Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biotechnology and Food Science

Brian Stengårdsbakken Aakre Juvik

Quality and acceptance of mushrooms cultivated on spent coffee grounds

Chemical composition analysis of grey oyster mushrooms (*Pleurotus ostreatus*) cultivated on spent coffee grounds, sensory evaluation of five common mushrooms and survey assessment of consumer attitudes towards mushrooms cultivated on coffee grounds

Master's thesis in Chemical Engineering and Biotechnology
Supervisor: Professor Jørgen Lerfall
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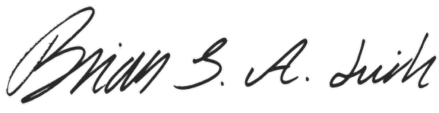
Preface

This Master's thesis was written and conducted by Brian Stengårdsbakken Aakre Juvik during spring 2022. It was performed at the Department of Biotechnology and Food Science, Faculty of Natural Sciences, at Norwegian University of Science and Technology (NTNU). The thesis was submitted in the course "TBT4900 - Biotechnology, Master's Thesis", concluding the 5-year Master's degree program in Chemical Engineering and Biotechnology.

In particular, I would like to thank PhD Candidate Sophie Kendler, for assistance with lipid extraction and total amino acid extraction methodologies. I would also like to thank Head Engineer Anne Kathrine Streitlien for instructions with regard to protein determination and Staff Engineer Martin Haider for assistance with freeze-drying, as well as Engineer Siri Stavrum for performing high-performance liquid chromatography for determination of free and total amino acid compositions. My thanks also go to Assistant Professor/PhD Candidate Lene Waldenstrøm for guidance regarding sensory methodologies.

Finally, I would like to give a special thanks to my supervisor, Professor Jørgen Lerfall, for excellent counselling throughout the period of my Master's project, and especially for providing extensive feedback on drafts of the written thesis.



Trondheim, 22.06.2022
Brian S. A. Juvik

Summary

Mushrooms are a popular as food around the world and are enjoyed because of their flavor, texture and desirable nutritional composition. Cultivating mushrooms on agro-industrial or urban waste is a way of reducing waste while simultaneously growing food. The main focus of the project originally was to compare chemical composition, sensory data and attitudinal data of *Pleurotus ostreatus* cultivated on spent coffee grounds, but due to the supplier having difficulties delivering sufficient amounts of such mushrooms, some parts of the project design had to be adapted. *Pleurotus ostreatus* is commonly known as grey oyster mushroom, which is a white rot fungus with the ability to grow on a multitude of lignocellulosic compounds, as well as urban waste like spent coffee grounds.

The contents of dry matter, ash, lipids and proteins in *Pleurotus ostreatus* cultivated on spent coffee grounds were found through drying, ashing, modified Bligh & Dyer method and modified Kjeldahl method respectively. Compositions of free and total amino acids were found through extraction of amino acids and high-performance liquid chromatography (HPLC), with the HPLC being performed by Siri Stavrum at Department of Biotechnology and Food Science, NTNU, Norway. A check-all-that-apply (CATA) test with 79 subjects was performed on the five common mushrooms *Pleurotus ostreatus* (grey oyster), *Pleurotus eryngii* (king oyster), *Lentinula edodes* (shiitake) and two types of *Agaricus bisporus* (white button mushroom and portobello mushroom), to investigate preference data, sensory attributes and the relation between them. An attitudinal questionnaire was created to investigate consumer attitudes towards mushrooms cultivated on coffee grounds, and it got 182 responses.

The chemical analyses showed significant difference at the 5% level of significance between some of the four types of *Pleurotus ostreatus* for dry matter, ash, lipid and protein content. Protein content was higher in *Pleurotus ostreatus* cultivated on high and low concentrations of spent coffee grounds compared to conventional, while ash content was lower when cultivated on low concentrations of spent coffee grounds compared to high concentration and conventional. For the four types of *Pleurotus ostreatus*, glutamine, glutamic acid and alanine were the most numerous free amino acids, while regarding total amino acids, all four had the most of glutamic acid, followed by aspartic acid and alanine. The results of the CATA showed significantly different mean liking scores at the 5% level, and white button mushroom was best liked, followed by portobello, king oyster, shiitake and grey oyster. Through penalty analysis, "Tastes like mushroom" was found to be a must have attribute, while "rich", "salty" and "dark in color" were nice to have terms, and "bitter", "tame", "soggy" and "earthy" were must not have terms. The results of the survey about consumer attitudes were used to categorize respondents into attitudinal groups, and 155 respondents were positive, 25 were neutral and 2 were negative to mushrooms cultivated on coffee grounds. Socio-demographic questions were used to determine that some groups were over represented among the respondents.

Sammendrag

Sopp er populært som mat i hele verden og nytes på grunn av sin smak, tekstur og gunstige ernæringsmessige sammensetning. Å dyrke sopp på agro-industrielt eller urbant avfall er en måte å redusere mengden avfall samtidig som man dyrker mat. Prosjektets hovedfokus var opprinnelig å sammenligne kjemisk sammensetning, sensorisk data og holdningsdata til *Pleurotus ostreatus* dyrket på kaffegrut, men på grunn av at leverandøren hadde problemer med å levere tilstrekkelige mengder av slik sopp, måtte noen deler av prosjektet bli tilpasset. *Pleurotus ostreatus* er vanligvis kjent som grå østerssopp, som er en hvitråtesopp med evnen til å vokse på en mengde lignocelluloseholdige forbindelser, i tillegg til urbant avfall, som kaffegrut.

Innholdet av tørrstoff, aske, lipider og proteiner i *Pleurotus ostreatus* dyrket på kaffegrut ble funnet gjennom henholdsvis tørking, asking, modifisert Bligh & Dyer metode og modifisert Kjeldahl metode. Sammensetninger av frie og totale aminosyrer ble funnet gjennom ekstraksjon av aminosyrer og høypresisjonsvæskekromatografi (HPLC), med HPLC utført av Siri Stavrum ved Institutt for Bioteknologi og Matvitenskap, NTNU, Norge. En kryss-av-for-alle-som-gjelder (CATA) test med 79 forsøkspersoner ble utført på de fem vanlige soppene *Pleurotus ostreatus* (grå østerssopp), *Pleurotus eryngii* (kongeøsterssopp), *Lentinula edodes* (shiitake) og to typer *Agaricus bisporus* (champignon og portobellosopp), for å undersøke preferansedata, sensoriske egenskaper og forholdet mellom dem. Det ble laget en spørreundersøkelse for å undersøke forbrukeres holdninger til sopp dyrket på kaffegrut, og den fikk 182 svar.

De kjemiske analysene viste signifikant forskjell på 5% signifikansnivå mellom noen av de fire typene av *Pleurotus ostreatus* for tørrstoff-, aske-, lipid- og proteininnhold. Proteininnholdet var høyere i *Pleurotus ostreatus* dyrket på høye og lave konsentrasjoner av kaffegrut sammenlignet med konvensjonell, mens askeinnholdet var lavere når soppen ble dyrket på lave konsentrasjoner av brukt kaffegrut sammenlignet med høy konsentrasjon og konvensjonell. For de fire typene *Pleurotus ostreatus* var glutamin, glutamat og alanin de mest tallrike frie aminosyrene, mens når det gjelder totale aminosyrer, hadde alle fire mest glutamat, etterfulgt av aspartat og alanin. Resultatene fra CATA viste signifikant forskjellige gjennomsnittlige preferanseverdier på 5% signifikansnivå, og champignon ble best likt, etterfulgt av portobellosopp, kongeøsterssopp, shiitake og grå østerssopp. Gjennom analyse av sammenhengen mellom egenskaper og preferanser, ble "Tastes like mushroom" funnet til å være en egenskap sopp må ha, mens "rich", "salty" og "dark in color" var egenskaper soppen fint kan ha, og "bitter", "tame", "soggy" og "earthy" var egenskaper soppen ikke burde ha. Resultatene fra undersøkelsen om forbrukerholdninger ble brukt til å kategorisere respondentene i holdningsgrupper, og 155 respondenter var positive, 25 var nøytrale og 2 var negative til sopp dyrket på kaffegrut. Sosiodemografiske spørsmål ble brukt for å fastslå at noen grupper var overrepresentert blant respondentene.

List of Abbreviations

NTNU	Norwegian University of Science and Technology
HPLC	High-performance liquid chromatography
CATA	Check-all-that-apply
SCG	Spent coffee grounds
ICP-MS	Inductive coupled plasma-mass spectrometry
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectroscopy
AD	Anno Domini
PAH	Polycyclic aromatic hydrocarbon
MSG	Monosodium glutamate
EU	European Union
EFSA	European Food Safety Authority
WCOT	Wall-coated open tubular
FSWC	Fused-silica wall-coated
SCOT	Support-coated open tubular
MS	Mass spectrometer
FID	Flame ionization detector
UV	Ultraviolet
WSP	Water-soluble proteins
rpm	Revolutions per minute
pH	Potential of hydrogen
AA	Amino acid
FAA	Free amino acids
TAA	Total amino acids
ANOVA	Analysis of variance

List of Figures

List of Tables

Contents

1 Introduction

Mushrooms are a mass marketed product worldwide, and the mushroom industry is expected to grown in the coming years due to the increasing vegan population requiring a protein-rich food source[1]. Lignocellulosic waste products are a growing environmental concern[2], which *Pleurotus ostreatus* (*P. ostreatus*) can help relieve. *P. ostreatus* have shown their ability to utilize multiple types of lignocellulosic materials from agro-industrial waste as substrate materials[3]. By doing this, it is possible to reduce waste while simultaneously turning the organic leftover materials in the waist into nutrient-rich foods, safe for human consumption. Cultivation of *P. ostreatus* on spent coffee grounds (SCG) have also shown that the mushroom can be used to recycle urban waste[4]. The effect of spent coffee grounds in substrate on the chemical composition and flavor profile of *P. ostreatus* will provide useful insight for further development of food products containing such mushrooms.

1.1 Project design and mushroom procurement difficulties

The project was originally supposed to investigate three aspects of spent coffee grounds in cultivation substrate of *P. ostreatus*, which were chemical composition of the fruiting bodies, how sensory attributes changes and consumer attitudes towards mushrooms cultivated on coffee grounds. The Norwegian mushroom farming company Toppsopp[5] was contacted, and they agreed to cultivate *P. ostreatus* on substrates containing different concentrations of spent coffee grounds. However, due to unforeseen circumstances, they were not able to supply sufficient amounts of mushrooms for the planned project design. Because of this, the sensory analysis design was adapted to use five commercially available mushrooms, which could provide useful information on a more general level about beneficial and detrimental attributes in mushrooms, while some chemical analyses had to be cut. The cut analyses were analysis of certain trace elements (arsenic, cadmium, mercury and lead) with inductive coupled plasma-mass spectrometry (ICP-MS) and analysis of volatile aromatic compounds with gas chromatography-mass spectroscopy (GC-MS). However, the executed experiments in the project provided substantial data for analysis within the scope.

1.2 Objectives and research aims

The adapted project design consisted of three main objectives. The first objective was to study the effect spent coffee grounds in mushrooms substrate had on chemical composition of *P. ostreatus*. This was done through determination of dry matter, ash, lipid and protein content in four types of *P. ostreatus*, three of which were cultivated on different substrates containing spent coffee grounds and one type being a conventional *P. ostreatus* from a grocery store. The second objective, which was adapted due to the lack of spent coffee ground mushrooms, was to investigate the appreciation and perceived attributes of the

five common mushrooms *Pleurotus ostreatus*, *Pleurotus eryngii*, *Lentinula edodes* and two types of *Agaricus bisporus* (white button mushroom and portobello mushroom). The third objective was to look into consumer attitudes towards mushrooms cultivated on coffee grounds to investigate the potential use of such mushrooms in the food industry.

2 Theoretical background

2.1 Edible mushrooms usage worldwide

Edible mushrooms are popular as food worldwide, well appreciated for their taste and aroma. The most cultivated mushrooms are button mushrooms (*Agricus bisporus*), oyster mushrooms (*Pleurotus spp.)* and shiitake mushrooms (*Lentinula edodes*)[6]. Data from 2007 to 2017, showed that Asia cultivated the most mushrooms on a worldwide basis (76.0%), followed by Europe (17.2%), America (5.9%), Oceania (0.6%) and Africa (0.2%)[7]. Historically, mushrooms were used both for food and medicine. Application of mushroom as a health food was documented in China as early as 100 AD. Mushrooms were used in herbal formulas, health tonics and health beneficial food dishes[8].

Mushrooms also have desirable nutritional value. Protein content is variable across mushroom species, but it is typically 19-35% of dry weight[9]. Mushrooms contain all essential amino acids, and they make up 25-40% of the amino acid profile. Mushroom contain little fat, with an estimate around 2-8% of dry weight. In general, mushrooms contain 85-95% water[10].

2.2 Oyster Mushrooms

Pleurotus ostreatus, also known as grey oyster mushrooms or pearl oyster mushrooms, are primary decomposers, and they typically grow on dead organic materials, like oak or maple[11]. *P. ostreatus* belong to the group white-rot fungi, which are effective lignin degraders[12]. Sánchez (2009)[13] presented a mechanism for the degradation of lignin by white-rot fungi, shown in figure 2.1. The mechanism shown by Sánchez (2009) in figure 2.1 is an updated version of the mechanism by Martìnes et al (2005)[14].

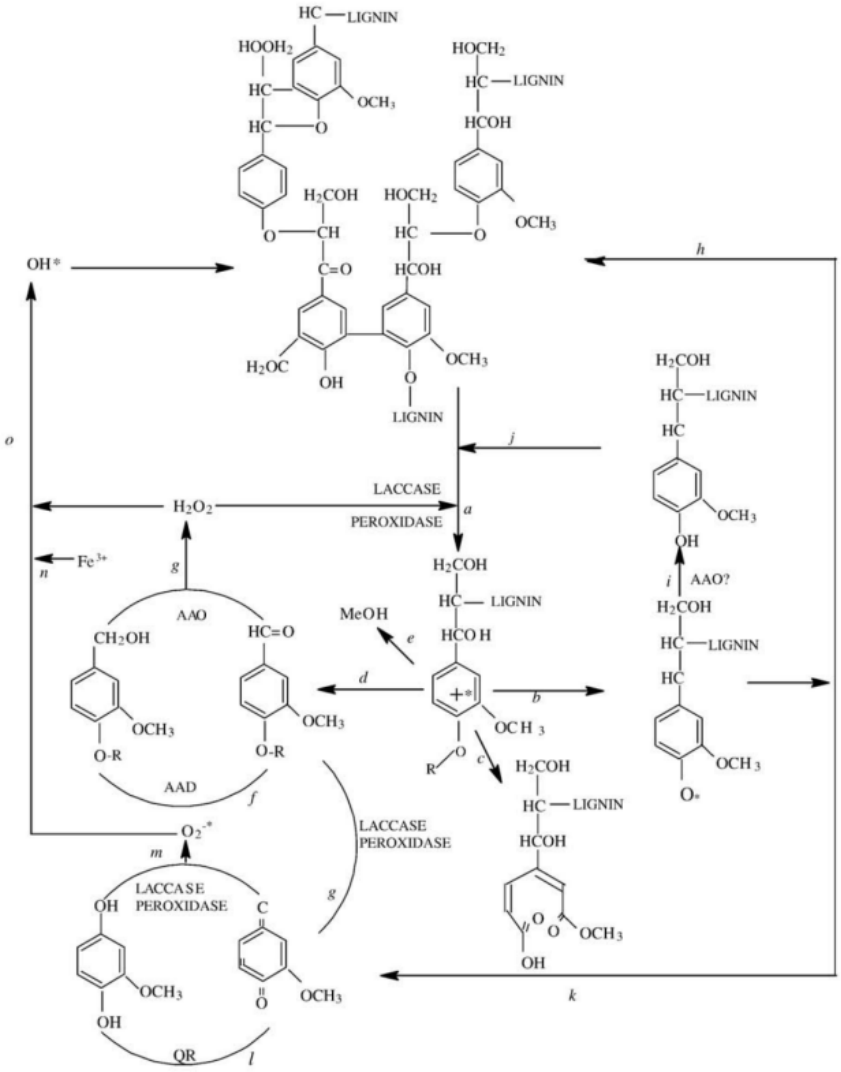


Figure 2.1: Mechanism for lignin degradation by white rot fungi. Figure by Sánchez (2009)[13], which is an updated version of the mechanism by Martìnes et al (2005)[14].

Oyster mushrooms are able to grow on many different types of substrates, which opens up for the possibility of utilizing waste products in the cultivation process[11]. They are able to metabolize polycyclic aromatic hydrocarbons (PAHs), which are components in coal tar, crude oil and creosote, that are of environmental concern because of their toxic and carcinogenic properties[15]. Carrasco-Cabrera et al (2019) studied the metabolism of caffeine in *P. ostreatus* cultivated on spent coffee grounds (SCG), and they proposed a pathway for caffeine degradation in fungi[4], which is shown in figure 2.2.

