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INTERACTIVE EFFECTS OF ISOLATE, CASING-LAYER THICKNESS, AND MOLYBDENUM ON WATER-SOLUBLE VITAMINS IN *AGARICUS BISPORUS* FRUITING BODIES

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Abstract:

Agaricus bisporus is widely cultivated for its nutritional value, although the combined effects of isolate identity and casing management on water-soluble vitamin accumulation remain poorly defined. This study examined the individual and interactive effects of mushroom isolate, casing-layer thickness, and molybdenum level on the vitamin profile of fruiting bodies. Two phenotypically distinct isolates, a white isolate (V1) and a brown isolate (V2) , were identified as *A. bisporus* by internal transcribed spacer sequencing. The experiment was conducted as a 2×3×3 factorial in a completely randomized design with three biological replicates. Casing-layer thicknesses were 3, 5, and 7 cm, while molybdenum was applied at 0, 60, and 120 mg Mo experimental unit^{-1} as sodium molybdate. Vitamins B1, B2, B3, B5, B6, B9, and C were quantified in first-flush fruiting bodies using high-performance liquid chromatography coupled with a diode-array detector and expressed as mg 100 g^{-1} dry weight. Treatment means were compared using Duncan's multiple range test at $P \leq 0.05$. Isolate identity produced the most consistent response, with V2 recording significantly higher concentrations of all seven vitamins than V1. Niacin (B3) and vitamin C reached 80.50 and 31.75 mg 100 g^{-1} dry weight in V2, compared with 55.19 and 16.02 mg 100 g^{-1} in V1, respectively. Responses to casing-layer thickness and molybdenum were vitamin-specific and non-linear. A 5-cm casing layer favored B1 and vitamin C, whereas a 7-cm layer favored B3 and B9. No significant casing-layer thickness × molybdenum interaction was detected. Among the three-way treatment combinations, V2 grown with a 5-cm casing layer and supplied with 60 mg Mo produced the highest concentrations of B1, B6, and vitamin C and the highest numerical concentration of B3. Vitamin concentrations were positively correlated ($r = 0.826–0.998$; $P \leq 0.001$). Overall, isolate selection was the main determinant of vitamin accumulation , while casing-layer thickness and molybdenum modified individual vitamin responses.

Keywords: Thiamine, riboflavin, niacin, HPLC-DAD, nutritional quality

INTRODUCTION

Edible mushrooms are widely valued as nutrient-dense foods because their fruiting bodies provide protein, dietary fiber, minerals, vitamins, and a range of bioactive compounds while generally containing relatively low amounts of fat and energy (Muszyńska et al., 2017 ; Kumar et al., 2021). *A. bisporus* is among the most extensively cultivated edible mushrooms and is important in commercial production because of its desirable sensory characteristics, adaptability to controlled cultivation, and nutritional value .

Water-soluble vitamins are important components of mushroom nutritional quality. Vitamins B1, B2, B3, B5, B6 and B9 function as vitamins or coenzyme precursors involved in energy metabolism, amino acid utilization, lipid and carbohydrate metabolism, and nucleic acid synthesis, whereas vitamin C contributes antioxidant activity and serves as a cofactor in several biological processes. Cultivated mushrooms are recognized as sources of riboflavin, niacin, and folate, and their thiamine and riboflavin concentrations vary among species and cultivation conditions (Mattila et al., 2001; Furlani and Godoy, 2008; Bernaś and Jaworska, 2016).

Vitamin accumulation in mushroom fruiting bodies can be influenced by both fungal material and the cultivation environment. Isolates within the same species may differ in mycelial growth, substrate utilization, nutrient acquisition & the allocation of metabolites during fruiting-body development. Differences among *A. bisporus* strains in crop performance and chemical composition have been reported under different compost formulations, while variation in growth substrate has also been associated with changes in thiamine, niacin, folate, and vitamin C contents in Agaricus spp. (Andrade et al., 2008; Rózsa et al., 2019) .

The casing layer is essential for the transition of *A. bisporus* from vegetative mycelial growth to primordium formation. It serves as a water reservoir, facilitates gas exchange, and provides a physical and microbial environment that supports fruiting. Consequently, water-holding capacity , water potential, porosity, and casing-layer thickness can influence crop development and fruiting-body quality (Baars et al. , 2020; Noble et al., 1999 ; Ghasemi et al., 2020; Wang et al., 2023a). Most previous studies, however, have focused on yield, biological efficiency, morphology, or overall chemical composition, whereas the response of individual water-soluble vitamins to casing-layer management remains less clearly defined.

