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Production of fungal biomass from oat flour for the use as a nutritious food source

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ABSTRACT

Fermentation can be a powerful tool for developing new sustainable foods with increased nutritional value and fermented microbial biomass derived from filamentous fungi is a promising example. This study investigates the nutritional profile of edible *Aspergillus oryzae* biomass produced under submerged fermentation (SmF) using oat flour as a substrate. The fermentation occurred in a $1m^3$ airlift bioreactor during 48 h at 35 °C and the nutritional profile of the produced fungal biomass in terms of amino acids, fatty acids, minerals (Fe, Zn, Cu, Mn), vitamins (E, D_2), and dietary fiber was compared to oat flour as well as pure fungal biomass grown on semi-synthetic medium. The total amount of amino acids increased from 11% per dry weight (dw) in oat flour to 23.5% dw in oat fungal biomass with an improved relative ratio of essential amino acids (0.37 to 0.42). An increase in dietary fibers, minerals (Fe, Zn, Cu), vitamin E, as well as vitamin D_2 were also obtained in the oat fungal biomass compared to oat flour. Moreover, the short chain omega-3 α-linolenic acid (ALA) and omega-6 linoleic acid (LA) values increased from 0.6 to 8.4 and 21.7 to 68.4 (mg/g dry weight sample), respectively, in oat fungal biomass. The results indicate that fungal biomass grown on oat flour could have a potential application in the food industry as a nutritious source for a wide variety of products.

1. Introduction

The rapid growth in global population and changing consumption patterns, followed by anthropogenic greenhouse gas (GHG) emissions put high demands on the global food production system [1]. According to United Nations Food and Agriculture Organization (FAO), contemporary agriculture animal farming is responsible for 14.5% of anthropogenic GHG emissions, and it is predicted that the environmental footprint will double by 2050 [2]. Therefore, optimizing current food systems and providing innovative products with reduced environmental impact are necessary preventive approaches. There are several strategies proposed as alternative food systems to replace conventional animal-based products [3,4].

Among the currently available alternatives are e.g., insects and artificial or in vitro meat. Regarding the latter, it is believed that artificial meat in the future can provide enough protein and reduce the environmental impact of meat production [2]. However, its biggest challenge is to fully mimic the muscle tissue of animal meat [5]. Insect protein could indeed be an alternative food source because of its high nutritional content and its environmentally sustainable production [6]. However, its use is still controversial, and it is far from universally acknowledged.

Plant-based foods, particularly legumes, and cereals, could also be a viable alternative to animal-based foods provided the proteins are concentrated with for example dry fractionation or pH-shift processing [7]. Apart from having a less negative environmental impact, they are high in unsaturated fats, dietary fibers and phytochemicals with antioxidant qualities [2]. However, plant-based products exhibit some nutritional deficiencies. They are not a complete source of protein as they do not have all essential amino acids in adequate portions [8]. Also, they contain antinutritional factors (e.g. lectins, tannins, saponins, and protease inhibitors) that can drastically decrease the bioavailability of many nutrients and interfere with their absorption [2].

Fungal biomass from edible filamentous fungi through a fermentation process is yet another promising alternative [9]. The filamentous fungal biomass not only provides a complete source of protein but is also

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a valuable source of bioactive compounds such as minerals, vitamins, polyunsaturated fatty acids, and antioxidants [10]. Fungal biomass are easily digested and their cell walls are good sources of dietary fibers, including chitin and β-glucan [11]. Hence, they may be categorized as a new alternative nutritious food source to supply a growing population with high protein content and with less environmental impact compared to e.g., red meat.

The ability of filamentous fungi to synthesize a variety of enzymes (amylase, invertase, cellulase, xylanase, lipase, and protease) allows them to grow on different type of substrates. This includes residues from food processing facilities such as thin stillage [12], pea by-product [13], brewers' spent grain [14], olive oil mill wastewater, and potato peel liquor [15,16]. However, choosing a viable substrate for producing fungal biomass intended for human consumption is challenging. The substrate should provide sufficient nutrients for good fungal growth as well as meet the safety requirements for human consumption. Cereals may represent an excellent choice due to their nutritional and widespread use. The cultivation of filamentous fungi on cereals makes a general improvement in nutritional value, protein digestibility [17], minerals bioavailability [18], and shelf life [19] compared to the cereals by themselves, and it also significantly lowers the content of determined antinutrients such as phytic acid, and tannins [20].

Fungal cultivation on cereals has traditionally been used for producing fermented products such as koji, mirin, sake and tapai which are the results of fermenting rice using *A. oryzae*. Among the cereals, oat has a high potential to be used as a substrate for producing fermented products due to its composition and documented health benefits [21]. Oats are known to be an excellent source of dietary fiber, antioxidants, minerals, and vitamins [22]. They are therapeutically active against diabetes, dyslipidaemia, hypertension, and inflammation (Londono et al). Also, oats are appropriate for people suffering from celiac disease as they are gluten-free [23]. Previous research studies regarding fermentation of *A. oryzae* on oat have been done for investigating the enrichment of phenolic compounds with antioxidant properties [24–26]. In addition, previous studies mainly comprise solid-state fermentation for fungal cultivation, while bacteria or yeasts have mostly been employed in submerged fermentation of oat in order to produce a prebiotic fermented oat beverage product [27]. Although solid-state fermentation requires low energy input, its scale up is difficult and thus mainly limited to small scale application for producing fermented food product. Additionally, solid-state fermentation results in a highly heterogeneous end-product made up of fungal mycelium and unconsumed substrate components [28]. Comparatively, submerged fermentation requires higher energy input, but is easier to scale up and currently applied in the majority of commercial food products obtained from filamentous fungus [29].