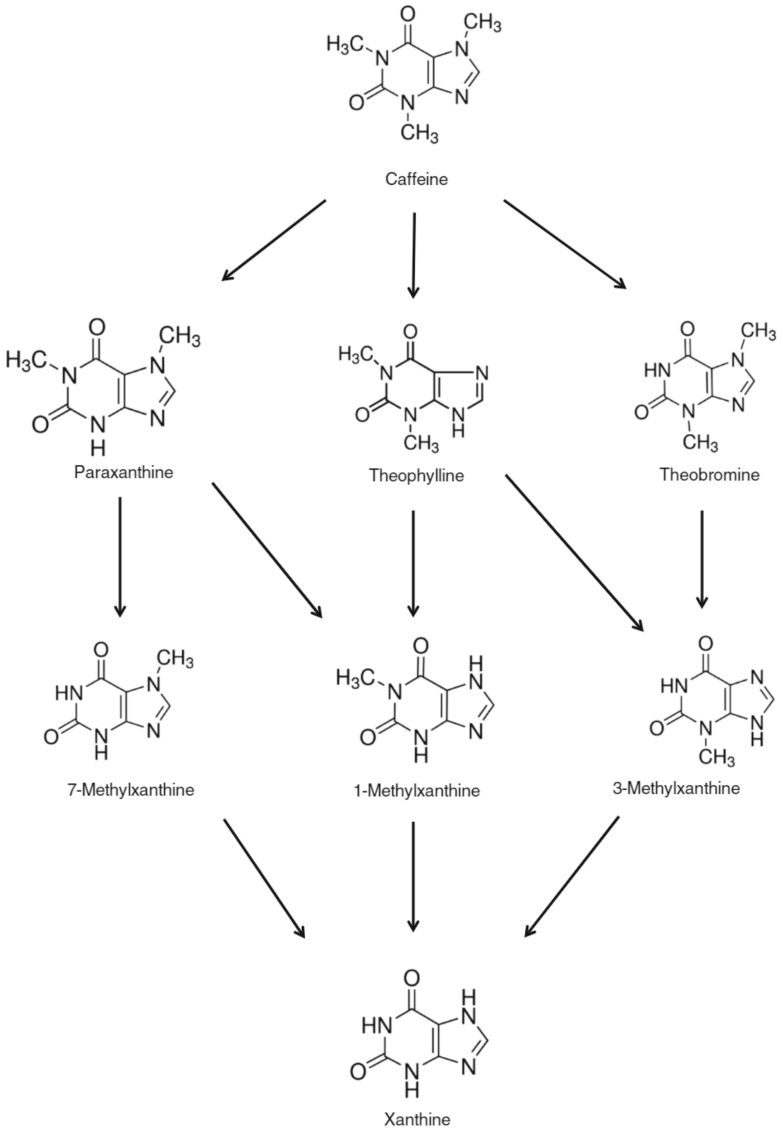


Figure 2.2: Proposed pathway for degradation of caffeine in fungi. Figure by Carrasco-Cabrera et al (2019) [4].

2.3 Substrate effect on mushrooms

The effect of various substrate compositions and waste products can affect the nutritional composition of mushrooms. It has been suggested that thiourea from cotton waste leads to mushrooms with favorable nutritional composition [16]. Using waste products such as coconut coir, pine sawdust and paper waste in mushroom substrate has also been found to lead to preferable properties in the mushrooms [17].

Biological efficiency and mushroom yield is also affected by the substrate. A study in Brazil showed that using local organic residues that are typically discarded, such as eucalyptus bark, lead to higher mushroom yield and more fruiting

bodies compared to the more conventional eucalyptus sawdust substrate. The study further suggests that having high contents of nitrogen and lignin might increase the performance of agro-industrial waste substrates[3]. Coffee residues are rich in nitrogen[18] and might serve as a good source for nitrogen in the substrate.

A risk of using waste in mushroom substrate is increasing the amount of toxic trace elements in the substrate that could accumulate in the mushrooms. Oyster mushrooms have shown high degree of accumulation of certain trace elements, like cadmium and lead[19]. Despite this, oyster mushrooms grown on different types of agro-industrial waste, including tea waste and wheat bran, have been found to be safe for human consumption[20].

2.4 Free amino acids and total amino acids

Free amino acids are important taste components for the flavor profiles of mushrooms. The amino acids are typically grouped into four classes based on what type of flavor they contribute to. These are sweet, bitter, MSG-like and tasteless. The MSG-like category contain amino acids that have chemical structure similar to that of monosodium glutamate (MSG), which is responsible for the umami taste[21]. The sweet and bitter categories relate directly to basic tastes, while the tasteless category contain amino acids that do not impact the taste. The amino acids characterised as sweet are alanine, glycine, proline, serine and threonine. Bitter amino acids are arginine, histidine, isoleucine, leucine, methionine, phenylalanine, tryptophan and valine. The MSG-like amino acids Aspartic acid and glutamic acid contribute to umami flavor. Cysteine, lysine and tyrosine are tasteless[22] [23]. The MSG-like amino acids are especially important for the typical mushroom taste[24]. In mushrooms, it has been found that the MSG-like and sweet components were taste-active, but the bitter amino acids were not. This might be due to the bitter taste being masked by sugars and the sweet components[25].

Total amino acids include the protein-bound amino acids in addition to the free amino acids. Insight in the composition of total amino acids is interesting since amino acids are important for synthesis of proteins in humans. Especially the essential amino acids that humans cannot synthesize are important to consume through the diet. There are nine essential amino acids, which are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine[26].

2.5 Volatile aromatic compounds

The aroma of mushrooms is an important characteristic which has big impact on mushroom quality. The main compounds in raw mushroom responsible for the aroma are compounds with carbon number 8 (C_8 compounds) and aliphatic alcohols[27]. Nyegue et al (2003) studied *Pleurotus ostreatus* from Cameroon and found that the major C_8 compound was octen-3-ol (59.3%), followed by octen-

3-one, octan-3-one, 3-octanol, n-octanal, (E)-2-octenal and n-octanol. They also found that benzaldehyde, benzyl alcohol and phenylethanol, which respectively are associated with almond odor, sweet-spicy odor and rose odor, may contribute to the flavor of *P. ostreatus*. Monoterpenes may also contribute to the oyster mushroom flavor profile[28].

Subjecting mushrooms to processing like sous-vide, drying or other treatments, has been reported to significantly change the compositions of volatile aromatic compounds[29]. It has also been reported for multiple mushrooms that cooking increased the intensity of sensory attributes. The differences perceived in sensory attributes between different strains are also bigger after cooking[30]. Cooked oyster mushrooms had lower levels of octen-3-ol, 3-octanol and 1-octanol, which are compounds associated with characteristic mushroom aroma, compared to raw oyster mushrooms. The concentration of 2-acetyl-1-pyrroline, which has a roasty, popcorn-like aroma, increased after cooking[31]. In *Agaricus bisporus L.*, pan-frying lead to decrease in octen-3-ol and octen-3-one concentrations. Like in oyster mushrooms, the concentration of 2-acetyl-1-pyrroline increased after cooking, but also the concentration of its analogue 2-propionyl-1-pyrroline increased[32].

2.6 Trace elements

Ingestion or inhalation of certain trace elements, including arsenic, cadmium, mercury and lead, can cause major detrimental effects in human bodies. Inorganic arsenic is a carcinogen and may lead to skin cancer, as well as cancer in liver, bladder or lungs. It can also reduce blood cell production and damage blood vessels. The most toxic forms of arsenic are As(III) and As(V), and Gonzálvez et al (2009) found that these forms made up all of the arsenic in most of the mushrooms they analyzed, including *Agaricus bisporus* and *Pleurotus eryngii*[33]. Cadmium may also cause cancer, and smokers are more exposed as high levels of cadmium could accumulate in the lungs. Mercury may cause various effects depending on if the mercury ingested is elemental, organic or inorganic. Some known effects are damage to nervous system, brain, kidneys, lung or increased blood pressure and heart rate. Lead has been suggested as a carcinogen that can cause cancer in every part of the human body, and it may also decrease functions of the nervous system and increase blood pressure[34][35][36]. Since certain trace elements can be extremely harmful to the human body, there are regulations on the maximum level of such contaminants in food. In the European Union (EU), the European Food Safety Authority (EFSA) have provided the framework for these limits[37]. The Norwegian Food Safety Authority follows the legislation provided by the EFSA for trace elements in food[38].

2.7 Methodological background

2.7.1 Dry weight and ash content

Dry weight, or dry matter, refers to all the compounds in a material except for water. When measuring and comparing different food products, the contents may be presented on dry weight basis. Dry matter can also be used for assessing quality of mushrooms[39] or determine if fruits are ripe before harvesting[40]. Dry matter content can be found by weighing a fresh sample, drying the sample by method of choice, weighing the sample after drying and then calculating the fraction or percentage of dry matter.

Ash is what is left after complete combustion of the sample. The ash is inorganic residue that primarily consists of minerals in the sample material. Ash content is determined through wet ashing or dry ashing. Wet ashing uses acids and oxidizing agents to oxidize the organic matter. In dry ashing the sample material is put in a specialized furnace at 500-600°C to evaporate water and volatile compounds and burn the organic matter[41]. The samples have to be weighed before and after ashing to determine ash content.

2.7.2 Lipid content determination - Bligh & Dyer method

Lipid content determination by Bligh & Dyer[42] is a popular methodology within food science[43]. The method consists of homogenizing the sample with water, methanol and chloroform. The lipids have high affinity for chloroform and will be in the chloroform phase. The water and chloroform phases are separated using a centrifuge, and the chloroform phase is extracted. After evaporating the solvent, the lipids are weighed[44]. Even though the Bligh & Dyer methodology is widely used, it has been suggested to be inaccurate in some cases due to high experimental error[45][46].

2.7.3 Protein content determination - Kjeldahl method

The Kjeldahl method for determination of nitrogen in organic matter[47] was published in 1883, and it has become a widely used method with considerable scope within analytical chemistry. The method consists of digestion of a sample through wet oxidation. Originally, sulfuric acid alone was used for the digestion, however, this was time consuming, and the method evolved to speed up the process through adding catalyst and heating up the sample. Typical catalysts include selenium, copper and mercury, but due to environmental concern, mercury is less used in recent years. After digestion, the sample is distilled, mixed with excess boric acid and nitrogen content is determined through back titration with alkaline solution[48][49]. Multiple systems have been developed to automate the steps in the method, including digestion, distillation and titration[50][51].

Conversion from nitrogen to protein is done by multiplying nitrogen content with a conversion factor. The factor 6.25 has been used as a universal factor, but nitrogen-to-protein factors vary between different food sources, and specific

conversion factors for products yield more accurate protein contents[52][53]. For mushrooms there is variation between species, but also within species. Fujihara et al (1995) reported a conversion factor of 4.15 for *Pleurotus ostreatus*[54], while Mattila et al (2002) found a conversion factor of 4.97[55]. A general conversion factor of 4.38 has been suggested for mushrooms and is commonly used[56].

2.7.4 Gas chromatography (GC) and High Performance Liquid Chromatography (HPLC)

Chromatography is a separation method based on the principle of repeated sorption and desorption as a mobile phase travels through a stationary phase. The compounds in the analyte have different affinities to the stationary phase, which leads to individual rates of sorption and desorption. Hence, the compounds will shift between being in the mobile phase and stationary phase at different rates. Typically, a detector records elution time and volume for the analyte, which gives rise to a chromatogram. The chromatogram can give both qualitative information, with identification based on comparing to known standards, and quantitative information with the area of the peak relating to the fraction of a certain compound in the analyte. Separation can also be observed as degree physical displacement on stationary phase. Gas chromatography (GC) and High performance liquid chromatography (HPLC) are popular variants of the method.[57]

Gas chromatography (GC) is an established method for analysis of volatile organic compounds[58]. Within food science it is commonly used to analyze aroma components, food composition and additives, either qualitatively or quantitatively[59]. In GC, the mobile phase is a chemically inert gas, such as hydrogen, helium or nitrogen. The mobile phase carries the sample through a column containing the stationary phase, which is typically a solid adsorbant or a liquid on an inert support. Some common columns are wall-coated open tubular column (WCOT), fused-silica wall-coated open tubular column (FSWC), support-coated open tubular (SCOT) or packed column. A detector measures the analyte as it elutes from the column. There are multiple types of detectors with varying applications, including mass spectrometer (MS) and flame ionization (FID) detectors.[60]

High performance liquid chromatography (HPLC) has a wide array of uses within food science, for instance analysis of amino acids, peptides, proteins, colorants, vitamins and many more[61]. In HPLC, the mobile phase is a liquid solvent, which also contains the analyte. Under pressure, the mobile phase travels through a column containing the stationary phase[62]. The columns can be packed with solids, commonly alumina or silica, or contain a liquid stationary phase. An ultraviolet (UV) absorption detector is frequently used as a HPLC detector[63].

2.7.5 Check-all-that-apply sensory analysis

Check-all-that-apply (CATA) is a methodology within sensory analysis to generate preference maps based on the relationship between descriptive terms and liking with consumers[64]. An advantage with CATA compared to traditional preference mapping, is that information on sensory attributes can be attained from a consumer panel rather than a trained panel, which makes the results more fit for use for product development with consumers in mind[65]. CATA can also be used to garner information about liking of different products directly and to figure out which version of a product is preferred by the consumer panel[66].

A CATA test typically consists of questions of overall liking, commonly with a 9-point hedonic scale[67], and a checklist of relevant sensory terms that the respondents should check if it applies for each product[64]. It is important that the list of terms is carefully crafted to fit the products tested[66]. The list of terms should also be concise and only contain relevant terms, as frequency of term usage has been shown to be lower if there are many suggested terms[68]. Primacy bias has been shown to affect the respondents use of terms, but randomizing the order of the terms reduces the effect of this bias[69]. Similarly, the presentation order of the products should be randomized to reduce positional bias[70]. There should be at least 60 consumers participation in the CATA for sufficient characterization of the different samples[71].

2.7.6 Questionnaire

Questionnaires are effective survey tools to gather information from people. When collecting data from large populations, questionnaires are more efficient and practical than more in-depth survey methods like interviews[72]. Since all questions and possibilities to answer are predetermined, it is important that a questionnaire is well designed, and the use of question types and scales should be considered carefully[73].

Questions can be either open or closed. Open questions allow each respondent to formulate their own answer to the question. This is good for attaining in-depth, qualitative data, but it is challenging to analyze all individual answers of big populations. With closed questions the respondents are presented with different alternatives that they have to choose between. Since closed questions give quantitative information, they make data analysis much easier than open questions. The trade-off is that less detailed information is obtained from each respondent.[72] [73]

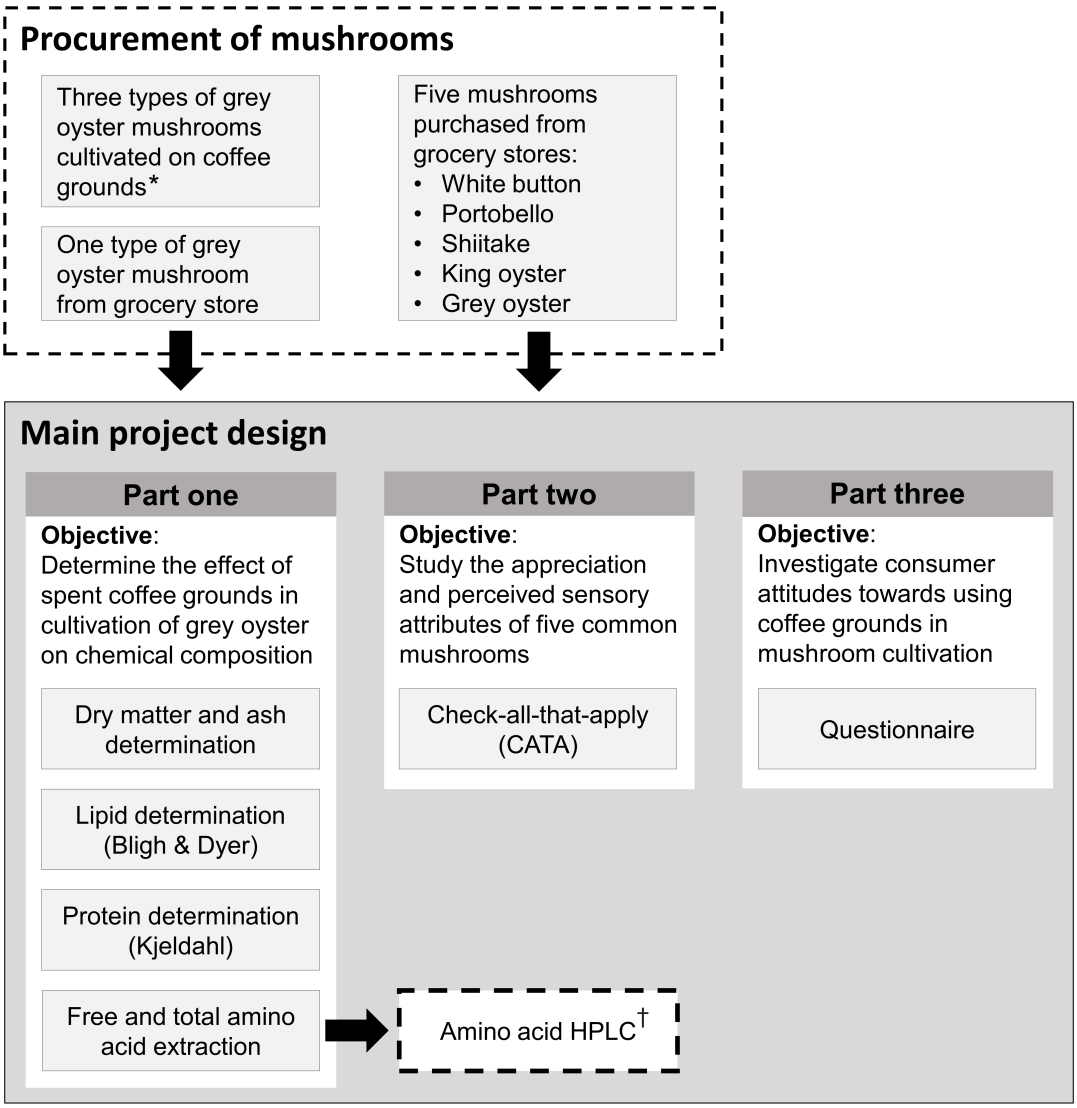
Answer options to closed questions may be in the form of different scales. Commonly, four types of scales are used, which are nominal, ordinal, interval or ratio scales. Each scale serves their own purpose, and in a questionnaire, multiple scales can be used in combination. Nominal scales are different categories, where no category is better or worse than the other. Ordinal scales introduce a rank to the categories, but the magnitude of the different between the categories is

unknown. Interval scales have set ranges, and thus do contain information of the magnitude between the categories, while ratio scales are similar to interval scales, but also have a defined zero point.[67]

