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Increasing the Nutritional Value of Camelina Meal via *Trametes versicolor* Solid-State Fermentation with Various Co-Substrates

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Abstract: Upcycling low-cost agricultural by-products into valuable and sustainable alternative feeding materials could secure human food-supply chains with a low carbon footprint. This study explored increasing the feeding value of camelina meal (CAM) mixed with wheat bran (WB), soybean hulls (SH), and rice hulls (RH) for monogastric animals via solid-state fermentation (SSF) using white rot fungus *Trametes versicolor*. Experiments evaluated fungal growth, amino acid profiles, structural carbohydrates, glucosinolates, phytate and in vitro dry matter digestibility (IVDMD). Weight loss analysis indicated that fungal growth was more active in WB/CAM and SH/CAM substrates than RH/CAM. Significant phytic acid degradation and near-complete glucosinolate elimination improved CAM feed quality across all substrates. Fermentation increased total and essential amino acids in the SH/CAM mixture, while reductions occurred in WB/CAM and RH/CAM mixtures. SH/CAM fermentation caused substantial cellulose and hemicellulose degradation, resulting in a 44% IVDMD increase. Conversely, RH/CAM fermentation decreased IVDMD despite a reduction in cellulose, possibly due to protein degradation. This study demonstrates the potential of *T. versicolor*-mediated SSF to enhance CAM and other agricultural residues' feeding value for monogastric animal applications.

Keywords: solid-state fermentation; oilseed cover crop; monogastric animal feed; bioprocess; glucosinolates; camelina meal; *Trametes versicolor*

Received: 1 January 2025
Revised: 29 January 2025
Accepted: 30 January 2025
Published: 4 February 2025

Citation: Boardman, K.; Sun, X.; Yao, D.; Chen, C.; van Lierop, L.; Hu, B. Increasing the Nutritional Value of Camelina Meal via *Trametes versicolor* Solid-State Fermentation with Various Co-Substrates. *Fermentation* **2025**, *11*, 77. https://doi.org/10.3390/fermentation11020077

1. Introduction

Camelina (*Camelina sativa*) is a Brassicaceae oilseed crop with cultivation dating back to 4000 B.C.E in Central Asia [1]. Recent years have seen growing interest in camelina due to its favorable agronomic traits, including germination capability at low temperatures, short life cycle, and frost tolerance [2]. These attributes have positioned camelina as a promising cash cover crop in the Upper Midwest. As a cover crop, camelina has demonstrated ecological benefits such as reducing nitrate leaching [3] and providing habitats for pollinators [4]. Camelina seeds are harvested in the springtime. Due to the high concentration of oil (30–40%), the seeds are processed through cold pressing, solvent extraction, or their combination to extract the oil which can be utilized for biofuel (biodiesel and sustainable aviation fuel) manufacturing and culinary applications [5].

The cake left after oil extraction is camelina meal (CAM), which has limited use as a feeding material for monogastric animals due to its high levels of antinutritional factors, specifically phytate (25.4–32.3 mg/kg) and glucosinolates (15.2–24.6 μmol/g) [6], regardless of its relatively low concentration of fiber (10%) [7] and high crude protein

content (36–40%) [8]. Glucosinolates, and their degraded products in particular, have growth suppressing effects on monogastric animals, resulting in a strict limitation of CAM in their diets [9]. Profitability analyses underscore the critical role of CAM's selling price in the widespread adoption of camelina across North America [5], emphasizing the necessity to develop strategies for producing nutritionally enhanced CAM.

Solid-state fermentation (SSF) is a bioprocessing technique that cultivates microorganisms on solid substrates without free flowing water and is commonly used for feed fermentation [10]. While the literature on CAM bioprocessing is limited, SSF has been shown to improve the nutritional quality of other oilseed meals from, e.g., soybean, canola, and pennycress, by increasing protein content [11], reducing structural carbohydrates [12,13], phytate, and glucosinolates [11,13]. The usage of fermented feed for monogastric animals is well documented and has been associated with lower levels of antinutritional factors and an increased bioavailability of nutrients [10]. Fermented soybean meal, for instance, has been successfully commercialized for non-ruminant young animals, improving growth performance and health compared to non-fermented meal [10]. Similarly, fermented canola meal has shown benefits in broiler chicken diets [14]. In contrast to soy and canola meals, the bioprocessing potential of CAM remains relatively unexplored. Fermenting CAM could significantly enhance its nutritional value, particularly by reducing glucosinolate levels, thereby allowing for greater inclusion in monogastric animal diets and enhancing economic returns from camelina cultivation as an oilseed cover crop.

In SSF, the substrate not only provides nutrients but also the physical structure to support microbial growth. Therefore, the physical properties of the substrate, such as porosity and density, play crucial roles in facilitating microbial growth and productivity [15]. Enhancing airflow within the substrate is vital at larger scales, where dense packing may hinder uniform airflow [16]. One strategy to improve airflow in the substrate is to conduct SSF with a co-substrate to act as a bulking agent [17]. Wheat bran (WB), soybean hulls (SH), and rice hulls (RH) are abundant agricultural residues rich in fiber, with a need for practical applications. These materials have previously been utilized as co-substrates in SSF experiments to enhance substrate porosity, stimulate fungal growth, and boost enzyme production [18–21]. SSF has proven effective in enhancing the nutritional quality of these residues by reducing fiber and phytate while enhancing digestibility [22,23].

Trametes versicolor, a white rot fungus belonging to the phylum Basidiomycota, is commonly known as the turkey tail mushroom due to its shape and colorful appearance. It has a worldwide distribution and can be found growing on various conifers and hardwood trees and plays an important role in wood degradation [24]. *T. versicolor* is the most studied white rot fungus due to its robust enzyme production, and it has significant applications in medicine, biotechnology, environmental remediation, and feed fermentation [24]. Although not commonly used in Western food systems, it has a long history of consumption in China without reported adverse safety effects [24].

