applied sciences

MDPI

Article

Application of Oyster Mushroom Cultivation Residue as an Upcycled Ingredient for Developing Bread

Hanieh Moshtaghian *, Mohsen Parchami, Kamran Rousta and Patrik R. Lennartsson

Swedish Centre for Resource Recovery, University of Borås, 50190 Borås, Sweden
* Correspondence: hanieh.moshtaghian@hb.se

Abstract: Oyster mushroom (OM) cultivation generates residue that needs to be managed; otherwise, it will be converted into waste. One of the substrates for OM cultivation is the food industry by-product, e.g., a mixture of the brewer's spent grain (BSG) and wheat bran. This study assesses the OM cultivation residue's physical and nutritional characteristics as a potential upcycled food ingredient and also considers developing bread from this cultivation residue. The OM was cultivated in a mixture of 55% BSG and 45% wheat bran. After the OM harvest, the cultivation residue (mixture of BSG, wheat bran and mycelium) had a lighter colour and a pleasant aroma compared to the initial substrate. It contained protein (10.8%) and had high niacin (42.4 mg/100 g), fibre (59.2%) and beta-glucan (6.6%). Thiamine, riboflavin and pyridoxine were also present in the cultivation residue. The bread was developed from 50% cultivation residue and 50% wheat flour, and its scores for darkness, dryness, sponginess, sour taste, bitter aftertaste, and aromatic aroma differed from white bread (p-value < 0.05). However, its overall acceptability and liking scores were not significantly different from white bread (p-value > 0.05). Therefore, this OM cultivation residue can be used as a nutritious ingredient; nevertheless, product development should be further explored.

Keywords: oyster mushroom; *Pleurotus ostreatus*; cultivation residue; brewer's spent grain; cereal-based food; upcycled food

Citation: Moshtaghian, H.; Parchami, M.; Rousta, K.; Lennartsson, P.R. Application of Oyster Mushroom Cultivation Residue as an Upcycled Ingredient for Developing Bread. *Appl. Sci.* **2022**, *12*, 11067. https://doi.org/10.3390/app122111067

Academic Editors: María José Jara-Palacios, Berta Baca-Bocanegra and M. Luisa Escudero-Gilete

Received: 26 September 2022
Accepted: 27 October 2022
Published: 1 November 2022

1. Introduction

Mushroom farming is a fast-growing industry. Mushroom production has increased over the last decade, and its global annual production is estimated to be around 9 million tons [1]. An increase in mushroom production generates a significant amount of mushroom cultivation residue, which is commonly disposed of by non-environmentally friendly methods [2]. Mushroom cultivation residue has several applications, including bioremediation, crop production, reuse in the cultivation of the mushroom, feed for animals and fish, pest management and as a source of renewable energy [3]. For example, its residue can be used for the removal of contaminants in water and soil, the cultivation of some vegetables, and the production of biofertilizer and cattle and ruminant feed [3]. *Pleurotus* species is one of the most cultivated mushroom species, contributing to more than 19% of global mushroom production [4]. The oyster mushroom (*Pleurotus ostreatus*) is an edible mushroom that grows on lignocellulosic materials produced in the forest or generated from agricultural and food industry practices [5].

Although the oyster mushroom can utilise the agricultural and food industry waste as a substrate to grow, its cultivation generates residue resulting in agricultural waste. The management of mushroom cultivation residue can be challenging. This residue, a combination of mycelium and lignocellulosic materials, can be disposed of by landfilling and incineration or recycled as compost, fertilizer and bio-fuel [2]. Oyster mushroom cultivation residue can also be used as livestock feed [6,7]. Some of these management options, such as incineration on the open field, have a negative environmental impact but others, such as composting, are more environmentally friendly [2].

Selecting a simple, feasible and environmentally friendly waste management action can help farmers manage mushroom cultivation residue. On the other hand, if the chosen waste management strategy results in the production of a value-added product and revenue generation, it can contribute to the circular economy and lead to farmers' financial gain. The characteristics of cultivation residue materials determine the waste management action and its potential for contribution to the circular economy. For example, if the mushroom is cultivated in agro-industrial residues, the cultivation residue can be used as animal feed [7]. If it is cultivated in the food industry by-products, it may have the potential to be used in upcycled foods. Upcycled foods utilise ingredients that otherwise would not have gone to human consumption [8,9]. These ingredients are unmarketable materials (e.g., by-products from the manufacturing of other food products or scraps of food preparation) that commonly exist in the food supply chain [9].

One of the substrates for oyster mushroom cultivation is a mixture of Brewer's Spent Grains (BSG) and wheat bran [10]. BSG is a by-product of wort production in the brewing industry [11], and wheat bran is the by-product of the conventional milling of wheat grains [12]. BSG consists of 20% protein and 80% partly lignified cell wall material rich in feruloylated (arabinoxylan polysaccharides) [13]. BSG and wheat bran have several nutritional benefits. BSG is high in protein (18%), essential amino acids, fibre [11] and beta-glucan [14]. Wheat bran is also high in fibre and a good source of beta-glucan [12]. Beta-glucan has several health benefits as it is a soluble fibre with functional and bioactive properties, including prebiotic effects [15,16]. After mushroom cultivation, the residue will also contain mycelium. The oyster mushroom mycelium has various nutrients and fibre, including beta-glucan [17,18]. Therefore, oyster mushroom cultivation residue (mixture of BSG, wheat bran and oyster mushroom mycelium) can serve as a nutritious food ingredient.

The mushroom cultivation residue containing BSG is currently used as animal feed [6] and has not been considered for human food production. BSG, wheat bran and oyster mushroom mycelium are edible materials and can be utilised in human food. Therefore, oyster mushroom cultivation residue has the potential to be used for upcycled foods; for example, as a nutritious ingredient in cereal-based products. To our knowledge, this is the first study that considers oyster mushroom cultivation residue as an upcycled food ingredient. This study assesses the physical property and nutritional characteristics of the oyster mushroom cultivation residue as a potential upcycled ingredient and also considers developing a bread prototype from this residue.