So far, no research has been reported on the nutritional profile of biomass obtained from oat after submerged fermentation using *A. oryzae*.

In this work we aimed to develop nutrient rich fungal biomass utilizing oat flour as substrate for *A. oryzae* under submerged fermentation conditions. In addition, to investigate the contribution of fungus to the final nutritional profile of biomass, the composition of pure fungal biomass grown on semi-synthetic medium was also examined. The obtained fungal biomass after fermentation had the potential to be developed further as a nutritious food source.

2. Material and methods

2.1. Substrate

Oat flour from AXA (Lantmännen Cerealia, Sweden) was used as the substrate. The oat flour was stored in a dark and dry place at room temperature until use. The compositional data of oat flour is presented in Table 1. The analytical procedures stated in this study were used to determine the composition of oat flour.

Table 1
Nutritional composition of oat flour, oat fungal biomass and pure fungal biomass. Results are given on a dry matter (dw) basis and are expressed as mean values ± SD ($n = 3$).

Component		Oat flour	Oat fungal biomass	Pure fungal biomass
Protein as TAA%		11.1 ± 2.63[1]	23.5 ± 1.9[2]	25.4 ± 1.44
Lipid %		7.7 ± 0.24[1]	22.8 ± 0.16[2]	8.4 ± 0.15
Dietary fiber %		12.3 ± 2.2[1]	34.7 ± 1.2[2]	37.3 ± 1.0
β-glucan		4.2 ± 0.03[1]	14 ± 0.06[2]	13.9 ± 0.02
Ash %		2.0 ± 0.03[1]	3.8 ± 0.13[2]	5.17 ± 0.07
Minerals	Cu	5.8 ± 0.2[1]	18.8 ± 0.3[2]	19.1 ± 0.3
(mg/kg dw)	Zn	38.3 ± 0.05[1]	43.3 ± 0.09[2]	98.5 ± 3.4
	Fe	44.8 ± 1.5[1]	57.3 ± 0.07[2]	31.6 ± 2.9
	Mn	56.5 ± 0.4[1]	26.5 ± 1.0[2]	8.6 ± 0.2
Vitamins (μg/g) (μg/100 g)	E	0.9 ± 0.4[1]	27.9 ± 2.4[2]	2.02 ± 1.3
	D_2	2.3 ± 0.1[1]	3.8 ± 0.9[2]	6.5 ± 0.4

Different numbers in the same row mean significant difference ($P < 0.05$) between the means at 95% confidence.

2.2. Fungal strain

The edible fungal strain *Aspergillus oryzae* CBS 819.72, was used in the current study.

The fungal strain was cultivated on potato dextrose agar (PDA) plates containing 20 g/L glucose, 15 g/L agar, and 4 g/L potato infusion, Pregrown PDA plates were flooded with 20 mL of sterile distilled water, and spores were distributed into solution using an L-shaped disposable plastic spreader. The new plates were then inoculated with 100 μL of spore solution, which was evenly spread across the agar surface with a similar plastic spreader, then incubated at 30 °C for 3–5 days before being stored at 4 °C until used.

2.3. Fungal fermentation

2.3.1. Producing fungal biomass on oat flour as substrate

Submerged fermentation was carried out according to Rousta et al. [11]. Three processes were used in the production of fungal biomass. First, the fungal spores were propagated from a culture that already existed. The spores (5.6×10^7 spores/mL) were then utilized to prepare a preculture in 1 L shake flasks, which were subsequently used to feed a 26 L bioreactor. Finally, the biomass from the 26 L bubble column bioreactor (Bioengineering, Switzerland) was used to inoculate the 1200 L airlift bioreactor. Before inoculation, the 1200 L bioreactor was sterilized in two steps. The first step involved in-situ sterilization by injection of steam at 120 °C for 20 min. Then 30 g/L oat flour mixture was transferred to the bioreactor and was sterilized through steam injection at 120 °C for 20 min. After cooling, the 20 L inoculum and 1 L vegetable oil as surfactant were added to the bioreactor. Cultivation was performed at 35 °C and using an aeration rate of 0.5 vvm (volume of air per volume of liquid per minute) and without a pH adjustment. The biomass was harvested after 48 h. To remove the liquid fraction, the suspension from the bioreactor was sieved using a vibrating screen (Russell Compact Sieve®, Russell Finex Ltd., UK). Before its analysis, the fungal biomass was freeze-dried at 0.05 bar and − 50 °C to a consistent weight and pulverized using a ball mill (Retsch MM 400, Haan, Germany) for periods of 25 s at a frequency of 30 Hz.

2.3.2. Producing pure fungal biomass on semi-synthetic media

To produce the pure fungal biomass, a semi-synthetic medium

containing 30 g/L glucose and 5 g/L yeast extract was prepared and cultivation was performed in a 26 L bubble column bioreactor. The medium was sterilized at 121 °C for 20 min in an autoclave (Systec, Germany) and the sterilization of the bioreactor was performed by injecting steam at 130 °C for 20 min. After sterilization, the 26 L bioreactor (2 m high × 15 cm diameter, Bioengineering AG, Wald, Switzerland) was dosed with 20 L of medium which had been inoculated with *A. oryzae* preculture biomass. This preculture had been previously grown in a shake flask with 1 L working volume for 24 h. The initial spore concentration during inoculation of the flask was 4.3×10^6 spores/mL.

