

## Original article

**Antiradical and functional properties of subcritical water extracts from edible mushrooms and from commercial counterparts**Paula Rodríguez-Seoane,<sup>1\*</sup> María Dolores Torres Perez,<sup>1</sup> Catalina Fernández de Ana,<sup>2</sup> Esteban Sinde-Stompel<sup>2</sup> & Herminia Domínguez<sup>1</sup><sup>1</sup> Chemical Engineering Department, Universidad de Vigo (Campus Ourense), Edificio Politécnico, As Lagoas, Ourense 32004, Spain<sup>2</sup> Hifas da Terra SL, Portomuiños, 7, Bora, Pontevedra 36154, Spain

(Received 29 June 2021; Accepted in revised form 28 September 2021)

**Summary** This study deals with the antioxidant and functional potential of subcritical water extracts from edible mushrooms, in comparison to commercial products. *Pleurotus citrinopileatus* extracts showed the highest phenolic content and antioxidant properties. Similar results were determined in commercial extracts of *Coriolus versicolor*. The highest growth inhibition in selected human carcinogenic cells was identified for the *P. citrinopileatus* extract obtained during heating up to 160 °C. Rheological studies confirmed that glucan-based hydrogels prepared with mushroom extracts exhibited enhanced viscoelastic properties compared to those formulated with commercial products. The extracts providing the strongest gels were obtained from *Pleurotus eryngii*, followed by *Hericium erinaceus* and *Pleurotus citrinopileatus*. No water syneresis for the proposed hydrogels was observed. The formulated hydrogels could be interesting for their application in the food sector.

**Keywords** Antioxidants, antitumoral, autohydrolysis, bioactives, glucans, rheology.

**Introduction**

Traditionally, edible mushrooms have been part of human diet for their nutritive and organoleptic properties, which convert them into an attractive source of high valuable compounds (Moon & Lo, 2014). Their use has recently expanded to the areas of pharmaceuticals, nutraceuticals and cosmeceuticals for their nutrition and human health benefits (Roselló-Soto *et al.*, 2016). Edible mushrooms could be the next generation food, since they are gluten-free, low caloric and fat content, and rich in water, minerals, proteins, fibres, carbohydrates and also can provide significant amounts of vitamins (Leong *et al.*, 2021). The presence of specific compounds confers to these raw materials biological activities, such as anti-fungal, anti-inflammatory, antitumor, antioxidant, antiviral, antibacterial, hepatoprotective, antidiabetic, hypolipidemic, antithrombotic and hypotensive (Rathore *et al.*, 2017; Gogoi *et al.*, 2019; Hao *et al.*, 2020; Calabretti *et al.*, 2021; Chu *et al.*, 2021; Patel *et al.*, 2021).

Among the bioactive compounds identified in mushrooms, polysaccharides have gained particular attention. The most widely studied are beta-glucans, consisting of a linear (1 → 3) backbone with (1 → 6)-

linked glucose branches. Mushroom β-glucans are structurally different compared with those of bacteria or plants. They also form heteroglycans involving arabinose, mannose, fucose, galactose, xylose, etc. The biological activity of beta-glucans depends on their shape, structure, and molecular weight (Pandya *et al.*, 2019; Leong *et al.*, 2021; Ruthes *et al.*, 2021; van Steenwijk *et al.*, 2021). β-glucans are responsible for most of the biological effects of mushrooms, in particular, immunomodulatory and antitumor actions (Rossi *et al.*, 2018; Patel *et al.*, 2021; Steenwijk *et al.*, 2021; Vetvicka *et al.*, 2021), they are also recognised as dietary fibres that may be used as prebiotics (Ciecierska *et al.*, 2019; Mitsou *et al.*, 2020; Ruthes *et al.*, 2021). Furthermore, the rheological properties of these biopolymers indicated that mushrooms beta-glucan can be an effective functional ingredient to incorporate in various food formulations, for example, as a thickener in food supplements (Ashraf Khan *et al.*, 2017; Rodríguez-Seoane *et al.*, 2021).

Minerals are also important for human health. Potassium could be associated with neuronal stimulatory function. Magnesium helps the proper function of muscles, bones and nerves, keeps the heart rate stable and maintains a healthy immune system. Calcium consumption is related to mineralisation and bone

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metabolism. Copper is essential for human health and iron deficiency leads to problems in thermoregulatory capacity and in thyroid hormone metabolism and the low sodium content in mushrooms makes them suitable for people with hypertension (Sinha *et al.*, 2021).