2. Materials and Methods

2.1. Oyster Mushroom Cultivation

The oyster mushroom was cultivated in the combination of wheat bran and BSG as a substrate based on a study conducted by Wang, Sakoda and Suzuki [10]. Wheat bran was bought from the market (Lantmännen, Sweden) and BSG was provided by Göteborgs Nya Bryggeri. Wet and hot BSG was collected from the brewery and air-dried. Oyster mushroom mycelium was obtained from the Svamphuset company (Höör, Sweden) to prepare the spawn. For spawn preparation, barley was cooked and sterilised in the autoclave (Systec VX-95, Linden, Germany) at 121 °C for 60 min. Then, oyster mushroom mycelium was transferred to flasks containing 50 g of cooked barley and produced a good quality spawn after two weeks.

In the next step, BSG (55 g) and wheat bran (45 g) were mixed in a cultivation bag. According to Wang, Sakoda and Suzuki [10], the cultivation of oyster mushrooms in this combination results in high biological efficacy. Then, 190 g distilled water was added to this bag and sterilised in the autoclave at 121 °C for 60 min. Thereafter, 15 g of spawn was added to the bag and left in the dark in the incubator (VWR, INCU-Line 150R, Sweden) for four weeks (23–24 °C, 97–98% humidity). When pinheads appeared, some holes were made in the bag to initiate the growth of the fruiting body and bags were placed in the light (16 °C, 97% humidity) for pinheads to turn into fruiting bodies. After 2–3 days, the oyster mushrooms' fruiting bodies were grown out of the bag and harvested (first flush).

For the second flush, the bags were kept in cold water at 5 °C for 24 h, followed by about a one-week waiting period for pinheads to appear again; thus, the process was repeated to get the second round of fruiting bodies.

The biological efficiency was calculated to assess the conversion of dry substrate to the fresh oyster mushroom [19]. For a biological efficacy assessment, the weight of the harvested fresh fruit bodies was divided by the substrate dry weight and expressed as a percentage [19,20]. The first flush biological efficacy and total biological efficacy (from the first and second flush) were calculated. Three bags after the first flush harvest and three bags after the second flush harvest were selected for the assessment.

2.2. Laboratory Analyses

For nutritional analyses, the cultivation residue was collected and dried in a freeze dryer at 0.05 bar and −50 °C (FreeZone, Labconco Co., Kansas City, MO, USA). The nutritional analyses were carried out on the initial substrate (the mixture of BSG and wheat bran) and the cultivation residue (mixture of BSG, wheat bran and mycelium). For the first flush cultivation residue, samples were collected from three bags after harvesting the first flush and were mixed in equal amounts. Similarly, the second flush cultivation residue was the mixture of residues from three bags after harvesting the second flush.

The accredited Eurofins laboratory (Lidköping, Sweden) determined the amino acid profile for the initial substrate and the second flush cultivation residue using the ISO 13903:2005 method. The total essential amino acids were calculated by summing up individual essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). Vitamin Bs (thiamine, riboflavin, niacin and pyridoxine), lutein, and zeaxanthin were also analyzed for the initial substrate and the second flush cultivation residue by the accredited Eurofins laboratory. Thiamine, riboflavin, pyridoxine and niacin were determined by EN 14122:2014, EN 14152:2014, EN 14164:2014 and EN 15652:2009 methods, respectively. The lutein and zeaxanthin content was assessed by AOAC 2005.07, AOAC 941.15/45.1.03 and AOAC 970.64 methods.

The crude protein, dietary fibre and beta-glucan were analysed for the initial substrate, as well as the first and second flush cultivation residues. The crude protein was assessed in duplicates based on nitrogen content quantification using Kjeldahl (Behr Labor-Technik, Düsseldorf, Germany) [21]. The nitrogen content was multiplied by 6.25 [22,23] for conversion to protein. The Total Dietary Fiber Assay Kit (K-TDFR-100A, Megazyme, Ireland) was used for total dietary fibre assessment. This kit quantifies the dietary fibre based on AOAC 991.43 and AACC 32-07.01 approved methods [24]. The Beta-Glucan Assay Kit (K-YBGL, Megazyme, Ireland) was used to determine the beta-glucan content of the samples [25].

2.3. Bread Prototype Development

The food development process has several stages, including concept generation, concept screening, product (prototype) development, product testing, package development, first product run and launch [26]. For the next step of this study, a bread prototype was developed from the oyster mushroom cultivation residue. For bread prototype development, the cultivation residue was milled (Pulverisette14, Fritsch GmbH, Idar-Oberstein, Germany) to turn into flour (particle size: 0.2 mm). This flour was used for bread prototype preparation. The bread was developed by the teacher and students of the bakery and the restaurant program at Almås High School in Borås.

Initially, the bread prototype was prepared with 100% cultivation residue flour; however, this amount of cultivation residue flour was found to be unsuitable for bread production due to unpalatability. In the next step, the bread prototype with 50% cultivation residue flour and 50% refined wheat flour was developed (Figure 1). For the purpose of this study, two types of bread were baked simultaneously in the same conditions: bread prototype (50% refined wheat flour and 50% cultivation residue flour) and white bread (100% refined wheat flour).