Previous SSF studies have found *T. versicolor* instrumental in enhancing the nutritional properties of fiber-rich agricultural residues by degradation of structural carbohydrates and enhancing protein and digestibility [25,26]. Given its robust enzyme production and capacity to degrade fibers [27], *T. versicolor* was selected based on results from a preliminary study, suggesting its potential to degrade glucosinolates and phytates effectively. Therefore, this study aims to investigate the impact of different initial substrate mixtures, including CAM blended with WB, SH, or RH, on the growth of *T. versicolor*, changes in anti-nutritional compounds, and the nutritional profiles of SSF.

2. Materials and Methods

2.1. Feedstocks

Camelina seeds (Joelle variety) were supplied by the University of Minnesota's Department of Agronomy and Plant Genetics (St. Paul, MN, USA). Seeds were harvested in the spring of 2022, milled, and subjected to oil extraction through a cold press process by the Agricultural Utilization Research Institute (AURI, Waseca, MN, USA). Soybean hull, rice hull, and WB were purchased from Republic Mills, Inc., Okolona, OH, USA. All feedstocks were stored in a cool, dry area upon receipt.

2.2. Solid-State Fermentation

The substrates were prepared as follows: 200 g of CAM was mixed with 200 g on a dry basis (d.b.) of either WB, SH, or RH in triplicates and placed in a sterilizable airflow spawn bag (Fungi Perfecti LLC, Olympia, WA, USA) which is made of polypropylene and measured 22.5 × 8.25 × 4.75 inches in size. Each bag is equipped with a microporous (0.3 microns) filter patch which allows gas exchange but precludes the passage of contaminants. The moisture content of each substrate mixture was adjusted to 60% using deionized (DI) water. The substrate in each bag was autoclaved at 121 °C for 45 min and allowed to cool to room temperature before inoculation. In a biosafety cabinet, the substrate was inoculated with 200 g (on a wet basis, 60% moisture content) of organic sorghum grains (Mycology Supply, Rancho Cordova, CA, USA) fully colonized with *T. versicolor*, according to supplier instructions (14 days post inoculation). The substrate and the inocula were mixed thoroughly, and the bags were sealed with an impulse sealer (Fungi Perfecti LLC, Olympia, WA, USA). One bag from each type of substrate mixture was immediately frozen at the 0 h time point to serve as the negative control. The remaining bags were incubated at 28 °C for 12 days, during which the bags were periodically observed to visually monitor the fungal colonization in different substrates. After fermentation, the bags were opened and transferred to a 60 °C air oven for 5 days until fully dried (moisture less than 5%).

2.3. Analytical Methods

Loss of dry matter (DM) in each fermentation bag was determined by weighing each bag prior to adding substrate and before and after fermentation. Moisture content and DM were calculated for the substrate before and after fermentation. The loss of DM was expressed as a percentage of the difference in DM between fermented and non-fermented substrate over non-fermented substrate.

Amino acid analysis was performed by hydrolyzing 0.05 g of the dried and ground sample in 1 mL of 6 N HCl solution in a 2 mL screw cap tube at 110 °C for 24 h, following AOAC Official Method 994.12 [28]. Prior to hydrolysis, each tube's headspace was purged with nitrogen gas for 10–20 s to prevent the oxidation of sulfur-containing amino acids (methionine, cysteine). The hydrolyzed sample was diluted 10 times with DI water, filtered through a 0.22 µm PTFE filter, and quantified by HPLC (1200 Infinity series, Agilent Technology, Santa Clara, CA, USA) using a ZORBAX Eclipse Plus C18 column (4.6 × 150 mm, 3.5 µm) and a diode array detector (Agilent Technologies, Inc., Santa Clara, CA, USA), as described by Henderson and Brooks [29].

Total and free phosphorus by dry mass were analyzed using the Phytic Acid Assay kit (Megazyme, Ltd., Bray, Ireland) following the manufacturer's protocol [30]. Phytic acid content was determined by subtracting free phosphorus from total phosphorus based on the same protocol mentioned above.

Glucosinolates in each sample were extracted following the method described by Berhow [31]. Briefly, 20 mg of dry ground sample was homogenized in 1 mL of 70% methanol using sonication (model 3510, Branson, Danbury, CT, USA) at room temperature

for 15 min. The solution was then shaken at 100 rpm overnight and centrifuged. The supernatant was filtered through a 0.22 µm PTFE filter. Analysis was performed using a Waters Acquity UPLC system coupled with a Xevo G2-S Q-TOF LC-MS equipped with a Water Acquity BEH C18 column (1.7 µm, 2.1 × 50 mm ID) (Waters Corporation, Milford, MA, USA). Chromatographic conditions involved mobile phase A (0.1% formic acid in 100% water) and mobile phase B (0.1% formic acid in 100% acetonitrile). The gradient program started with 0.5% B elevated to 20% B over 4 min, followed by an increase to 95% B over the next 4 min. The gradient reached 100% B at 8.1 min and maintained this level until 9 min before returning to 0.5% B by 10 min for re-equilibration. The flow rate was set at 0.5 mL/min, and the column temperature was maintained at 40 °C. The autosampler temperature was 4 °C. For mass spectrometry, the Xevo G2-S Q-TOF was calibrated before analysis using sodium formate solution to ensure an accurate mass measurement. Capillary voltage was set to 0.2 kV, sampling cone voltage to 40 V, and source offset voltage to 80 V. ESI source and desolvation temperature were 120 °C and 350 °C, respectively. Cone gas flow rate was 50 L/h, and desolvation gas flow rate was 800 L/h. The MS was operated in the negative mode and ions were scanned in the range of m/z from 50 to 1200. Data processing and quantification were performed using QuanLynx software V4.2 (Waters).

In vitro dry matter digestibility (IVDMD) was assessed using a sequential enzymatic hydrolysis method to simulate gastric and small intestine digestion, following the procedure outlined by Jang [32]. A quantity of 1 g of each dry grounded sample was hydrolyzed in a pepsin solution (pre-adjusted to pH 2.0) in a 500 mL Erlenmyer flask for 2 h at 39 °C, which mimicked gastric digestion. Subsequently, a solution of NaOH, phosphate buffer, and pancreatin was added, adjusting the pH to 6.8. The samples underwent an additional 4 h hydrolysis at the same temperature to simulate small intestine digestion. After completion, the residual solids from each reaction mixture were vacuum-filtered, washed, and dried. The calculation of IVDMD was based on the change in dry mass before and after the sequential enzymatic hydrolysis steps.