The aeration rate was set at 0.5 vvm, and the cultivation was performed at 35 °C without a pH adjustment. After 48 h, the biomass was harvested and sieved for removing excess water with a vibrating screen (Russell Compact Sieve®, Russell Finex Ltd., UK). Before its analysis, the fungal biomass was freeze-dried at 0.05 bar and − 50 °C to a consistent weight and pulverized using a ball mill (Retsch MM 400, Haan, Germany) for periods of 25 s at a frequency of 30 Hz.

2.4. Analytical methods

2.4.1. Determination of amino acid composition

Four mL of 6 M HCl was added to ~30 mg dried sample in glass tubes and the headspace was flushed with nitrogen gas before incubation at 110 °C for 22 h. After the acid hydrolysis, the solution was filtered (0.45 μm PES, Fisherbrand) and then diluted ten times with 0.2 M acetic.

acid before amino acid composition was analyzed with LC/APCI-MS as described by [30]. Quantification was done using Amino Acid Standard H (Thermo Scientific) as external standard. Results are expressed as the anhydro-amino acid values in mg per 100 g on a dry weight (dw) basis as well as percent of total amino acids excluding tryptophane, which is not detected by acid hydrolysis.

2.4.2. Determination of total lipids and fatty acid composition

Total fatty acid analysis was performed according to Hinchcliffe et al. [31]. Total lipids were extracted from ~0.2 g dry sample with 5 mL chloroform:methanol (2:1). Samples were incubated in a rotation shaker for 60 min in total, with 5 min sonication every 30 min. Phase separation was obtained by addition of 1.5 mL milli-Q water and centrifugation (2000 ×*g*, 6 min). The chloroform phase was collected and pooled with a second extract using 4 mL chloroform. Total lipids were determined gravimetrically after evaporation of the chloroform under nitrogen gas. Results are expressed as g lipid per dry weight basis.

Composition of fatty acids was determined in extracted lipids re-dissolved in toluene. To 1 mL toluene sample, 0.3 mg heptadecanoic acid (C17:0; Larodan, 10–1700-13) was added as internal standard. Methylation of fatty acids was done by adding 1 mL of 10% acethyl-chloride in methanol and incubating the samples at 70 °C for 2 h. Milli-Q water (0.2 mL) was added to stop the reaction and 2.5 mL petroleum ether:diethyl ether (4:1) was used as extraction solvent for fatty acid methyl esters (FAMEs). After centrifugation (2000 ×*g*, 6 min), the ether phase was evaporated under nitrogen and FAMEs were re-dissolved in isooctane. FAMEs were analyzed using GC–MS (7890A GC System and 5975C triple-axis MS detector, Agilent Technologies) equipped with a VF-VAX column (30 cm × 0.25 mm × 0.25 μm) as described by Cavonius et al. [32]. The reference standard GLC-463 (Nu-Check Prep, Inc.) was used for identification and quantification. Results are expressed as mg FA per g dry sample and percent FA of total FA.

2.4.3. Determination of Total dietary fiber and Beta glucan

The enzymatic method AOAC 991.43 was used with Total Dietary Fiber, K-TDFR 06/0 to determine total dietary fibers as soluble and insoluble fractions (Megazyme, Ireland). The Beta Glucan Assay kit (K-YBGL, megazyme, Ireland) was used to determine β-glucan as a soluble fiber. Results are expressed as as percent dietary fiber on a dry weight basis.

2.4.4. Determination of tocopherols

α-, γ-, and δ-tocopherol was measured using LC with fluorescence detection (LC Jasco 2089 Plus and fluorescence detector Shimadzu RF-551) according to ([33,34] Results are expressed as μg per g on a dry weight basis.

2.4.5. Determination of vitamin D

Vitamin D was analyzed according to [33] with the following modifications. LCMS system used was Sciex 6500+ QTRAP triple-quadrupole mass spectrometer (AB Sciex, 11,432 Stockholm, Sweden) which was equipped with an APCI source and operated in the positive-ion mode. Chromatographic separations were performed on a Phenomenex Kinetix Core-Shell C18 (2.1, 100 mm, 1.7 μm 100 Å) UPLC column with SecurityGuard ULTRA Cartridges (C18 2.1 mm ID). Mobile phases used was A) 0.1% formic acid in milli-Q water and B) 0.1% formic acid in methanol for gradient elution. The column flow rate was 0.4 mL/min and the column temperature was 50 °C, the autosampler was kept at 4 °C. LC starting conditions at 50% B, held for 1 min, 2 min 60% B ramping linearly to 85% B at 5 min, then to 100% B at 10 min. Followed by recondition (50% B), total runtime 13 min. The MRM transitions were optimized one by one by direct infusion of vitamin D (D2-Sigma Aldrich 95,220) containing 1 μg/mL. The Q1/Q3 pairs were used in the MRM scan mode to optimize the collision energies for each analyte, and the two most sensitive pairs per analyte were used for the subsequent analyses. The retention time window for the scheduled MRM was 1 min for each analyte. The two MRM transitions per analyte, the Q1/Q3 pair that showed the higher sensitivity was selected as the MRM transition for quantitation. The other transition acted as a qualifier for the purpose of verification of the identity of the molecule. UPLC/MRM-MS data was acquired in the "scheduled MRM" mode using the Analyst 1.5 software and data processing was performed using the MultiQuant 3.0.3 software (AB Sciex, 11,432 Stockholm, Sweden)[33]. Results are expressed as μg per 100 g on a dry weight basis.