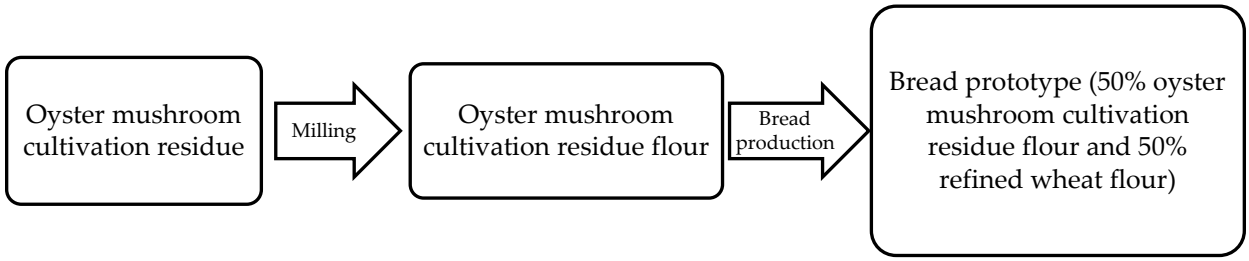

Figure 1. Bread prototype development.

The bread prototype ingredients were 200 g refined wheat flour, 200 g cultivation residue flour, 240 g water, 15 g salt, 15 g rapeseed oil and 20 g yeast. The same ingredients were used for baking white bread, except for the flour, where only refined wheat flour was used. All the ingredients were mixed in a dough mixer for dough preparation to produce a smooth dough. Then, the dough was kneaded lightly and covered with a plastic bag and rested for 5 min. In the next step, the dough was rounded and fermented to reach at least double its size. The fermentation took about 30 min for the bread prototype dough and 40 min for the white bread dough (the bread prototype dough fermented faster). Then, the doughs were baked at 210 °C in the oven for 20 min.

In the next step, the preliminary sensory assessment of the bread prototype (appearance, texture, flavour and aroma) was carried out. The participants of this study were researchers from the University of Borås. Five participants were asked to describe the bread prototype attributes to identify the main sensory characteristics of this bread. Thereafter, 11 participants were asked to assess the sensory characteristics of the bread prototype and white bread. The participants were seven men and four women with postgraduate educations (Master's or PhD degree). They scored the bread prototype and white bread's main sensory characteristics and liking from 0 to 5 (0 = not present, 1 = very low, 2 = low, 3 = neither high nor low, 4 = high, 5 = very high).

2.4. Statistical Analyses

The biological efficacy of each cultivation bag was calculated using Excel 2016 (Microsoft Corp., Redmond, WA, USA), and data were presented as mean and standard deviation (SD). For crude protein analyses, samples were analysed in duplicates, and the mean (SD) was calculated using Excel 2016. Fibre and beta-glucan content of samples was calculated in the Megazyme Mega-Calc^{TM} data calculator for total dietary fibre [27] and beta-glucan [28] assessment. To compare the sensory and liking scores of the bread prototype and white bread, a Wilcoxon Signed Ranks Test was carried out in SPSS (Version 27, SPSS Inc., Chicago, IL, USA). The statistical significance was set at p-value < 0.05.

3. Results

3.1. Mushroom Production and Properties of Cultivation Residue

The mean (SD) of oyster mushroom biological efficacy after harvesting the first flush was 30.2% (3.7), and the total biological efficacy after harvesting two flushes was 82.5% (17.2). It is worth mentioning that no contamination was observed in the cultivation bags. Regarding the physical properties of cultivation residue, this residue contained white oyster mushroom mycelium and had a pleasant aromatic smell. Although the substrate (mixture of BSG and wheat bran) had a dark brown colour, the cultivation residue (mixture of BSG, wheat bran and mycelium) had a lighter brown colour compared to the substrate.

3.2. Nutritional Characteristics

The results of the initial substrate and oyster mushroom cultivation residue's analyses for protein, amino acids, selected vitamin Bs, lutein, zeaxanthin, fibre and beta-glucan are as follows:

3.2.1. Protein and Amino Acid Profile

The protein analyses, as Kjeldahl crude protein, showed an increase in mean (SD) protein content from 18.2% (0.3) in the initial substrate to 20.8% (0.2) and 23.3% (0.1) in cultivation residue after harvesting the first and second flush, respectively. The amino acid profile of the initial substrate and cultivation residue after harvesting the second flush is presented in Table 1. The total amino acid and total essential amino acid content of the initial substrate were 13.7% and 5.2%, respectively. The cultivation residue's total amino acid amount and total essential amino acid amount were 10.8% and 4.2%, respectively. The most abundant amino acid in both samples was glutamic acid, followed by aspartic acid. The amino acids of the initial substrate decreased after oyster mushroom cultivation (except for arginine and threonine), and the highest reduction was observed in glutamic acid.

Table 1. The amino acid profile of initial substrate and cultivation residue after harvesting the second flush of oyster mushrooms.

	Substrate (g/100 g)	Cultivation Residue after 2nd Flush (g/100 g)
Tryptophane	0.26	0.12
Alanine	0.82	0.74
Arginine	0.88	0.91
Aspartic acid	1.17	1.16
Glutamic acid	2.54	1.34
Glycine	0.77	0.72
Histidine	0.37	0.30
Hydroxyproline	<0.20	<0.20
Isoleucine	0.51	0.46
Leucine	1.00	0.78
Lysine	0.72	0.51
Ornithine	<0.05	<0.05
Phenylalanine	0.71	0.56
Proline	0.94	0.60
Serin	0.63	0.57
Threonine	0.58	0.63
Tyrosine	0.45	0.28
Valine	0.79	0.68
Cysteine + Cystine	0.34	0.31
Methionine	0.27	0.18

3.2.2. Vitamin Bs, Lutein and Zeaxanthin

The selected vitamin Bs, lutein and zeaxanthin content of the initial substrate and cultivation residue (after harvesting the second flush) are presented in Table 2. The thiamine and pyridoxine content of the initial substrate decreased after cultivation, whereas its riboflavin and niacin content increased. The niacin content of cultivation residue increased from 3 mg/100 g to 42 mg/100 g after cultivation. The quantity of lutein and zeaxanthin was negligible in both samples.