Trace elements may also influence fungal metabolism through their involvement in enzyme systems. Molybdenum becomes biologically active after incorporation into the molybdenum cofactor (Moco), a prosthetic group required by several molybdenum-dependent oxidoreductases. In fungi, nitrate reductase is a Moco-dependent enzyme involved in nitrate assimilation, suggesting that molybdenum availability may indirectly affect nitrogen-related metabolic processes associated with vitamin biosynthesis or accumulation (Mendel & Oliphant , 2024; Ringel et al., 2013). A simple dose-dependent response is not necessarily expected, because the basal substrate may already contain physiologically adequate molybdenum and individual metabolic pathways may respond differently to supplementation.

Mushroom research conducted in Iraq has examined the effects of substrates and casing materials on fruiting-body productivity and quality, optimization of growth media, molecular identification of locally obtained edible-mushroom isolates, and nutritional applications of edible fungi (Abdulrazzaq et al., 2017a; Abdulrazzaq et al., 2017b; Mardiana et al., 2021; Farhan and Chechan, 2026 ; Al-Kanain et al., 2026). However, limited information is available on the simultaneous effects of isolate identity, casing-layer thickness, and molybdenum supplementation on individual water-soluble vitamins in *A. bisporus*. This study therefore evaluated the main and interactive effects of a white isolate (V1) and a brown isolate (V2), casing-layer thicknesses of 3, 5, 7 cm, and molybdenum levels of 0, 60, 120 mg Mo experimental unit^{-1} on the concentrations of vitamins B1, B2, B3, B5, B6, B9, and C in first-flush fruiting bodies.

MATERIALS AND METHODS

Experimental site and cultivation conditions

The experiment was conducted during the 2025-2026 production season at the Mushroom Production Station, Department of Horticulture and Landscape Engineering, College of Agriculture, Diyala University, iraq. Cultivation was carried out in a 4×7 m production room equipped with mushroom-growing shelves. Before the experiment, the room, shelves, and cultivation equipment were cleaned and disinfected. Temperature, relative humidity, and ventilation were adjusted according to the requirements of each cultivation stage . Ventilation was regulated to limit carbon dioxide accumulation while avoiding direct airflow over the cultivation bags.

Mushroom isolates and molecular identification

Two phenotypically distinct *A. bisporus* isolates were obtained from a local mushroom-producing company. The white isolate was designated V1 and corresponded to sample B in the molecular identification report, whereas the brown isolate was designated V2 and corresponded to sample A.

Species identity was verified by sequencing the internal transcribed spacer (ITS) region of ribosomal DNA. The ITS region was amplified and sequenced using primers ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′). The resulting sequences were compared with reference sequences available in the National Center for Biotechnology Information nucleotide database using the Basic Local Alignment Search Tool (BLAST).

The V1 sequence showed 100.00% identity, with 729 identical nucleotides among 729 aligned positions, and 94% query coverage with an *A. bisporus* reference sequence under accession number PP140826.1; the E-value was 0.0. The V2 sequence showed 99.33% identity, with 743 identical nucleotides among 748 aligned positions, and 97% query coverage with an *A. bisporus* reference sequence under accession number PP140817.1; the E-value was 0.0. These results verified both isolates as *A. bisporus*. Accession numbers PP140826.1 and PP140817.1 refer to the closest matching reference sequences in the database and were not assigned to the isolates examined in this study.

Experimental design and treatments

The experiment was arranged as a 2×3×3 factorial in a completely randomized design with three biological replicates per treatment combination. The evaluated factors were mushroom isolate, casing-layer thickness, and molybdenum level. The isolate factor included V1 and V2. Casing-layer thicknesses were 3, 5, 7 cm, while molybdenum was applied at 0, 60, 120 mg Mo experimental unit^{-1} using sodium molybdate as the molybdenum source. The factorial arrangement comprised 18 treatment combinations and 54 experimental units. Each experimental unit consisted of one polyethylene cultivation bag containing 5 kg of wet compost.

Compost preparation and pasteurization

Approximately 1 t of compost was prepared from wheat straw and poultry manure. Agricultural gypsum ($CaSO_4 \cdot 2H_2O$) was added to improve compost structure and reduce compaction, while urea was included as an

additional nitrogen source. The ingredients were thoroughly mixed and gradually moistened until the compost moisture content reached approximately 60-70%. The mixture was formed into an aerated pile and subjected to primary fermentation for 14 days. During fermentation, the pile was turned every 3 days, and its internal temperature was maintained between 60 and 70°C. After primary fermentation, the compost was pasteurized with steam at 60°C for 6 h and then cooled to approximately 25°C before spawning. Compost preparation and pasteurization followed established procedures for *A. bisporus* cultivation, with minor adaptation to local production conditions (Ahlawat, 2011; Gerrits, 1974).