2.4.6. Determination of minerals

Minerals were analyzed according to [34]. One mL nitric acid and 0.2 mL hydrochloric acid was added to ~0.3 g dry sample in Teflon buckets. Samples were microwave digested (Milestone microwave laboratory system Ethos Plus Sorisole, Italy) at 180 °C for 20 min. Minerals were analyzed using atomic absorption spectroscopy (Agilent Technologies 200 Series 240FS). Standard curves of iron (Sigma TraceCERT), manganese (Reagecon), zinc (Assurance SpexCertiPrep) and copper (Fluka Chemica) were used for quantification. Results are expressed as mg per kg on a dry weight basis.

2.4.7. Determination of ash

The ash content was determined by combustion of ~200 mg dry sample at 550 °C for 3 h according to the Approved Method of the American Association of Cereal Chemists, Method 08–01(AACC, 2000). Samples were oven dried at 105 °C over-night before ash determination. Results are expressed as percent ash per dry weight basis.

2.5. Statistical analyses

All compositional data were analyzed on triplicate from one sample obtained from pilot scale. The data were statically analyzed using MINITAB 17 statistical software (Version 17.1.0, Minitab Inc., State college, PA, USA). The analysis of variance (ANOVA) using general linear model was performed on results and a confidence interval of 95% plus the pairwise comparisons between the data using Turkey's test were considered.

3. Results and discussion

In this study, oat fungal biomass was produced through submerged fermentation of oat flour using *A. oryzae*. The fermentation occurred in

1 m3 airlift bioreactor for 48 h. The nutritional profile of oat flour in terms of amino acids and fatty acids profile, minerals (Fe, Mn, Cu, Zn), and vitamins (E, D_2) was analyzed before and after fermentation. The composition of pure fungal biomass produced on semi-synthetic medium was also analyzed to evaluate the contribution of fungus to the final nutritional profile of biomass. The nutritional profile of oat fungal biomass produced in this study was investigated to determine its potential for use as a nutritious food source. Nutritional characterization of the oat flour and oat fungal biomass, as well as pure fungal biomass, are presented in Tables 1–3.

3.1. Characterization of oat flour and pure fungal biomass

Oat flour is a good candidate as a substrate for fermentation. It is nutritious and due to its milled structure, it has a high surface area and hence an increased contact surface between substrate and fungi. This in turn facilitates the accebility of nutrients to the fungi [2].The physiochemical characteristics of different oat flours can vary due to soil composition, climate condition and cultural practices [35]. The oat flour in this study was mainly composed of starch, 63.2% and had a total amino acid (TAA) content of 11.1% dw, lipid content of 7.7% dw and dietary fiber content of 12.3% dw (Table 1). The amino acid and fatty acid profiles of oat flour are presented in Tables 2 and 3.

The pure fungal biomass, cultivated on semi-synthetic media, was analyzed for the same nutritional parameters as that of oat flour and oat fungal biomass. The pure fungal biomass was used as a control to determine the contribution of *A. oryzae* to the nutritional profile of oat fungal biomass. Pure fungal biomass had a total amino acid content of 25.4% dw and was composed of 8.4% dw lipid and 37.3% dw dietary fiber (Table 1). Tables 2 and 3 show the amino acid and fatty acid profile, respectively, of pure fungal biomass.

3.2. Fungal fermentation using oat flour as substrate

3.2.1. Protein content and amino acid profile

Analysis of amino acids of oat fungal biomass revealed a significant increase in the total amino acid (TAA) content (110%) and in the relative amount of essential amino acids (EAA) (140%) compared to the oat substrate. An increase in the protein content of oat (104.7%) has also earlier been reported after fermentation by *R. oryzae* [2]. The EAA/TAA ratio was improved by 15% which represents the improvement in the quality of protein in oat fungal biomass compared to substrate. The individual amino acids in oat fungal biomass increasing the most were lysine, threonine and methionine which are all EAA. Lysine is an important essential, but limiting, amino acid in oat and its level increased 3.4 times in fermented biomass compared to the substrate oat flour. Lysine is important for proper growth and muscle turnover. It also plays an essential role in the production of carnitine, a nutrient responsible for converting fatty acids into energy and helping to lower cholesterol as well as promoting the uptake of trace metals such as calcium, iron, and zinc [36]. Threonine, the second limiting amino acid in oat flour, increased 2.6 times in biomass after fermentation, i.e. compared to the oat substrate. Threonine affects the energy metabolism, and immunity responses [37]. Even though oat, compared to other cereals, has some lysine and threonine, the amount is not high enough to be considered as a complete source of protein. It was shown in this study that fermentation of oat flour by *A. oryzae* greatly improves the quality of protein with a significant increase in EAA. Furthermore, methionine increased 2.8 times in oat fungal biomass after fermentation. Improving this sulphur amino acid content is also important because methionine acts as the substrate for other amino acids such as cysteine and its derivative taurine as well as for versatile compounds such as SAM-e, and the important antioxidant, glutathione. Methionine, as well as other sulphur-containing AAs have also been related to support the characteristic umami flavor in food [38]. Similar observations were reported regarding an increase in lysine, threonine, and methionine after fermentation of bread, brewers spent grain, and grass pea seeds by *N. intermedia*, *Rhizopus* sp., and *A. oryzae,* respectively [39,33,40]. The increment in amino acids could be explained as during fermentation carbohydrates serve as an energy source for fungal growth, and some of them may have been bio converted into protein [41]. Furthermore, a reduction in tannin concentration has been documented during fermentation due to the activity of a tannase that fungi may contain. Therefore, protein availability and digestibility may be improved because tannins bind proteins and form tannin-protein complexes [17].