Table 2. Selected vitamin Bs, lutein and zeaxanthin in the initial substrate and cultivation residue after harvesting the second flush of oyster mushrooms.

	Substrate (mg/100 g)	Cultivation Residue after 2nd Flush (mg/100 g)
Thiamine	0.47	0.04
Riboflavin	0.14	0.57
Niacin	3.01	42.4
Pyridoxine	0.47	0.24
Lutein	<0.02	<0.02
Zeaxanthin	<0.02	<0.02

3.2.3. Fibre and Beta-Glucan

The total dietary fibre content of the initial substrate was 45.6% and increased to 57.9% and 59.2% in cultivation residue after harvesting the first and second flush, respectively. The beta-glucan content of cultivation residue after harvesting the first flush (6.5%) and the second flush (6.6%) was higher than the initial substrate (4.8%).

3.3. Bread Prototype Development

The bread prototype had a golden-brown colour, was darker, and had less puffiness than white bread (Figure 2). The common sensory characteristics of the bread prototype reported by the sensory panel were darkness, low sponginess, dryness, sour taste, bitter aftertaste and aromatic and sour smell. The sensory scores for the bread prototype and white bread are presented in Figure 3. The bread prototype's median score for darkness, dryness, sour taste, bitter aftertaste and aromatic and sour smell was higher than white bread, and for sponginess was lower than white bread. There was a statistically significant difference between these two types of bread in terms of darkness, dryness, sponginess, sour taste, bitter aftertaste and aromatic smell (p-value < 0.05).

(**a**) (**b**)

Figure 2. Bread prototype (**a**) consists of 50% oyster mushroom cultivation residue and 50% refined wheat flour. White bread (**b**) consists of 100% refined wheat flour.

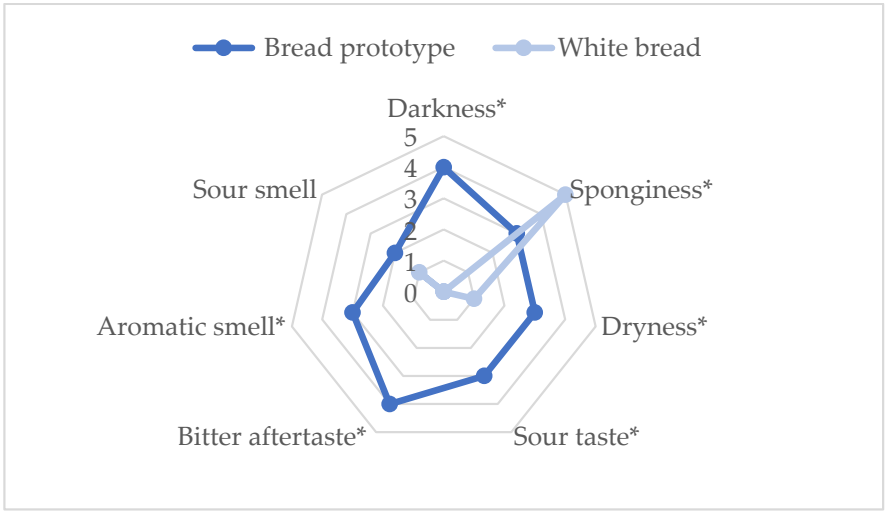

Figure 3. The median score for common sensory characteristics of the bread prototype and white bread (n = 11). The scores were from 0 to 5 (0 = not present, 1 = very low, 2 = low, 3 = neither high nor low, 4 = high, 5 = very high). * Significant difference between bread prototype and white bread: p-value < 0.05.

The liking scores for sensory characteristics and acceptability are shown in Table 3. The bread prototype's median scores for texture, flavour and aroma liking and overall liking and acceptability were lower than for white bread. There was a statistically significant difference between the bread prototype and white bread's flavour liking scores (p-value = 0.019). The overall liking and acceptability scores were not statistically different between these two types of bread (p-value > 0.05).

Table 3. The median scores [1] for the liking and acceptability of the bread prototype and white bread, and the comparison of these two types of bread (n = 11).

	Bread Prototype Median (Min–Max)	White Bread Median (Min–Max)	p-Value *
Appearance liking	4.00 (3–5)	4.00 (3–5)	0.792
Texture liking	3.00 (2–5)	4.00 (2–5)	0.366
Flavour liking	3.00 (1–4)	4.00 (2–5)	0.019
Aroma liking	3.00 (1–4)	4.00 (2–5)	0.072
Overall liking	3.00 (2–5)	4.00 (2–5)	0.141
Acceptability	3.00 (2–5)	4.00 (3–5)	0.248

[1] The scores were from 0 to 5 (0 = not present, 1 = very low, 2 = low, 3 = neither high nor low, 4 = high, 5 = very high). * p-value for comparison between bread prototype and white bread assessed by the Wilcoxon Signed Ranks Test.

4. Discussion

In this study, the oyster mushroom is cultivated in the BSG and wheat bran mixture, and the cultivation residue is used as an ingredient for baking bread. The cultivation residue had high fibre, beta-glucan and niacin content. It also contained protein, thiamine, riboflavin and pyridoxine. The development of bread from 50% cultivation residue and 50% wheat flour resulted in a golden-brown bread with different sensory characteristics in terms of colour, texture, taste and smell compared to white bread. Although the bread prototype's sensory characteristics scores differed from white bread, its overall liking and acceptability scores were not significantly different from white bread.

According to the findings of this study, the biological efficacy of oyster mushroom cultivation in the BSG and wheat bran mixture can be considered good, as the biological efficacy of this type of mushroom varies between less than 30% and more than 100%, depending on the substrate [29]. For example, the oyster mushroom cultivation in maize stalks and wheat straw had a biological efficiency of 27% and 115%, respectively [29]. It is worth mentioning that Wang, Sakoda and Suzuki [10] calculated the biological efficacy after harvesting one flush based on the weight of dried oyster mushrooms; therefore, their results cannot be directly compared to our findings, despite the same substrate being used.

