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Original article

Drying of extracts from *Coriolus versicolor* using the lyophilisation technique and their application in new industrially produced dehydrated soups

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Summary This research aimed to produce water and ethanol extract of the medicinal mushroom *Coriolus versicolor*, to lyophilise the extracts and to determine the influence of the lyophilised extracts, as a complete substitute for the MSG additive, on the sensory properties of industrially produced dehydrated vegetable soup. Based on SEM analysis, it can be observed that there are differences in the microstructure between the water and ethanol extracts. In the water extract, a great heterogeneity is observed in terms of structural characteristics. Water extract showed identical antimicrobial activity (15.7 mm) against *Enterococcus faecalis* compared to tetracycline, while the ethanol extract had identical antimicrobial activity (14.0 mm) compared to chloramphenicol. Both extracts can be competitive ($P < 0.05$) with BHT in terms of capturing DPPH radicals. According to the results of the sensory analysis, it can be noted that all analysed soups were well received (quality <70%) by the evaluators.

Keywords Bio-soup, chemical additives substitution, extracts, lyophilisation, novel food.

Introduction

Coriolus versicolor (*Trametes versicolor* (L.: Fr) Lloyd, 1920) grows on coniferous and deciduous trees in summer and autumn. It is considered an extremely medicinal mushroom, which has been confirmed during years of research by Chinese and Japanese researchers, which is the reason why numerous clinical trials have recently been conducted to evaluate and identify the main biologically active compounds extracted from this mushroom (Cruz *et al*., 2016).

The choice of appropriate extraction techniques, solvent and extraction parameters plays a crucial role in obtaining high-quality extracts with the desired bioactive compounds. The extraction technique chosen can significantly affect the quality and content of the extract. Lyophilisation is the most commonly used method of encapsulation based on the dehydration by sublimation of a frozen sample (Pudziuvelyte *et al*., 2020).

Traditional methods of food protection try to ensure its safety, by applying more effective synthetic preservatives. However, it was found that these procedures have a lot of drawbacks. In this direction, industrially produced soups usually contain additives to improve the colour and enhance the aroma, and among them, the most commonly used additive is monosodium glutamate (E621). Although it is very effective as a flavour enhancer, it is quite tasteless on its own, giving rise to the famous umami taste. Studies have shown that the human body uses the amino acid glutamate as a neurotransmitter because there are areas in the brain that respond to their impulse. All of these can lead to neurological diseases that have been demonstrated in laboratory animals (Wang *et al*., 2019). Blaylock (1998) indicated that monosodium glutamate is associated with many side effects, including obesity, visual impairment, headache, nausea and depression. Therefore, today numerous studies are increasingly focused on finding different types of supplements that could replace MSG, in industrial products, such as dehydrated soups, without causing negative, unwanted health effects (Farzana *et al*., 2017). Consumers are increasingly interested in bioactive food ingredients that have positive effects on human health and that reduce the risk of various diseases. One such food additive is mushrooms (Kumar, 2015).

The aim of this research was to produce water and ethanol extract of the medicinal mushroom *Coriolus*

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versicolor, to perform lyophilisation of the extracts and to determine the influence of the lyophilised extracts, as a complete substitute for the MSG additive, on the sensory properties of industrially produced dehydrated vegetable soup.

Material and methods

Coriolus versicolor (L.) Lloyd, a medicinal mushroom, was used, collected from Maleševska mountain – Klepalo, at an altitude of 1340 m, in a beech forest (*Fagetum*), from the trunk of *Fagus sylvestris* (coordinates: 41.6648759, 22.9863181).

Preparation of water extract and ethanol extract

The water extract was prepared according to Slawińska *et al.* (2013) and Ribeiro *et al.* (2015). The ethanol extract was prepared according to Vidović (2011).

Scanning electron microscopy (SEM)

Extracts were imaged with an OXFORD Instruments X-Act scanning electron microscope, 51-ADD0007. The samples were placed on a sample support previously coated with double-layer adhesive carbon tapes. The extracts were then sputtered with gold powder at a speed of 100 s/30 mA. The samples were taken under different magnifications.