According to the FAO/WHO recommendations, dietary requirements of protein by healthy human adults are 0.8 g per kg body weight per day [42]. Based on that, 100 g of dry fungal biomass produced in this study

Table 2
Amino acid profile of oat flour, oat fungal biomass and pure fungal biomass. Results are given on a dry matter (dw) basis and are expressed as mean values ± SD (n = 3).

	mg AA/g dw sample			AA (% of total AA)		
	Oat flour	Oat fungal biomass	Pure fungal biomass	Oat flour	Oat fungal biomass	Pure fungal biomass
Amino acid						
Glycine	5.62 ± 0.2	11.2 ± 1.1	12.1 ± 0.7	5.0 ± 0.1	4.7 ± 0.2	4.7 ± 0.1
Alanine	5.33 ± 0.1	14.6 ± 1.3	16.6 ± 0.7	4.7 ± 0.0	6.2 ± 0.2	6.5 ± 0.1
Serine	4.95 ± 0.2	11.4 ± 0.8	12.2 ± 0.0	4.4 ± 0.2	4.8 ± 0.0	4.8 ± 0.1
Proline	6.60 ± 0.3	12.1 ± 0.7	11.6 ± 0.5	5.9 ± 0.1	5.1 ± 0.0	4.5 ± 0.1
Valine*	6.57 ± 0.3	15.3 ± 1.4	16.2 ± 0.9	5.8 ± 0.1	6.5 ± 0.2	6.3 ± 0.1
Threonine*	4.59 ± 0.1	11.9 ± 0.5	14.7 ± 0.5	4.1 ± 0.0	5.1 ± 0.1	5.8 ± 0.0
Isoleucine*	4.95 ± 0.1	11.4 ± 0.8	12.7 ± 0.6	4.4 ± 0.0	4.8 ± 0.0	4.9 ± 0.1
Leucine*	9.52 ± 0.6	20.4 ± 1.4	20.7 ± 0.9	8.5 ± 0.4	8.6 ± 0.1	8.1 ± 0.1
Aspartic acid	9.51 ± 0.1	23.2 ± 1.1	25.8 ± 0.3	8.5 ± 0.2	9.8 ± 0.3	10.1 ± 0.2
Lysine*	5.11 ± 0.1	17.5 ± 1.3	25.3 ± 1.2	4.5 ± 0.0	7.4 ± 0.1	9.9 ± 0.2
Glutamic acid	25.24 ± 0.4	38.1 ± 3.2	32.6 ± 0.7	22.5 ± 0.2	16.1 ± 0.4	12.8 ± 0.0
Methionine*	1.70 ± 0.05	4.8 ± 0.1	4.3 ± 0.1	1.5 ± 0.0	2.0 ± 0.0	1.6 ± 0.0
Histidine*	2.37 ± 0.1	5.3 ± 0.4	5.8 ± 0.3	2.1 ± 0.1	2.2 ± 0.0	2.2 ± 0.0
Phenylalanine*	6.33 ± 0.07	12.3 ± 0.8	12.4 ± 0.3	5.6 ± 0.1	5.2 ± 0.0	4.8 ± 0.0
Arginine	6.28 ± 0.2	13.1 ± 0.6	16.0 ± 0.3	5.6 ± 0.1	5.5 ± 0.0	6.2 ± 0.2
Tyrosine	5.90 ± 0.1	10.5 ± 0.4	13.6 ± 0.6	5.2 ± 0.1	4.5 ± 0.4	5.3 ± 0.3
Cystein	1.23 ± 0.07	1.3 ± 0.04	1.6 ± 0.0	1.1 ± 0.0	0.5 ± 0.0	0.6 ± 0.0
TAA[a]	111.87 ± 2.6[1]	235.1 ± 1.9[2]	254.9 ± 1.4	100.00	100.00	100.00
TEAA[a]	41.17 ± 1.3[1]	99.3 ± 6.6[2]	112.4 ± 4.9	36.7 ± 0.4	42.2 ± 0.3	44.0 ± 0.6
TEAA/TAA	0.36[1]	0.42[2]	0.44			

Different numbers in the same row mean significant difference ($P < 0.05$) between the means at 95% confidence.
* Rows with asterisk indicate essential amino acids.
[a] AA: amino acids, TEAA: total essential amino acids, and TAA: total amino acids (excluding tryptophane).

Table 3
Fatty acid profile of oat flour, oat fungal biomass and pure fungal biomass. Results are given on a dry matter (dw) basis and are expressed as mean values ± SD ($n = 3$).

