis, extraction, purification, structural characteristics, bioavailability, biofunctional attributes, industrial utilization, and global trade, *Nutrients*, 2024, **16**, 1–35.

14 M. B. K. Choudhury, *Pleurotus ostreatus* improves lipid profile of obese hypertensive nondiabetic males, *Bangladesh J. Med. Biochem.*, 2013, **7**, 37–44.

15 I. Kajaba, R. Simoncic, K. Frecerova and G. Belay, Clinical studies on the hypolipidemic and antioxidant effects of selected natural substances, *Bratisl. Lek. Listy*, 2008, **109**, 267–272.

16 K. Khatun, H. Mahtab, P. A. Khanam, M. A. Sayeed and K. A. Khan, Oyster mushroom reduced blood glucose and cholesterol in diabetic subjects, *Mymensingh Med. J.*, 2007, **16**, 94–99.

17 M. A. Sayeed, A. Banu, K. Khatun, P. A. Khanam, T. Begum, H. Mahtab and J. A. Haq, Effect of edible mushroom (*Pleurotus ostreatus*) on type-2 diabetics, *Ibrahim Med. Coll. J.*, 2014, **8**, 6–11.

18 W. I. Abd-alwahab, F. K. Al-dulaimi and A. T. Abdulqader, Effect of mushroom cooked in olive oil on some physiological and biochemical parameters of human, *EurAsian J. BioSci.*, 2018, **12**, 393–397.

19 M. Blumfield, K. Abbott, E. Duve, T. Cassettari, S. Marshall and F. Fayet-Moore, Examining the health effects and bioactive components in Agaricus bisporus mushrooms: A scoping review, *J. Nutr. Biochem.*, 2020, **84**, 108453.

20 I. Mirończuk-Chodakowska and A. M. Witkowska, Evaluation of Polish wild mushrooms as beta-glucan sources, *Int. J. Environ. Res. Public Health*, 2020, **17**, 1–18.

21 I. Roncero-Ramos, M. Mendiola-Lanao, M. Pérez-Clavijo and C. Delgado-Andrade, Effect of different cooking methods on nutritional value and antioxidant activity of cultivated mushrooms, *Int. J. Food Sci. Nutr.*, 2017, **68**, 287–297.

22 M. Sari, A. Prange, J. I. Lelley and R. Hambitzer, Screening of beta-glucan contents in commercially cultivated and wild growing mushrooms, *Food Chem.*, 2017, **216**, 45–51.

23 B. V. McCleary and A. Draga, Measurement of β-glucan in mushrooms and mycelial products, *J. AOAC Int.*, 2016, **99**, 364–373.

24 Food Standards Australia New Zealand, Fruits and vegetables measures program, 2024, **https://www.foodstandards.gov.au/science-data/monitoringnutrients/ausnut/foodmeasures/fruits-and-vegetable-measures-program**, Access date is 01/03/2024.

25 H. J. Kim and P. J. White, Optimizing the molecular weight of oat β-glucan for in vitro bile acid binding and fermentation, *J. Agric. Food Chem.*, 2011, **59**, 10322–10328.

26 A. R. Bird, S. Usher, B. May, D. L. Topping and M. K. Morell, in *Dietary Fiber and Health*, ed. S. S. Cho and N. Almeida, CRC Press, 1st edn, 2012, ch. 5, pp. 41–56.

27 Food Standards Australian New Zealand, AUSNUT 2011–13 – Australian Food Composition Database, **https://www.foodstandards.gov.au/science-data/food-composition-databases/ausnut-2011-13/ausnut-datafiles**.

28 Hort Innovation, *Mushroom Fund. Annual Investment Plan 2020/21*, Sydney, 2020/21, Access date is 01/03/2024.

29 K. Pogoń, G. Jaworska, A. Duda-Chodak and I. Maciejaszek, Influence of the culinary treatment on the quality of *Lactarius deliciosus*, *Foods*, 2013, **2**, 238–253.
30 I. Schneider, G. Kressel, A. Meyer, U. Krings, R. G. Berger and A. Hahn, Lipid lowering effects of oyster mushroom (*Pleurotus ostreatus*) in humans, *J. Funct. Foods*, 2011, **3**, 17–24.
31 A. J. Weigand-Heller, P. M. Kris-Etherton and R. B. Beelman, The bioavailability of ergothioneine from mushrooms (*Agaricus bisporus*) and the acute effects on antioxidant capacity and biomarkers of inflammation, *J. Prev. Med.*, 2012, **54**, S75–S78.
32 K. Khatun, H. Mahtab, P. A. Khanam, M. A. Sayeed and K. A. Khan, Oyster mushroom reduced blood glucose and cholesterol in diabetic subjects, *Mymensingh Med. J.*, 2007, **16**, 94–99.
33 Australian Bureau of Statistics, Australian Health Survey: Nutrition and Physical Activity, 2011–12. 4324055002 – Microdata: Australian Health Survey, **https://www.abs.gov.au/AUSSTATS/abs@.nsf/Lookup/4324.0.55.002Main+Features682011-12?OpenDocument**.
34 National Health and Medical Research Council, *Eat for Health: Australian Dietary Guidelines*, Canberra, 2013.