In addition to the substrate type, the biological efficiency depends on the number of flushes and substrate supplementation. The biological efficiency of oyster mushroom cultivation in sugarcane bagasse after harvesting one flush was 50% but increased to 115% after harvesting three flushes [30]. The substrate supplementation with other lignocellulosic materials may also improve the biological efficacy [31]. In this study, total biological efficacy was calculated after harvesting two flushes of mushrooms, and BSG was supplemented with 45% wheat bran.

Some of the cultivation residue's properties, such as a pleasant smell, can be due to the presence of mycelium. Oyster mushroom mycelium contains diverse aromatic compounds, and its olfactory impact is related to *Pleurotus* aroma [32]. The main aromatic compound in oyster mushroom mycelium is octan-3-one, followed by octan-3-ol [32]. The octan-3-one odour has been described as sweet, fruity or mildewy and is responsible for the lemon-like odour [32]. The octan-3-ol contributes to the hazelnut and herbaceous sweet note [32]. Other aromatic compounds are oct-1-en-3-one (cooked mushroom note), octanal (honey-orange like odour) and octanol (orange-rose like odour) [32]. The *Pleurotus* aroma is probably responsible for the bread prototype's aromatic smell, which is one of the sensory characteristics that differentiated this bread from the white bread.

The nutritional assessment of cultivation residue showed an increase in the crude protein from 18% in the initial substrate to 23% in the cultivation residue (after the second flush harvest). In contrast, data from amino acid analyses demonstrated a decrease in protein content (from 13.7% to 10.8%). This inconsistency is due to the protein assessment method. In the crude protein assessment, the total nitrogen content of the product is measured by Kjeldahl, and protein is calculated based on nitrogen content [33]. Therefore, any nitrogen from non-protein sources results in overestimating the protein content [33]. However, calculating the protein content by summing up the amino acids (resulting from protein hydrolysis) does not have this limitation [33]. It is worth mentioning that the crude protein content of wheat flour varies between 10.5% to 13.5% [34]. Thus, the protein content of oyster mushroom cultivation residue is not less than wheat flour.

According to Wang, Sakoda and Suzuki [10], the total amino acid content of the oyster mushroom (grown in BSG and wheat bran mixture) was 34.8 g/100 g, and its essential amino acid content was 12.7 g/100 g. The aspartic acid, glutamic acid, alanine, valine, leucine, lysine and arginine content of the oyster mushroom was more than 2 g/100 g, and its glutamic acid content (5.3 g/100 g) was higher than other amino acids [10]. In fact, glutamic acid is the most abundant amino acid in oyster mushroom [35]. This can justify the decrease in the glutamic acid content of the cultivation residue. The comparison of amino acids between the initial substrate and cultivation residue indicated a decrease in most amino acids (except for arginine and threonine), particularly glutamic acid. It is worth mentioning that when the mushroom grows, it uses the available protein in the substrate and also produces its own protein. Therefore, the change is due to the different amino acids composition in the substrate and the mushroom proteins. In terms of essential amino acids in cultivation residue, although some of them were slightly higher (e.g., lysine, threonine) or lower (e.g., leucine) than wheat flour, the total essential amino acids content of this residue is similar to wheat flour [36].

Regarding the vitamin content of the cultivation residue, the niacin content of the substrate noticeably increased after oyster mushroom cultivation. Since the oyster mushroom has high niacin (90 mg/100 g) content [10], it can be presumed that the substrate is naturally fortified with niacin after cultivating oyster mushrooms due to the presence of mycelium. Although niacin has several health benefits [37], its upper limit of intake is 35 mg per day, and excess intake can result in vasodilatory effects (flushing) [38]. Since 100 g of oyster mushroom cultivation residue contains 42 mg of niacin (above the daily upper limit), this quantity of cultivation residue should not be consumed in a day. The niacin content of wheat flour is 1.6 mg/100 g [34]; thus, developing a bread prototype from a mixture of wheat flour and cultivation residue flour reduces the niacin content of this bread to the safe level.

The cultivation residue's thiamine, riboflavin and pyridoxine content have slightly changed compared to the initial substrate; however, the quantity of these vitamins is not noticeably different from wheat flour [34]. Regarding lutein and zeaxanthin, although wheat bran and barley malt contain lutein and zeaxanthin [39,40], the quantity of these nutrients was negligible in the cultivation residue. Since lutein and zeaxanthin were observed in brewery trub [41] and were not detected in cultivation residue, it can be presumed that they have been lost during the beer production process.

The total dietary fibre and beta-glucan content of cultivation residue were higher than wheat flour. The cultivation residue's dietary fibre was 59%, whereas wheat flour's dietary fibre is 3% [34]. Therefore, the fibre content of cultivation residue is higher than wheat flour, and this residue can be considered a good source of dietary fibre. In terms of beta-glucan, the cultivation residue's beta-glucan was more than 6%, whereas wheat beta-glucan is less than 0.7% [42]. Thus, the beta-glucan content of cultivation residue is noticeably higher than wheat flour. Dietary fibre has several health benefits, and beta-glucan, as a soluble dietary fibre, contributes to colorectal cancer prevention, gut microflora growth, insulin resistance prevention and lowering cholesterol [43]. After oyster mushroom cultivation, the increase in initial substrate fibre and beta-glucan content can be due to mycelium growth.

The oyster mushroom mycelium has high dietary fibre content, including soluble and insoluble fibres [44].