Structural carbohydrates (glucan, xylan, arabian, mannan, and galactan) for fermented and non-fermented CAM were determined using the NREL (National Renewable Energy Laboratory, Golden, CO, USA) two-step acid hydrolysis method [33] followed by sugar analysis via HPLC (1200 Infinity series, Agilent Technologies, Santa Clara, CA, USA) [34]. Cellulose concentration was expressed as glucan, and hemicellulose concentration was calculated as the sum of xylan, arabinan, galactan, and mannan concentrations by dry mass.

Crude protein, lignin, neutral detergent fiber (NDF), and acid detergent fiber (ADF) were analyzed by Cumberland Valley Analytical Services (Waynesboro, PA, USA) [35–38].

2.4. Statistical Analysis

Differences between groups were analyzed using a Student's *t*-test comparison of the means with a confidence level of 95% ($p < 0.05$) in JMP Pro 16 (SAS Institute Inc., Cary, NC, USA). Statistical comparisons were made between day 0 and day 12 for each substrate mixture.

3. Results and Discussion

3.1. Compositions of Unfermented Meal, CAM and Its Comparison with Canola Meal (CM), Soybean Meal (SM), Wheat Bran (WB), and Rice Hull (RH)

The components of non-fermented camelina meal were analyzed and compared with canola and soybean meals from the literature (Table 1). Soybean meal is the current gold standard for monogastric animal feed due to its high protein content reported between 44 and 50%, low levels of fiber (5–10% ADF, 10–21% NDF), phytate (0.4%), and glucosinolates (0.21 µmol/g) [39–42]. Canola is a Brassicaceae oilseed crop and is related to camelina [43].

Compared to soybean meal, both canola meal (CM) and CAM are considered inferior feedstocks due to their lower protein levels and higher levels of fiber, phytate, and glucosinolates. CM has similar protein, phytate, and fiber as camelina but has lower glucosinolate levels due to selective breeding programs [44]. For CAM to compete with soybean meal, the total protein content should be improved while phytate, fiber, and glucosinolate levels are reduced.

Table 1. Chemical composition of camelina meal (CAM) in this study as compared to canola meal (CM) and soybean meal (SM) in the literature.

Parameter [a]	CAM (This Study)	CM [45–47]	SM [39–42]
Total Lipids, % DM basis	5.7	3.7	0.8
Crude Protein, % DM basis	42.2	36.5–42.1	43.9–50.4
Phytate, % DM basis	2.5	2.7	0.4
Lignin, % DM basis	6.0	5–8	0.4–1.2
ADF, % DM basis	20.7	18.2–20.0	5.0–10.2
NDF, % DM basis	29.0	24.9–26.0	10.0–21.4
GLN, µmol/g DM basis	25.3 *	3.5–13.5	0.21

[a] DM, dry matter; ADF, acid detergent fiber; NDF, neutral detergent fiber; GLN, glucosinolates. * Total glucosinolate concentration as determined by LC-MS analysis.

In SSF, the substrate provides not only the nutrients but also the physical structure needed to support microbial growth. Therefore, the physical properties of the substrate are very important and will influence the microbial growth and productivity [15]. Porosity and density are important physical properties in the substrate that require greater consideration at larger scales as the substrate becomes more compact and it becomes increasingly important to promote uniform airflow [16]. One strategy to improve airflow in the substrate is to conduct SSF with a co-substrate to act as a bulking agent and improve porosity [17]. Good candidates for bulking agents are agricultural residues produced in abundance with a need for applications that also have the potential to improve the morphological characteristics of CAM.

Another key feature of the CAM is its high content of mucilage, as some references describe around (~10%) in camelina seeds, even though the composition of the mucilages depends on the source. Mucilage is a soluble fiber found in the outermost layer (mucous epidermis) of the seed and is commonly composed of a mixture of polysaccharides, for example, flaxseed mucilage comprises neutral arabinoxylans and acidic rhamnose-containing polysaccharides [48]. Due to its polysaccharide nature, CAM flour tends to aggregate together when moisturized, and fungal cells barely grow due to the limitations of poor porosity. Three agricultural residues with the potential to increase the C/N ratio and porosity when mixed with CAM are wheat bran, soybean hull, and rice hull. Wheat bran (WB) is the by-product of the wheat milling process and 150 million tons/year are produced world-wide [49]; it also has applications in livestock feed [49]. Previous SSF studies have incorporated WB as a co-substrate as it has been shown to improve the porosity of a substrate when mixed with rice bran [18]. Another study found that a 50/50 mixture of wheat bran and soybean meal improved protease production in the SSF of Aspergillus niger more than soybean meal alone [20]. Soybean hull (SH) is the largest by-product of soybean refineries. Its production is estimated to reach 29.6–39.7 million tons by 2030 and it is commonly used in animal feed. It is typically high in fiber; however, its composition varies significantly based on the efficiency of dehulling [50]. SH has been shown to increase porosity and promote fungal growth when used as a co-substrate in SSF with distiller's dried grains with solubles (DDGS) [21]. Rice hull (RH) is the by-product of rice production, accounting for 20% of the annual worldwide production of 680 million tons of rice produced per year. It has a high lignin concentration and low protein levels (Table 2) [51]. A previous study mixed rice hull with soybean meal to increase porosity and improve oxygen transfer in SSF [19].

Table 2. Relevant compositional components of WB, SH, and RH.