Spawning and mycelial incubation

The cooled compost was inoculated with mushroom spawn at 2% of the wet compost weight. Spawn from each isolate was mixed uniformly throughout the compost. The inoculated compost was then distributed into perforated polyethylene bags measuring 60×40×18 cm, with each bag containing 5 kg of wet compost. The perforations allowed gas exchange during mycelial growth. Bags were incubated in darkness at 23 ± 2°C and a relative humidity of at least 70% for 14-18 days or until the compost was fully colonized by the mycelium.

Casing-layer and molybdenum application

After complete mycelial colonization, the upper portion of each cultivation bag was removed, and peat moss was evenly applied over the compost surface as the casing material. The casing layer was adjusted to the assigned thickness of 3, 5, or 7 cm. Excessive compaction was avoided to maintain suitable water retention, porosity, and gas exchange.

Molybdenum was applied 3 days after casing using sodium molybdate. The amounts required to provide 60 or 120 mg Mo experimental unit^{-1} were separately dissolved in 250 mL of distilled water and sprayed uniformly over the casing surface. Control units received 250 mL of distilled water without sodium molybdate. An equal liquid volume was applied to all experimental units to minimize variation associated with water addition.

Casing colonization, pinning, and fruiting

Following casing application, the production-room temperature was maintained at 24 ± 2°C, and relative humidity was kept at no less than 70% to promote mycelial colonization of the casing layer. Once mycelial aggregates became visible beneath the casing surface, the temperature was gradually reduced to approximately 16°C. Relative humidity was increased to approximately 90% using a mist humidifier to promote primordium formation. During pinning and fruiting, ventilation was adjusted to reduce carbon dioxide accumulation and support normal fruiting-body development. Mushrooms were harvested at marketable maturity. Only fruiting bodies collected from the first flush were used for chemical analysis.

Sample collection and drying

Fresh fruiting bodies were collected separately from each experimental unit. Peat-moss residues and other adhering materials were removed, and the samples were cut into small pieces. A 100-g fresh subsample from each experimental unit was dried in a forced-air oven at 60°C until constant weight. The dried samples were ground into a fine, homogeneous powder and stored in airtight containers protected from light and moisture until extraction.

Determination of water-soluble vitamins

Vitamins B1, B2, B3, B5, B6, and B9 in *A. bisporus* fruiting bodies were determined by high-performance liquid chromatography coupled with a diode-array detector (HPLC-DAD) , following the analytical approach described by Mateeva et al. (2023), with minor modifications appropriate for mushroom samples. One gram of finely ground dried material was extracted with 25 mL of extraction solution. The mixture was sonicated for 30 min, centrifuged, and filtered before injection into the HPLC system.

Chromatographic separation was performed on a C18 column using a mobile phase composed of potassium phosphate solution and methanol. The flow rate was maintained at 1.0 mL min^{-1}, and the injection volume was 20 µL. Vitamins were identified by comparing their retention times and absorption spectra with those of pure reference standards. Concentrations were calculated from individual calibration curves prepared for each vitamin.

Vitamin C was extracted separately under acidic conditions. The extract was protected from light and analyzed immediately by HPLC-DAD according to Klimczak and Gliszczyńska-Świgło(2015), with minor modifications to sample weight and extraction volume. Concentrations of all vitamins were expressed as mg 100 g^{-1} dry weight (DW).

Statistical analysis

Data were analyzed as a 2 × 3 × 3 factorial experiment arranged in a completely randomized design. Three-way analysis of variance was used to evaluate the main effects of mushroom isolate, casing-layer thickness, and molybdenum level, as well as their two-way and three-way interactions. When significant differences were detected, treatment means were separated using Duncan's multiple range test at $P \leq 0.05$ (Duncan, 1955). Significant interactions were interpreted before the corresponding main effects.

Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA). Results were expressed as means of three independent biological replicates, with one cultivation bag considered the experimental unit. Relationships among vitamin concentrations were evaluated using Pearson's correlation coefficient (r), with statistical significance assessed at $P \leq 0.001$.