Lyophilisation of extracts

The lyophilisation procedure of the extracts was performed using a Labconco FreeZone 2.5 (US) lyophiliser (Labcono, Kansas City, MO, USA). Extracts were frozen at a temperature of −80 °C for 24 h. The dish was then subjected to a freezing program at a temperature of −50 °C for 2 h. In the following, the primary phase of vacuum drying was carried out, with the aim of faster expulsion of air molecules, easier and faster penetration of heat through the sample, and faster evaporation of an unnecessary amount of water. This phase was performed under a pressure of 0.01 mBar, at a temperature of 5 °C, for 10 h. Finally, in the phase of secondary drying of the sample, the temperature was 40 °C for 5–7 h.

Antimicrobial potential of lyophilised extracts

The antimicrobial activity of mushroom extracts was determined using the disc-diffusion method (Klaus *et al.*, 2015).

Antioxidant potential of lyophilised extracts

The antioxidant activity of water and ethanol extracts was determined using two methods: the ability to capture-free DPPH radicals and antioxidant activity in the linolenic acid system according to Stojanova *et al.* (2021).

Production of dehydrated soups enriched with lyophilised water and ethanol mushroom extracts

Dehydrated soups were produced in the industry in North Macedonia. The control variant was produced according to the following recipe: mixed dried vegetables (carrot, parsnip, onion and parsley); salt; sugar; oil; monosodium glutamate and pasta. The recipe was modified so that instead of monosodium glutamate, lyophilised water and ethanol extracts were added, separately. Due to the characteristic taste, compensating for the absence of monosodium glutamate and improving the sensory properties of the final product, dried and ground boletus (*B. edulis*) commercially available was added. After production, the soups were packed in 45 g bags and stored at room temperature until further analysis.

The realisation of the planned research was carried out by designing three types of dehydrated soup:

- Type 1 – control variant;
- Type 2 – dehydrated vegetable soup without monosodium glutamate, enriched with lyophilised water extract of the medicinal mushroom *Coriolus versicolor*;
- Type 3 – dehydrated vegetable soup without monosodium glutamate, enriched with lyophilised ethanol extract of the medicinal mushroom *Coriolus versicolor*.

Sensory analysis of dehydrated soups enriched with lyophilised mushroom extracts

The sensory properties of the finished product were evaluated according to the scoring method (Radovanović & Popov-Raljić, 2001). The evaluation was attended by 20 consumers. Mineral water and slices of bread were also available to the raters to neutralise the taste between the ratings of all samples (Sharif *et al.*, 2016). The assessment was made on a scale from 0 to 5. The percentage of the maximum possible quality is calculated, which is the ratio of the weighted average value (WAV) and the maximum score (5):

$$\mathrm{WAV}/5 \times 100$$

Statistical analysis

To determine the statistically significant differences ANOVA test *post hoc* Tuckey's test ($P < 0.05$) was made, using SPSS 20.

Results and discussion

Scanning electron microscopy (SEM)

SEM is a powerful research technique that uses a focused electron beam to obtain complex magnified images (up to 500 000 times) of the surface topography of a sample. This enables the estimation of quantitative parameters of the sample: number of connected pores, average pore length, average pore diameter and specific surface area (Khaund & Joshi, 2014; Milojković, 2015).

Based on the images obtained by SEM analysis, it can be observed that there are differences in the microstructure between the water and ethanol extracts. In the water extract (Fig. 1), a great heterogeneity is observed in terms of structural characteristics. Namely, the presence of rod-like structures that form a network and are the result of hydrogen bonds formed between polysaccharide molecules is observed. On the other hand, a certain porosity of the structure is observed, the pores of which are of different sizes. Pore formation is mainly due to N-H bonds of protein molecules or C-O bonds of polysaccharides (Widjanarko *et al*., 2011). A similar microstructure was observed in the ethanol extract (Fig. 2).

Different mushroom treatments in terms of the use of extractants and process temperature have a key influence on the formation of the microstructure of the final products (Tian *et al*., 2016).

Morphological changes of polysaccharides in mushroom extracts can be qualitatively analysed with SEM imaging (Zhang *et al*., 2019). According to the authors, SEM analysis (×500) of the water extract of the *P. baumii* mushroom showed that the extract has a fragmented honeycomb structure. At higher magnification (×1000), an irregular morphological structure was observed, as well as the appearance of lumps of different sizes.