Subcritical water extraction, SWE, (also referred to as autohydrolysis) uses water as solvent at temperatures above the boiling point (100–374 °C) and pressure (1–22.1 MPa) sufficient to keep it at liquid state (Roselló-Soto *et al.*, 2016; Morales *et al.*, 2019; Leong *et al.*, 2021). This environmentally friendly technique facilitated the extraction of both polysaccharides and antioxidant compounds from mushrooms (Rodríguez-Seoane *et al.*, 2018; Hwang *et al.*, 2019; Morales *et al.*, 2019; Zhang *et al.*, 2019).

This work is focused on the subcritical water extraction of bioactives from different edible mushrooms (*Pleurotus eryngii*, *Pleurotus citrinopileatus* and *Hericiium erinaceus*) and further comparison to commercial products. The main aim of this work is to obtain extracts with  $\beta$ -glucan content similar to commercial ones previously reported and with other bioactives, such as phenolics, and to introduce them in gelling matrices with interesting rheological properties that could be used in food applications. In order to extend their potential applications, a parallel objective is the preliminary assessment of the cytotoxic activity of the above extracts for different human colon carcinoma cell lines.

## Materials and methods

### Raw materials

The mushrooms used in this work were kindly provided by Hifas da Terra S.L. (Pontevedra, Spain). *Pleurotus eryngii* (P.ery), *Pleurotus citrinopileatus* (P.cit) and *Hericiium erinaceus* (H.eri) dried samples were milled to powder size using a Moulinex grinder MC3001 and stored in airtight bags before use.

Commercial extracts from *Coriolus versicolor* (Corio), *Agaricus blazei* (Sol), *Lentinula edodes* (Shii), *Hericiium erinaceus* (Leo) and a combined preparation of *Ganoderma lucidum*, *Grifola frondosa* and *Lentinula edodes* (Mix) were also provided by Hifas da Terra S.L.

### Subcritical water extraction

Subcritical water extraction was carried out in a 0.6 L reactor (Parr Instr. Co., Illinois, USA). Mushrooms (P.ery, P.cit and H.eri) were mixed with water at a liquid to solid ratio 12 (w/w) and heated up to maximum temperatures of 160 and 190 °C, selected based on a previous work of these authors to balance the recovery of antioxidant compounds without jeopardising the mechanical properties of the glucans (Rodríguez-Seoane *et al.*, 2021). After extraction, liquid and solid

phases were separated by filtration, and the aqueous extracts were lyophilised (Christ Alpha 2-4 LD plus, Osterode).

### Soluble extracts features

Lyophilised extracts were dissolved in water at a concentration of 2 g L<sup>-1</sup>, and further characterised. Total glucan content was assayed using a mushroom and yeast beta-glucans kit (Megazyme, K-YBGL, Ireland). Beta-glucan content was calculated as the difference between total glucans and alfa-glucans, and total phenolic content was determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965) and expressed as gallic acid equivalents (GAE), using gallic acid (Sigma) as standard. This procedure consists of adding to 0.5 mL of liquid extract: 3.75 mL of distilled water, 0.25 mL of Folin–Ciocalteu's reagent (1:1) and 0.5 mL of sodium carbonate (10%, w/v). After incubation of samples in the darkness for 1 h, absorbance at 765 nm was measured in a Thermo Scientific Evolution 201 spectrophotometer. The antioxidant capacity was determined against ABTS and DPPH radicals. Trolox equivalent antioxidant capacity (TEAC) was calculated according to the Re *et al.* (1999) method. A diluted solution of ABTS<sup>+</sup> (1.0 mL) with phosphate-buffered saline (PBS) was added to the extracts (10  $\mu$ L). After 6 min incubation in a 30 °C water bath, absorbance was measured at 734 nm, and data expressed as equivalents of Trolox (Sigma). The DPPH ( $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl) radical scavenging assay was determined in accordance with the method described by von Gadov *et al.* (1997). To 50  $\mu$ L of extract, 2 mL of a 3.6 10<sup>-5</sup> M methanolic solution of DPPH was added. After 16 min, absorbance was measured at 515 nm. Results are expressed as inhibition percentage.

Total nitrogen assay was conducted on a FlashEA 1112 Elemental analyser (Thermo, Barcelona, Spain) with Helium as carrier and reference gas at 130 and 100 mL min<sup>-1</sup>, respectively. The temperatures of the oxidation and reduction ovens were 900 and 680 °C, respectively, and the oxygen flow was 250 mL min<sup>-1</sup>. Protein content was calculated using the factor 4.38 (FAO & INFOODS, 2012).