RESULTS AND DISCUSSION

Main effects of isolate, casing-layer thickness, and molybdenum level

The concentrations of vitamins B1, B2, B3, B5, B6, B9, C in *A. bisporus* fruiting bodies are presented in Tables 1 and 2 and expressed as mg 100 g^{-1} DW. Isolate identity produced the most consistent main effect. The brown isolate V2

contained significantly higher concentrations of all seven vitamins than the white isolate V1. Concentrations of B1, B2, B3, B5, B6, B9, and vitamin C in V2 were 1.63, 5.07, 80.50, 40.67, 4.20, 0.88, and 31.75 mg 100 g^{-1} DW, respectively, compared with 1.18, 3.12, 55.19, 23.89, 2.85, 0.66, and 16.02 mg 100 g^{-1} DW in V1.

Casing-layer thickness produced smaller and vitamin-dependent responses. The 5-cm layer (C2) resulted in the highest B1 and vitamin C concentrations, while C2 and the 7-cm layer (C3) were statistically similar and superior to the 3-cm layer (C1) for B2. C3 produced the highest B3 and B9 concentrations, whereas C1 produced the highest B5 and B6 means. Molybdenum showed a similarly variable response among vitamins. M0 and M60 shared the highest statistical group for B1, whereas M0 and M120 shared the highest group for B2. The highest B3 and B6 means were recorded under M0, B5 was highest under M60, and B9 reached its highest value under M120. Vitamin C concentrations were statistically similar under M0 and M60 and lower under M120.

Isolate × casing-layer thickness interaction

The isolate × casing-layer thickness interaction showed that V2 maintained higher vitamin concentrations across the tested casing thicknesses. V2C2 produced the highest B1 and vitamin C concentrations, reaching 1.69 and 32.10 mg 100 g^{-1} DW, respectively. For B2, B3, and B6, V2C1, V2C2, and V2C3 were statistically similar. V2C1 produced the highest B5 mean, whereas V2C3 produced the highest B9 mean.

Isolate × molybdenum level interaction

For the isolate × molybdenum interaction, V2M0, V2M60, and V2M120 were statistically similar for B1, B2, B3, B5, and vitamin C, and all remained above the corresponding V1 combinations. A more distinct response to molybdenum was observed for B6 and B9. For B6, V2M0 and V2M60 were higher than V2M120, whereas V2M120 produced the highest B9 mean and did not differ significantly from V2M0.

Casing-layer thickness × molybdenum level interaction

No significant differences were detected among the casing-layer thickness × molybdenum combinations for any of the seven vitamins. Thus, when averaged across isolates, the response to molybdenum was not consistently modified by casing-layer thickness within the tested ranges.

Three-way interaction

The isolate × casing-layer thickness × molybdenum interaction showed that the treatment producing the highest concentration depended on the vitamin. V2C2M60 produced the highest B1, B6, and vitamin C concentrations, reaching 1.79, 4.34, and 32.33 mg 100 g^{-1} DW, respectively. The same treatment also produced the highest numerical B3 concentration at 81.37 mg 100 g^{-1} DW, although it shared the highest statistical group with several other V2 combinations. The highest numerical B2 concentration was recorded under V2C3M120 at 5.18 mg 100 g^{-1} DW and did not differ significantly from V2C2M60 at 5.15 mg 100 g^{-1} DW. B5 reached its highest value under V2C1M120 at 41.24 mg 100 g^{-1} DW, whereas B9 was highest under V2C3M0 at 0.96 mg 100 g^{-1} DW and was statistically similar to V2C1M120 at 0.95 mg 100 g^{-1} DW.

Correlation among water-soluble vitamins

All vitamin pairs were positively and highly significantly correlated (Table 3). Pearson correlation coefficients ranged from r = 0.826 for B1 × B9 to r = 0.998 for B5 × vitamin C. Other particularly strong relationships were observed between B2 and vitamin C (r = 0.997), B2 and B5 (r = 0.996), and B6 and vitamin C (r = 0.989).

Table 1. Effects of mushroom isolate, casing-layer thickness, molybdenum level, and their interactions on the B1, B2, B3, and B5 contents of fruiting bodies (mg 100 g^{-1} DW).