Antimicrobial potential of lyophilised extracts

Numerous studies indicate the antimicrobial effect of mushrooms and their extracts against Gram-positive, and Gram-negative bacteria, as well as against

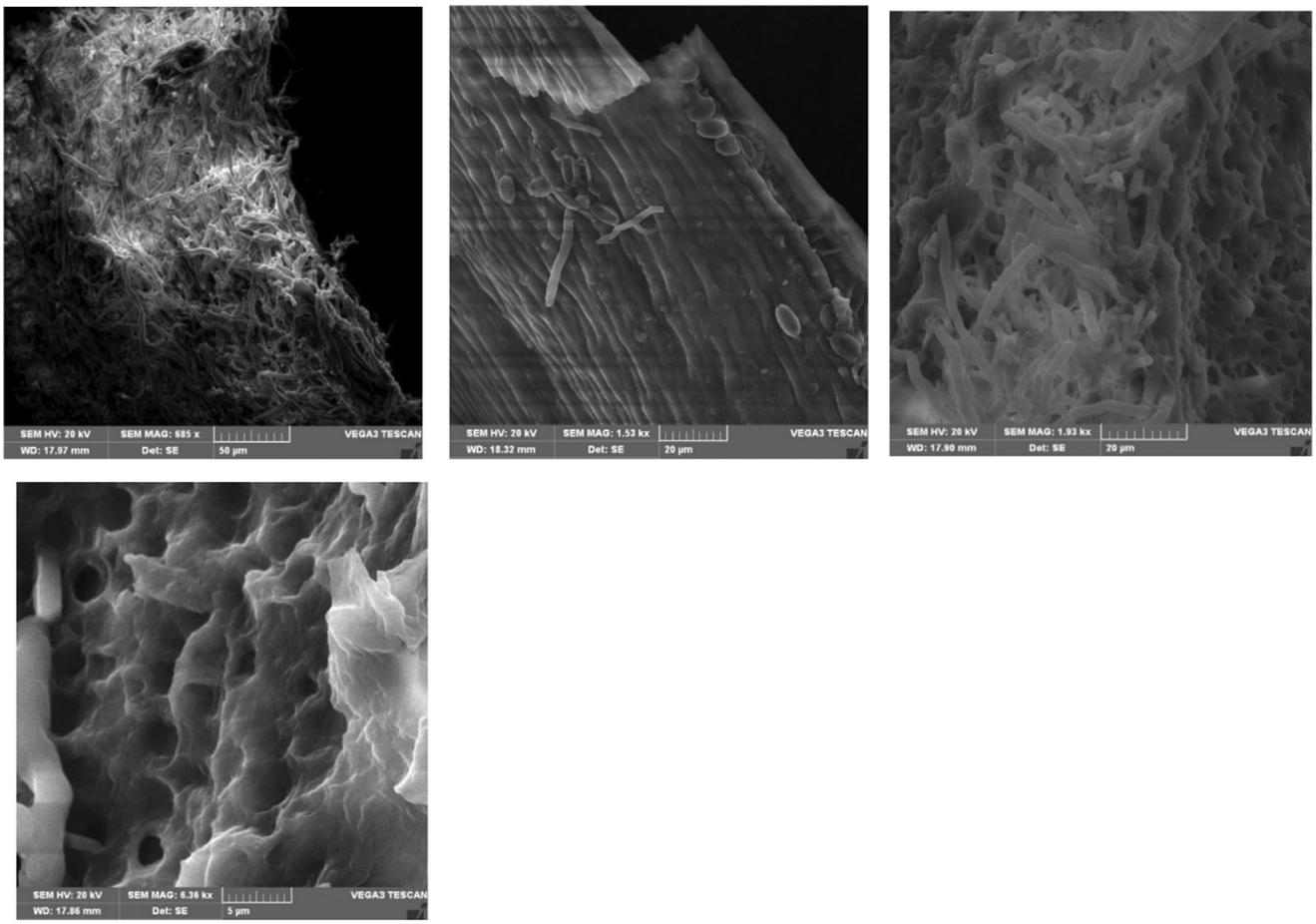

Figure 1 SEM analysis of *Coriolus versicolor* water extract.