The Ca, Mg, Fe, B, Cu, Cd and Pb contents in the extracts were determined by atomic absorption, whereas Na and K were analysed by ICP-OES (inductively coupled plasma optical emission spectrometry) using Indium as internal standard. Hg was analysed by CVAAS (cold vapour-atomic absorption spectrophotometry) after microwave-assisted acid digestion.

### Antitumoral analysis

The cytotoxic activity of extracts was evaluated in different human colon carcinoma cell lines (HT-29, HCT-

116 and CACO-2) at 10 mg mL<sup>-1</sup>, as previously reported (Rodríguez-Seoane *et al.*, 2021). Preliminary tests were made at lower concentrations (0.01, 0.1 and 1 mg mL<sup>-1</sup>) in order to establish the most adequate assay conditions. Cisplatin was used as a control. CACO-2 cells were grown on DMEM (Dulbecco Modified Eagle's Medium), whereas HCT-116 and HT-29 were cultivated on McCoy's 5a Medium Modified. All systems were supplemented with 10% Fetal Bovine Serum (10%, FBS, Sigma) in a controlled air atmosphere (95% air/5% CO<sub>2</sub>, 37 °C). The cell growth inhibition was evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide, Sigma) trials (10 000 cells well<sup>-1</sup> for 24 h). Subsequently, extracts dissolved in Milli-Q water were incorporated and incubated (95% air/5% CO<sub>2</sub>, 37 °C, 72 h). The MTT (10 µL) was added and the mixture was incubated for 4 h followed by the addition of sodium dodecyl sulphate (100 µL, SDS) and incubation for 12–14 h. Then, absorbance was read at 595 nm (Tecan Infinite M1000 Pro, Grödig, Austria) and the results presented as growth inhibition (%).

### Rheology

Glucan-based hydrogels were prepared at 20% extract for the lyophilised extracts (P.cit, H.eri) and the commercial ones (Corio, Leo, Mix, Shii, Sol) and at 7.5% for lyophilised P.ery. Note here that biopolymer content was selected after preliminary tests, where different amounts (1–25%) were used to formulate glucan-based gelled matrices with rheological values within those commonly used in food gelled matrices (Torres *et al.*, 2013). In all cases, a neutral biopolymer as gelatine (1%) (Scharlau, CAS number 9000-70-8) was incorporated into the above matrices in order to guarantee the full glucan-matrices gelation. Both extracts and gelatine were mixed and heated (10 min, 90 °C) with continuous stirring. Then, systems were cooled down to room temperature (1 h) and cold stored (24 h) to allow complete gels maturation. Note here that preliminary time sweeps (12 h, 4 °C, 15 Pa) were made on the prepared matrices to ensure that the selected cold storage time was enough to allow full gels formation (<5 h in all cases). Before rheological testing, glucan-based hydrogels were equilibrated at room temperature for 60 min. The viscoelastic features of the above hydrogels were determined using small amplitude oscillatory shear measurements (SAOS), in terms of elastic modulus,  $G'$ , and viscous modulus,  $G''$ . Rheological trials were performed on a controlled-stress rheometer (MCR302, Anton Paar Physica, Austria) using sandblasted parallel plate (25 mm diameter), which allows to avoid possible samples slippage. All systems were carefully placed on the measuring system with a 1 mm gap and were rested (5 min) to

favour the structural and thermal homogenisation. After the linear viscoelastic region was delimited (<20 Pa) using stress sweep tests (from 0.1 to 100 Pa, 1 Hz, 25 °C), the monitoring of the elastic ( $G'$ ) and viscous ( $G''$ ) moduli was performed through the mechanical spectra (from 0.1 to 10 Hz at 15 Pa and 25 °C).

### Statistical analysis

All above measurements were performed at least in triplicate. Data were assessed using one-factor analysis of variance, ANOVA, using PASW Statistics (v.22, IBM SPSS Statistics, New York, USA). When the variance analysis presented differences among means, a post-hoc Scheffé test was performed to differentiate means with 95% confidence ( $P < 0.05$ ).