	mg FA/g dw sample			FA (% of total FA)		
	Oat flour	Oat fungal biomass	Pure fungal biomass	Oat flour	Oat fungal biomass	Pure fungal biomass
Fatty acid						
C14:0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
C15:0	0.0 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
C16:0	8.2 ± 0.1	28.4 ± 0.3	9.8 ± 0.0	16.7 ± 0.1	16.7 ± 0.0	17.8 ± 0.1
C16:1n7	0.1 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.6 ± 0.0
C18:0	0.4 ± 0.0	2.8 ± 0.0	4.3 ± 0.0	0.9 ± 0.0	1.7 ± 0.0	7.8 ± 0.1
C18:1n9	17.2 ± 0.2	56.2 ± 0.8	25.9 ± 0.1	35.0 ± 0.1	33.0 ± 0.2	47.1 ± 0.1
C18:1n7	0.4 ± 0.1	2.7 ± 0.1	0.2 ± 0.0	0.9 ± 0.0	1.6 ± 0.0	0.4 ± 0.0
C18:2n6*	21.7 ± 0.5	68.4 ± 1.5	12.6 ± 0.1	44.1 ± 0.6	40.2 ± 0.2	22.9 ± 0.3
C18:3n6	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.0
C18:3n3*	0.60 ± 0.0	8.4 ± 0.1	0.2 ± 0.0	1.2 ± 0.0	4.9 ± 0.1	0.4 ± 0.0
C20:0	0.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.0
C20:1n9	0.3 ± 0.0	0.7 ± 0.0	0.1 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.2 ± 0.0
C20:2n6	0.0 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C22:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
C24:0	0.0 ± 0.0	0.9 ± 0.1	0.8 ± 0.1	0.0 ± 0.0	0.5 ± 0.0	1.5 ± 0.1
Total FA[a]	49.0 ± 0.9[1]	170.1 ± 2.7[2]	55.1 ± 0.2	100.0	100.0	100.0
Total SFA[a]	8.8 ± 0.1[1]	33.0 ± 0.3[2]	15.5 ± 0.2	18.0 ± 0.3	19.4 ± 0.1	28.1 ± 0.2
Total MUFA[a]	18.0 ± 0.2[1]	60.1 ± 0.9[2]	26.6 ± 0.1	36.6 ± 1.2	35.3 ± 0.8	48.2 ± 0.4
Total PUFA[a]	22.3 ± 0.6[1]	77.0 ± 1.6[2]	13.0 ± 0.1	45.4 ± 0.3	45.2 ± 0.7	23.6 ± 1.1

Rows with asterisk indicate essential fatty acids.
Different numbers in the same row mean significant difference ($P < 0.05$) between the means at 95% confidence.
[a] FA: Fatty acids, SFA: Saturated fatty acids, MUFA: monounsaturated fatty acid, and PUFA: polyunsaturated fatty acids.

can meet 42% of the protein requirement of healthy adult with 70 kg body weight.

While the effects of fermentation on total protein content of various substrates have earlier been established [43,44,45] less is known about the impact of fermentation on the amino acid profile, something which may vary based on the kind of fungi, fermentation, and substrate employed. For example, the content of lysine, methionine, and threonine increased after solid state fermentation of bread by *N. intermedia* while a decrease in selected AA, mainly glutamine, proline, and phenylalanine was observed [33]. In another study comprising SmF of bread by *R. delemar*, a decrease in the contents of cysteine, glutamine and proline was defined beside an increase in the content of other amino acids [46]. However, in the current study, all amino acids increased in amount in the *A. oryzae* biomass after SmF.

In this study, pure fungal biomass of *A. oryzae* was also compared with biomass obtained after fermentation of oat flour. The protein content of this pure biomass, measured as TAA (25.4% dw) was nearly close to that of oat fungal biomass (23.5% dw). In addition, the ratio between TEAA/TAA in the pure fungal biomass and the oat fungal biomass stayed relatively similar (Table 2). The results show that the protein content and amino acids profile of oat fungal biomass was mainly derived from the *A. oryzae* rather than from oat flour.

3.2.2. Lipid content and fatty acid profile

After fermentation, the total lipid content increased from 7.7% in oat flour to 22.8% in the fermented biomass. Even though the amount of all individual fatty acids increased after fermentation, the largest amount was ascribed to polyunsaturated fatty acids (PUFA). The biomass was composed of 45.2% PUFA, 35.3% MUFA, and 19.41% SFA with a nearly similar relative ratio to oat flour. The most abundant fatty acid in the fermented biomass was linoleic acid (C18:2), followed by oleic acid (C18:1), and also palmitic acid (C16:0), which almost tripled after fermentation compared to the oat flour. A similar pattern has also been reported when bread was fermented by *N. intermedia* [33]. In addition, through fermentation of vinasse and sweet potato with *A. oryzae* [47,48] and thin stillage and sweet potato with *N. intermedia* [47,49], linoleic, oleic, and palmitic acid increased as primary fatty acids in fermented fungal biomass. The rise in total lipid content could be attributed to the high carbohydrate content of oat flour, which encourages the synthesis of mycelial fat. [50]. The high amylase activity of *A. oryzae* (industrially used for amylase production) [51] makes glucose more accessible for fungus during cultivation. Therefore, more elevated glucose or C/N ratio in the nutrient medium triggers the fatty acids synthesis pathway in filamentous fungal cells and leads to an increased fat content [52]. In one study, the lipid content of *A. oryzae* increased with increasing C/N ratio. The increase was from 1.8% at the C:N 20:1 to 37% at C:N 300:1 [52]. As for protein, the profile of fatty acids synthesized by fungi varies depending on the type of fungi, fermentation condition, and substrate used. For example, increased fat content during cultivation of *R. delemar* on bread was aligned with increased SFA and decreased MUFA and PUFA [46]. However, in the current study, PUFA was the major group of fatty acids in oat fungal biomass, which may be connected to certain health benefits. Essential fatty acids including linoleic (LA; C18:2n6) and α-linolenic acid (ALA; C18:3n3) have been proved to play a significant role in regulating blood pressure, inflammatory responses, protection against fatal heart disease, prevention of diabetes and certain types of cancer [53] which supports the importance of ensuring adequate intake through the diet. The oat fungal biomass in this work consisted of 6.83% dw linoleic acid and 0.83% dw α-linolenic acid, which was a 3- and 13-fold increase, respectively, compared to oat flour before fermentation. The recommended dietary intake (RDI) reports the adequate intake of linoleic acid for women and men to be between 11 and 14 g/day respectively [54]. In addition to this, the optimal intake for α-linolenic acid is estimated at 0.8 to 1.1 g/day [55]. According to this information, 100 g of oat fungal biomass produced in this study meets nearly 50% of the daily requirement of linoleic acid and 100% of α-linolenic acid (Table 4). The amount of linolenic acid obtained in oat fungal biomass was remarkably higher relative to when using substrates such as, vinasse, rice bran, potato peel liquor, stale bread, and thin stillage to cultivate *A. oryzae*, *R. oryzae*, *R. delemar*, *N. intermedia* respectively [56,33,48]. Even though increase in fat content has been observed in different studies after fermentation [47,12,33,46], some authors have reported a decrease in fat content after fermentation of rice bran and tepary beans by *Rhizopus* sp. This is likely due to the ability of some fungi such as *R. oligosporus*, to acquire energy from released fatty acids during fermentation [56,57].