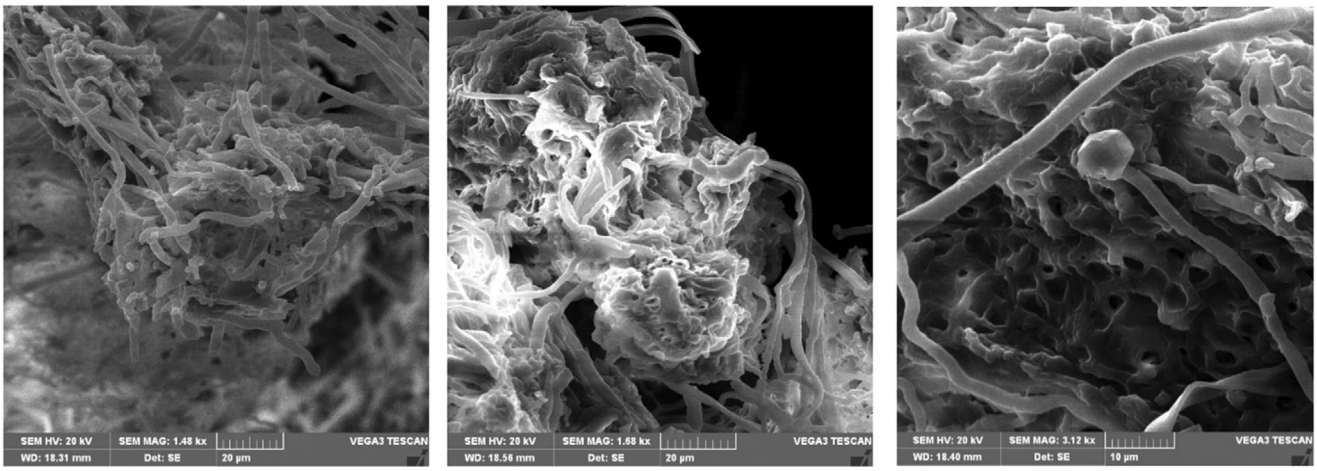

Figure 2 SEM analysis of *Coriolus versicolor* ethanol extract.

pathogenic fungi. Mushroom extracts generally show better antimicrobial activity against Gram-positive bacteria compared to Gram-negative bacteria (Stojanova *et al*., 2024).

According to data presented in Table 1 can be seen that water extract was characterised by higher ($P < 0.05$) antimicrobial potential against most of the tested microorganisms compared to the ethanol

Table 1 Antimicrobial activity of lyophilised water and ethanol extracts of *Coriolus versicolor* (mm)

Microorganism	*n*	Water extract $\bar{x} \pm$ SD	Ethanol extract $\bar{x} \pm$ SD	Tetracycline 30 µg disc^{-1} $\bar{x} \pm$ SD	Chloramphenicol 30 µg disc^{-1} $\bar{x} \pm$ SD	Bifonazole 30 µg disc^{-1} $\bar{x} \pm$ SD
Staphylococcus aureus ATCC 25923	3	14.5 ± 0.00^{a}	9.2 ± 0.03^{b}	30.0 ± 0.02^{c}	21.5 ± 0.02^{d}	n.d.
Bacillus cereus ATCC 10876	3	9.3 ± 0.00^{a}	10.7 ± 0.02^{b}	11.8 ± 0.01^{c}	19.0 ± 0.04^{d}	n.d.
Listeria monocytogenes ATCC 19115	3	7.1 ± 0.01^{a}	14.0 ± 0.01^{b}	15.0 ± 0.03^{c}	14.1 ± 0.01^{b}	n.d.
Listeria ivanovii ATCC 19119	3	9.0 ± 0.02^{a}	7.0 ± 0.01^{b}	14.9 ± 0.06^{c}	14.0 ± 0.03^{d}	n.d.
Enterococcus faecalis ATCC 29212	3	15.7 ± 0.02^{a}	14.8 ± 0.01^{b}	16.0 ± 0.02^{a}	17.7 ± 0.02^{d}	n.d.
Salmonella Enteritidis ATCC 13076	3	17.8 ± 0.02^{a}	14.5 ± 0.01^{b}	21.4 ± 0.01^{c}	25.2 ± 0.02^{d}	n.d.
Escherichia coli ATCC 11230	3	5.6 ± 0.01^{a}	8.0 ± 0.06^{b}	11.0 ± 0.02^{c}	12.5 ± 0.02^{d}	n.d.
Yersinia enterocolitica ATCC 27729	3	14.2 ± 0.02^{a}	13.5 ± 0.02^{a}	27.1 ± 0.01^{c}	26.8 ± 0.02^{c}	n.d.
Shigella sonnei ATCC 29930	3	10.9 ± 0.01^{a}	9.2 ± 0.03^{a}	11.8 ± 0.01^{c}	13.1 ± 0.01^{d}	n.d.
Proteus vulgaris ATCC 8427	3	10.4 ± 0.01^{a}	13.8 ± 0.03^{b}	18.7 ± 0.01^{c}	16.3 ± 0.01^{d}	n.d.
Candida albicans ATCC 10259	3	19.2 ± 0.07^{a}	19.0 ± 0.02^{a}	n.d.	n.d.	33.5 ± 0.01^{b}
Cryptococcus neoformans ATCC 76484	3	n.d.	n.d.	n.d.	n.d.	28.0 ± 0.03