## Results and discussion

### Fundamental chemical features of the soluble extracts

Table 1 shows the content in total glucan,  $\alpha$ -glucans and  $\beta$ -glucans of the extracts obtained at the tested extraction temperatures. The increase in extraction temperature induced a positive effect on total glucan content in all samples. The total glucan content in subcritical water extracts of *Cantharellus tubaeformis* also increased with temperature reaching the highest value at 210 °C (13.7%) (Rodríguez-Seoane *et al.*, 2018). Among the glucans,  $\beta$ -glucans are the most interesting. The highest content of  $\beta$ -glucans was observed in P.ery extracts, above 30%, followed by H.eri and P.cit extracts, below 17%. Similar  $\beta$ -glucan content was found in commercial extracts of Leo (16.8%), Sol (11.9%), Shii (24.8%), Corio (30.9%) and "Mix" (20.6%) according to values shown on commercial labels. Calabretti *et al.* (2021) reported that wild-type *P. eryngii* isolates had a higher  $\beta$ -glucan content (30%) compared to the commercial ones (20%).

**Table 1** Total glucan content,  $\alpha$ -glucans,  $\beta$ -glucans and protein content of subcritical water mushroom extracts

	Total glucans (%)	$\alpha$ -glucans (%)	$\beta$ -glucans (%) <sup>†</sup>	Protein (%)
H.eri 160	12.06 ± 0.45 <sup>e</sup>	1.24 ± 0.13 <sup>d</sup>	10.82 <sup>e</sup>	19.5 ± 0.5 <sup>a</sup>
H.eri 190	17.68 ± 0.47 <sup>c</sup>	1.03 ± 0.01 <sup>e</sup>	16.65 <sup>c</sup>	14.8 ± 0.3 <sup>b</sup>
P.cit 160	11.63 ± 0.03 <sup>e</sup>	4.62 ± 0.12 <sup>b</sup>	7.00 <sup>f</sup>	20.3 ± 0.6 <sup>a</sup>
P.cit 190	16.26 ± 0.09 <sup>d</sup>	3.97 ± 0.31 <sup>c</sup>	12.29 <sup>d</sup>	15.2 ± 0.4 <sup>b</sup>
P.ery 160	37.20 ± 1.19 <sup>b</sup>	6.56 ± 0.08 <sup>a</sup>	30.64 <sup>b</sup>	16.4 ± 0.1 <sup>c</sup>
P.ery 190	47.57 ± 1.30 <sup>a</sup>	0.41 ± 0.01 <sup>f</sup>	47.15 <sup>a</sup>	10.6 ± 0.3 <sup>d</sup>

Data are presented as mean ± standard deviation. Data values in a column with different superscript letters are significantly different at the  $P \leq 0.05$  level.

<sup>†</sup>Calculated as difference between the previous columns.

Mitsou *et al.* (2020) reported comparable or higher  $\beta$ -glucan content in several fungal strains grown on different substrates, 27–35% in *P. ostreatus*, 38–42% in *P. eryngii*, 15–20% in *Hericium erinaceus* and 33–37% in *C. cylindracea*.

Protein content (Table 1) for three edible mushrooms varied between 13.6% and 20.3%, with the highest values identified in extracts from P.cit followed by those of H.eri and P.ery. A statistical decrease was observed with increasing temperatures. These values are consistent with those previously reported for other mushrooms species (Rodrigues *et al.*, 2015; Bach *et al.*, 2017). Since an adequate mineral content can produce beneficial effects for health, composition in the lyophilised extracts was determined (Table 2). Potassium was the most abundant element in the extracts, with slightly higher values than those found in fruiting bodies (Brzezicha-Cirocka *et al.*, 2019). Iron content varied among species, being the highest in P.cit (154.6–167.0 mg kg<sup>-1</sup>) and the lowest content P.ery (9.2–16.8 mg kg<sup>-1</sup>). Similarly, Brzezicha-Cirocka *et al.* (2019) reported great variations in Fe content among species. Sodium, calcium and magnesium exhibited similar values in all samples. Calcium content is consistent with the mean values reported by Mleczek *et al.* (2021) in *Pleurotus citrinopileatus* (554 mg kg<sup>-1</sup>) and *P. eryngii* (652 mg kg<sup>-1</sup>) whereas sodium and magnesium contents are higher in the samples herein analysed. Heavy toxic metals were under detection limits.