3 Materials and methods

The design of the project was split into three main parts, and a flowchart explaining the process is shown in figure 3.1. Part one consisted of different chemical analyses to determine composition and some flavor-active components in three types of *Pleurotus ostreatus* cultivated on spent coffee grounds, as well as one conventional type. This included determination of dry matter, ash, lipid and protein content, as well as composition of free amino acids and total amino acids. Part two focused on the difference in perceived sensory attributes of different common mushrooms sold in grocery stores. These mushrooms were *Pleurotus ostreatus*, *Pleurotus eryngii*, *Lentinula edodes* and two types of *Agaricus bisporus* (white button mushroom and portobello mushroom). Sensory analysis was done through a check-all-that-apply (CATA) test. In part three, consumer attitudes towards mushrooms cultivated on waste products and spent coffee grounds (SCG) were studied through a questionnaire.

Due to unforeseen obstacles for the supplier, there were difficulties in procurement of mushrooms grown partially on spent coffee grounds. Because of this, the project design was altered to accommodate for lacking raw material. The biggest change was that the CATA test in part two could not be performed on *Pleurotus ostreatus* cultivated on spent coffee grounds, which was originally planned. An alternate approach using common edible mushrooms was adapted to gather general sensory data that was relevant to the project. Some other planned analyses also had to be cut, including trace element analysis with inductive coupled plasma mass spectrometry (ICP-MS) and analysis of volatile aromatic compounds with gas chromatography-mass spectroscopy (GC-MS).



*Grey oyster mushrooms cultivated on spent coffee grounds were supplied by Toppsopp [5].
†HPLC was performed by Siri Stavrum at Department of Biotechnology and Food Science, NTNU, Trondheim, Norway [74].

Figure 3.1: Flowchart explaining the project design, with procurement of mushrooms and the three different parts of the main project design. HPLC is short for high-performance liquid chromatography.

3.1 Part one: Chemical composition of *Pleurotus ostreatus*

3.1.1 Mushroom storage and handling

Pleurotus ostreatus cultivated on various fractions of spent coffee grounds and other products were procured from Toppsopp [5] and a type of conventional mushroom was bought from a local grocery store. The supplier of conventional mushrooms was not able to provide information of the substrate composition, but it was decided to include the mushroom to assess how the mushrooms grown on substrates containing waste products were perceived compared to widely sold

conventional mushrooms. The mushrooms cultivated on spent coffee grounds were assigned unique one-letter codes to make experimental marking simpler, these are shown in table 3.1. The three types of mushrooms grown partially on spent coffee grounds were marked as mushroom X (25% coffee grounds, 25% oak sawdust and 50% rapeseed straw), mushroom Y (25% coffee grounds, 25% faba bean hulls and 50% oak sawdust) and mushroom Z (50% coffe grounds and 50% oak sawdust).

Table 3.1: Cultivation substrate compositions and assigned codes for each type of *Pleurotus ostreatus* cultivated on spent coffee grounds and conventional *Pleurotus ostreatus*.

Assigned one-letter code	Mushroom cultivation substrate
Conventional*	-
X	25% spent coffee grounds, 25% oak sawdust and 50% rapeseed straw
Y	25% spent coffee grounds, 25% faba bean hulls and 50% oak sawdust
Z	50% spent coffee grounds and 50% oak sawdust

*Conventional oyster mushroom had unknown cultivation substrate composition.

Three types of *P. ostreatus* that were partially cultivated on spent coffee grounds and one type of conventional *P. ostreatus* were collected on the same day. The oyster mushrooms were stored in a dry, dark and refrigerated room at 4°C with good aeration. They were stored in their original packaging, which for the coffee grounds mushrooms consisted of a cardboard tray and a plastic lid, and for the conventional mushrooms consisted of a cardboard tray and a plastic bag. After three days, some of each type of oyster mushroom were frozen in preparation for freeze-drying before extraction of total amino acids. They were put in a dark, well aerated and dry freezer room at -20°C. An overview of the storage times for the mushrooms in part 1 is shown in table 3.2.

Table 3.2: Overview of the duration the mushrooms were in 4°C storage before the beginning of an analytical method or change in storage conditions.

Analytical method	Duration of 4°C storage prior to method
Dry matter and ash determination	0 days
Lipid determination	1 day
Protein determination	7 days*
Free amino acid extraction	5 days*
Total amino acid extraction	3 days†

*Mushrooms for protein determination and free amino acid extraction were homogenized on day 3.
†Mushrooms were frozen after this (-20°C) in preparation for freeze-drying before extraction of total amino acids.

3.1.2 Dry matter and ash content determination

Dry matter and ash content determination were performed on three types of *P. ostreatus* cultivated on substrates containing spent coffee grounds and one

conventional type of *P. ostreatus*. Three experimental parallels were analyzed for each of the four types of *P. ostreatus*. For each parallel, 5g of mushroom was weighed exactly and put in already weighed porcelain crucibles. The crucibles were put in a Termaks TS 8056 heating cupboard at 105°C for 24 hours. Each crucible was weighed after this step, and the percentage of dry weight was calculated by equation 3.1:

$$\text{Dry matter content } [\%] = \frac{m_{\text{dry}}}{m_{\text{fresh}}} \cdot 100\%, \tag{3.1}$$

where m_{fresh} was the weight of fresh mushrooms and m_{dry} was the weight of mushrooms after drying.

To determine ash content, the crucibles containing dried mushrooms were put in a Nabertherm ashing furnace[75] at 550°C for 24 hours. After ashing, the crucibles were weighed again. To prevent rehydration that could impact the results, the crucibles were kept in a glass desiccator while cooling down after drying and ashing. Ash content on fresh mushroom basis was calculated by equation 3.2:

$$\text{Ash content } [\%] = \frac{m_{\text{ash}}}{m_{\text{fresh}}} \cdot 100\%, \tag{3.2}$$

where m_{fresh} was the weight of fresh mushrooms and m_{ash} was the weight of mushrooms after ashing. To convert this into unit for mg ash per 100g of fresh mushroom, equation 3.3 was used,

$$\text{Ash content } [\text{mg}/100\text{g}_{\text{fresh}}] = \frac{\text{Ash content}[\%]}{100\%} \cdot \frac{100\text{g}}{100\text{g}_{\text{fresh}}} \cdot \frac{1000\text{mg}}{1\text{g}}. \tag{3.3}$$

3.1.3 Lipid determination

Lipid determination was performed with a modified version of the methodology by Bligh & Dyer (1959)[42]. The method was performed on three types of *P. ostreatus* cultivated on substrates containing spent coffee grounds and one conventional type of *P. ostreatus*. Three parallels were analyzed for each of the four types of *P. ostreatus*. For each parallel, 2g of mushroom were weighed exactly and put in a plastic centrifuge tube, that was chloroform resistant and had a screw cap. The tubes were put on ice during the next steps to prevent evaporation of solvent. To each tube, 4.0mL of deionized water, 10.0mL of methanol and 5.0mL of chloroform was added. The contents in each tube were homogenized for 60 seconds, using a Kinematica Polytron™ PT3100D homogenizer motor[76] with a Kinematica™ 12mm W-Design Aggregate[77]. Then, 5.0mL of chloroform was added and each tube was homogenized for 20 seconds. Following that, 4.0mL of deionized water was added and each tube was homogenized again for 20 seconds. While repeating the steps for the remaining parallels, the completed tubes were put on ice. The tubes were centrifuged in a Kubota 1700 centrifuge[78] at

7000rpm and 4°C for 10 minutes. After centrifugation, more than 4mL of the chloroform phase was extracted and put in a test tube, while avoid also extracting solid residues or water phase. Using volumetric pipettes, two separate volumes of 2.00mL were transferred to two different Kimax tubes that were already weighed. The chloroform was evaporated with nitrogen for 15 minutes, leaving only the lipids. The Kimax tubes were then weighed again to find lipid content. The percentage of lipid content was found for each sample by using equation 3.4:

$$\text{Lipid content } [\%] = \frac{(m_{\text{lipids + kimax}} - m_{\text{empty kimax}}) \cdot V_{\text{chloroform added}}}{m_{\text{sample}} \cdot V_{\text{chloroform taken out}}} \cdot 100\%, \quad (3.4)$$

where $m_{\text{lipids + kimax}}$ was the weight of the kimax tube containing extracted lipids, $m_{\text{empty kimax}}$ was the weight of the empty kimax tube, m_{sample} was the weight of homogenized mushroom added for that sample, $V_{\text{chloroform added}}$ was the total volume of chloroform added (10.0mL) and $V_{\text{chloroform taken out}}$ was the volume of chloroform phase taken out for lipid extraction (2.00mL). To convert this into unit for g lipid per 100g of fresh mushroom, equation 3.5 was used,

$$\text{Lipid content } [\text{g}/100\text{g}_{\text{fresh}}] = \frac{\text{Lipid content } [\%]}{100\%} \cdot \frac{100\text{g}}{100\text{g}_{\text{fresh}}}. \quad (3.5)$$

3.1.4 Protein determination

Kjeldahl method[47] for nitrogen content and protein determination was performed with a modified setup, which combined steps of Kjeldahl procedures by Buchi for dairy[79] and meat[80]. The method was performed on three types of *P. ostreatus* cultivated on substrates containing spent coffee grounds and one conventional type of *P. ostreatus*. The design consisted of three parallels for each of the four types of *P. ostreatus*, where three of them were cultivated on substrates containing spent coffee grounds and one was conventional *P. ostreatus*, as well as two blanks and two references of D-tryptophan. Oyster mushrooms of each type were homogenized four days prior to analysis, using an InVite 800W immersion blender. For each type of homogenized mushroom, 2g were weighed on nitrogen-free weighing paper and put in glass digestion tubes, while noting exact weight. Nitrogen-free weighing paper was also added to glass digestion tubes for blanks and references, and in the references, 0.2g of D-tryptophan was added, with exact weight being noted. To each tube, 15mL of sulfuric acid (98%) and two BUCHI Kjeldahl tablets ECO (3.998 g K_2SO_4 / 0.002 g $CuSO_4 \cdot 5\ H_2O$)[81] were added. The tubes were placed in a stand and put in a KjelDigester K-449[82] digestion unit, which was connected to a Scrubber K-415[83]. The digestion program was as follows: the unit was preheated to 280°C, after inserting the sample rack, the temperature was increased to 320°C for 20 minutes, followed by a temperature increase to 420°C for 100 minutes, and then the samples were cooled down for 35 minutes. After digestion, the tubes were put in a system with KjelMaster

K-375 and KjelSampler K-376[84] for distillation, titration and protein content calculation. The titration type was boric acid titration, with potentiometric sensor and 4.65 as endpoint pH. The nitrogen to protein conversion factor 4.38[56] was entered into the system, which automatically calculated the protein content from the found nitrogen content.

3.1.5 Extraction of free amino acids

Analysis of free amino acid composition was performed on three types of *P. ostreatus* cultivated on substrates containing spent coffee grounds and one conventional type of *P. ostreatus*. The four types of oyster mushrooms were homogenized two days prior to extraction. Water-soluble proteins (WSP) were extracted using a modified version of the methodology described by Anderson et al (1968)[85]. For each type of homogenized mushroom, approximately 2g (weighed exactly) were mixed with 25.0mL of KH_2PO_4 buffer (0.05M) in a plastic centrifugation tube. The tubes were centrifuged at 10 000rpm for 20 minutes in a Kubota 1700 centrifuge[78]. The supernatant of each tube was decanted through a Whatman® (grade 589/1 black ribbon) ashless filter paper[86] and transferred to a test tube.

The methodology for extraction of free amino acids was adapted from Osnes and Mohr (1985)[87]. For each mushroom type, three separate volumes of 1mL WSP extract were pipetted into three different 1.5mL Eppendorf tubes, creating three parallels. To each tube, 0.25mL of 10% sulphosalicylic acid were added (in effect also diluting 1:1.25), and the tubes were vigorously shaken, before the tubes were put in a 4°C refrigerator for 30 minutes. The chilled Eppendorf tubes were centrifuged at 10 000rpm for 10 minutes in a Thermo Scientific™ Heraeus™ Megafuge™ 8R benchtop centrifuge[88], and the supernatant of each tube was transferred to 4mL glass tubes and stored at -80°C for 12 days. Each sample was thawed, diluted by a factor 1:25 with deionized water and filtered through a VWR 25mm syringe filter with 0.22μm polyethersulfone membrane[89]. For each parallel, 0.205mL was transferred to a glass vial, and the glass vials were stored at -80°C until high-performance liquid chromatography (HPLC) was performed.

3.1.6 Extraction of total amino acids

Analysis of total amino acid composition was performed on three types of *P. ostreatus* cultivated on substrates containing spent coffee grounds and one conventional type of *P. ostreatus*. Prior to extraction of total amino acids, the four different types of *P. ostreatus* were freeze-dried. Approximately 35g of each type of mushrooms, that were cooled to -20°C in a freezer room, were freeze-dried using a FreeZone 12 Liter -50C Console Freeze Dryer (Model nr. 7754030)[90]. The mushrooms were freeze-dried for 70 hours, and exact weights before and after freeze-drying were noted to calculate the yield. The freeze-dried mushrooms were put in zip-lock bags with respect to the mushroom type and crushed to homogenize the mushrooms.

The methodology for extraction of total amino acids was adapted from Blackburn (1978)[91]. For three parallels per mushroom type, 2.5g of freeze-dried mushrooms were weighed into glass tubes with screw caps, noting exact weight. To each glass tube, 1mL hydrochloric acid (HCL, 6M) was added, and the samples were hydrolyzed at 105°C for 22 hours in a Termaks TS 8056 heating cupboard. After cooling the glass tubes, the samples were poured into 20mL beakers, and the tubes were rinsed with deionized water to collect as much of the contents as possible. The samples were titrated to pH 7 (acceptable range: 6.50-7.50) using sodium hydroxide solutions of varying concentrations. A MeterLab™ PHM210 Standard pH meter was used to measure pH during titration. After titration, each sample was filtered through a Whatman GF/C glass microfiber filter[92] using suction and poured into a 10.00mL measuring flask, which was then filled to 10.00mL using deionized water. For one sample, the volume accidentally exceeded 10.00mL, so the sample was then transferred to a 25.00mL measuring flask and deionized water was added to make up 25.00mL. The samples were then diluted by a factor 1:250, filtered through VWR 25mm syringe filters with 0.22μm polyethersulfone membrane[89] and 0.205mL of each sample was transferred to a glass vial in preparation for HPLC. The glass vials were stored at -40°C until HPLC.

3.1.7 High-performance liquid chromatography (HPLC) of free and total amino acids

High-performance liquid chromatography (HPLC) of free amino acids and total amino acids were performed by Siri Stavrum at Department of Biotechnology and Food Science, NTNU, Trondheim, Norway[74]. The instrument used was a Dionex/Thermo Scientific UltiMate 3000 HPLC system[93] with accompanying pump, column heater, auto sampler and an RF 2000 detector. The column used was a Waters Nova-Pak C18 Column, 3.9mm x 150mm[94], and the derivatization reagent used was a P0532 Phthaldialdehyde Reagent by Sigma-Aldrich (subsidiary of Merck KGaA)[95]. For each sample, 20.00μL was injected into the HPLC system. Standards of amino acids were used to identify the amino acids in the samples, and the amino acids identifiable by the method are noted in table 3.3, presented with respective molecular weights (M_{AA}), which were used for calculations. The molecular weights were found on the website of Thermo Fisher Scientific[96], with the exception of α-aminobutyric acid which was found on the website of National Library of Medicine[97].