^{a,b,c,d}Values for the different extract and the same microorganism marked with different letters are statistically significantly different, ANOVA, post hoc Tukey's test ($P < 0.05$); n.d., not determined.

extract: *Staphylococcus aureus* (14.5 mm), *Listeria ivanovii* (9.0 mm), *Enterococcus faecalis* (15.7 mm), *Salmonella* Enteritidis (17.8 mm), *Yersinia enterocolitica* (14.2 mm), *Shigella sonnei* (10.9 mm) and *Candida albicans* (19.2 mm).

What is particularly important to emphasise is that the water extract showed identical antimicrobial activity (15.7 mm) against *Enterococcus faecalis* compared with the antibiotic tetracycline, while the ethanol extract had identical antimicrobial activity (14.0 mm) compared to the antibiotic chloramphenicol.

The obtained data on the antimicrobial activity of the examined extracts in this research largely coincide with the literature data. Many authors report that water extracts show strong antimicrobial activity. It is believed that the presence of carbohydrates (especially glucan), proteins, phenols, terpenes and other compounds, as well as their forms, influence the antimicrobial and antifungal activity of the mushroom (Ishmael *et al*., 2017). These components are also called secondary metabolites, and their primary function is to protect the fungus from negative external influences.

But on the other hand, they act identically to synthetic antibiotics and show strong antimicrobial activity (Özcan & Ertan, 2018). Zaidi *et al*. (2013) investigated the antimicrobial effect and biological potential of the water extract of the mushroom *C. versicolor*. The authors point out that the lowest MIC values (3 and 6 mg mL^{-1}) were shown by the extracts against pathogenic *S. pyogenes* and *Microsporum gypsum*, respectively. Hleba *et al*. (2014) investigated the antimicrobial effect of methanol extracts of *G. lucidum* and *C. versicolor* mushrooms. The authors explain that the greatest antimicrobial effect of the investigated extracts was determined against the yeast *S. cerevisiae* with MIC50 value of 24 μg mL^{-1} for the extract of *C. versicolor*.

Antioxidant potential of lyophilised extracts

Fungi can accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. Numerous authors confirm that their phenolic compounds show outstanding antioxidant activity and are not mutagenic (Ivone *et al*., 2016).

In terms of the ability to capture DPPH radicals (Fig. 3), it can be seen that slightly higher values were obtained for the water extract (79.05% at 10 mg mL^{-1}), compared with the ethanol extract (78.63% at 10 mg mL^{-1}). Nevertheless, it can be seen that both extracts can be competitive ($P < 0.05$) with BHT, but none of them had higher values than alpha-tocopherol.

According to Fig. 3 (right) can be seen that slightly higher values were obtained for the ethanol extract (75.07% at 10 mg mL^{-1}), compared with the water extract (74.71% at 10 mg mL^{-1}). Both extracts had higher values compared to ascorbic acid and alpha-tocopherol as a positive control.