### Antioxidant features of the soluble extracts

Mushrooms are a natural source of dietary antioxidants and help to prevent oxidative stress. Moreover, the antioxidant capacity of mushrooms is naturally associated with other biological properties (Adebayo *et al.*, 2018). The total phenolic content (TPC) and antioxidant capacity against ABTS and DPPH radicals of the subcritical water extracts and commercial products are shown in Fig. 1. The highest TPC was found in P.cit extract (3.6 g GAE per 100 g), comparable to the value found in Corio (4 g GAE per 100 g extract), which was higher than in the other commercial extracts. A similar tendency was observed for the TEAC value of the extracts (P.cit: 8 g Trolox eq. per

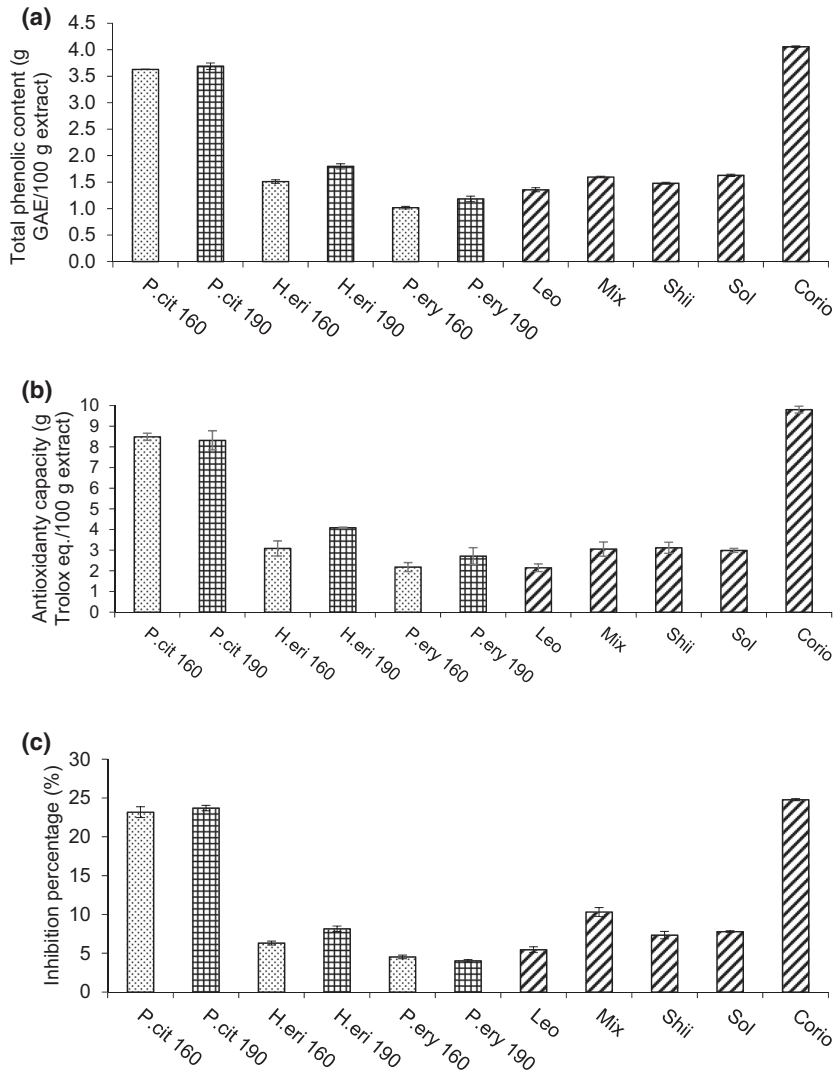
100 g; Corio: 9.8 g Trolox eq. per 100 g and others: 2–4 g Trolox eq. per 100 g) and for the inhibition percentage determined against the DPPH radical (P.cit: 23%; Corio: 24% and others: 4–10%). A slight increase in the antiradical properties with extraction temperature was observed. Comparable trends in phenolic content (1.9 and 4.0 g GAE per 100 g) and TEAC value (3.8 and 6.0 g Trolox eq. per 100 g) have been reported for *Cantharellus tubaeformis* extracts produced at 170 °C and at 240 °C, respectively (Rodríguez-Seoane *et al.*, 2018). Calabretti *et al.* (2021) reported a TPC of 3.2 mg g<sup>-1</sup> and an ABTS antiradical capacity of 28.5 mg mL<sup>-1</sup> in commercial *P. eryngii* isolates and Gogoi *et al.* (2019) have reported inhibition percentages against the DPPH radical in the range 55–70% for water extracts and 52–87% for ethanol extracts obtained with ultrasound assistance from *P. citrinopileatus*.