	WB [49,52]	SH [50]	RH [51]
Hemicellulose % DM basis	28	10–25	12
Cellulose % DM basis	10–11	29–51	36
Lignin % DM basis	3	1–4	15
Crude protein % DM basis	13.2–18.4	11–15	2

3.2. Fungal Growth

Visual observation showed that the fungus *T. versicolor* colonized in all three tested substrate mixtures after 12 days (Figure 1). The colonization of *T. versicolor* in substrate RH/CAM was less dense than in WB/CAM and SH/CAM substrates. Weight loss analysis (Figure 2) demonstrated a higher loss of DM in SH/CAM (32%) and WB/CAM (24%) than in RH/CAM (15%), which corresponds to the visual observation because the DM loss is positively correlated with fungal growth and activity. The more growth, the more substrate carbon being metabolized into volatile compounds such as carbon dioxide and ammonia gas. Therefore, the co-substrates WB and SH supported more extensive fungal growth than RH.

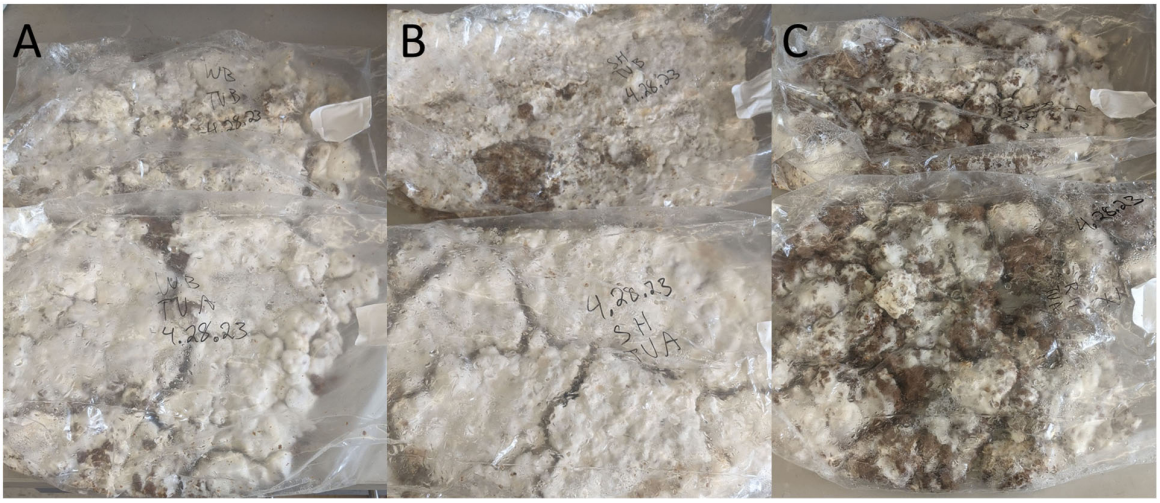

Figure 1. Photographs of mycelial growth after 12 days of incubation with *T. versicolor* in substrate mixture (**A**) WB/CAM, (**B**) SH/CAM, and (**C**) RH/CAM (WB: wheat bran; SH: soybean hull; RH: rice hull; CAM: camelina meal).

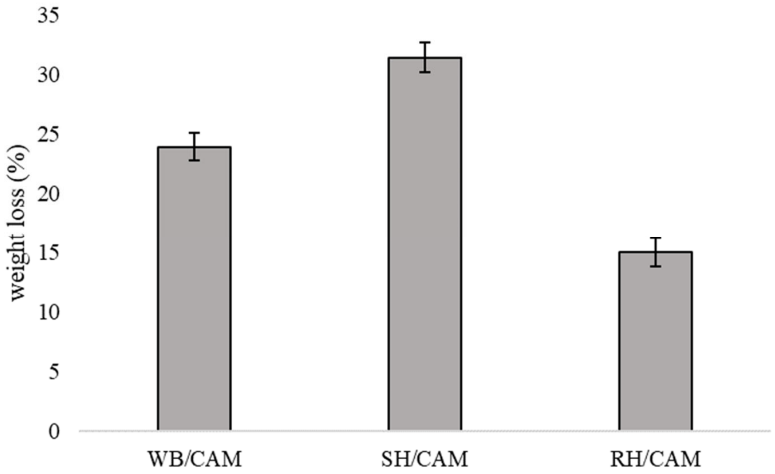

Figure 2. Dry weight loss due to fermentation expressed as a percentage of differences between fermented and non-fermented substrate over non-fermented substrate for each co-substrate mixture.

As a white rot fungus, *T. versicolor* effectively degrades lignin, cellulose, and hemicellulose indiscriminately and its ability to degrade fiber can be negatively affected if the substrate was diluted with non-fiber materials [13]. This indicates that high fiber substrates induce the growth of *T. versicolor*. The initial substrate mixtures used in this present study were different in fiber content, with SH/CAM being the highest (48% of dry mass) in a combined concentration of cellulose and hemicellulose (Figure 3), which could induce the activity of *T. versicolor* and therefore more substrate DM loss. Lignin and ash content may also contribute to the different growth rate in each substrate mixture. Higher ash and lignin contents were found in RH compared to WB and SH (Table 2) [50–52]. Lignin is a complex polymer and crosslinked with cellulose and hemicellulose, making it difficult for fungi to break down. High ash content can create an imbalanced nutrient environment less conducive to fungal proliferation [53,54]. Physical properties such as bulk density, particle density, water retention, and porosity can cause different gas exchange capacities in substrates, which directly affect oxygen levels available to fungus [15]. Therefore, the substrate chemical and physical characteristics in SSF could directly affect the fermentation efficiency.