In terms of bread prototype development, some of the sensory characteristics of the bread, such as taste, require further improvement. In previous studies, incorporating BSG in the development of cereal bars [45], cookies [46], pasta [47] and bread [48] modified the sensory characteristics of these foods. For example, the BSG containing foods have a dark colour due to BSG protein content that initiates the Maillard reaction [46]. The bitter aftertaste also relates to BSG polyphenol content [48]. Hence, the quantity of BSG influences the sensory characteristics of upcycled foods, e.g., cookies containing 50% BSG had lower sensory and acceptability scores compared to those with 25% or less BSG [46]. Therefore, decreasing the amount of BSG in the upcycled food results in more similarity between cereal-based upcycled foods and conventional foods. Since using up to 10% BSG in bread produces a sensory-acceptable food [48], making bread from 10% cultivation residue should be investigated in future research.

The incorporation of food by-products in bread production has been investigated in several studies [49–51]. For example, replacing wheat flour with 5% to 10% mushroom stalk or red beetroot powder [49] or 6% cumin and caraway by-products produced sensory-acceptable bread [51]. However, an increase in the percentage contribution of by-products influences the bread's sensory characteristics, such as its sponginess [50]. It appears that replacing flour with $\leq$10% food by-products produces sensory-acceptable bread.

To our knowledge, this was the first study that assessed the oyster mushroom cultivation residue's physical and nutritional properties and investigated its potential as a cereal-based upcycled ingredient for bread production. This study provided an example of a circular economy where BSG, as a by-product of the food industry, was not only used for producing the mushroom but also reused as a nutritious ingredient for the production of bread. The entire process was designed and implemented on a small scale, and it appears to be viable on a large scale; however, its feasibility on a large scale requires further investigation.

Despite the mentioned strengths of this study, there are some limitations. For example, selected nutrients of interest were assessed for the cultivation residue; thus, a complete nutrient profiling is required if this ingredient is to be intended for food production. Moreover, the effect of various quantities of cultivation residue on bread and other cereal-based products' sensory characteristics should be assessed. Furthermore, the acceptability score of the bread prototype was lower than white bread, although this difference was not statistically significant. Therefore, some sensory characteristics of the prototype require improvement. Since this study was a feasibility study, no laboratory measurement for sensory characteristics (e.g., colour and texture determination) was conducted and the sensory data was based on participants' observations. The inclusion of laboratory measurements for sensory characteristics should be taken into consideration in future studies. This study was a preliminary sensory assessment of the bread prototype and although the sample size of the sensory panel was similar to other studies [47,48], a larger group of consumers is needed to evaluate the final product to ensure acceptability.

5. Conclusions

This study presented a feasible strategy that provides an opportunity for farmers to upcycle oyster mushroom cultivation residue for cereal-based food production. Cultivating oyster mushroom in the mixture of wheat bran and BSG provided an upcycled ingredient with higher niacin, fibre and beta-glucan and a similar protein, thiamine, riboflavin and pyridoxine content compared to the wheat flour. Therefore, oyster mushroom cultivation residue can be a nutritious ingredient for human food production, such as cereal-based products. The incorporation of cultivation residue in bread production resulted in the development of golden-grown bread that differed from white bread in terms of sensory characteristics. Although the bread prototype's overall liking and acceptability were not significantly different from white bread, there is a need for improvement in its sensory

characteristics. Future studies should evaluate the incorporation of this upcycled ingredient in different quantities for various cereal-based products and assess its impact on the sensory characteristics of the final food product. Furthermore, the laboratory quantitative sensory assessment of the bread prototype should be taken into consideration to facilitate acceptability.

Author Contributions: Conceptualization, H.M., K.R. and P.R.L.; formal analysis, H.M.; investigation, H.M.; laboratory analyses, H.M. and M.P.; writing—original draft preparation, H.M.; writing—review and editing, H.M., M.P., K.R. and P.R.L.; funding acquisition, P.R.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Swedish Agency for Economic and Regional Growth (Ways2Taste [20201656]).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical reasons.