### Antiproliferative potential of the extract

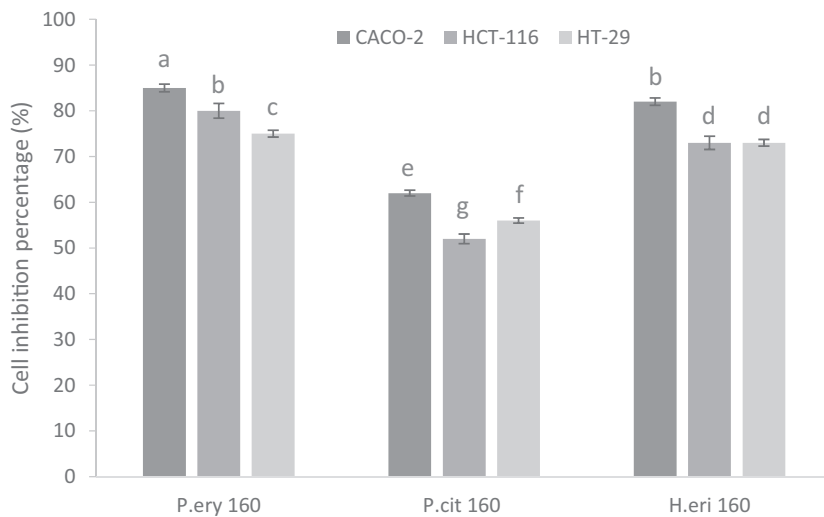
The antitumoral potential of mushroom metabolites in relation to the reduced incidence of tumorigenesis and in the increased chemotherapeutic effects is well known (He *et al.*, 2017), and topical studies have suggested their ability as natural antiproliferative agents (Nowacka-Jechalke *et al.*, 2021). In this context, extracts involved in the gelled matrices with the most adequate viscoelastic features (H.eri 160, P.cit 160, P.ery 160) for food applications were selected to further study their cell growth inhibitory potential on different human colon carcinoma cell lines (HCT-116, Caco-2 and HT-29). Figure 2 presents the corresponding antiproliferative activity results. Note here that cisplatin (used as control) exhibited percentages of cellular inhibition around 63% (IC<sub>50</sub>, 4  $\mu$ M). Regardless of the tested carcinoma cell line, at an extract content of 10 mg mL<sup>-1</sup>, P.ery 160 extracts were the most potent for cell growth inhibition followed by H.eri 160 and P.cit 160. The best results were identified for Caco-2 without notable differences between HT-29 and HCT-116. The IC<sub>50</sub> (mg mL<sup>-1</sup>) were high, ranging from 5.5 (P.ery) and 7.9 (H.eri 160 and P.cit 160) for Caco-2, and from 2.6 (P.ery) and 9.3 (H.eri 160 and P.cit 160) for HCT-116 and HT-29. Antitumor activity of mushrooms and their extracts was also confirmed by other studies. Sharif *et al.* (2018) determined in mushrooms aqueous extracts, IC<sub>50</sub> values against HT-29 colon cancer cells around 100  $\mu$ g mL<sup>-1</sup>, whereas other mushroom extracts provided reduction of 20–40% in cell viability. Hu *et al.* (2018) reported lower IC<sub>50</sub> values for a phenolic-rich *P. eryngii* extract against HCT-116 cells, being 48.8 and 36.9  $\mu$ g mL<sup>-1</sup> at 48 and 72 h, respectively. Lavi *et al.* (2010) reported that in HT-29, Caco2, and HCT-116 cells, the growth inhibition caused by glucan form *Pleurotus*