Akgul *et al*. (2017) found that the ethanol extract of *C. versicolor* had higher DPPH activity (26.77%) compared to the ethanol extract of *A. auricula* (24.50%) at a concentration of 1 mg mL^{-1}, which is a lower value compared with the ethanol extract of *C. versicolor* in this study. The authors also point out that the ability to capture free DPPH radicals in the ethanol extract of the mushroom is directly proportional to the concentration, which is in agreement with the results obtained for all the ethanol extracts of this research.

Knezević *et al*. (2018) researched several parameters of selected *Trametes* mushroom species from Serbia. Based on the FRAP test authors obtained a value of 0.037 mM for the ethanol extract from *C. versicolor*. Considering that lyophilisation is a method whose sole objective is to remove water, without the use of high temperature and with the application of controlled

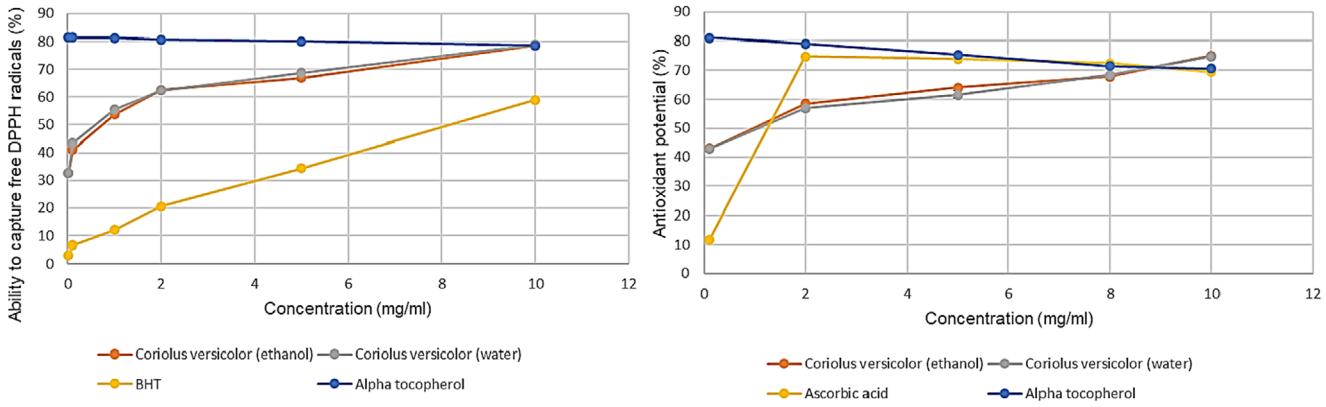

Figure 3 Ability of lyophilised extracts to capture DPPH radicals (left) and ($\bar{x} \pm$ SD) antioxidant activity of the lyophilised extracts in the system of linoleic acid (right) ($\bar{x} \pm$ SD).

pressure, the chemical composition, antimicrobial and antioxidant potential of the final product may be unchanged or may increase in value, as a result of the reduced amount of water and concentration present chemical and nutritional parameters, which fully matches the results obtained in this research. During lyophilisation, the water content decreases, without changing the stability of the present components and their activity (Fissore & Piano, 2015).

Sensory analysis of dehydrated soups enriched with lyophilised mushroom extracts

Quality properties of food are the sum of characteristics that are acceptable to consumers. Sensory evaluation is a key component in the development of new food products by setting standards for testing, analysing and interpreting sensory results. Appearance is the first characteristic perceived by human senses and plays an important role in the identification and final choice of food. The first impression of a meal is the most influential in stimulating appetite resulting in satisfaction or rejection of the product (Sharif *et al.*, 2016).

In Tables 2 and 3, the results of the different sensory properties of Bio soups are shown. According to these values, it can be concluded that the control soups on the 0th day of production were characterised by the highest WAV (3.91) and the highest possible quality

Table 2 Average values of sensory analysis of Bio-soups (day 0 of production)