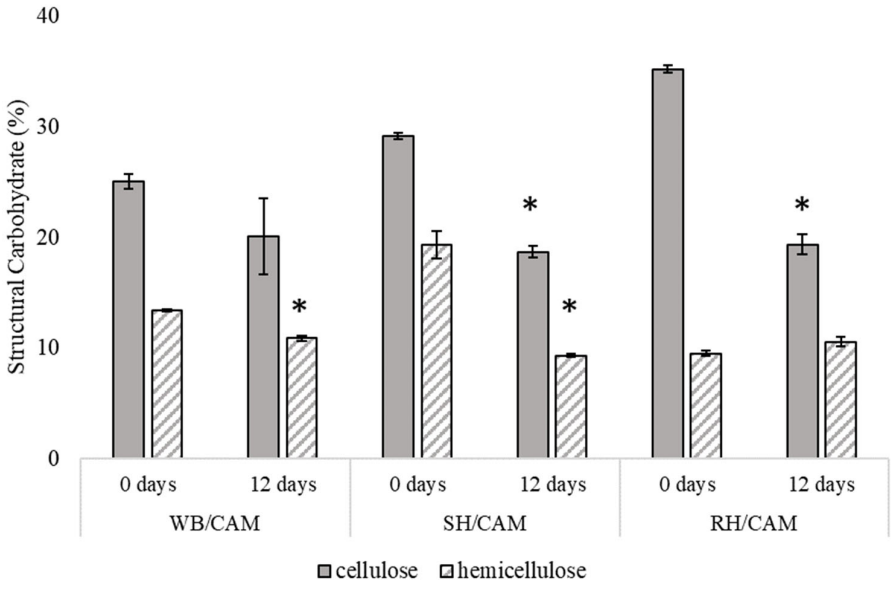

Figure 3. Cellulose and hemicellulose fractions (% DM) of CAM mixed with co-substrates WB, SH, and RH at 0 and 12 days of fermentation. * Significant decrease in concentration due to 12 days of fermentation ($p < 0.05$).

3.3. Fermented Meal Composition

The compositional components of the samples were analyzed by Cumberland Valley Analytical Services and are shown in Table 3. Total crude protein was found to increase by 33 and 36% in CAM/WB and CAM/SH, respectively, while maintaining its same concentration in CAM/RH. The increase in crude protein can be attributed to growth of fungal biomass resulting in conversion of non-organic nitrogen to organic nitrogen as well as selective degradation of non-protein components [55] such as non-fiber carbohydrates (CAM/WB) and structural carbohydrates (CAM/SH). The soluble protein was increased in fermentation with each co-substrate. This is likely due to protease activity, which has previously been reported in *T. versicolor* [27]. Protease hydrolyzes insoluble proteins to soluble components such as small peptides and free amino acids which are often highly digestible [56].

Table 3. Compositional analysis of various components in the nonfermented (0 day) and fermented (12 days) samples from each substrate mixture.

	CAM/WB		CAM/SH		CAM/RH	
	0 Days	**12 Days**	**0 Days**	**12 Days**	**0 Days**	**12 Days**
Crude Protein % DM basis	21.8	29.1	22.7	30.8	20.3	20.2
Soluble protein % DM basis	7.8	16.8	6.2	17.9	6.2	10.7
Lignin % DM basis	4.47	4.8	3.04	4.77	8.29	12.6
NFC % DM basis	41.8	34.0	27.2	39.6	29.0	14.5
Ash % DM basis	4.27	5.85	4.11	6.10	8.15	12.15
Crude Fat % DM basis	8.59	7.64	10.7	6.14	7.6	6.36

3.4. Effect of Fermentation on Amino Acid Profile

Total amino acid concentration was significantly reduced ($p < 0.05$) in the WB/CAM and RH/CAM mixtures by 20% and 12%, respectively, after fermentation. In contrast, the SH/CAM fermentation resulted in a significant 7% increase ($p < 0.05$) in total amino acid concentration. Specifically, several key amino acids showed significant increases ($p < 0.05$) in the SH/CAM mixture: threonine (by 25%), valine (by 17%), isoleucine (by 12%), and leucine (by 4%). Conversely, significant decreases ($p < 0.05$) were noted for all essential amino acids except for threonine in the WB/CAM mixture, and for arginine, methionine, isoleucine, and leucine in the RH/CAM mixture. The increased concentration of amino acids in SH/CAM compared to WB/CAM and RH/CAM after fermentation could be due to higher fiber reduction and less protein degradation.

The amino acid profiles of the fermented co-substrates vary significantly after fermentation. The reduction in total amino acids in WB/CAM and RH/CAM mixtures (Table 4) suggested extensive conversion of these amino acids to volatile compounds (e.g., ammonia) through the fungal metabolization of the substrate protein regardless of fungal protein syntheses or their conversion to other compounds [57]. The degradation of the substrate protein into ammonia after an extended period of fermentation had been reported in corn distiller grains and soluble fermentation with *Rhizopus oryzae* [34]. The substrate with a higher C/N (carbon to nitrogen) ratio resulted in less degradation of amino acids into ammonia than substrate with a lower C/N ratio [34]. The higher combined concentration of cellulose and hemicellulose in SH/CAM indicated a higher C/N ratio than in WB/CAM and RH/CAM, resulting in less degradation of the total amino acid concentration and a final elevated concentration of the total amino acids due to the concentrating effect of fiber reduction in SH/CAM. The SH/CAM mixture's increase in specific essential amino acids such as threonine, valine, isoleucine, and leucine occurred due to the synthesis of fungal protein, which is rich in essential amino acids, as reported earlier [58]. This suggests that *T. versicolor* fermentation of SH/CAM could enhance the nutritional profile of the substrate for monogastric animals [15].

Improving essential amino acids is critical for enhancing the nutritional value of animal feed, especially for monogastric animals that cannot synthesize these amino acids endogenously [59]. The observed increase in essential amino acids in the SH/CAM fermentation are favorable as they align with the balanced amino acid profile found in soybean meal, a high-quality protein source for monogastric animals [60]. A 25% increase in threonine was observed due to the fermentation of the SH/CAM mixture. Threonine is the second limiting amino acid in swine diets, playing a crucial role in maintaining gut health, enhancing immune function, and improving nutrient absorption. Adequate levels of threonine in the diet can significantly enhance growth performance and feed efficiency by supporting gut barrier function and modulating the gut microbiota, making it indispensable for optimal swine nutrition [61]. However, significant decreases were observed in the

majority of the essential amino acids in the WB/CAM and RH/CAM mixtures, suggesting that fermentation did not have a beneficial effect on the amino acid profiles.

Table 4. Concentration of essential and total amino acids in each co-substrate mixture at 0 and 12 days of fermentation expressed as mg/g DM.