Acknowledgments: We would like to thank the teachers and students of the bakery and restaurant program at Almås High School (Almåsgymnasiet) in Borås for their contribution to bread prototype development.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kumla, J.; Suwannarach, N.; Sujarit, K.; Penkhrue, W.; Kakumyan, P.; Jatuwong, K.; Vadthanarat, S.; Lumyong, S. Cultivation of Mushrooms and Their Lignocellulolytic Enzyme Production Through the Utilization of Agro-Industrial Waste. *Molecules* **2020**, *25*, 2811. [CrossRef] [PubMed]
2. Wan Mahari, W.A.; Peng, W.; Nam, W.L.; Yang, H.; Lee, X.Y.; Lee, Y.K.; Liew, R.K.; Ma, N.L.; Mohammad, A.; Sonne, C.; et al. A review on valorization of oyster mushroom and waste generated in the mushroom cultivation industry. *J. Hazard. Mater.* **2020**, *400*, 123156. [CrossRef] [PubMed]
3. Rinker, D.L. Spent mushroom substrate uses. *Edible Med. Mushrooms Technol. Appl.* **2017**, *1*, 427–454.
4. Royse, D.J.; Baars, J.; Tan, Q. Current Overview of Mushroom Production in the World. In *Edible and Medicinal Mushrooms*; Wiley: Hoboken, NJ, USA, 2017; pp. 5–13.
5. Sánchez, C. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Appl. Microbiol. Biotechnol.* **2010**, *85*, 1321–1337. [CrossRef]
6. Muthukumarappan, K.; Misra, N. Sustainable Brewing. In *Sustainable Food Processing*; Wiley Online Library: Hoboken, NJ, USA, 2013; pp. 295–312.
7. Philippoussis, A. Production of Mushrooms Using Agro-Industrial Residues as Substrates. In *Biotechnology for Agro-Industrial Residues Utilisation*; Springer: Dordrecht, The Netherlands, 2009; pp. 163–196.
8. Moshtaghian, H.; Bolton, K.; Rousta, K. Challenges for Upcycled Foods: Definition, Inclusion in the Food Waste Management Hierarchy and Public Acceptability. *Foods* **2021**, *10*, 2874. [CrossRef]
9. The Upcycled Foods Definition Task Force. Defining Upcycled Foods. Available online: https://www.chlpi.org/wp-content/uploads/2013/12/Upcycled-Food_Definition.pdf (accessed on 15 January 2021).
10. Wang, D.; Sakoda, A.; Suzuki, M. Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. *Bioresour. Technol.* **2001**, *78*, 293–300. [CrossRef]
11. Fărcaş, A.; Tofană, M.; Socaci, S.; Mudura, E.; Scrob, S.; Salanţă, L.; Mureşan, V. Brewers' spent grain—A new potential ingredient for functional foods. *J. Agroaliment. Process. Technol.* **2014**, *20*, 137–141.
12. Stevenson, L.; Phillips, F.; O'Sullivan, K.; Walton, J. Wheat bran: Its composition and benefits to health, a European perspective. *Int. J. Food Sci. Nutr.* **2012**, *63*, 1001–1013. [CrossRef]
13. Jay, A.J.; Parker, M.L.; Faulks, R.; Husband, F.; Wilde, P.; Smith, A.C.; Faulds, C.B.; Waldron, K.W. A systematic micro-dissection of brewers' spent grain. *J. Cereal Sci.* **2008**, *47*, 357–364. [CrossRef]
14. Steiner, J.; Procopio, S.; Becker, T. Brewer's spent grain: Source of value-added polysaccharides for the food industry in reference to the health claims. *Eur. Food Res. Technol.* **2015**, *241*, 303–315. [CrossRef]
15. El Khoury, D.; Cuda, C.; Luhovyy, B.L.; Anderson, G.H. Beta glucan: Health benefits in obesity and metabolic syndrome. *J. Nutr. Metab.* **2012**, *2012*, 851362. [CrossRef] [PubMed]
16. Lam, K.-L.; Chi-Keung Cheung, P. Non-digestible long chain beta-glucans as novel prebiotics. *Bioact. Carbohydr. Diet. Fibre* **2013**, *2*, 45–64. [CrossRef]
17. Golak-Siwulska, I.; Kałużewicz, A.; Spiżewski, T.; Siwulski, M.; Sobieralski, K. Bioactive compounds and medicinal properties of *Oyster mushrooms* (sp.). *Folia Hortic.* **2018**, *30*, 191–201. [CrossRef]