			Type 1 (control)		Type 2		Type 3	
Sensory properties	***n***	**CI**	**O**	**C** $\bar{x} \pm$ **SD**	**O**	**C** $\bar{x} \pm$ **SD**	**O**	**C** $\bar{x} \pm$ **SD**
Colour	20	3	3.40	10.20 ± 0.05^{a}	3.27	9.81 ± 0.20^{b}	3.31	9.93 ± 0.07^{b}
Smell	20	4	4.11	16.44 ± 0.01^{a}	3.78	15.12 ± 0.08^{b}	3.05	12.20 ± 0.35^{c}
Taste	20	5	3.90	19.50 ± 0.10^{a}	3.05	15.25 ± 0.03^{c}	3.19	15.95 ± 0.12^{c}
Consistency	20	3	4.07	12.21 ± 0.09^{a}	4.00	12.00 ± 0.01^{a}	4.17	$12.51 \pm 0,09^{b}$
Appearance	20	4	3.90	15.60 ± 0.31^{a}	4.28	17.12 ± 0.02^{b}	4.21	16.84 ± 0.15^{c}
Overall acceptability	20	1	4.35	4.35 ± 0.09^{a}	4.30	4.30 ± 0.02^{a}	4.12	4.12 ± 0.10^{a}
Total CI		20						
WAV				3.91 ± 0.02^{a}		3.68 ± 0.06^{b}		3.58 ± 0.02^{c}
% maximum possible quality				78.30 ± 0.01^{a}		73.60 ± 0.06^{b}		71.55 ± 0.05^{c}

[a,b,c]Values for the different soup types and the same sensory property marked with different letters are statistically significantly different, ANOVA, post hoc Tukey test ($P < 0.05$). C, corrected grade; CI, coefficient of importance; O, average grade; WAV, weighted average value.

Table 3 Average values of sensory analysis of Bio-soups (day 90 of production)

			Type 1 (control)		Type 2		Type 3	
Sensory properties	***n***	**CI**	**O**	**C** $\bar{x} \pm$ **SD**	**O**	**C** $\bar{x} \pm$ **SD**	**O**	**C** $\bar{x} \pm$ **SD**
Colour	20	3	3.20	9.60 ± 0.09^{a}	3.06	9.18 ± 0.27^{b}	3.00	9.00 ± 0.21^{c}
Smell	20	4	3.70	14.80 ± 0.05^{a}	3.72	14.88 ± 0.19^{a}	3.09	12.36 ± 0.19^{b}
Taste	20	5	3.55	17.75 ± 0.11^{a}	3.62	18.10 ± 0.24^{b}	3.17	15.85 ± 0.05^{c}
Consistency	20	3	3.80	11.40 ± 0.10^{a}	3.91	11.73 ± 0.13^{b}	4.00	12.00 ± 0.05^{c}
Appearance	20	4	3.69	14.76 ± 0.09^{a}	4.02	16.08 ± 0.06^{b}	4.13	16.52 ± 0.10^{c}
Overall acceptability	20	1	4.17	4.14 ± 0.22^{a}	4.12	4.12 ± 0.28^{a}	3.99	3.99 ± 0.19^{b}
Total CI		20						
WAV				3.62 ± 0.06^{a}		3.70 ± 0.03^{b}		3.48 ± 0.09^{c}
% maximum possible quality				72.48 ± 0.06^{a}		74.09 ± 0.08^{b}		69.72 ± 0.09^{c}

[a,b,c]Values for the different soup types and the same sensory property marked with different letters are statistically significantly different, ANOVA, post hoc Tukey test ($P < 0.05$). C, corrected grade; CI, coefficient of importance; O, average grade; WAV, weighted average value.

(78.30%) compared with the soups from type 2 (3.68; 76.30%) and type 3 (3.55; 71.55%).

On the other hand, on the 90th day of production, the values for WAV decreased in the order: type 2 (3.70) > type 1 (3.62) > type 3 (3.48), and accordingly the values for maximum possible quality are in order: type 2 (74.09%) > type 1 (72.48%) > type 3 (69.72%). Because the new Bio soups have been evaluated with the highest possible quality <70%, it can be concluded that they are well received by the evaluators. According to the results of the sensory analysis, it can be noted that all the analysed soups had a constant quality during the monitoring period, which was also confirmed by the tasters' ratings for the investigated properties.

Aroma is one of the most important sensory properties of a product to which mushrooms have been added. Aromatic compounds are released during the cooking process of food (Sharif *et al*., 2016).