	WB/CAM		SH/CAM		RH/CAM	
	0 Days	**12 Days**	**0 Days**	**12 Days**	**0 Days**	**12 Days**
Thr	8.67 ± 0.06	7.94 ± 0.42	7.01 ± 0.03	8.73 ± 0.32 *	6.00 ± 0.13	5.69 ± 0.08
Arg	17.82 ± 0.10	9.47 ± 0.44 †	12.08 ± 0.02	8.24 ± 0.70 †	9.99 ± 0.34	5.69 ± 0.03 †
Met	2.82 ± 0.07	2.06 ± 0.13 †	2.14 ± 0.03	2.03 ± 0.08	2.10 ± 0.10	1.50 ± 0.01 †
Lys	6.58 ± 0.09	5.25 ± 0.40 †	5.77 ± 0.19	5.98 ± 0.31	3.69 ± 0.08	4.46 ± 0.21
Val	12.91 ± 0.13	11.29 ± 0.52 †	10.32 ± 0.14	12.03 ± 0.15 *	8.65 ± 0.12	8.47 ± 0.06
Ile	9.13 ± 0.13	7.84 ± 0.30 †	7.72 ± 0.28	8.66 ± 0.18 *	6.65 ± 0.19	5.93 ± 0.00 †
Leu	17.03 ± 0.08	14.66 ± 0.13 †	14.66 ± 0.21	15.25 ± 0.11 *	14.08 ± 0.10	11.51 ± 0.03 †
Total	207.88 ± 1.15	165.47 ± 6.33 †	165.08 ± 3.02	176.70 ± 2.46 *	139.25 ± 1.11	121.77 ± 0.63 †

* Significantly higher than correlating non-fermented control. † significantly lower than correlating non-fermented control ($p < 0.05$).

The balance of branched-chain amino acids leucine, isoleucine, and valine is crucial to prevent adverse effects on nitrogen retention in swine [62]. The fermentation process slightly elevated the valine-to-leucine and isoleucine-to-leucine ratios in each co-substrate mixture, suggesting that the fermentation did not result in excess leucine production, which is beneficial for maintaining a balanced amino acid profile.

3.5. Change in Structural Carbohydrates

The effect of fermentation on cellulose and hemicellulose concentration depended significantly on the initial co-substrate mixture (Figure 3). The combined cellulose and hemicellulose accounts for around 38% dry mass of WB/CAM, 48% dry mass of SH/CAM, and 43% dry mass of RH/CAM. In the WB/CAM and SH/CAM mixtures, fermentation led to notable decreases ($p < 0.05$) in hemicellulose (19% and 52%, respectively). Significant decreases ($p < 0.05$) in cellulose were observed in SH/CAM and RH/CAM mixtures (36% and 45%, respectively). Therefore, a significant reduction ($p < 0.05$) of both cellulose and hemicellulose occurred in SH/CAM (Figure 3), which is reflected as higher DM loss in SH/CAM than WB/CAM and RH/CAM (Figure 2). In addition, the more significant reduction in fiber in SH/CAM was reflected in the greater concentration of amino acids, as shown in Table 1.

White rot fungi are well known for their ability to degrade plant structural components [24]. *T. versicolor* secretes cellulases and hemicellulases, among other enzymes, crucial for cellulose and hemicellulose degradation [26]. The impact of fermentation on these components varied based on the co-substrate used. For instance, degradation of cellulose in WB/CAM and hemicellulose in RH/CAM, respectively, was not significant ($p > 0.05$) (Figure 3). In contrast, the SH/CAM mixture exhibited significant degradation ($p < 0.05$) of both cellulose and hemicellulose (36% and 52%, respectively). The robust fungal growth observed on this substrate (Figures 1 and 2), which contains a higher combined concentration of cellulose and hemicellulose, likely induced and better facilitated the production of cellulases and hemicellulases, thereby enhancing degradation efficiency.

Interestingly, the RH/CAM mixture showed significant cellulose degradation (45%) but unexpectedly increased hemicellulose concentration (10%), which could be due to the low hemicellulose degradation and concentrating effect of protein and cellulose degradation (Table 4 and Figure 3). In this study, *T. versicolor* degraded the fiber components (cellulose and hemicellulose) differently based on the different substrate mixtures. This is in agreement

with many other studies where hemicellulose and cellulose were degraded differently by fermenting fungi, including *T. versicolor* [26,63]. In addition, studies using SSF have consistently shown that enzyme production and substrate degradation outcomes are influenced by various factors, including substrate composition and fermentation duration [64].

Furthermore, our findings align with previous research indicating that the substrate type heavily influences the extent of cellulose degradation by white rot fungi in SSF. For example, a study demonstrated that *Pleurotus florida* significantly decreased acid detergent fiber (ADF, comprising cellulose and lignin) in rice straw, wheat straw, and barley straw, but showed no change in ADF when fermented on soybean straw, canola straw, pea straw, or rice hull [55]. This study underscores a pattern where substrates with higher initial concentrations of cellulose or hemicellulose tend to exhibit more potent degradation of these components during fermentation.

3.6. Change in Glucosinolate Concentration

The total glucosinolates, including 9-MSG, 10-MSG (glucocamelinin), and 11-MSG, were quantified using LC-MS analysis, revealing an initial concentration of 32.5 µmol/g of total glucosinolates on a dry basis in the original meal. After accounting for the dilution of CAM with each co-substrate and inoculum (5:5:2 mix ratio on a dry basis), and degradation caused by autoclaving and drying process, the total glucosinolates concentration in the WB/CAM, SH/CAM and RH/CAM mixtures decreased by 83%, 73%, and 75%, respectively, prior to fermentation. After 12 days of fermentation, there was a near-complete degradation (>99%) of total glucosinolates in all co-substrate mixtures, indicating a minimal variability among the substrates regarding degradation efficiency (Figure 4).