**Table 2** Mineral content of the obtained extracts

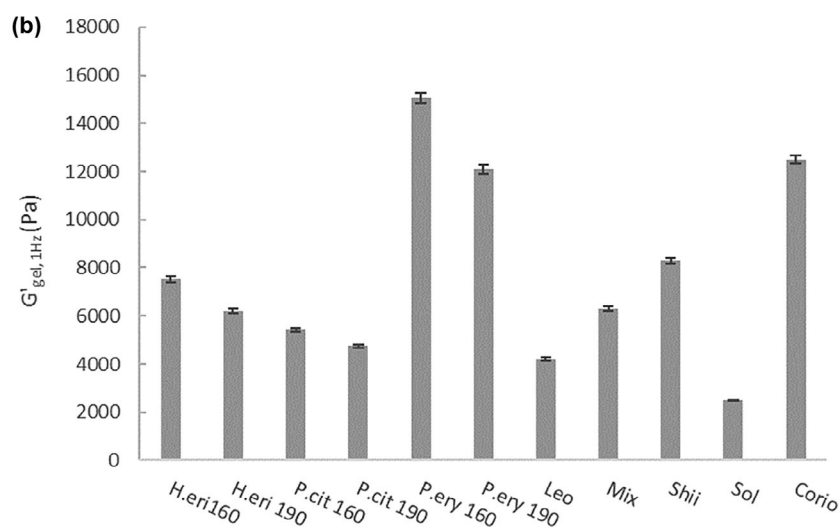
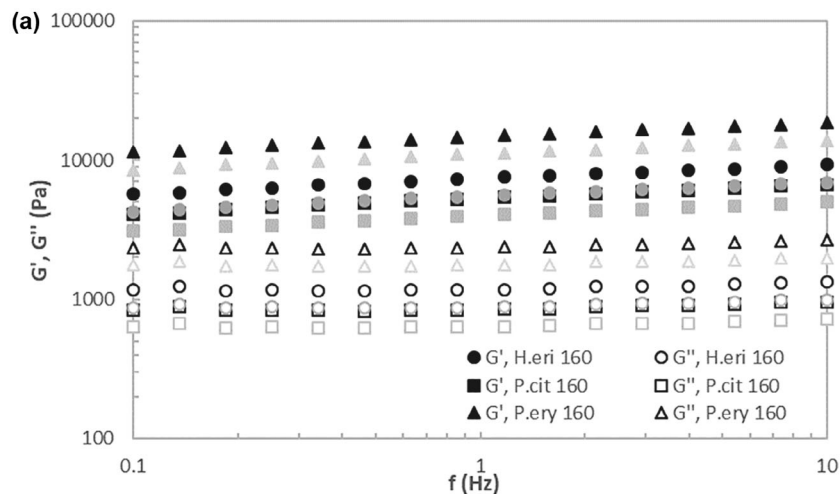
	Na	K	Ca	Mg	Fe	B	Cu	Cd	Pb	Hg
	g kg <sup>-1</sup>				mg kg <sup>-1</sup>					
H.eri 160	1.1	57.5	0.5	1.9	88.0	18.8	<2	<2	<6	<0.04
H.eri 190	1.3	52.3	0.6	1.9	105.5	17.7	<2	<2	<6	<0.04
P.cit 160	1.4	57.3	0.4	3.4	154.6	31.2	2.0	<2	<6	<0.04
P.cit 190	1.8	51.0	0.6	3.0	167.0	38.2	2.0	<2	<6	<0.04
P.ery 160	2.6	55.7	0.4	2.5	16.8	38.8	<2	<2	<6	<0.04
P.ery 190	1.6	34.1	0.5	1.8	9.2	33.6	2.4	<2	<6	<0.04



**Figure 1** Comparison of total phenolic content (a), antioxidant capacity (b) and inhibition percentage (c) between autohydrolysis extracts obtained at 160 °C (dotted), at 190 °C (squared) and commercial extracts (lined).



**Figure 2** Cell growth inhibition percentage (%) for selected extracts (H.eri 160, P.cit 160, P.ery 160) at 10 mg mL<sup>-1</sup>. Note here that cisplatin (used as control) showed cellular inhibition percentages about 63% (IC<sub>50</sub>, 4 μM).



**Figure 3** (a) Mechanical spectra determined using small amplitude oscillatory shear measurements of glucan-based hydrogels prepared with representative extracts and (b) overview of the viscoelastic features ( $G'_{\text{gel}, 1 \text{ Hz}}$ , Pa) for all formulated glucan gelled matrices. Note here that grey symbols in plot (a) correspond with their commercial counterparts.

*pulmonarius* glucan was lower than for other cells, requiring more prolonged times of higher concentration (up to 0.5%). The high  $\text{IC}_{50}$  values have been reported for other water soluble mushroom extracts, such as those of *G. tsugae* on C6, Hep 3B, and HL-60 cells, being 1.13, 2.73, and 2.60  $\text{mg mL}^{-1}$ , respectively, whereas those of baby *G. tsugae* were 1.87, 2.63, and 3.12  $\text{mg mL}^{-1}$ , respectively. In addition, the filtrates of *G. tsugae* on C6 and Hep 3B cells were 2.81 and 2.80  $\text{mg mL}^{-1}$ , respectively.