The umami taste of mushrooms is one of the key factors for the formation of the taste and aroma of the final product, and is mainly due to the presence of sodium salts, glutamic (Glu) and aspartic (Asp) amino acids, as well as 5′-nucleotides (Zhang *et al*., 2013). Thus, Mau (2005) divided mushrooms into four groups based on equivalent concentrations for umami taste (EUC): >1000 g, 100–1000 g, 10–100 g and < 10 g MSG/100 g d.m. According to this classification, dried (hot air) fruiting bodies of the mushroom *S. graoulatus* have an EUC value of 274 g MSG/100 g d.m. (Zhao *et al*., 2020).

More and more manufacturers in different countries are putting MSG out of use, one of the main components responsible for the acceptable taste of the end products, due to the growing number of studies that indicate its negative impact on the health of consumers. In this direction, Wang *et al*. (2019) suggest that a suitable replacement for MSG in dehydrated soups may be 0.1% mushroom concentrate, 0.025% yeast extract or 0.2% tomato concentrate. In doing so, identical sensory effects in terms of taste are obtained, as when using 0.1% MSG (Farzana *et al*., 2017; Wang *et al*., 2019).

Given that there is no data in the literature so far about soups that are enriched with *C. versicolor* mushroom extracts, the results obtained in this scientific paper were compared with publications about soups that have the most similar characteristics to this research.

Lawal *et al*. (2018) produced dehydrated vegetable soup with the addition of leaves of traditional Asian plants: marugbo (*Clerodendrum volubile*), tete (*Amaranthus hybridus*) and ila (*Abelmoschus esculentus* L.), and concluded that all three varieties showed antimicrobial activity. Namely, the soup with the addition of ila, marguba and tea, showed antimicrobial activity against the bacteria *E. coli* (11.87, 15.50 and 13.00 mm, respectively), as well as against the bacteria *S. aureus*. From the perspective of the DPPH test, the soup with the addition of theta leaves showed the strongest antioxidant effect (31.00%).

The study by Farzana *et al*. (2017) investigated the formulations of dehydrated soup with the addition of soy flour, moringa leaf and mushroom (*P. ostreatus*). From the aspect of sensory analysis, several properties were evaluated, such as colour (average score 8.5), texture (average score 8.3), smell (average score 8.4), taste (average score 8.6), consistency (average score 8.5) and overall acceptability (average score 8.5).

Conclusion

According to the obtained results of this research, it can be concluded that the water and ethanol lyophilised extract of *Coriolus versicolor* is suitable to be a complete substitute for the monosodium glutamate additive in vegetable dehydrated broth produced in industrial conditions.

Based on the images obtained by SEM analysis, it can be observed that there are differences in the microstructure between the water and ethanol extracts. In the water extract of the mushroom *C. versicolor*, a great heterogeneity is observed in terms of structural characteristics. The presence of rod-like structures that form a network and are the result of hydrogen bonds formed between polysaccharide molecules is observed. Water extract showed identical antimicrobial activity (15.7 mm) against *Enterococcus faecalis* compared with tetracycline, while the ethanol extract had identical antimicrobial activity (14.0 mm) compared with chloramphenicol. Both extracts had higher values compared to ascorbic acid and alpha-tocopherol in the linoleic acid system test, while both extracts can be competitive ($P < 0.05$) with BHT in terms of capturing DPPH radicals. Because the new Bio soups have been evaluated with the highest possible quality <70%, it can be concluded that they are well received by the evaluators. According to the results of the sensory analysis, it can be noted that all the analysed soups had a constant quality during the monitoring period.

Because Bio-soup is the first industrial product of its kind, it opens up new possibilities for replacing synthetic additives with natural components, and also for enriching industrial products with biologically active components. This paves the way for the industrial method of obtaining completely healthy, high-quality and safe products, which leads to the encouragement of consumer habits for daily consumption of healthy products, which in the long term affect the health and well-being of the population.

Author contributions

Monika Stojanova: Investigation; writing – original draft; formal analysis; data curation; methodology.

Milena Pantic: Conceptualization; methodology; supervision; writing – review and editing. **Blazo Boev:** Formal analysis. **Dragana Mihajlovic:** Methodology; writing – review and editing. **Marina Todor Stojanova:** Methodology; formal analysis. **Miomir Niksic:** Supervision; writing – review and editing; methodology.

Conflict of interests

The authors declare there is no conflict of interest.

Ethical declaration

Ethical 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.

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