Comparing the fatty acids profile of oat fungal biomass with pure fungal biomass, it was specified that without considering the oat as substrate, *A. oryzae* is able to produce palmitic, oleic, linoleic, and α-linolenic acids. However, the concentration of the fatty acids (Table 3) and the lipid content of pure biomass (8.4%) were in much lesser

Table 4
Comparison of the amount of nutrients in oat fungal biomass with dietary recommended daily intake (RDI) [58].

	RDI for adult		Nutrient amount in oat fungal biomass
Nutrient	Males unit/day	Females unit/day	Unit/100 g dw
Iron	16 mg	12 mg	5.7 ± 0.07 mg
Zinc	11	8	4.3 ± 0.09
Copper	0.9	0.9	1.8 ± 0.3
Manganase	2.3	1.8	2.6 ± 1.0
Vitamin E	15	15	2.7 ± 2.4
Vitamin D_2	15 μg	15 μg	3.78 ± 0.9 μg
Linoleic acid (ω6)	14 g	11 g	6.8 ± 1.5 g
α-Linolenic acid (ω3)	0.8–1.1	0.8–1.1	0.8 ± 0.1
Dietary fiber	28	28	34.7 ± 1.2

amount relative to when *A. oryzae* was grown on oat flour (22.8%). It shows that fat accumulation rate by *A. oryzae* has been affected by the type of substrate.

3.2.3. Micronutrients

SmF of oat flour by *A. oryzae* significantly improved the content of iron, zinc, and copper compared to oat flour. Along with this, the ash content was increased in the final fermented biomass (3.8 g/100 g) (Table 1). The highest increase was obtained in copper content ($P = 0.00$) while a slight but significant increase was observed in iron and zinc content after fermentation ($P < 0.05$). Similarly, studies by Sadh et al. [59] and Chawla et al. [60] reported an increase in the mineral content (iron and zinc) in peanut oil cake and black eye pea after fermentation with *A. oryzae*. Likewise, fermentation of bread with *N. intermedia* resulted in an increase in total ash content, comprising increases in minerals such as iron, zinc, and copper [33].

Despite the increase of the above-mentioned minerals, the manganese content decreased in oat fungal biomass. The decrease in manganese may be attributed to high consumption of this mineral in fungal metabolic processes. Manganese acts as a cofactor for pyruvate carboxylase which converts pyruvate to oxaloacetate. Oxaloacetate is a key intermediate of the citric acid cycle and involved in many important anabolic processes such as fatty acids and amino acid synthesis [61]. According to recommended dietary intake for iron, zinc, copper, and manganese [58], 100 g of dry oat fungal biomass produced in this study can meet around 50% requirement of iron and zinc and 100% to that of copper and manganese.

Bioavailability of minerals in cereals is negatively influenced by the presence of anti-nutritional factors such as phytate, phytic acid, and polyphenols [62]. During fermentation, the interaction of minerals with phytate and the phenolic compound is disrupted by phytase and makes the minerals free and accessible for the digestive system [63]. Increased bioavailability of iron and zinc has been reported after fermentation of peanut and black- eyed pea seed flour with *A. oryzae* [60,59].

A. oryzae takes up minerals from the environment and their concentration in the produced fungal biomass varies depending upon the amount of each mineral in the fermentation medium [64]. Different substrates such as oat flour and semi-synthetic media provide different initial concentrations of iron, zinc, copper, and manganese, which leads to varying concentrations of minerals in cultivated fungal biomass (Table 1).

A substantial increase in the fat-soluble antioxidant tocopherol (α-, δ- and ɣ-tocopherol), i.e., vitamin E was observed after fermentation resulting in 27.8 ± 0.3 μg/g dw in oat-fungal biomass. A similar result has been reported after the fermentation of stale bread by *N. intermedia* [33]. The recommended dietary intake (RDI) for vitamin E for males and females aged 14 years and older is 15 mg daily. Accordingly, 100 g of dry fungal biomass produced in this study meets 15% of daily requirement of vitamin E (Table 4). Most of the vitamin E in oat fungal biomass consisted of α- and ɣ-tocopherol which are the two major forms of this vitamin. The main sources of vitamin E in the human diet are vegetable oils and plant seeds where the ɣ-tocopherol form is predominant [65]. However, α-tocopherol is the most biologically active form of vitamin E for the human body [66] and the only form of vitamin E in vitamin supplements [67]. In this study, fermentation significantly increased the α-tocopherol content of oat flour from 0.3 μg/g to 12.5 μg/g dw in final biomass. The increase in the vitamin E content of biomass along with the increase in PUFA may prevent peroxidation of the PUFA and thereby protecting the fungal cell from oxidative damage [68]. Vitamin E prevents or delays certain chronic diseases associated with free radicals such as cardiovascular disease and cancer due to its function as an antioxidant [69]. It also has an anti-inflammatory effect and enhances the immune system when consumed in the recommended amounts [70].