Table 3.3: The amino acids that were identifiable with the high-performance liquid chromatography setup. Short names used for the different amino acids and molecular weights are also displayed. The molecular weights were found on the website of Thermo Fisher Scientific [96], with the exception of α-aminobutyric acid which was found on the website of National Library of Medicine [97]. Glycine and arginine could be identified, but they could not be separated by the experimental setup used.

Short name	Amino acid	Molecular weight (M_{AA}) [g/mol]
Asp	Aspartic acid	133.1
Glu	Glutamic acid	147.1
Ser	Serine	105.1
Thr	Threonine	119.1
Ala	Alanine	89.1
Gly/Arg*	Glycine/Arginine	-
His	Histidine	155.2
Met	Methionine	149.2
Val	Valine	117.1
Phe	Phenylalanine	165.2
Ile	Isoleucine	131.2
Leu	Leucine	131.2
Asn†	Asparagine	132.1
Gln†	Glutamine	146.2
Tyr	Tyrosine	181.2
Lys	Lysine	146.2
Aba	α-aminobutyric acid	103.1

*Glycine and arginine could not be separated by the experimental methodology and values were not reported.

†Due to non-enzymatic deamidation of asparagine and glutamine under acidic condition, determination of asparagine and glutamine content was not possible with the total amino acid methodology used [98] [99].

For each run a chromatogram was generated, containing information about retention time and also amount of each amino acid, which was automatically integrated into a concentration measurement (C_{AA}) with the unit μmol/L. These values were recalculated into the unit mg amino acid per g dry mushroom by using the molar mass for each respective amino acid (M_{AA}), the sample weight of mushrooms added (m_{sample}), dry matter content fraction for each respective mushroom (f_{dry}) and by taking dilutions and conversion factors into consideration. For free amino acids (FAA), equation 3.6 was used,

$$\mathrm{FAA}\ [\mathrm{mg/g_{dry}}] = \frac{C_{\mathrm{AA}} \cdot M_{\mathrm{AA}} \cdot V_{\mathrm{KH_2PO_4}}}{m_{\mathrm{sample}} \cdot f_{\mathrm{dry}}} \cdot 1.25 \cdot 25 \cdot \frac{1\mathrm{mg}}{1000\mu\mathrm{g}}, \tag{3.6}$$

where $V_{KH_2PO_4}$ was the volume of KH_2PO_4 buffer used (0.0250L), multiplication with the factors 1.25 and 25 adjusted for dilution and a conversion from μg to mg was used. For total amino acids (TAA), equation 3.7 was used,

$$\text{TAA } [\text{mg/g}_{\text{dry}}] = \frac{C_{\text{AA}} \cdot M_{\text{AA}} \cdot V_{\text{titration}} \cdot Y_{\text{freeze-dry}}}{m_{\text{sample}} \cdot f_{\text{dry}}} \cdot 250 \cdot \frac{1\text{mg}}{1000\mu\text{g}}, \tag{3.7}$$

where $V_{\text{titration}}$ was the volume after titration and dilution to 10.00mL (one sample was diluted to 25.00mL), $Y_{\text{freeze-dry}}$ was the freeze-drying yield of each respective mushroom type (calculations of freeze-drying yields are shown in appendix A), multiplication with the factor 250 adjusted for dilution and a conversion from μg to mg was used.

3.2 Part two: Mushroom preference mapping

3.2.1 Mushroom storage and handling

Since the supplier was not able to deliver more mushrooms cultivated on substrates containing spent coffee grounds, an alternate approach using conventional mushrooms was adapted. Five types of common mushrooms were bought from grocery stores, which were grey oyster (*Pleurotus ostreatus*), king oyster (*Pleurotus eryngii*), shiitake (*Lentinula edodes*), white button (*Agaricus bisporus*) and portobello (*Agaricus bisporus*). All types of mushrooms were purchased one day prior to analysis, and they were stored in their original packaging in a 4°C refrigerator until preparation.

3.2.2 Check-all-that-apply (CATA)

Check-all-that-apply (CATA) was performed on pan-fried *Pleurotus ostreatus*, *Pleurotus eryngii*, *Lentinula edodes* and two types of *Agaricus bisporus* (white button mushroom and portobello mushroom). For each type of mushroom, 750g were fried in three batches of approximately 250g. Each batch was fried in 1 tablespoon of rapeseed oil, and after frying, 1/8 teaspoon of salt and freshly ground black pepper was added to each batch. The batches for each mushroom were combined in one aluminum container, and the containers were placed on heating trays to keep the prepared mushrooms warm.

CATA was performed at Norwegian University of Science and Technology (NTNU) Gløshaugen in Trondheim, Norway, with a consumer panel of 81 subjects. The respondents were mostly students or employees at the university. The sensory and consumer research software EyeQuestion® [100] was used to deliver the CATA questions to the respondents, who answered on their smartphones. Each subject was given a tray containing a serving of each of the five types of pan-fried mushrooms, which were coded with their respective three-digit code, as shown in table 3.4. The codes were randomly assigned in EyeQuestion®, and to reduce positional bias, the software also provided a serving order for each participant.

Table 3.4: The five mushroom types and species used in the check-all-that-apply (CATA) test with corresponding randomized three-digit codes.

Mushroom type	Species	three-digit code
Grey oyster	*Pleurotus ostreatus*	558
King oyster	*Pleurotus eryngii*	950
Shiitake	*Lentinula edodes*	150
White button	*Agaricus bisporus*	266
Portonello	*Agaricus bisporus*	810

For each sample the subjects tasted, they were asked to answer about how well they liked it on a 9-point hedonic scale and then check of all attributes that applied to that sample. The terms presented to the subjects were related to appearance, flavor and texture. Some terms were general sensory terms (such as sweet, salty, bitter, etc.), while others were related to information found in literature about some of the mushrooms or expectations to mushroom flavor and texture. The 26 terms used in the CATA are shown in table 3.5.

Table 3.5: The 26 terms used in check-all-that-apply test, categorized under flavor, appearance and texture. A short description for each term is also presented.

Term	Description
Flavor	
Flavorful (Rich)	Product has much flavor
Flavorless (Tame)	Product has little flavor
Salty	Tastes salty
Sweet	Tastes sweet
Bitter	Tastes bitter
Sour	Tastes sour
Umami	Tastes umami
Savoury	Product perceived as savoury
Fatty	Product perceived as fatty
Earthy	Product perceived as earthy
Stale	Product has taste associated with being stale
Tastes like mushroom	Perceived taste associated with mushroom
Tastes like almonds*	Perceived taste associated with almonds
Tastes like seafood†	Perceived taste associated with seafood
Tastes like anise (black licorice)†	Perceived taste associated with anise or black licorice
Tastes like black pepper‡	Perceived taste associated with black pepper
Appearance	
Yellow	Color perceived as yellow
Brown	Color perceived as brown
White	Color perceived as white
Light in color	Product is light in color
Dark in color	Product is dark in color
Texture	
Firm	Texture of the product is firm
Soft	Texture of the product is soft
Soggy	Texture of the product is soggy
Chewy	Texture of the product is chewy
Meat-like texture	Product has texture similar to meat

*Benzaldehyde in grey oyster mushrooms[101] may cause almond-like taste[102].
†Grey oyster mushrooms and king oyster mushroom may have a slight seafood or anise-like flavor[103].
‡Term used since black pepper was added as an ingredient.

3.3 Part three: Questionnaire for attitudes towards mushrooms cultivated on waste products

To investigate consumer attitudes towards the use of coffee grounds in mushroom cultivation substrates, a survey in the form of a questionnaire was created. The questionnaire was made with Google Forms[104], and it was distributed on the social media platform Facebook[105], to groups of university students, as well as to personal connections to the author. The questions, statements and answer options were created by the author in Norwegian language, and they were translated to English by the author for the purpose of this report. All questions with original Norwegian wording and English translations are shown in appendix C.

After accepting answers for 13 days, the questionnaire had been answered by 182 respondents.

The questionnaire consisted of 16 questions in total, all of which were closed questions. To contextualize the answers with socio-demographic factors, the first eight questions asked for background information, including gender, age, education, yearly income, family situation and work situation. These were all answered using either ordinal scales or ratio scales. One question asked about the frequency of eating mushrooms, which was intended to serve as sorting criteria for possible product relevance. The respondents were also asked to consider seven statements, which compared mushrooms grown on waste substrates and coffee grounds to conventional mushrooms, with respect to environmental concern, healthiness, taste, price, safety and product interest. These were answered using a 5-point scale, where 1 meant totally disagree to the statement and 5 meant totally agree.

3.4 Data analysis

3.4.1 Chemical mushroom composition calculation and analysis

Dry matter, ash, lipid and protein content were calculated using Microsoft® Excel® for Microsoft 365 MSO (Version 2205)[106]. Calculation examples are shown in appendix B. The data for each experiment was analyzed by one-way analysis of variance (ANOVA) with Tukey's post hoc test for significant difference at the 5% level of significance in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0)[107].

The compositions of free and total amino acids in the mushrooms were received from HPLC sampling with the unit μmol L^{-1} (μmol amino acid per litre in analyzed sample), which was calculated to mg g^{-1} (mg amino acid per g of dry weight mushroom) in Microsoft® Excel® for Microsoft 365 MSO (Version 2205) Example calculations and explanations of calculation steps are shown in appendix B. The amino acids glycine and arginine were excluded, as the method was not able to distinguish between them. Due to non-enzymatic deamidation of asparagine and glutamine under acidic condition, total amino acid determination of asparagine and glutamine content was not possible with the methodology used[98] [99] Data analysis to find mean values and standard deviations of the parallel experiments was performed in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0).

3.4.2 Analysis of check-all-that apply (CATA) data

There were CATA responses received from 81 subjects, however, two sets of answers were removed as they were incomplete and did not give answers for all mushroom types. Thus, CATA data analysis was performed with 79 subjects. Preference data was analysed with one-way ANOVA and Tukey's post hoc test for significant difference at the 5% level of significance in the statistical software

platform IBM® SPSS® Statistics (Version 27.0.0.0). The term usage and their between groups significance were analyzed with Cochran's Q test with McNemar procedure for multiple pairwise comparisons using XLSTAT statistical software with tools for sensory analysis (Version 2022.2.1)[108]. XLSTAT was also used to generate a penalty analysis, through defining the most liked sample (white button mushroom) as an ideal product and observing changes in mean liking when terms are checked for tested product, but not for ideal and vice versa. The terms were categorized as "must have", "nice to have", "does not harm" or "must not have", based on changes in mean liking when the terms were differently checked compared to ideal product, and the threshold for term usage was set to 10%.

3.4.3 Questionnaire data analysis

Five of the statements the respondents were asked to consider could be directly linked to the respondents attitude towards mushrooms cultivated on coffee grounds. These statements are presented in table 3.6 (translated from Norwegian to English by the author).

Table 3.6: Statements used to determine respondents attitudes towards mushrooms cultivated on coffee grounds. The respondents answered how much they agreed or disagreed to each statement on a 5-point scale (1 = totally disagree, 5 = totally agree). The statements were translated from Norwegian to English by the author.

Statements regarding mushrooms cultivated on coffee grounds
The use of waste products (such as coffee grounds) in mushroom production is good for the environment.
Mushrooms cultivated on coffee grounds are healthier than conventional mushrooms from grocery stores.
Mushrooms cultivated on waste products (such as coffee grounds) are not natural.*
I find it safe to eat mushrooms cultivated on waste products (such as coffee grounds).
If it was available in grocery stores, I would buy mushrooms cultivated on coffee grounds.

*Since the statement was worded in a negative way with regard to attitude towards coffee grounds, the answer scores were reversed for attitudinal analysis.

The respondent attitudes towards mushrooms cultivated on coffee grounds were analyzed and grouped into three attitudinal groups based on their mean scores for the five statements, which were found using Microsoft® Excel® for Microsoft 365 MSO (Version 2205). Mean scores under 2.5 were designated to the negative group, between 2.5 and 3.5 to the neutral group and above 3.5 to the positive group. The mean scores within each group was found by ANOVA in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0). The custom table function in the same software was used to create a table comparing the count of respondents in each attitudinal group to the different socio-demographic factors. Some socio-demographic scales were merged to make the information presented in the table easier to interpret, including increasing the age scales to 20 year intervals instead of 10 years. The other changes were that for number of persons in the household, the categories 5 and 6 or more were merged to 5 or more, and similarly for number of underage children in the household, the

categories 3 and 4 or more were merged to 3 or more. The age range over 80 years was not represented by any respondents and was therefore not present in the table. For some questions, the respondents were able to enter their own answer, if the answer options did not suffice. These entered answers were sorted as "other" or put into a fitting category as decided by the author. All original answers are presented in appendix C.

3.4.4 Significant digits

The experimental data for content of dry matter, ash, lipid and protein, as well as free and total amino acids, was reported as mean values with standard deviations. The standard deviations were as a rule rounded to the first decimal, and the mean data was presented with the same number of decimal places as the rounded standard deviation. If the first digit of the standard deviation was 1, then an additional digit was reported for both the standard deviation and the mean value. The mean values and standard deviations reported from CATA preference data and questionnaire mean attitudes were based on hedonic scales, and the answers were related to the respondents personal opinions. Because of this, it was chosen to report values with two decimal places for CATA liking and questionnaire attitude means.

4 Results

4.1 Effect of cultivation substrate on chemical composition in *Pleurotus ostreatus*

Dry matter, ash, lipid and protein content of *Pleurotus ostreatus* grown on four different cultivation substrates are shown in table 4.1. The four oyster mushroom types will be referred to as conventional, mushroom X (25% coffee grounds, 25% oak sawdust and 50% rapeseed straw), mushroom Y (25% coffee grounds, 25% faba bean fiber and 50% oak sawdust) and mushroom Z (50% coffee grounds and 50% oak sawdust). All results were analyzed using one-way analysis of variance (ANOVA) with Tukey's post hoc test for significant difference at the 5% level of significance was used to analyze the results in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0).

Dry matter content was found through drying three parallels of each mushroom type in a heating cupboard at 105°C for 24 hours, weighing before and after. Mushroom Z had the highest dry matter content with 12±1.1% of fresh mushroom weight. This was followed by mushroom X (10.8±0.6%), conventional oyster mushroom (9.27±0.07%) and mushroom Y (6±2%). Mushrooms Z and X had significantly higher dry matter content than mushroom Y based on Tukey's post hoc test at the 5% level of significance.

Ash content was determined through ashing of the previously dried samples used to find dry matter content at 550°C for 24 hours, weighing before and

after. Mushroom Z had the highest ash content with 9.37±0.18mg per 100g of fresh mushroom. Following this was conventional oyster mushroom (8.9±0.5mg), mushroom X (7.5±0.4mg) and mushroom Y (7.3±0.23mg). Mushroom Z and conventional oyster mushroom had significantly higher ash content compared to mushrooms X and Y based on Tukey's post hoc test at the 5% level of significance.

A modified approach to Bligh & Dyer was used to determine lipid content. Three experimental parallels were run for each mushroom type, and each original parallel was separated into two new parallels for more accurate lipid content determination. Mushrooms Z and X had the highest lipid contents, with respectively 0.54±0.05g and 0.51±0.03g per 100g of fresh mushroom. Based on Tukey's post hoc test at the 5% level of significance, they had significantly higher lipid content than mushroom Y and conventional oyster mushroom, which had 0.37±0.04g and 0.360±0.019g per 100g of fresh mushroom respectively.

Kjeldahl method for nitrogen determination was performed with three experimental parallels, and a conversion factor of 4.38 was used to calculate the protein content. The protein content in the different mushrooms were all significantly different at the 5% level of significance. Mushroom Z had the highest protein content with 3.69±0.03g per 100g of fresh mushroom. Mushroom Y, mushroom X and conventional oyster mushroom had respectively 2.37±0.09g, 2.18±0.10g and 1.98±0.014g per 100g of fresh mushroom.

Table 4.1: Content of dry matter, ash, lipids and protein in *Pleurotus ostreatus* cultivated on four different growth substrates. The values are all given per 100g of fresh mushroom weight and are presented with standard deviation. Data analysis was done using one-way analysis of variance (ANOVA) with Tukey's post hoc test for significant difference at the 5% level of significance in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0), and statistical subsets are indicated in superscript. The p-value for between groups comparison of each terms is also presented.