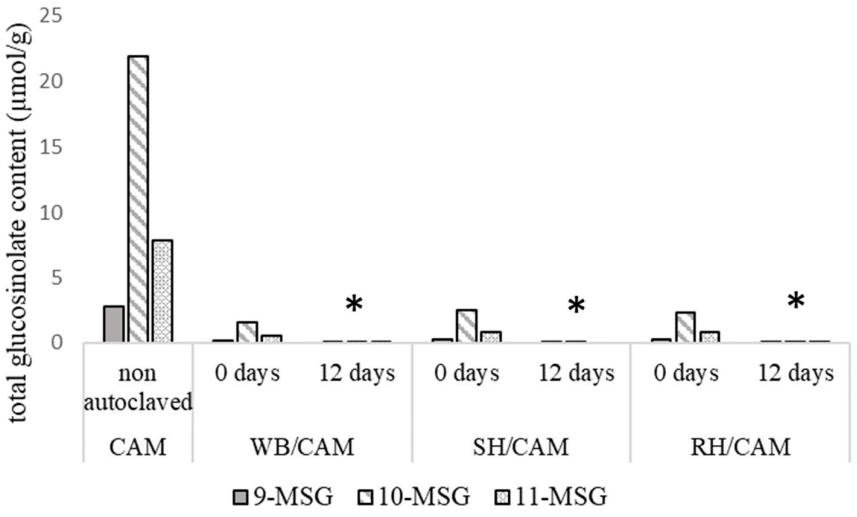

Figure 4. Concentration of the three glucosinolates (µmol/g DM) present in CAM (9-MSG, 10-MSG, 11-MSG) in each co-substrate mixture at 0 and 12 days of fermentation, as determined by LC-MS analysis. * Significant decrease in concentration due to 12 days of fermentation ($p < 0.05$).

Glucosinolates have been shown to inhibit thyroid function, resulting in reduced growth, lower fertility, and increased mortality rates. Due to these effects, glucosinolate levels should not exceed 0.78 µmol/g for swine and 5.4 µmol/g for poultry [9]. A significant reduction in glucosinolate concentration was observed following autoclaving and drying, consistent with previous reports [65]. Similarly, the decrease in glucosinolate levels due to fermentation aligns with findings from other studies [11,66]. Due to the effects of autoclaving, fermentation, and drying, the end glucosinolate concentration is acceptable for swine and poultry diets.

While these methods effectively reduce glucosinolates in CAM, understanding the nature of their breakdown products is crucial for assessing meal detoxification. Research has indicated that heat treatment primarily yields nitriles as the major breakdown product of sulfur-containing aliphatic glucosinolates, present in CAM [67]. Nitrile consumption in monogastric animal diets has been associated with physiological effects such as hypertrophy and hyperplasia [68], suggesting that heat treatment alone may not sufficiently reduce toxicity.

In contrast, fungal fermentation offers promise for detoxifying glucosinolate breakdown products. Fungi utilize glucosinolates for carbon and energy yet must manage toxic metabolites with known fungicidal effects [66]. Recent reviews highlight fungal enzymatic pathways capable of detoxifying a variety of glucosinolate breakdown products, underscoring the versatility and efficiency of fungal metabolism [66].

Studies on *T. versicolor* have identified a nitrilase enzyme (NitTv1) similar to plant nitrilases, suggesting potential interactions between fungal and plant-derived nitriles. NitTv1 exhibits robust activity, degrading nitrile metabolites into less toxic amides and carboxylic acids with excellent pH stability [69]. This enzymatic capability supports the hypothesis that SSF with *T. versicolor* can effectively detoxify glucosinolate breakdown products. However, further research employing chemometric analysis of fermentation products is essential to fully comprehend the detoxification mechanisms and ensure the safety and efficacy of using SSF with *T. versicolor* to enhance the nutritional quality of CAM in animal feed applications.

3.7. Changes in Phytic Acid Concentration and Free Phosphorous

The changes in phytic acid and free P are depicted in Figure 5. The initial substrate of WB/CAM showed almost twice as much phytic acid as it was in SH/CAM and RH/CAM, indicating the existence of phytic acid in WB. The fermentation resulted in significant decreases ($p < 0.05$) in phytic acid concentration by 83%, 84%, and 66% in WB/CAM, SH/CAM, and RH/CAM, respectively. Phytic acid degradation can release free P. Correspondingly, free P increased by 273%, 409%, and 222% in WB/CAM, SH/CAM, and RH/CAM.

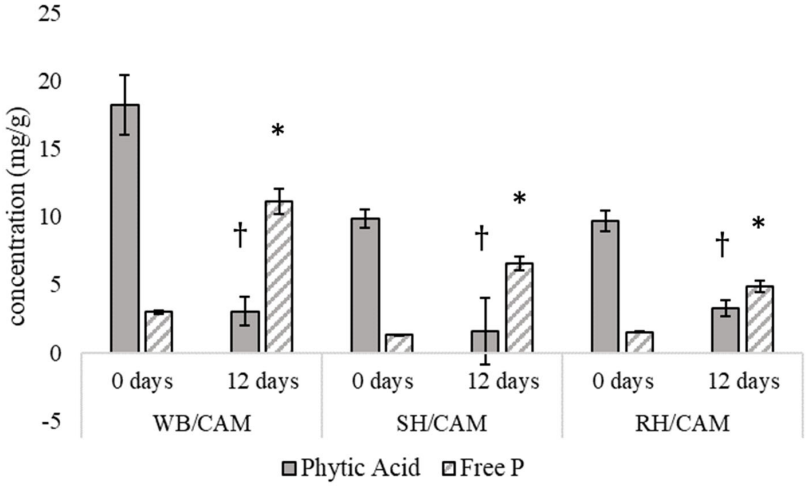

Figure 5. Concentration of phytic acid and free phosphorus (mg/g DM) in each co-substrate mixture at 0 and 12 days of fermentation. * Significant increase in concentration due to 12 days of fermentation ($p < 0.05$). † Significant decrease in concentration due to 12 days of fermentation ($p < 0.05$).