Oat flour is not a good source of vitamin D which is commonly fortified to satisfy human requirements. Filamentous fungi can produce vitamin D_2 (ergocalciferol) from their precursor ergosterol in their cell wall. As much as 3.78 ± 0.92 μg/100 g dry weight of vitamin D_2 was obtained in the fermented biomass in this study. Gmoser et al. [33] reported an even higher concentration of vitamin D_2 in biomass after bread fermentation; 0.89 ± 0.1 μg/g dw. This could be due to exposing the fungi to UV light during fermentation which triggers the vitamin D synthesis. The recommended dietary intake for vitamin D is 15 μg/day; accordingly, 100 g of dried fermented biomass obtained in this study can meet 25% of the daily requirement (Table 4). Getting enough vitamin D is vital for the typical growth and development of bones and teeth as well as for improved resistance to certain diseases. Since major sources of vitamin D are either sunlight or animal products such as fatty fish, fungal products as a natural food source of vitamin D for vegetarians/vegans is highly interesting.

The oat fungal biomass contained much higher levels of vitamin E compared with the pure fungal biomass, but lower levels of vitamin D_2 (Table 1). The presence of very high amount of vitamin E in final biomass compared to pure fungal biomass shows the significance of using oat flour as a substrate for the cultivation of *A. oryzae*. The results prove that oat flour as a substrate provides enough precursors for the synthesis of high levels of vitamin E during fermentation. For vitamin D_2, the highest level was found in pure fungal biomass and the lowest level in oat flour substrate, which indicates that vitamin D_2 is added in oat fungal biomass from fungus itself which is a rich source of vitamin D [71].

3.2.4. Total dietary fiber and Beta Glucan

Even though oat flour is a good source of dietary fiber and Beta Glucan (*β*-glucan) [22], submerged fermentation of oat flour using *A. oryzae* converts it to an excellent source of dietary fiber and *β*-glucan with 2.81 and 3.3 fold increase, respectively (Table 1). A diet rich in fiber has been gaining interest due to its multiple functional and bioactive properties. Fibers are essential for keeping the digestive system healthy and are also related to lower blood pressure and reduced heart problems, diabetes, and obesity [72]. *β*-glucan as a soluble fiber has a great potential to form highly viscous solutions in the human gut and it may constitute the basis of their health benefits [73].

According to the USDA (United States Department of Agriculture) the recommended dietary fiber intake should be 28 g for men and women based on the obtained results and recommendations, 75.6 g of dry fungal product can satisfy the daily requirement of dietary fiber in human (Table 4). An increase in dietary fiber to 22% has been reported after the fermentation of bread, quinoa, and BSG by *N. intermedia* and *R. oligosporus* respectively [39,33,74]. In comparison to the above-mentioned studies, and also with a fungal product available in the market such as Quorn (17.8%) [33], the fungal biomass produced in this study provides more fibers.

Comparing the pure fungal biomass with oat fungal biomass proves that the dietary fiber content in oat fungal biomass (34.7%) is mainly

contributed by the fungus itself (37.3%) rather than from oats. Similarly, comparing the β-glucan content of pure fungal biomass (13.9%) with oat fungal biomass (14%) also represented the main contribution of fungus to the final β-glucan content of oat biomass (Table 1). It has also been reported earlier that fungal biomass is responsible for the increase in dietary fiber following fermentation [75]. The cell wall of filamentous fungi is mainly composed of β-glucan, a polymer of glucose in addition to chitin around 10 to 15% [76]. These two components of the cell wall have been classified as dietary fibers in food products.

4. Conclusion

Providing innovative products through fermentation using edible filamentous fungi is a promising approach in producing sustainable and healthy food products. SmF of edible filamentous fungus *A. oryzae* successfully improved the nutritional value of oat flour and converted it to a nutrient-rich product. The quantity and the quality of the protein was improved after fermentation with a significant increase in the essential amino acids, especially lysine and threonine as limiting amino acids. The increase in fat content mainly was aligned with increased polyunsaturated fatty acids, specifically α-linolenic acid compared to the substrate. In addition, SmF enhanced vitamin E, D_2, mineral levels (Cu, Fe, Zn), and dietary fiber in the fermented biomass. The nutrient-rich fungal biomass obtained in this study has a high potential for being utilized in the food industry. However, in vitro digestion studies are recommended as future work to investigate the digestibility/availability of key nutrients in fungal biomass.

Ethical statement - studies in humans and animals

The research presented does not involve any animal or human study.

CRediT authorship contribution statement

Neda Rousta: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft, Writing - Review & Editing. **Karin Larsson:** Formal analysis, Validation, Writing - original draft, Writing - Review & Editing. **Rikard Fristedt:** Formal analysis, Validation, Writing - original draft, Writing - Review & Editing. **Ingrid Undeland:** Validation, Writing - Review & Editing. **Swarnima Agnihotri:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Writing - Review & Editing. **Mohammad J. Taherzadeh:** Funding acquisition, Project administration, Supervision, Validation, Writing - Review & Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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