Analyzed value		Conventional	Mushroom X	Mushroom Y	Mushroom Z	P-value
Dry matter	[%]	9.27 ± 0.07^{ab}	10.8 ± 0.6^{b}	6 ± 2^{a}	12.3 ± 1.1^{b}	0.003^{**}
Ash	[mg/100g$_{fresh}$]	8.9 ± 0.5^{b}	7.5 ± 0.4^{a}	7.3 ± 0.3^{a}	9.37 ± 0.18^{b}	$<0.001^{**}$
Lipid	[g/100g$_{fresh}$]	0.360 ± 0.019^{a}	0.51 ± 0.03^{b}	0.37 ± 0.04^{a}	0.54 ± 0.05^{b}	$<0.001^{**}$
Protein	[g/100g$_{fresh}$]	1.984 ± 0.014^{a}	2.18 ± 0.10^{b}	2.37 ± 0.09^{c}	3.69 ± 0.03^{d}	$<0.001^{**}$

**Significant difference between groups at the 1% level of significance.

Free amino acids were extracted from water-soluble protein solution in three experimental parallels and measured using HPLC (performed by Siri Stavrum at Department of Biotechnology and Food Science, NTNU, Norway). One-way ANOVA with Tukey's post hoc test for significant difference at the 5% level of significance in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0) was used to analyze the means and standard deviations of the free amino acids, which are presented in table 4.2. All the types of *P. ostreatus* contained the most of the same three free amino acids, which were glutamine, glutamic acid and alanine, but the order differed between all types. In conventional *P. ostreatus*,

mushroom X and mushroom Y, most free amino acids analyzed belonged to the tasteless group, followed by the bitter, sweet and MSG-like groups. In mushroom Z, there were most tasteless and sweet amino acids, followed by bitter and MSG-like. The sum of free amino acids were highest in mushroom Y, followed by mushroom Z, conventional *P. ostreatus* and lowest in mushroom X, and the sums of essential amino acids follow the same order.

Table 4.2: Composition of free amino acids in conventional *Pleurotus ostreatus* and three different types, grown on different substrates containing waste products. The amino acids are shown as mg g^{-1} of dry mushrooms. In the table, the free amino acids are categorized by which taste they contribute to, and the sums of the categories are also presented. Sums of essential and non-essential amino acids, as well as the sums of free amino acids are shown for each mushroom type. Three experimental parallels were analyzed for each type of mushrooms. One-way analysis of variance (ANOVA) with Tukey's post hoc test for significant difference at the 5% level of significance in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0) was used to find means, standard deviations and between groups differences. Different subsets are indicated in superscript.

Amino acid	Conventional	Mushroom X	Mushroom Y	Mushroom Z	P-value
MSG-like					
Asp	10.2 ± 0.3^{b}	6.4 ± 0.4^{a}	9.9 ± 0.2^{b}	5.90 ± 0.12^{a}	$<0.001^{**}$
Glu	14.5 ± 0.4^{a}	14.1 ± 0.8^{a}	21.5 ± 1.2^{c}	18.53 ± 0.14^{b}	$<0.001^{**}$
Sweet					
Ser	6.4 ± 0.2^{a}	6.1 ± 0.5^{a}	6.9 ± 0.4^{a}	8.7 ± 0.3^{b}	$<0.001^{**}$
Thr†	6.56 ± 0.10^{b}	5.8 ± 0.2^{a}	7.6 ± 0.2^{c}	7.60 ± 0.11^{c}	$<0.001^{**}$
Ala	15.0154 ± 0.0016^{a}	14.4 ± 0.7^{a}	18.9 ± 0.7^{b}	24.8 ± 0.2^{c}	$<0.001^{**}$
Bitter					
His†	4.9 ± 0.6^{b}	2.9 ± 1.0^{a}	7.4 ± 0.7^{c}	3.5 ± 0.6^{ab}	$<0.001^{**}$
Met†	3.832 ± 0.008^{b}	3.12 ± 0.17^{a}	4.1 ± 0.2^{b}	2.97 ± 0.03^{a}	$<0.001^{**}$
Val†	6.47 ± 0.02^{b}	5.5 ± 0.3^{a}	7.4 ± 0.2^{c}	7.44 ± 0.09^{c}	$<0.001^{**}$
Phe†	7.01 ± 0.03^{b}	5.3 ± 0.3^{a}	7.0 ± 0.3^{b}	8.26 ± 0.10^{c}	$<0.001^{**}$
Ile†	5.43 ± 0.06^{b}	4.6 ± 0.2^{a}	6.3 ± 0.4^{c}	6.30 ± 0.07^{c}	$<0.001^{**}$
Leu†	9.49 ± 0.06^{b}	7.7 ± 0.4^{a}	11.4 ± 0.6^{c}	11.24 ± 0.15^{c}	$<0.001^{**}$
Tasteless					
Asn	4.47 ± 0.06^{bc}	3.7 ± 0.2^{a}	4.14 ± 0.14^{b}	4.72 ± 0.06^{c}	$<0.001^{**}$
Gln	14.7 ± 0.2^{a}	19.6 ± 1.1^{b}	38.9 ± 1.4^{c}	15.9 ± 0.2^{a}	$<0.001^{**}$
Tyr	11.1 ± 0.2^{c}	5.6 ± 0.3^{a}	7.9 ± 0.6^{b}	11.5 ± 0.2^{c}	$<0.001^{**}$
Lys†	7.8 ± 0.3^{b}	6.1 ± 0.3^{a}	9.3 ± 0.3^{c}	8.38 ± 0.09^{b}	$<0.001^{**}$
Aba	1.20 ± 0.03^{a}	1.26 ± 0.09^{ab}	1.53 ± 0.07^{c}	1.49 ± 0.14^{bc}	0.005^{**}
Sum MSG-like	24.7 ± 0.5^{b}	20.4 ± 0.9^{a}	31.4 ± 1.3^{c}	24.43 ± 0.19^{b}	$<0.001^{**}$
Sum sweet	28.0 ± 0.2^{a}	26.3 ± 0.9^{a}	33.4 ± 0.8^{b}	41.2 ± 0.4^{c}	$<0.001^{**}$
Sum bitter	37.2 ± 0.6^{b}	29.2 ± 1.2^{a}	43.5 ± 1.1^{c}	39.8 ± 0.7^{bc}	$<0.001^{**}$
Sum tasteless	39.3 ± 0.4^{ab}	36.3 ± 1.2^{a}	61.8 ± 1.6^{c}	42.0 ± 0.4^{b}	$<0.001^{**}$
Sum essential	51.6 ± 0.7^{b}	41.2 ± 1.2^{a}	60.4 ± 1.1^{c}	55.7 ± 0.7^{bc}	$<0.001^{**}$
Sum non-essential	77.6 ± 0.6^{a}	71.1 ± 1.7^{a}	110 ± 2^{c}	91.6 ± 0.6^{b}	$<0.001^{**}$
Sum free amino acids	129.1 ± 0.9^{b}	112 ± 2^{a}	170 ± 2^{d}	147.3 ± 0.9^{c}	$<0.001^{**}$

**Significant difference between groups at the 1% level of significance.
†Essential amino acids.

Total amino acids were extracted by hydrolyzing freeze-dried oyster mushroom samples in three experimental parallels, and they were measured using HPLC (performed by Siri Stavrum at Department of Biotechnology and Food Science, NTNU, Norway). One-way ANOVA with Tukey's post hoc test for significant difference at the 5% level of significance in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0) was used to analyze the means and standard deviations of the total amino acids, which are presented in table 4.3. For all four types of *P. ostreatus*, glutamic acid was the most numerous amino acid, followed by aspartic acid and alanine. The sum of total amino acids was highest in mushroom Z, followed by mushroom Y, mushroom X and the lowest in conventional *P. ostreatus*, and the same order applies to the sums of essential amino acids, though mushroom X and conventional have approximately the same amount of essential amino acids.

Table 4.3: Composition of total amino acids in conventional *Pleurotus ostreatus* and three different types, grown on different substrates containing waste products. The amino acids are shown as mg g^{-1} of dry mushrooms. Sums of essential and non-essential amino acids, as well as the sums of total amino acids are shown for each mushroom type. Three experimental parallels were analyzed for each type of mushrooms. One-way analysis of variance (ANOVA) with Tukey's post hoc test for significant difference at the 5% level of significance in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0) was used to find means, standard deviations and between groups differences. Different subsets are indicated in superscript.

Amino acid	Conventional	Mushroom X	Mushroom Y	Mushroom Z	P-value
Asp	31.8 ± 0.9^{a}	28.8 ± 1.1^{a}	27.53 ± 0.13^{a}	51 ± 6^{b}	$<0.001^{**}$
Glu	44.8 ± 1.2^{a}	79 ± 6^{b}	98 ± 6^{c}	72 ± 7^{b}	$<0.001^{**}$
Ser	9.9 ± 0.5^{a}	11.6 ± 1.0^{ab}	13.8 ± 0.2^{b}	20 ± 3^{c}	$<0.001^{**}$
Thr†	11.1 ± 0.3^{a}	12.5 ± 0.7^{ab}	14.4 ± 0.8^{b}	22 ± 2^{c}	$<0.001^{**}$
Ala	16.1 ± 0.4^{a}	21.4 ± 1.6^{ab}	25.3 ± 1.9^{b}	35 ± 4^{c}	$<0.001^{**}$
His†	6.7 ± 0.6^{ab}	6.2 ± 1.9^{a}	9.7 ± 1.9^{ab}	10.2 ± 0.6^{b}	$<0.019^{*}$
Met†	2.66 ± 0.19^{a}	3.1 ± 0.5^{a}	3.9 ± 0.6^{a}	5.9 ± 1.0^{b}	0.001^{**}
Val†	9.4 ± 0.2^{a}	9.8 ± 0.7^{a}	11.3 ± 0.6^{a}	19 ± 2^{b}	$<0.001^{**}$
Phe†	9.6 ± 0.4^{a}	8.9 ± 0.5^{a}	9.4 ± 0.3^{a}	17.4 ± 1.8^{b}	$<0.001^{**}$
Ile†	7.9 ± 0.2^{a}	7.9 ± 0.4^{a}	9.1 ± 0.2^{a}	15.2 ± 1.5^{b}	$<0.001^{**}$
Leu†	14.5 ± 0.4^{a}	14.3 ± 0.7^{a}	16.3 ± 0.5^{a}	27 ± 3^{b}	$<0.001^{**}$
Tyr	5.77 ± 0.10^{a}	6.1 ± 0.3^{a}	6.56 ± 0.15^{a}	12.5 ± 1.3^{b}	$<0.001^{**}$
Lys†	13.1 ± 0.3^{a}	12.2 ± 0.4^{a}	13.7 ± 0.9^{a}	24 ± 3^{b}	$<0.001^{**}$
Aba	0.91 ± 0.08^{a}	1.18 ± 0.11^{ab}	1.5 ± 0.2^{ab}	1.9 ± 0.7^{b}	$<0.055^{*}$
Sum essential	75.0 ± 1.0^{a}	75 ± 2^{a}	88 ± 3^{a}	141 ± 6^{b}	$<0.001^{**}$
Sum non-essential	109.2 ± 1.6^{a}	148 ± 7^{b}	173 ± 7^{bc}	191 ± 10^{c}	$<0.001^{**}$
Sum total amino acids	184.2 ± 1.9^{a}	223 ± 7^{ab}	261 ± 7^{b}	332 ± 12^{c}	$<0.001^{**}$

*Significant difference between groups at the 5% level of significance (for aba when rounding to 2 decimals).
**Significant difference between groups at the 1% level of significance.
†Essential amino acids.

4.2 Sensory characteristics of the five edible mushrooms grey oyster, king oyster, shiitake, white button and portobello

CATA with 79 subjects was performed on the five common edible mushrooms grey oyster, king oyster, shiitake, white button and portobello. The subjects judged the liking of each sample on a 9-point hedonic scale (1 = do not like at all, 9 = like a lot) and checked terms they perceived in the product. The statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0) was used to perform one-way ANOVA with Tukey's post hoc test for significant difference at the 5% level of significance on the preference data. Means for liking are shown in table 4.4, presented with standard deviation for each mean and subsets indicated by subscript letters. White button mushroom had the highest mean liking, with 7.15±1.51, and was significantly better liked than grey oyster, king oyster and shiitake at the 5% level. Portobello had the second highest score, with a mean liking of 7.05±1.96, and it was significantly different from grey oyster and shiitake. King oyster and shiitake had the mean scores 6.25±1.94 and 5.91±1.99 respectively, both being significantly higher liking means than grey oyster. Grey oyster had the lowest mean liking score with 4.38±2.14, and it was the only mushroom to score on the lower half of the 9-point hedonic scale.

Table 4.4: Mean liking of the different mushroom types in CATA (n=79), presented with standard deviation. Liking was indicated using a 9-point hedonic scale (1 = do not like at all, 9 = like a lot) for each sample. The CATA was performed on pan-fried mushrooms of the five varieties, and the preference data was analyzed with one-way ANOVA and Tukey's post hoc test at the 5% level of significance in IBM® SPSS® Statistics (Version 27.0.0.0). The different subsets are presented by superscript letters. The p-value for between groups comparison of each terms is also presented.

Grey oyster	King oyster	Shiitake	White button	Portobello	P-value
4.38 ± 2.14^{a}	6.25 ± 1.94^{bc}	5.91 ± 1.99^{b}	7.15 ± 1.51^{d}	7.05 ± 1.96^{cd}	$<0.001^{**}$

**Significant difference between groups at the 1% level of significance.

The sensory terms in the CATA were analyzed in XLSTAT statistical software with tools for sensory analysis (Version 2022.2.1), using Cochran's Q test with McNemar procedure for multiple pairwise comparisons was used to identify subsets at the 5% level of significance. Table 4.5 shows the usage of the terms for each mushrooms with p-value for between groups difference, and statistical subsets are indicated by subscript letters. At the 1% level of significance, the following 15 terms were used differently for the mushrooms: "flavorful (rich)", "flavorless (tame)", "bitter", "savoury", "earthy", "stale", "tastes like mushroom", "tastes like black pepper", "brown", "white", "light in color", "dark in color", "firm", "soft" and "chewy". At the 5% level of significance, the terms "salty", "sweet" and "yellow" were also used significantly different to describe the products.

Table 4.5: Term usage from CATA (n=79) with grey oyster, king oyster, shiitake, white button and portobello mushrooms. Data analysis was performed in XLSTAT statistical software with tools for sensory analysis (Version 2022.2.1), and Cochran's Q test with McNemar procedure for multiple pairwise comparisons was used to identify subsets at the 5% level of significance. The p-value for between groups comparison of each terms is also presented.

Term	Grey oyster	King Oyster	Shiitake	White button	Portobello	P-value
Flavor						
Flavorful (Rich)	13^{a}	22^{ab}	14^{a}	30^{b}	30^{b}	0.001^{**}
Flavorless (Tame)	13^{ab}	10^{ab}	25^{b}	10^{ab}	5^{a}	$<0.001^{**}$
Salty	12^{ab}	19^{ab}	11^{a}	18^{ab}	27^{b}	0.011^{*}
Sweet	1^{a}	9^{ab}	7^{ab}	13^{b}	10^{ab}	0.012^{*}
Bitter	20^{b}	3^{a}	7^{ab}	2^{a}	4^{a}	$<0.001^{**}$
Sour	10^{a}	8^{a}	4^{a}	3^{a}	6^{a}	0.153
Umami	10^{a}	17^{a}	19^{a}	22^{a}	19^{a}	0.076
Savoury	8^{a}	21^{ab}	12^{ab}	25^{b}	25^{b}	$<0.001^{**}$
Fatty	6^{a}	10^{a}	9^{a}	17^{a}	10^{a}	0.108
Earthy	39^{b}	15^{a}	16^{a}	17^{a}	19^{a}	$<0.001^{**}$
Stale	11^{a}	5^{a}	1^{a}	1^{a}	4^{a}	0.002^{**}
Tastes like mushroom	19^{a}	36^{b}	40^{b}	51^{b}	46^{b}	$<0.001^{**}$
Tastes like almonds	9^{a}	5^{a}	6^{a}	3^{a}	3^{a}	0.271
Tastes like seafood	10^{a}	10^{a}	6^{a}	3^{a}	5^{a}	0.167
Tastes like anise (black licorice)	3^{a}	3^{a}	0^{a}	2^{a}	3^{a}	0.493
Tastes like black pepper	13^{ab}	15^{ab}	9^{a}	28^{b}	25^{b}	0.001^{**}
Appearance						
Yellow	2^{a}	9^{a}	2^{a}	5^{a}	1^{a}	0.011^{*}
Brown	21^{ab}	11^{a}	17^{ab}	25^{ab}	32^{b}	0.001^{**}
White	2^{ab}	14^{b}	4^{ab}	3^{ab}	0^{a}	$<0.001^{**}$
Light in color	19^{b}	31^{b}	18^{b}	17^{b}	3^{a}	$<0.001^{**}$
Dark in color	10^{a}	3^{a}	14^{a}	7^{a}	41^{b}	$<0.001^{**}$
Texture						
Firm	13^{a}	31^{b}	18^{ab}	23^{ab}	16^{ab}	0.006^{**}
Soft	13^{ab}	9^{a}	32^{c}	22^{abc}	28^{bc}	$<0.001^{**}$
Soggy	21^{a}	13^{a}	20^{a}	14^{a}	14^{a}	0.235
Chewy	31^{ab}	36^{b}	27^{ab}	24^{ab}	17^{a}	0.010^{**}
Meat-like	10^{a}	17^{a}	12^{a}	8^{a}	17^{a}	0.127

*Significant difference between groups at the 5% level of significance.
**Significant difference between groups at the 1% level of significance.