### Functional hydrogels

Figure 3a presents the viscoelastic properties of glucan-based matrices prepared with representative lyophilised extracts (H.eri 160, P.ery 160, P.cit 160). Note here that no notable differences in the tendencies of the mechanical spectra were identified when

compared with those performed with their commercial counterparts. In all cases, the elastic modulus was larger than the viscous one and almost frequency independent, which indicates a typical gel character as reported elsewhere (Sovrani *et al.*, 2017). The developed matrices exhibited intermediate gel strength, being the strongest gel features identified for P.ery 160 followed by H.eri 160 and P.cit 160. This effect was noteworthy taking into account the lower extract content in the matrices with P.ery (7.5%) compared to other extracts (25%). This behaviour could be explained by the highest glucan content determined in P.ery 160 and the possible glucan-protein complexes formed, which can be related to the strength of gelled matrices (Rodrigues *et al.*, 2015; Ahmed *et al.*, 2016). For comparative purposes, Fig. 3b shows an overview of the viscoelastic features, in terms of  $G'_{0,1 \text{ Hz}}$ , of the matrices prepared with all tested mushrooms extracts.

The gels made with extracts previously treated at mild autohydrolysis conditions (160 °C) displayed higher viscoelastic modulus (about 1.2 times) than those prepared with extract obtained at 190 °C. Matrices formulated with commercial mushrooms extracts featured weaker gel behaviour. These trends are consistent with those previously reported for bio-hydrogels made with *Hericium erinaceus* enriched in *Paulownia elongata* × *fortunei* (Rodríguez-Seoane *et al.*, 2021). Another advantage of hydrogels prepared with the subcritical water extracts is that they did not present water syneresis for 15 days of cold storage, whereas those made with commercial extracts led to water release (about 10%) after 72 h of refrigerated storage. Overall, the developed gelled matrices could be attractive alternatives bases to formulate savoury/sweet gelled desserts (Torres *et al.*, 2013) or edible films (Larotonda *et al.*, 2016) with application in the food-stuff sector. Food technologists are trying to formulate mushroom beta-glucan fortified functional foodstuffs such as bread, cookies, soups or dairy food products to use not only its potential health benefits but also its gel-forming capacity (Kaur *et al.*, 2019), where the proposed gelled matrices could have another interesting application field.

## Conclusions

Hydrothermal processing with subcritical water was a suitable technique for the extraction of bioactive fractions from edible mushrooms without compromising the viscoelastic features of the corresponding hydrogels. The extracts obtained from different edible mushrooms showed  $\beta$ -glucan content comparable to commercial products and also interesting antioxidant properties, similar to those determined from commercial extracts and even higher in the case of *Pleurotus citrinopileatus* extracts. The hydrogels formulated with these extracts offer advantages in terms of the enhancement of the viscoelastic properties and absence of water release regarding those prepared with commercial extracts. Overall, the subcritical water extraction of valuable fractions from mushrooms promoted the integral valorisation of these natural sources in an environmentally friendly mode that could be applied to other underused resources of interest such as agroforestry, agroalimentary or marine field.

## Acknowledgements

The authors are grateful to FEDER-INTERCONECTA (GALICIA) 2015 (Fungitech-Onco). PRS acknowledges to the Ministry of Economy, Industry and Competitiveness of Spain her PIF grant (BES-2016-076840). M.D.T. thanks the Ministry of Science, Innovation and

Universities of Spain for her postdoctoral grant (RYC2018-024454-I).

## Conflicts of interest

The authors declare no conflicts of interest.

## Author contribution

**Paula Rodríguez-Seoane:** Formal analysis (equal); Investigation (equal); Writing-original draft (equal). **Maria Dolores Torres Perez:** Formal analysis (equal); Investigation (equal); Supervision (equal); Writing-original draft (equal). **Catalina Fernández de Ana:** Conceptualization (equal); Writing-review & editing (equal). **Esteban Sinde-Stompel:** Conceptualization (equal); Writing-review & editing (equal). **Hermínia Dominguez:** Conceptualization (equal); Supervision (equal); Writing-review & editing (equal).

## Ethical approval

Ethics approval was not required for this research.

## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## References

- \*This recent article determined bioactive substances in twelve isolated *Pleurotus eryngii*, including phenolic compounds,  $\beta$ -glucans, and antioxidant activity. This work is interesting to discuss our results.
  - \*This recent article was cited by its relevance within the rheological field of gelled matrices prepared with natural sources, and the potential application of proposed gels in our work to the described edible films.
  - \*This is a review focused in conventional and current applications of edible mushrooms in food industry
  - \*In this research the edible mushroom *Cantharellus tubaeformis* was extracted by the same extraction method that in this current manuscript and the characterization of extracts obtained was also carried out.
  - \*This reference had a relevant impact on the current manuscript, setting the base of the development of glucan-based hydrogels formulated with soluble extracts from hydrothermal treatment of comparable edible mushrooms.
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