18. Deepalakshmi, K.; Sankaran, M. *Pleurotus ostreatus*: An oyster mushroom with nutritional and medicinal properties. *J. Biochem. Technol.* **2014**, *5*, 718–726.
19. Chang, S.T.; Lau, O.W.; Cho, K.Y. The cultivation and nutritional value of Pleurotus sajor-caju. *Eur. J. Appl. Microbiol. Biotechnol.* **1981**, *12*, 58–62. [CrossRef]
20. Madan, M.; Vasudevan, P.; Sharma, S. Cultivation of Pleurotus sajor-caju on different wastes. *Biol. Wastes* **1987**, *22*, 241–250. [CrossRef]
21. Bradstreet, R.B. *The Kjeldahl Method for Organic Nitrogen*; Academic Press: Cambridge, MA, USA, 1965.
22. Arauzo, P.J.; Du, L.; Olszewski, M.P.; Meza Zavala, M.F.; Alhnidi, M.J.; Kruse, A. Effect of protein during hydrothermal carbonization of brewer's spent grain. *Bioresour. Technol.* **2019**, *293*, 122117. [CrossRef]
23. Mathias, T.R.d.S.; Alexandre, V.M.F.; Cammarota, M.C.; de Mello, P.P.M.; Sérvulo, E.F.C. Characterization and determination of brewer's solid wastes composition. *J. Inst. Brew.* **2015**, *121*, 400–404. [CrossRef]
24. Megazyme. Total Dietary Fiber Assay Procedure. Available online: https://www.megazyme.com/documents/Assay_Protocol/K-TDFR-200A_DATA.pdf (accessed on 2 March 2022).
25. Megazyme. Mushroom and Yeast Beta Glucan Assay Procedure. Available online: https://www.megazyme.com/documents/Assay_Protocol/K-YBGL_DATA.pdf (accessed on 2 March 2022).
26. Rudder, A.; Ainsworth, P.; Holgate, D. New food product development: Strategies for success? *Br. Food J.* **2001**, *103*, 657–671. [CrossRef]
27. Megazyme. Total, Soluble and Insoluble Dietary Fibre (K-TDFR)-Determination. Available online: https://view.officeapps.live.com/op/view.aspx?src=https%3A%2F%2Fwww.megazyme.com%2Fdocuments%2FData_Calculator%2FK-TDFR-200A_CALC.xlsx&wdOrigin=BROWSELINK (accessed on 2 March 2022).
28. Megazyme. Yeast and Mushroom β-Glucan (K-YBGL)-Instructions. Available online: https://view.officeapps.live.com/op/view.aspx?src=https%3A%2F%2Fwww.megazyme.com%2Fdocuments%2FData_Calculator%2FK-YBGL_CALC.xlsx&wdOrigin=BROWSELINK (accessed on 2 March 2022).
29. Ritota, M.; Manzi, P. *Pleurotus* spp. Cultivation on Different Agri-Food By-Products: Example of Biotechnological Application. *Sustainability* **2019**, *11*, 5049. [CrossRef]
30. Tarko, D.B.; Sirna, A.M. Substrate optimization for cultivation of *Pleurotus ostreatus* on lignocellulosic wastes (coffee, sawdust, and sugarcane bagasse) in Mizan Tepi University, Tepi Campus, Tepi Town. *J. Appl. Biol. Biotechnol.* **2018**, *6*, 14–20.
31. Fanadzo, M.; Zireva, D.; Dube, E.; Mashingaidze, A.B. Evaluation of various substrates and supplements for biological efficiency of Pleurotus sajor-caju and *Pleurotus ostreatus*. *Afr. J. Biotechnol.* **2010**, *9*, 2756–2761.
32. Kabbaj, W.; Breheret, S.; Guimberteau, J.; Talou, T.; Olivier, J.; Sobal, M.; Bensoussan, M.; Roussos, S. Pleurotus Ostreatus Volatile Aroma Compounds Identified from Fruit-Body and from Mycelium Grown in Submerged and Solid-State Cultures. In *New Horizons in Biotechnology*; Springer: Berlin/Heidelberg, Germany, 2003; pp. 371–377.
33. Tontisirin, K.; MacLean, W.C.; Warwick, P. *Food Energy: Methods of Analysis and Conversion Factors: Report of a Technical Workshop, Rome, 3–6 December 2002*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2003.
34. U.S. Department of Agriculture. Flour, Wheat, All-Purpose, Unenriched, Unbleached. Available online: https://fdc.nal.usda.gov/fdc-app.html#/food-details/790018/nutrients (accessed on 11 January 2022).
35. Chirinang, P.; Intarapichet, K.-O. Amino acids and antioxidant properties of the oyster mushrooms, *Pleurotus ostreatus* and Pleurotus sajor-caju. *Sci. Asia* **2009**, *35*, 326–331. [CrossRef]
36. McDermott, E.E.; Pace, J. The content of amino-acids in white flour and bread. *Br. J. Nutr.* **1957**, *11*, 446–452. [CrossRef]
37. MacKay, D.; Hathcock, J.; Guarneri, E. Niacin: Chemical forms, bioavailability, and health effects. *Nutr. Rev.* **2012**, *70*, 357–366. [CrossRef]
38. United States Institute of Medicine. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*; The National Academies Press: Washington, DC, USA, 1998; p. 592.
39. U.S. Department of Agriculture. Wheat Bran, Crude. Available online: https://fdc.nal.usda.gov/fdc-app.html#/food-details/169722/nutrients (accessed on 29 March 2022).
40. U.S. Department of Agriculture. Barley Malt Flour. Available online: https://fdc.nal.usda.gov/fdc-app.html#/food-details/169740/nutrients (accessed on 29 March 2022).
41. Mattioli, S.; Castellini, C.; Mancini, S.; Roscini, V.; Cartoni Mancinelli, A.; Cotozzolo, E.; Pauselli, M.; Dal Bosco, A. Effect of trub and/or linseed dietary supplementation on in vivo oxidative status and some quality traits of rabbit meat. *Meat Sci.* **2020**, *163*, 108061. [CrossRef]
42. Havrlentova, M.; Kraic, J. Content of β-D-glucan in cereal grains. *J. Food Nutr. Res.* **2006**, *45*, 97–103.
43. Ahmad, A.; Anjum, F.M.; Zahoor, T.; Nawaz, H.; Dilshad, S.M.R. Beta Glucan: A Valuable Functional Ingredient in Foods. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 201–212. [CrossRef]
44. Laforteza, J.C.; Reyes, R.G.; Trinidad, T.P. Dietary Fiber Contents and Its Fermentability In Vitro of *Pleurotus ostreatus* cv. Florida Mycelia (Agaricomycetes). *Int. J. Med. Mushrooms* **2020**, *22*, 651–657. [CrossRef]
45. Stelick, A.; Sogari, G.; Rodolfi, M.; Dando, R.; Paciulli, M. Impact of sustainability and nutritional messaging on Italian consumers' purchase intent of cereal bars made with brewery spent grains. *J. Food Sci.* **2021**, *86*, 531–539. [CrossRef]
46. Petrovic, J.; Pajin, B.; Tanackov-Kocic, S.; Pejin, J.; Fistes, A.; Bojanic, N.; Lončarević, I. Quality properties of cookies supplemented with fresh brewer's spent grain. *Food Feed Res.* **2017**, *44*, 57–63. [CrossRef]

47. Schettino, R.; Verni, M.; Acin-Albiac, M.; Vincentini, O.; Krona, A.; Knaapila, A.; Cagno, R.D.; Gobbetti, M.; Rizzello, C.G.; Coda, R. Bioprocessed Brewers' Spent Grain Improves Nutritional and Antioxidant Properties of Pasta. *Antioxidants* **2021**, *10*, 742. [CrossRef]
48. Waters, D.M.; Jacob, F.; Titze, J.; Arendt, E.K.; Zannini, E. Fibre, protein and mineral fortification of wheat bread through milled and fermented brewer's spent grain enrichment. *Eur. Food Res. Technol.* **2012**, *235*, 767–778. [CrossRef]
49. El-Maaty, A.; El-Nemr, S.E.; El-Shourbagy, G.; Galal, G.A. Effect of Addition Oyster Mushroom and Red Beet Root by-Products on Quality of Pan Bread. *Zagazig J. Agric. Res.* **2016**, *43*, 507–517. [CrossRef]
50. Kowalczewski, P.Ł.; Walkowiak, K.; Masewicz, Ł.; Duda, A.; Poliszko, N.; Różańska, M.B.; Jeżowski, P.; Tomkowiak, A.; Mildner-Szkudlarz, S.; Baranowska, H.M. Wheat bread enriched with raspberry and strawberry oilcakes: Effects on proximate composition, texture and water properties. *Eur. Food Res. Technol.* **2019**, *245*, 2591–2600. [CrossRef]
51. Sayed Ahmad, B.; Talou, T.; Straumite, E.; Sabovics, M.; Kruma, Z.; Saad, Z.; Hijazi, A.; Merah, O. Protein Bread Fortification with Cumin and Caraway Seeds and By-Product Flour. *Foods* **2018**, *7*, 28. [CrossRef]