Penalty analysis on the CATA data was performed using white button mushroom as an ideal reference product in XLSTAT statistical software with tools for sensory analysis (Version 2022.2.1), with a term usage threshold of 10%. The penalty analysis is presented as two plots in figure 4.1, showing mean change in liking as a function of proportion of respondents when (a) a term was not checked for the tested product, but it was checked for the ideal product, and (b) a term was checked for the tested product, but it was not checked for the ideal product. "Tastes like mushroom" was the only must have term, while the three terms "salty", "rich" and "dark in color" were nice to have terms. On the other hand, "tame", "soggy", "earthy" and "bitter" were must not have terms. The remaining terms belonged to the does not harm category.

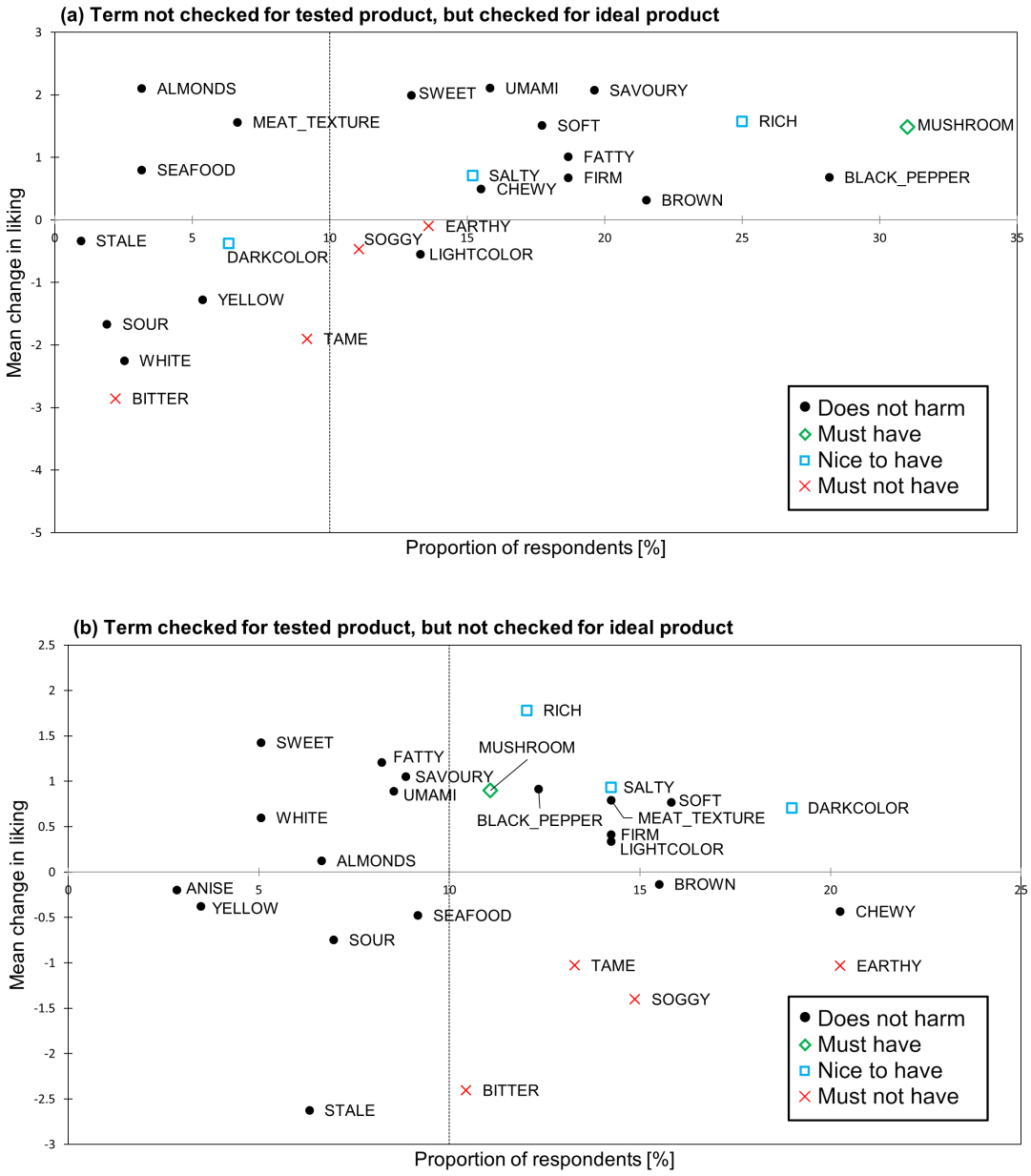


Figure 4.1: Penalty analysis of CATA (n=79) with the five edible mushrooms grey oyster, king oyster, shiitake, white button and portobello. The plots show mean change in liking as a function of proportion of respondents when (a) term was not checked for tested product, but was checked for ideal product, and (b) term was checked for tested product, but it was not checked for ideal product. XLSTAT statistical software with tools for sensory analysis (Version 2022.2.1) was used for data analysis, with white button mushroom chosen as ideal product due to its high liking scores, and the threshold for term usage was set to 10%. "Tastes like mushroom" was a must have term, while "Rich", "salty" and "dark in color" were nice to have terms, and "Earthy", "soggy", "tame" and "bitter" were must not have terms. The remaining terms were does not harm terms.

4.3 Consumer attitudes toward mushrooms cultivated on substrates containing coffee grounds

A questionnaire collecting socio-demographic information and 5-point scores on statements relating to attitude towards cultivating mushrooms on substrates containing coffee grounds received 182 responses. Mean scores on the five relevant statements were used to calculate a mean attitude score, which was the basis for categorization of respondents into the attitudinal categories positive (mean > 3.5), neutral ($2.5 <$ mean < 3.5) or negative (mean < 2.5). Data analysis was done with one-way ANOVA in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0). The counts of respondents belonging to each category and mean attitudes within each group are shown in table 4.6. The positive group was the largest, with 155 respondents belonging there, and it had a mean score of 4.16±0.35. In the middle was the neutral group, with 25 respondents and a mean score of 3.17±0.27, and the negative group was smallest with 2 respondents and a mean score of 2.30±0.14. The total mean score of all 182 respondents was 4.00±0.51.

Table 4.6: Attitudinal categories with their set mean limits, group count and mean attitude scores from questionnaire studying attitudes towards mushrooms cultivated on coffee grounds. The data was analyzed with one-way ANOVA in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0).

Attitudinal category	Category mean limit	Group count	Mean attitudes
Positive	>3.5	155	4.16 ± 0.35
Neutral	2.5-3.5	25	3.17 ± 0.27
Negative	<2.5	2	2.30 ± 0.14
Total		182	4.00 ± 0.51

The attitudinal groups were investigated in relation to different socio-demographic factors, which is shown in table 4.7. The table was created using the custom table function in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0). There were more women (62.09%) than men (36.81%) among the respondents, and the majority of the respondents were 21-40 years old (77.47%). More than half the respondents also belonged to the group with yearly income below 600 000 NOK (56.59%), while 160 respondents (87.91%) did not have any underage children in their household. Regarding occupational status, 106 (58.24%) were students and 66 (36.26%) were working. Most respondents reported that they eat mushrooms either monthly (44.51%) or weekly (35.16%). 130 (71.43%) respondents belonged to the group with positive attitude towards mushrooms cultivated on coffee grounds and reported that they eat mushrooms monthly, weekly or daily.

Table 4.7: Results of questionnaire studying attitudes towards mushrooms cultivated on coffee grounds, presented as count and percentage of respondents belonging to the different attitudinal groups (positive, neutral and negative) and total respondents. The results are sorted by various socio-demographic factors. Data analysis was performed using the custom table function in the statistical software platform IBM® SPSS® Statistics (Version 27.0.0.0)

Socio-demographic factor	Total (%)	Positive (%)	Neutral (%)	Negative (%)
Gender				
Woman	113 (62.09)	100 (64.52)	12 (48.00)	1 (50.00)
Man	67 (36.81)	54 (34.84)	12 (48.00)	1 (50.00)
Other	2 (1.10)	1 (0.65)	1 (4.00)	0 (0.00)
Age				
<20	2 (1.10)	2 (1.29)	0 (0.00)	0 (0.00)
21-40	141 (77.47)	118 (76.13)	22 (88.00)	1 (50.00)
41-60	30 (16.48)	27 (17.42)	2 (8.00)	1 (50.00)
61-80	9 (4.95)	8 (5.16)	1 (4.00)	0 (0.00)
Highest finished education				
High school	67 (36.81)	58 (37.42)	8 (32.00)	1 (50.00)
Master's degree	60 (32.97)	54 (34.84)	6 (24.00)	0 (0.00)
Bachelor's degree	44 (24.18)	34 (21.94)	10 (40.00)	0 (0.00)
Doctorate degree	2 (1.10)	2 (1.29)	0 (0.00)	0 (0.00)
Other / Do not wish to answer	9 (4.95)	7 (4.52)	1 (4.00)	1 (50.00)
Yearly income [NOK/year]				
<600 000	103 (56.59)	88 (56.77)	14 (56.00)	1 (50.00)
600 000 - 999 999	28 (15.38)	23 (14.84)	5 (20.00)	0 (0.00)
1 000 000 - 1 400 000	23 (12.64)	21 (13.55)	2 (8.00)	0 (0.00)
>1 400 000	22 (12.09)	19 (12.26)	2 (8.00)	1 (50.00)
Do not wish to answer	6 (3.30)	4 (2.58)	2 (8.00)	0 (0.00)
Number of persons in the household				
1	54 (29.67)	46 (29.68)	8 (32.00)	0 (0.00)
2	70 (38.46)	58 (37.42)	10 (40.00)	2 (100.00)
3	23 (12.64)	20 (12.9)	3 (12.00)	0 (0.00)
4	20 (10.99)	19 (12.26)	1 (4.00)	0 (0.00)
5 or more	15 (8.24)	12 (7.74)	3 (12.00)	0 (0.00)
Number of underage children in the household				
0	160 (87.91)	138 (89.03)	21 (84.00)	1 (50.00)
1	12 (6.59)	11 (7.10)	1 (4.00)	0 (0.00)
2	5 (2.75)	3 (1.94)	1 (4.00)	1 (50.00)
3 or more	5 (2.75)	3 (1.94)	2 (8.00)	0 (0.00)
Main buyer of food in the household				
Me	80 (43.96)	69 (44.52)	10 (40.00)	1 (50.00)
Evenly distributed	79 (43.41)	65 (41.94)	13 (52.00)	1 (50.00)
My partner	13 (7.14)	12 (7.74)	1 (4.00)	0 (0.00)
My parents	10 (5.49)	9 (5.81)	1 (4.00)	0 (0.00)
Occupational status				
Student	106 (58.24)	94 (60.65)	11 (44.00)	1 (50.00)
Working	66 (36.26)	52 (33.55)	13 (52.00)	1 (50.00)
Retired	8 (4.40)	7 (4.52)	1 (4.00)	0 (0.00)
Disabled	2 (1.10)	2 (1.29)	0 (0.00)	0 (0.00)
Frequency of eating mushrooms in meals				
Less than once a year	5 (2.75)	2 (1.29)	3 (12.00)	0 (0.00)
1-3 times a year	9 (4.95)	5 (3.23)	3 (12.00)	1 (50.00)
4-6 times a year	21 (11.54)	18 (11.61)	3 (12.00)	0 (0.00)
Monthly	81 (44.51)	74 (47.74)	7 (28.00)	0 (0.00)
Weekly	64 (35.16)	54 (34.84)	9 (36.00)	1 (50.00)
Daily	2 (1.10)	2 (1.29)	0 (0.00)	0 (0.00)

5 Discussion

5.1 Substrate effect on dry matter, ash, lipid and protein content in *Pleurotus ostreatus*

The results of dry matter, ash, lipid and protein content determination are shown in table 4.1. BAMA Gruppen AS is a Norwegian distributor of vegetables and fruits, and on their website they report that grey oyster mushrooms contain 2g of protein and 0.3g of fat per 100g of fresh mushroom[109]. This matches well with the results for conventional *P. ostreatus* in this projects, with protein content of 1.98g and slightly higher lipid content of 0.360g per 100g of fresh mushroom. Mushroom Y also has similar protein content (2.37g per 100g) and lipid content (0.37g per 100g), while mushroom X has similar protein content (2.18g per 100g), but higher lipid content (0.51g per 100g), and mushroom Z has higher content of both protein (3.69g per 100g) and lipid (0.54g per 100g) compared to the values reported by BAMA Gruppen AS. Sopanrao et al (2010) studied the nutritional value of *Pleurotus ostreatus* cultivated on six different substrates with lignocellulosic wastes, and they reported dry matter content to range between 10.1-11.5g, protein content between 2.06-2.83g and fat between 0.261-0.323g, all per 100g of fresh mushrooms (converted from dry mushroom basis to fresh by the author)[110]. The dry matter found in this project varies more, with results both above and below the values Sopanrao et al (2010) found. The protein content matches well for mushrooms X and Y, with conventional being a bit below the values found by Sopanrao et al (2010), but Mushroom Z was found to have higher protein content. The lipid contents were higher for all mushrooms in this project compared to the results by Sopanrao et al (2010).

The dry matter contents of mushroom X and mushroom Z were significantly higher than for mushroom Y, while the dry matter content of the conventional *P. ostreatus* was not significantly different from any of the other types at the 5% level of significance. This shows that spent coffee grounds (SCG) in the cultivation substrate did not cause differences in dry matter content. The ash contents in conventional *P. ostreatus* and mushroom Z are significantly higher than in mushroom X and mushroom Y at the 5% level of significance. Hence, low concentrations of SCG in the substrate lead to lower ash content, while high concentration of SCG resulted in similar ash content compared to conventional *P. ostreatus*. The lipid content was significantly higher in Mushroom X and mushroom Z compared to conventional mushroom and mushroom Y at the 5% level of significance. This could point towards that SCG in the substrate lead to higher lipid content in *P. ostreatus*, though the effect was not observed for mushroom Y, which also had 25% coffee grounds in the cultivation substrate. Mushrooms X, Y and Z all had significantly higher protein contents than conventional *P. ostreatus* at the 5% level of significance. This showed to coffee grounds in cultivation substrates for *P. ostreatus* lead to higher protein content. Moreover, mushroom Z, with the highest percentage of coffee grounds in the substrate, also had significantly higher protein content than mushrooms X and Y, indicating that higher

concentrations of coffee grounds could lead to higher protein content. This could be due to coffee residues being a good source of organic nitrogen[18], and nitrogen is an essential component in amino acids[111], which are the building blocks of proteins[112]. Fan et al (2000) found a slight increase in protein content for *P. ostreatus* cultivated on spent coffee grounds, which supports the findings of this project[113]. On the other hand, Alsanad et al (2021) found that addition of spent coffee grounds to cultivation substrate decreased the protein content, however, the reference mushrooms were grown on wheat straw substrate, which could have lead to higher protein content compared to rapeseed straw, oak sawdust or faba bean hulls, which was used in this project. Regarding fat content, Alsanad et al (2021) found that spent coffee grounds in high concentration in the substrate lead to an increase in fat content, which is in line with the findings of this project[114].

5.2 Substrate effect on free and total amino acid composition in *Pleurotus ostreatus*

The results of free amino acid compositions, presented in table 4.2, show that glutamic acid, glutamine and alanine were the three most numerous free amino acids in all types of mushrooms. This is in line with the findings of Yamauchi et al (2019), who reported that alanine, glutamic acid, arginine and glutamine were the four most abundant free amino acids in *P. ostreatus*[115]. Hong et al (1989) found the three most abundant free amino acids to be serine, threonine and glutamic acid in the pileus and threonine, alanine and glutamic acid in the stipe of *P. ostreatus*, but they did not report the amount of glutamine[116]. Mushroom Y contained the highest amount of free amino acids, followed by mushroom Z, conventional *P. ostreatus* and the least in mushroom X, and the amount of essential amino acids followed the same trend. This does not show a definite trend for change in free amino acids with respect to spent coffee grounds in the substrate. Of the taste-active categories, most amino acids belonged to the bitter category for conventional *P. ostreatus*, mushroom X and mushroom Y, followed by sweet and MSG-like. Tagkouli et at reported the same order of taste-active groups in *P. ostreatus*[117]. For mushroom Z, most amino acids belonged to the sweet category, followed by the bitter and MSG-like. This points towards high concentration of spent coffee grounds in the cultivation substrate leading to more sweet amino acids. The original project plan was to perform a CATA test on these mushrooms, but since the supplier could not deliver enough mushrooms, it was not possible. Such sensory analysis could provide insight in the relation between free amino acid composition and perceived taste of *P. ostreatus* cultivated on substrates with spent coffee grounds.