Phytic acid was decreased in each co-substrate mixture after 12 days of fermentation in alignment with previous research showing that *T. versicolor* produces phytase when used in SSF [70]. A decrease in phytic acid is desirable for a higher quality feed as phosphorus has low digestibility in monogastric animals, leading to increased phosphorus excretion in manure production [12]. Beyond its impact on phosphorus bioavailability, phytate (salt

of phytic acid) undergoes negative charge modification in the digestive tract, forming complexes with digestible nutrients such as lipids, starch, and proteins, thereby reducing their digestibility as well [71]. The lack of phosphorus digestion in phytate results in additional phosphorus supplement in the feeding diets while wasting phosphorus via its excretion into the environment, causing significant environmental pollution through animal farming and contributing to eutrophication [60,72].

3.8. Effect of Fermentation on In Vitro Digestibility of Camelina Meal/Co-Substrate Mixtures

After 12 days of fermentation, the IVDMD increased by 31% in WB/CAM, 44% in SH/CAM, and decreased by 19% in RH/CAM. This could be due to the mixed effects of different concentrations of amino acids (Table 4), structural carbohydrates (Figure 3), and phytic acid (Figure 5) in the fermented substrate mixtures.

The pepsin (endopeptidase) and pancreatin (mixture of amylase, lipase, and protease) used in the digestibility analysis primarily digested proteins, poly-peptides, starches, and lipids in the solid samples. The decreases in structural carbohydrates concentration between the controls and fermented WB/CAM and SH/CAM reduced the crystallinity and polymerization of these structural carbohydrates with protein, starch, and lipid, making them more susceptible to the sequential enzymatic hydrolysis, thereby improving overall IVDMD [73]. However, the digestibility of the RH/CAM mixture decreased due to fermentation (Figure 6), which could be due to various factors. Although there was a significant decrease in cellulose due to fermentation, the hemicellulose concentration increased slightly (Figure 3), which could negatively influence the digestibility as the highly branched molecular structure of hemicellulose block or make it less accessible for the enzymes to the other digestible components (e.g., protein, starch, lipids). Rice hull contains significantly higher lignin concentrations than WB and SH (15%, 3%, 1–4%, respectively). Moreover, the relatively high proportion of phytic acid in fermented RH/CAM (Figure 5) could also reduce the digestibility of protein and starch because phytate can form complexes with protein and starch, making them less digestible [71]. Because substrates with different physical conditions and chemical compositions influence fermentation efficiency, the resulting digestibility of the fermented substrates can also vary as supported by many studies [74–76].

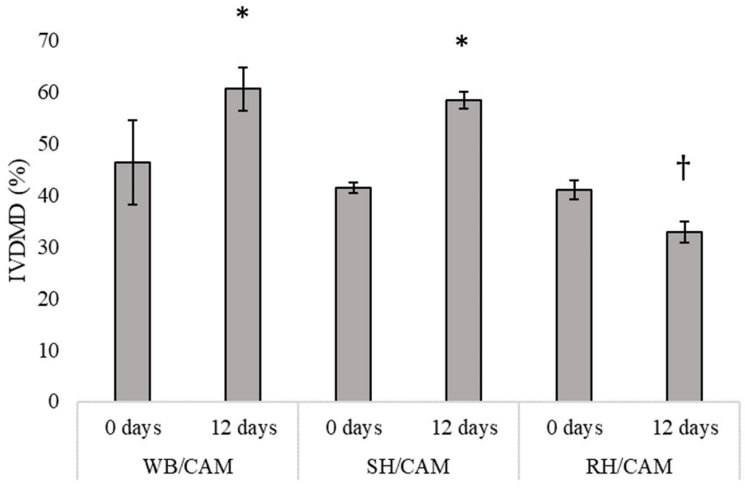

Figure 6. The in vitro dry matter digestibility (IVDMD) in each co-substrate mixture at 0 and 12 days of fermentation. * Significant increase in concentration due to 12 days of fermentation. † Significant decrease in concentration due to 12 days of fermentation ($p < 0.05$).

Based on the results shown in Figure 4, high pressure appears to have a greater degradation effect on glucosinolates in CAM. However, the authors' experiments were conducted after high-pressure sterilization followed by inoculation. In production, especially

for large-scale processing in tons, high-pressure sterilization may not be feasible due to cost considerations. Therefore, it is uncertain whether the current results can be extrapolated to industrial applications.

4. Conclusions

The fermentation of CAM with different co-substrates using *T. versicolor* resulted in significant reductions in phytic acid and the near-complete elimination of glucosinolates across all mixtures. The impact of fermentation on protein and carbohydrate components varied depending on the co-substrate used. Among the mixtures, SH/CAM fermentation demonstrated the most substantial nutritional enhancement, with notable increases in total and essential amino acid concentrations. Additionally, structural carbohydrates decreased by 42%, corresponding with a 44% increase in digestibility compared to the non-fermented control.

These findings highlight the potential of *T. versicolor*-mediated SSF to improve the nutritional value of CAM and other agricultural residues. By adjusting substrate composition and fermentation conditions, SSF can be optimized to enhance fungal growth and amino acid profiles, thereby increasing the economic and nutritional value of agricultural by-products for use in animal feed [74–76].

Future research should focus on optimizing SSF parameters, including substrate formulations and fermentation conditions, to maximize nutritional benefits and minimize processing costs. Additionally, chemometric analysis of fermentation products to determine the glucosinolate metabolites, which will help assess the extent of glucosinolate detoxification due to autoclave treatment and fermentation [66,67]. Investigating the effects of fermented products on animal health, growth performance, and nutrient utilization efficiency will further validate their potential as sustainable alternatives in animal feed formulations [9,57,66,70].

Author Contributions: K.B.: Investigation, writing—original draft preparation, writing—review and editing, data curation, methodology, formal analysis. X.S.: Conceptualization, writing—review and editing, methodology. D.Y.: Validation, Data curation, methodology. C.C.: Validation, writing—review and editing. L.v.L.: Writing—review and editing. B.H.: Funding acquisition, conceptualization, supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by UMN Forever Green Initiative with an Agriculture and Food Research Initiative Competitive Grant (No. 2019-69012-29851) and Minnesota Environment and Natural Resources Trust Fund (2022-303).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: We would like to acknowledge Matthew Ott, James Anderson and David Marks for providing camelina seeds and the Agricultural Utilization Research Institute for processing the meal.

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

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