The results of total amino acid compositions are presented in table 4.3, and the most numerous amino acid was glutamic acid, followed by aspartic acid and alanine for all mushroom types. These findings are supported by Mattila et al (2002)[55], Mendez et al (2005)[118], Chirinang et al (2009)[119] and Manzi et al

(1999)[120], who all reported that glutamic acid was the most abundant amino acid in *P. ostreatus*, with aspartic acid, arginine, lysine, leucine and alanine being other major amino acids. Mushroom Z contained the highest amount of total amino acids, followed by mushroom Y, mushroom X and the least in conventional *P. ostreatus*. This was the same trend as was observed for protein content in the different mushroom types. Mushroom Z had the highest amount of essential amino acids, followed by mushroom Y, while conventional *P. ostreatus* and mushroom X contained approximately the same amount of essential amino acids. Overall, this showed that amino acid content was higher in *P. ostreatus* cultivated on substrates containing spent coffee grounds, and essential amino acid content was especially high in *P. ostreatus* cultivated on substrate with high concentration of spent coffee grounds.

5.3 Reflection on experimental design of part 1 and suggested improvements

Assessing the actual effect of spent coffee grounds in *Pleurotus ostreatus* cultivation substrate was difficult, as the substrate combinations provided by supplier were not ideal for this purpose. The substrates contained combination of four different products (coffee grounds, rapeseed straw, oak sawdust and faba bean hulls), which varied in a way that assessing the effect of individual parameters was difficult. For this project, the supplier changed some substrates compared to what was originally planned, including the addition of faba bean hulls to one substrate and rapeseed straw to another. The negative control, with respect to the effect of coffee grounds, could also be improved, as the cultivation substrate was not known, and it was cultivated and distributed by a separate supplier compared to the coffee grounds mushrooms. Using a 2^3 factorial design, as suggested by Leardi, could improve the experimental design and clarify which effects are caused by which factors as well as the interactions between them[121]. In practice this could be performed with high and low concentrations of coffee grounds in substrates mixed with high and low concentrations of two commonly used lignocellulosic materials, such as oak sawdust and rapeseed straw. All mushrooms in the experiment should also be cultivated by the same supplier to minimize other factors affecting the results.

The standard deviations are high for some analyzed values. This is especially notable for dry matter content in mushroom Y and mushroom Z, as well as multiple total amino acid measurements for mushrooms X, Y and Z. As the standard deviations are consistently lower for conventional *P. ostreatus* than the other types, there might be high degrees of variation in the mushrooms cultivated on spent coffee grounds. To further investigate if this was the case, or the varying values were due to experimental errors, the analyzes in question could have been repeated, potentially with more experimental parallels. However, due to the aforementioned lack of mushrooms cultivated on spent coffee grounds, repeating these experiments as a part of this project was not possible.

5.4 CATA with the five mushroom types grey oyster, king oyster, shiitake, white button and portobello

CATA with 79 subjects showed significant difference between the five edible mushrooms, and liking order from best to worst was: white button, portobello, king oyster, shiitake and grey oyster. Grey oyster mushroom was the only product to receive a mean liking score below 5, which means it was disliked more than it was liked by the subjects. It was rational that white button mushroom and portobello mushroom performed the best with respect to liking, as they are both *Agaricus bisporus*, which is the most commonly consumed mushroom species in the world[122]. Though the grey oyster mushroom product was purchased one day prior to CATA preparations, and the product was not past the printed expiration date, it was observed by the author to seem inferior in quality compared to the conventional oyster mushrooms used for chemical composition determination in part 1 of the project. Thus, the overall dislike of grey oyster mushroom may have been caused by the specific mushroom used coming from a bad batch or being handled poorly prior to purchase.

Penalty analysis with a 10% threshold for term usage was used to study the effect each attribute had on liking scores. "Tastes like mushroom" was the only must have term, and it was used significantly less for grey oyster mushroom than all the other products. Describing that something "tastes like mushroom" is an abstract concept, however, this meant the subjects had certain expectations of taste from the mushrooms, which lead to lower liking scores if not fulfilled. The terms "rich", "salty" and "dark in color" were nice to have terms, which were more frequently used for the well liked products. "Rich" was significantly more used for white button and portobello than grey oyster and shiitake, "salty" was significantly more used for portobello compared to shiitake and "dark in color" was significantly more used for portobello compared to all other products. On the negative side, the terms "tame", "earthy", "bitter" and "soggy" were must not have terms. "Tame" was significantly more used for shiitake than portobello, and "earthy" and "bitter" were significantly more used for grey oyster compared to all other products. The term "soggy" was not used significantly different to describe the products. The remainder of the terms were does not harm terms.

Some foods are better liked due to their chemical compositions, and personal preferences also contribute to deciding which foods are better liked than others. However, it is also interesting to consider other factors that may play a role in why certain foods are preferred. Familiarity with food are important for the perception of products[123], and Pliner et al (1993) found that liking of novel foods were lower compared to liking of familiar foods[124]. The products in the CATA were coded and tested blind, but the subjects might have still sensed familiarity through appearances, textures or flavors when testing. White button mushroom (*Agaricus bisporus*) is the most commonly eaten mushroom in the world[122], and of the mushrooms in the CATA, it is likely that the majority of the subjects were the most familiar with white button. The familiarity with

white button mushroom, and by extension portobello mushrooms as they are the same species, may have influenced the subjects liking in a positive manner. Boin et al (2018) have studied mushroom consumption behaviour in Portugal, and they found that *Agaricus Bisporus* was the most frequently eaten cultivated mushroom species, followed by *Lentinula edodes*, but most respondents were not familiar with *Pleurotus ostreatus* and *Pleurotus eryngii*[125]. This lacking familiarity could perhaps partly explain why the *Pleurotus spp.* mushrooms, which have delicacy status in multiple parts of the world[126], scored lower than the *Agaricus bisporus* mushrooms, especially in the case of *Pleurotus ostreatus*, which had the the lowest liking score of all the products.

The CATA results can be directly applied for product development. Market analysis predicts a growth in market size the next years, mainly driven by the increasing popularity of veganism, which requires meatless foods that are rich in protein[1]. To meet this demand, food companies will have to develop new products containing mushrooms that cater to this group. Insight on preference data and which attributes are necessary or should be avoided, which has been gained in this project, is crucial for development of successful products that are well liked by consumers. The CATA data also points towards white button mushroom and portobello mushroom being the most well liked, and because of this, new products with these mushrooms are more likely to be well received than products with grey oyster mushroom or shiitake. The data is especially relevant for companies operating in the Norwegian market or where food culture is similar, as most of the CATA subjects were from Norway.

5.5 Consumer attitudes towards mushrooms cultivated on coffee grounds waste

From the questionnaire with 182 respondents, it was found that most respondents were positive (n = 155) towards mushrooms cultivated on substrate containing coffee grounds, while some were neutral (n = 25) or negative (n = 2) based on the attitudinal sorting criteria used. It is important to consider how well the results of the questionnaire sample the attitudes of consumers in general, and this can be done by studying the socio-demographic groupings shown in table 4.7. Women were over represented in the questionnaire (62.09%), as well as the age group 21-40 years (77.47%). Hence, the survey results do not represent the attitudes of men, other gender identities or other age groups as well. Two other over represented groups are the yearly income group that reported to earn <600 000 NOK per year (56.59%) and the occupational status group student (58.24%), and the high representation of these groups was expected as the survey was shared in social media groups for students. From a product development point of view, it would be important that the groups who commonly eat mushrooms have a positive attitude, and 130 (71.43%) respondents were positive towards the mushrooms cultivated on coffee grounds and answered that they eat mushroom monthly, weekly or daily. Hence, there is a market interest for mushrooms cul-

tivated on coffee grounds or products containing such mushrooms based on the survey results.

It is also important to consider how some biases may have affected the score. Bernard et al (2005) wrote an article about the many biases that can influence the answers to a questionnaire, including ambiguous questions, uncommon wording and the mind-set of the respondents[127]. Ambiguous questions were avoided by using explicit statements of different situations to consider. The questionnaire was created in Norwegian language to circumvent uncommon wording for the respondents, and the questions phrasing was short and precise. The state of mind of the respondents is important to consider carefully in this case, as the survey was distributed in social networks, meaning it primarily reached respondents with some social connection to the author. Some of these respondents might have been in a supportive mind-set, which could have influenced them to answer the questionnaire in a more positive manner. Because of this, the overall answers might be more positively skewed compared to a randomly selected representative sample group of the consumer market.

The attitudinal groups were formed based on the mean score of 5-point scale answers to five statements related to mushrooms cultivated on substrates containing coffee grounds. There were seven statements in the questionnaire, which are all presented in appendix C, but two of them were not used to determine mean attitude score since they did not explicitly state anything about mushrooms grown on coffee grounds or waste substrates. The statements were related to sustainability in mushroom cultivation, and the answers would have been interesting to include in the main analysis if they had been worded in a way that directly referenced mushrooms cultivated on waste products or coffee grounds specifically. None of the five used attitude statements explicitly bring up the taste of mushrooms cultivated on coffee grounds, which could have been included to broaden the perspective of the attitudinal results.

6 Conclusions

Cultivation of *Pleurotus ostreatus* on spent coffee grounds (SCG) did not significantly influence dry matter content based on the results of this project. The ash content was lower in *P. ostreatus* cultivated on low concentration of SCG (25%) compared to conventional *P. ostreatus*, while high concentration of SCG (50%) gave similar ash content as conventional. The effect of SCG on lipid content was difficult to conclude, as it was higher for two out of three *P. ostreatus* cultivated on SCG compared to conventional, but the other SCG mushroom had similar lipid content as conventional. The protein content was higher in *P. ostreatus* cultivated on SCG substrates compared to conventional *P. ostreatus*, and high concentration of SCG (50%) in substrate resulted in higher protein content than low concentration of SCG (25%). The three most numerous free amino acids were glutamine, glutamic acid and alanine for all four types of *P. ostreatus* analyzed. The group of sweet amino acids constituted a bigger part of the free amino acid composition in *P. ostreatus* cultivated on high concentration of SCG (50%) compared to the three other types. With regard to total amino acid composition, all four types of *P. ostreatus* contained the most of glutamic acid, followed by aspartic acid and alanine.

CATA with 79 subjects was performed on five common mushrooms, and the results were that white button mushroom was best liked, followed by portobello, king oyster, shiitake and grey oyster. Penalty analysis on the CATA data revealed that "tastes like mushroom" was a must have attribute for mushrooms, while "rich", "salty" and "dark in color" were nice to have attributes and "soggy", "earthy", "tame" and "bitter" were must not have attributes.

Through attitudinal analysis of a questionnaire regarding consumer attitudes towards mushrooms cultivated on coffee grounds, it was found that 155 out of 182 respondents were positive, while 25 were neutral and 2 were negative. In a group of respondents that consume mushrooms daily, weekly or monthly, 71.43% had a positive attitude towards mushrooms cultivated on coffee grounds. This showed possible product interest from a consumer point of view, though certain biases, like state of mind bias, may have skewed the results towards the positive side.

7 Further perspectives

Multiple aspects regarding the effect of spent coffee ground (SCG) in cultivation of *Pleurotus ostreatus* and the social acceptance for mushrooms cultivated on substrates containing SCG are still unsettled. Conducting new experiments with chemical composition determination, in a more systematic manner with regards to the composition of the substrates used, would provide supplementary information on how spent coffee grounds affect the composition in mushrooms. In such experiments, all mushrooms used should be cultivated by the same supplier, and ideally, the only variable between the substrates should be the concentration of SCG. Combining such experiments with sensory analysis of the same types of mushrooms cultivated on SCG would offer a unique perspective on the relationship between flavor characteristics and chemical components in connection with SCG in substrate. To assess consumer attitudes towards mushrooms cultivated on SCG or waste substrates more reliably, a new survey could be created. The main improvements compared to the survey in this project should be inclusion of more aspects, like taste, as well as having a representative consumer panel answer the survey.

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A Appendix A: Calculation of freeze-drying yields for compositions of total amino acids

Extraction of total amino acids was performed for four types of *Pleurotus ostreatus*, of which three were cultivated on substrates containing spent coffee grounds and one was a conventional type. To calculate the freeze-drying yield of each *Pleurotus ostreatus* type, the samples were weighed before and after freeze-drying. The measurements and the freeze-drying yield of each mushroom type are presented in table A.1, which uses the mushroom codes defined in table 3.1.

Table A.1: Weight of fresh mushrooms (m_{fresh}), weight of mushrooms after freeze-drying ($m_{\text{freeze-dry}}$) and calculated freeze-drying yield ($Y_{\text{freeze-dry}}$) for the three *Pleurotus ostreatus* cultivated on coffee grounds and the conventional type. The mushroom types are coded corresponding to table 3.1.

Mushroom type	m_{fresh} [g]	$m_{\text{freeze-dry}}$ [g]	$Y_{\text{freeze-dry}}$
Conventional	37.2361	4.7122	0.12655
X	35.8245	4.3524	0.12149
Y	36.3082	3.4051	0.093783
Z	37.3049	5.9974	0.16077

For each mushroom type, the freeze-drying yield ($Y_{\text{freeze-dry}}$), was calculated by using equation A.1,

$$Y_{\text{freeze-dry}} = \frac{m_{\text{freeze-dry}}}{m_{\text{fresh}}}, \tag{A.1}$$

where m_{fresh} was the weight of the fresh mushrooms prior to freeze-drying and $m_{\text{freeze-dry}}$ was the weight of the freeze-dried mushrooms. An example calculation for conventional *Pleurotus ostreatus* is shown in equation A.2,

$$Y_{\text{freeze-dry, conventional}} = \frac{4.7122\text{g}}{37.2361\text{g}} = 0.12655. \tag{A.2}$$

B Appendix B: Calculation examples for chemical compositions

In this appendix, example calculations for dry matter content, ash content, lipid content, free amino acids and total amino acids will be shown using equations presented in section 3.

B.1 Dry matter content example calculation

To demonstrate an example calculation for dry matter content, a parallel of conventional *Pleurotus ostreatus* was used, and the relevant parameters to calculate the dry matter content are shown in table B.1.

Table B.1: Relevant values for calculation of dry matter content for one parallel of conventional *Pleurotus ostreatus*. The variables are weight of fresh mushroom in the sample (m_{fresh}) and weight after drying (m_{dry}).

Mushroom type	m_{fresh} [g]	m_{dry} [g]
Conventional (one parallel)	5.1175	0.4742

An example calculation using equation 3.1, with the values for a parallel of conventional *Pleurotus ostreatus*, is shown in equation B.1:

$$\text{Dry matter content} = \frac{0.4742\text{g}}{5.1175\text{g}} \cdot 100\% = 9.266\%. \tag{B.1}$$

The dry matter content presented in the report is a mean of three parallels for each type of *Pleurotus ostreatus*.

B.2 Ash content example calculation

To demonstrate an example calculation for ash content, a parallel of conventional *Pleurotus ostreatus* was used, and the relevant parameters to calculate the dry matter content are shown in table B.2.

Table B.2: Relevant values for calculation of ash content for one parallel of conventional *Pleurotus ostreatus*. The variables are weight of fresh mushroom in the sample (m_{fresh}) and weight after ashing (m_{ash}).

Mushroom type	m_{fresh} [g]	m_{ash} [g]
Conventional (one parallel)	4.8356	0.0429

An example calculation using equation 3.2, with the values for a parallel of conventional *Pleurotus ostreatus*, is shown in equation B.2:

$$\text{Ash content} = \frac{0.0429\text{g}}{4.8356\text{g}} \cdot 100\% = 0.887\%. \tag{B.2}$$

Equation 3.3 was then used to change the unit to mg per 100g fresh mushrooms, with en example shown in equation B.3:

$$\text{Ash content} = \frac{0.887\%}{100\%} \cdot \frac{100\text{g}}{100\text{g}_{\text{fresh}}} \cdot \frac{1000\text{mg}}{1\text{g}} = 8.87\text{mg}/100\text{g}_{\text{fresh}}. \tag{B.3}$$

The ash content presented in the report is a mean of three parallels for each type of *Pleurotus ostreatus*.

B.3 Lipid content example calculation

To demonstrate an example calculation for lipid content, a parallel of conventional *Pleurotus ostreatus* was used, and the relevant parameters to calculate the dry matter content are shown in table B.3.

Table B.3: Relevant values for calculation of lipif content for one parallel of conventional *Pleurotus ostreatus*. The variables are weight of lipids and kimax tube ($m_{\text{lipids + kimax}}$), weight of emtpy kimax tube ($m_{\text{empty kimax}}$), sample weight of fresh mushrooms added (m_{sample}), total volume of chloroform added ($V_{\text{chloroform added}}$) and volume of chloroform phase extracted for evaporation and weighing of lipids ($V_{\text{chloroform taken out}}$).

Mushroom type	$m_{\text{lipids + kimax}}$ [g]	$m_{\text{empty kimax}}$ [g]	m_{sample} [g]	$V_{\text{chloroform added}}$ [mL]	$V_{\text{chloroform taken out}}$ [mL]
Conventional (one parallel)	12.5558	12.5542	2.2203	10.0	2.00

An example calculation using equation 3.4, with the values for a parallel of conventional *Pleurotus ostreatus*, is shown in equation B.4:

$$\text{Lipid content} = \frac{(12.5558\text{g} - 12.5542\text{g}) \cdot 10.0\text{mL}}{2.2203\text{g} \cdot 2.00\text{mL}} \cdot 100\% = 0.360\%, \tag{B.4}$$

Equation 3.5 was then used to change the unit to g per 100g fresh mushrooms, with en example shown in equation B.5:

$$\text{Lipid content} = \frac{0.360\%}{100\%} \cdot \frac{100\text{g}}{100\text{g}_{\text{fresh}}} = 0.360\text{g}/100\text{g}_{\text{fresh}}. \tag{B.5}$$

For lipid content determination, three experimental parallels were done per type of mushrooms, and two chloroform phases were extracted per original parallel. Hence, the lipid content presented in the report is a mean of in total six parallel measurements for each type of *Pleurotus ostreatus*.

B.4 Free amino acids example calculation

To demonstrate an example calculation for free amino acids, a parallel of conventional *Pleurotus ostreatus* was used with respect to aspartic acid (asp), and the relevant parameters to calculate the dry matter content are shown in table B.4.

Table B.4: Relevant values for example calculation of composition of free amino acids, with respect to aspartic acid, for one parallel of conventional *Pleurotus ostreatus*. The variables are concentration of aspartic acid from HPLC (C_{asp}), molar mass of aspartic acid (M_{asp}), volume of KH_2PO_4 buffer the sample was mixed with ($V_{KH_2PO_4}$), weight of homogenized mushroom sample used in the parallel (m_{sample}) and fraction of dry matter content in the mushroom (f_{dry}).

Mushroom type	C_{asp} [μmol/L]	M_{asp} [g/mol]	$V_{KH_2PO_4}$ [L]	m_{sample} [g]	f_{dry}
Conventional (one parallel)	17.8954	133.1	0.0250	1.9867	0.09270

An example calculation using equation 3.6, with the values for a parallel of conventional *Pleurotus ostreatus* with respect to aspartic acid (asp), is shown in equation B.6:

$$\text{FAA}_{\text{asp}} = \frac{17.8954\mu\text{mol/L} \cdot 133.1\text{g/mol} \cdot 0.0250\text{L}}{1.9867\text{g} \cdot 0.09270} \cdot 1.25 \cdot 25 \cdot \frac{1\text{mg}}{1000\mu\text{g}} = 10.1\text{mg/g}_{\text{dry}}. \tag{B.6}$$

The content of free amino acids presented in the report is a mean of three parallels for each type of *Pleurotus ostreatus* per amino acid analyzed.

B.5 Total amino acids example calculation

To demonstrate an example calculation for total amino acids, a parallel of conventional *Pleurotus ostreatus* was used with respect to aspartic acid (asp), and the relevant parameters to calculate the dry matter content are shown in table B.5.

Table B.5: Relevant values for example calculation of composition of total amino acids, with respect to aspartic acid, for one parallel of conventional *Pleurotus ostreatus*. The variables are concentration of aspartic acid from HPLC (C_{asp}), molar mass of aspartic acid (M_{asp}), volume after titration and dilution to 10.00mL ($V_{\text{titration}}$), yield of freeze-drying ($Y_{\text{freeze-dry}}$) , weight of freeze-dried mushroom sample used in the parallel (m_{sample}) and fraction of dry matter content in the mushroom (f_{dry}).

Mushroom type	C_{asp} [μmol/L]	M_{asp} [g/mol]	$V_{\text{titration}}$ [L]	$Y_{\text{freeze-dry}}$	m_{sample} [g]	f_{dry}
Conventional (one parallel)	3.3646	133.1	0.01000	0.12655	0.0476	0.09270

An example calculation using equation 3.7, with the values for a parallel of

conventional *Pleurotus ostreatus* with respect to aspartic acid (asp), is shown in equation B.7:

$$\begin{aligned} \mathrm{TAA_{asp}} &= \frac{3.3646\mu\mathrm{mol/L} \cdot 133.1\mathrm{g/mol} \cdot 0.01000\mathrm{L} \cdot 0.12655}{0.0476\mathrm{g} \cdot 0.09270} \cdot 250 \cdot \frac{1\mathrm{mg}}{1000\mu\mathrm{g}} \\ &= 32.1\mathrm{mg/g_{dry}} \end{aligned} \quad \text{(B.7)}$$

The content of total amino acids presented in the report is a mean of three parallels for each type of *Pleurotus ostreatus* per amino acid analyzed.

C Appendix C: Questionnaire

The questionnaire to assess consumer attitudes towards mushrooms cultivated on coffee grounds was created in Google forms[104] and distributed mainly through the social media platform Facebook[105]. The 16 questions and statements were created by the author and are presented in table C.1 in original Norwegian wording, as well as with English translations (translated by the author).

Table C.1: All 16 questions and statements used in the questionnaire regarding attitudes towards mushrooms cultivated on coffee grounds. The questions are presented in original Norwegian wording and with English translation by the author.

Q1	Hvilket kjønn identifiserer du som?
English translation	What gender do you identify as?
Q2	Hvor gammel er du?
English translation	How old are you?
Q3	Hva er ditt høyeste fullførte utdanningsnivå?
English translation	What is your highest level of completed education?
Q4	Hva er den total inntekten i husstanden før fradrag og skatt? (NOK per år)
English translation	What is the total income of the household before deductions and taxes? (NOK per year)
Q5	Hvor mange personer er det i husholdningen (inkludert deg)?
English translation	How many people live in the household (including you)?
Q6	Hvor mange mindreårige barn (under 18 år) er det i husholdningen?
English translation	How many underage children (under 18 years) live in the household?
Q7	Hvem i husstanden er hovedansvarlig for dagligvarehandel?
English translation	Who in the household is the main responsible for grocery shopping?
Q8	Hva er din arbeidsstatus?
English translation	What is your occupational status?
Q9	Hvor ofte spiser du mat der sopp er en ingrediens?
English translation	How often do you eat food where mushroom is an ingredient?
Q10 (statement)*	Bruk av avfallsstoffer (slik som kaffegrut) i produksjon av sopp er bra for miljøet.
English translation	The use of waste products (such as coffee grounds) in mushroom production is good for the environment.
Q11 (statement)*	Sopp dyrket på kaffegrut er sunnere enn konvensjonell sopp fra dagligvarebutikk.
English translation	Mushrooms cultivated on coffee grounds are healthier than conventional mushrooms from grocery stores.
Q12 (statement)	Det er viktigere for meg at soppen smaker godt enn at den er produsert på en bærekraftig måte.
English translation	It is more important to me that the mushroom tastes good than that it is cultivated in a sustainable way.
Q13 (statement)	Jeg er villig til å betale mer for sopp som er produsert på en bærekraftig måte enn konvensjonell sopp fra dagligvarebutikk.
English translation	I am willing to pay more for mushrooms that are cultivated in a sustainable way than conventional mushrooms from the grocery store.
Q14 (statement)*	Sopp dyrket på avfallsstoffer (slik som kaffegrut) er ikke naturlig.
English translation	Mushrooms cultivated on waste products (such as coffee grounds) are not natural.
Q15 (statement)*	Jeg opplever det som trygt å spise sopp dyrket på avfallsstoffer (slik som kaffegrut).
English translation	I find it safe to eat mushrooms cultivated on waste products (such as coffee grounds).
Q16 (statement)*	Hvis det var tilgjengelig i dagligvarebutikker ville jeg kjøpt sopp dyrket på kaffegrut.
English translation	If it was available in grocery stores, I would buy mushrooms cultivated on coffee grounds.

*Statements that were used to determine attitudinal categories (positive, neutral and negative) towards mushrooms cultivated on coffee grounds..

C.1 Results of questions and statements

In this section, the results of questions 1-9 are presented with all submitted answers, including those entered by the respondents. In the report, these answers were usually collected as "other" when analyzing, unless they were found to be fitting within another another category.

The responses to question 1 ("What gender do you identify as?") are presented in table C.2.

Table C.2: The answers to question 1 ("What gender do you identify as?").

Q1: What gender do you identify as?		
Original answer (Norwegian)	Translated answer (English)	Number of answers
Kvinne	Woman	113
Mann	Man	67
Ønsker ikke oppgi	Do not wish to disclose	1
Lol	Lol	1
Total		182

The responses to question 2 ("How old are you?") are presented in table C.3.

Table C.3: The responses to question 2 ("How old are you?).

Q2: How old are you?		
Original answer (Norwegian)	Translated answer (English)	Number of answers
20 år eller yngre	20 years or younger	2
21-30 år	21-30 years old	133
31-40 år	31-40 years old	8
41-50 år	41-50 years old	14
51-60 år	51-60 years old	16
61-70 år	61-70 years old	5
71-80 år	71-80 years old	4
81 år eller eldre	81 years or older	0
Total		182

The responses to question 3 ("What is your highest level of completed education?") are presented in table C.4.

Table C.4: The responses to question 3 ("What is your highest level of completed education?").

Q3: What is your highest level of completed education?		
Original answer (Norwegian)	Translated answer (English)	Number of answers
Videregående skole	High school	67
Bachelorgrad	Bachelor's degree	44
Mastergrad	Master's degree	59
Doktorgrad	Doctoral degree	2
Annet / Ønsker ikke oppgi	Other / do not wish to disclore	6
Snart mastergrad	Soon master's degree	1
Folkehøgskole	Folk high school	1
Årsstudium	One-year study	1
Høyskole	University college	1
Ungdomsskole	Middle school	0
Total		182

The responses to question 4 ("What is the total income of the household before deductions and taxes? (NOK per year)") are presented in table C.5.

Table C.5: The responses to question 4 ("What is the total income of the household before deductions and taxes? (NOK per year)")

Q4: What is the total income of the household before deductions and taxes? (NOK per year)		
Original answer (Norwegian)	Translated answer (English)	Number of answers
Mindre enn 600 000	Less than 600 000	103
600 000 - 999 999	600 000 - 999 999	28
1 000 000 - 1 400 000	1 000 000 - 1 400 000	23
Mer enn 1 400 000	More than 1 400 000	22
Ønsker ikke oppgi	Do not wish to disclose	6
Total		182

The responses to question 5 ("How many people live in the household (including you)?") are presented in table C.6.

Table C.6: The responses to question 5 ("How many people live in the household (including you)?")

Q5: How many people live in the household (including you)?		
Original answer (Norwegian)	Translated answer (English)	Number of answers
1	1	54
2	2	70
3	3	23
4	4	20
5	5	11
6 eller flere	6 or more	4
Total		182

The responses to question 6 ("How many underage children (under 18 years) live in the household?") are presented in table C.7.

Table C.7: The responses to question 6 ("How many underage children (under 18 years) live in the household?")

Q6: How many underage children (under 18 years) live in the household?		
Original answer (Norwegian)	Translated answer (English)	Number of answers
Ingen	None	160
1	1	12
2	2	5
3	3	2
4 eller flere	4 or more	3
Total		182

The responses to question 7 ("Who in the household is the main responsible for grocery shopping?") are presented in table C.8.

Table C.8: The responses to question 7 ("Who in the household is the main responsible for grocery shopping?")

Q7: Who in the household is the main responsible for grocery shopping?		
Original answer (Norwegian)	Translated answer (English)	Number of answers
Meg	Me	79
Min partner	My partner	13
Jevnt fordelt	Evenly distributed	79
Mine foreldre	My parents	10
Alle hver for seg	Everyone for themselves	1
Total		182

The responses to question 8 ("What is your occupational status?") are presented in table C.9.

Table C.9: The responses to question 8 ("What is your occupational status?").

Q8: What is your occupational status?		
Original answer (Norwegian)	Translated answer (English)	Number of answers
I jobb (fulltid eller deltid)	Working (full time or part time)	66
Student / skoleelev / Lærling	Student / pupil / apprentice	106
Pensjonert	Retired	7
Ufør (kan ikke arbeide)	Disabled (cannot work)	2
Litt arbeid, men pensjonist	A little work, but retired	1
Total		182

The responses to question 9 ("How often do you eat food where mushroom is an ingredient?") are presented in table C.10.

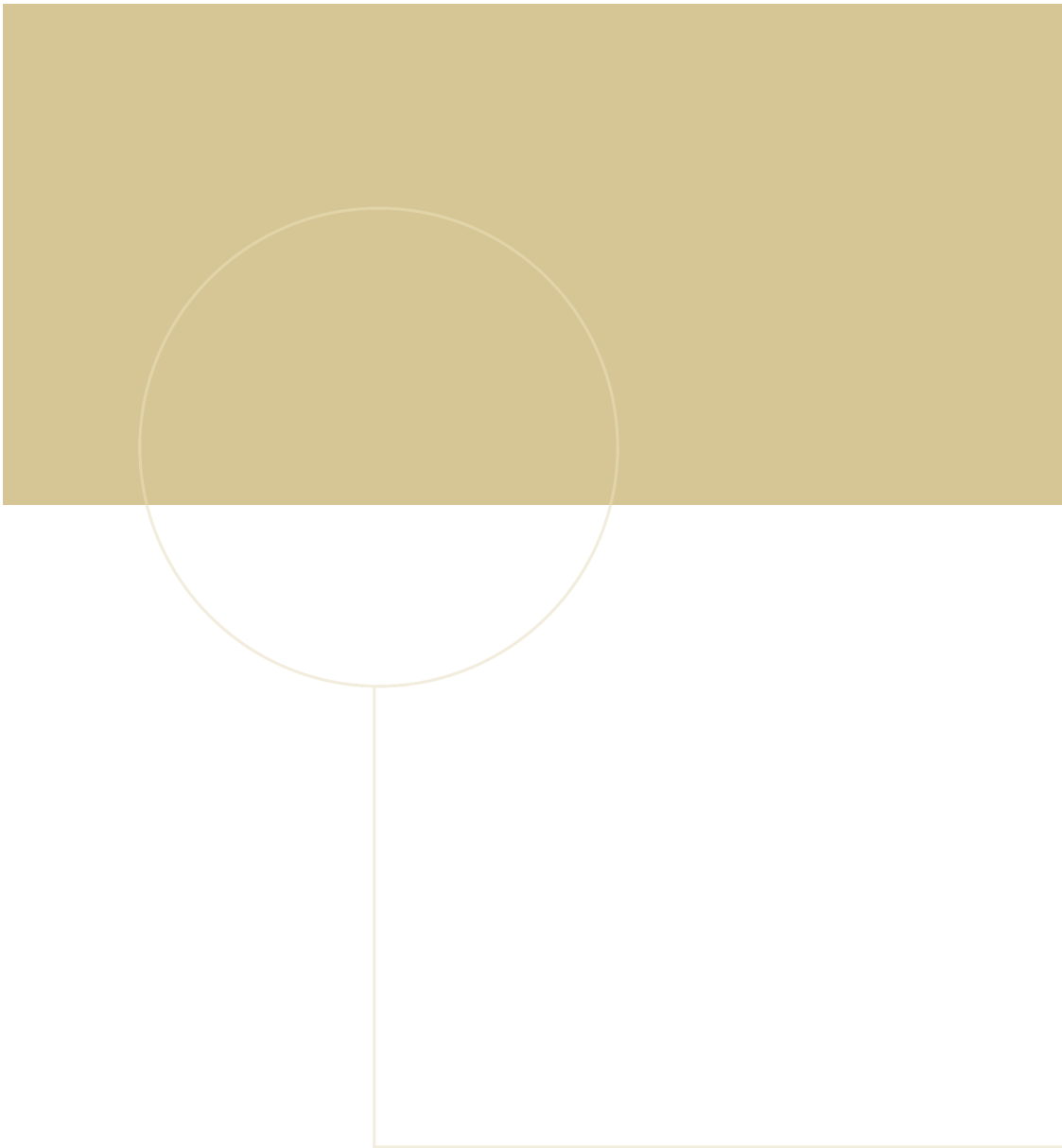
Table C.10: The responses to question 9 ("How often do you eat food where mushroom is an ingredient?").

Q9: How often do you eat food where mushroom is an ingredient?		
Original answer (Norwegian)	Translated answer (English)	Number of answers
Sjeldnere enn 1 gang i året	Less often than 1 time a year	5
1-3 ganger i året	1-3 times a year	9
4-6 ganger i året	4-6 times a year	21
Månedlig	Monthly	81
Ukentlig	Weekly	64
Daglig	Daily	2
Total		182

The results of the statements are presented in table C.11, and are represented by their number assigned in table C.1 (e.g. the statement "The use of waste products (such as coffee grounds) in mushroom production is good for the environment." is referred to as "Q10"). Answers were given on a 5-point hedonic scale (1 = totally disagree, 5 = totally agree).

Table C.11: The responses to the statements in the questionnaire (Q10-Q16). The answers were given on a 5-point hedonic scale (1 = totally disagree, 5 = totally agree).

5-point scale answer	Q10	Q11	Q12	Q13	Q14	Q15	Q16
1	0	9	11	18	106	4	6
2	1	17	46	34	50	3	4
3	23	135	53	46	17	26	50
4	43	12	51	64	7	61	61
5	115	9	21	20	2	88	61
Total	182	182	182	182	182	182	182



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