Treatment	B1	B2	B3	B5
Single factors				
V1	1.18 B	3.12 B	55.19 B	23.89 B
V2	1.63 A	5.07 A	80.50 A	40.67 A
C1 (3 cm)	1.41 B	4.06 B	67.51 B	32.39 A
C2 (5 cm)	1.43 A	4.12 A	67.52 B	32.27 AB
C3 (7 cm)	1.38 C	4.11 A	68.50 A	32.18 B
M0 (0 mg)	1.41 A	4.11 A	68.81 A	32.19 B
M60 (60 mg)	1.42 A	4.06 B	67.45 B	32.40 A
M120 (120 mg)	1.39 B	4.12 A	67.28 B	32.25 AB
Two-way interaction: V x C				
V1C1	1.20 c	3.08 c	54.35 c	23.82 c
V1C2	1.17 c	3.16 b	54.03 c	23.89 c
V1C3	1.17 c	3.14 bc	57.17 b	23.96 c
V2C1	1.62 b	5.04 a	80.68 a	40.97 a
V2C2	1.69 a	5.09 a	81.01 a	40.66 ab
V2C3	1.58 b	5.07 a	79.82 a	40.39 b
Two-way interaction: V x M				
V1M0	1.18 b	3.18 b	57.64 b	23.88 b

Treatment	B1	B2	B3	B5
V1M60	1.18 b	3.06 c	53.92 c	23.95 b
V1M120	1.18 b	3.14 b	54.00 c	23.83 b
V2M0	1.65 a	5.04 a	79.97 a	40.50 a
V2M60	1.65 a	5.06 a	80.98 a	40.85 a
V2M120	1.60 a	5.10 a	80.56 a	40.67 a
Two-way interaction: C x M				
C1M0	1.40 a	4.11 a	67.55 a	32.03 a
C1M60	1.43 a	4.01 a	67.23 a	32.56 a
C1M120	1.40 a	4.05 a	67.77 a	32.58 a
C2M0	1.41 a	4.08 a	67.62 a	32.42 a
C2M60	1.50 a	4.14 a	67.62 a	32.40 a
C2M120	1.39 a	4.14 a	67.32 a	32.01 a
C3M0	1.43 a	4.13 a	71.25 a	32.12 a
C3M60	1.32 a	4.01 a	67.49 a	32.25 a
C3M120	1.39 a	4.17 a	66.75 a	32.17 a
Three-way interaction: V x C x M				
V1C1M0	1.18 m	3.12 ef	54.94 f	23.51 h
V1C1M60	1.23 j	3.02 g	53.83 fg	24.01 fgh
V1C1M120	1.19 lm	3.08 f	54.29 fg	23.93 fgh
V1C2M0	1.10 o	3.16 e	54.23 fg	23.75 gh
V1C2M60	1.21 k	3.14 ef	53.87 fg	24.14 fg
V1C2M120	1.21 kl	3.17 e	53.99 fg	23.77 gh
V1C3M0	1.25 i	3.25 d	63.75 e	24.38 f
V1C3M60	1.11 o	3.01 g	54.04 fg	23.70 gh
V1C3M120	1.15 n	3.16 e	53.72 g	23.80 gh
V2C1M0	1.62 de	5.10 b	80.16 bc	40.55 cd
V2C1M60	1.64 c	5.01 c	80.62 abc	41.11 ab
V2C1M120	1.60 f	5.01 c	81.25 ab	41.24 a
V2C2M0	1.71 b	5.00 c	81.00 ab	41.08 ab
V2C2M60	1.79 a	5.15 ab	81.37 a	40.65 bcd
V2C2M120	1.56 g	5.12 b	80.64 abc	40.25 de
V2C3M0	1.61 ef	5.02 c	78.76 d	39.86 e
V2C3M60	1.52 h	5.02 c	80.94 ab	40.79 abc
V2C3M120	1.63 d	5.18 a	79.77 c	40.53 cd

* V1 = white isolate; V2 = brown isolate; C1 = 3 cm casing layer; C2 = 5 cm casing layer; C3 = 7 cm casing layer; M0 = 0 mg Mo experimental unit^{-1}; M60 = 60 mg Mo experimental unit^{-1}; M120 = 120 mg Mo experimental unit^{-1}; DW = dry weight.

* Within each factor or interaction section and column, means followed by different letters differ significantly according to Duncan's multiple range test at $P \leq 0.05$.

Table 2. Effects of mushroom isolate, casing-layer thickness, molybdenum level, and their interactions on the B6, B9, and vitamin C contents of fruiting bodies (mg 100 g^{-1} DW).

Treatment	B6	B9	Vit. C
Single factors			
V1	2.85 B	0.66 B	16.02 B
V2	4.20 A	0.88 A	31.75 A
C1 (3 cm)	3.57 A	0.74 C	23.85 B
C2 (5 cm)	3.53 B	0.77 B	24.02 A
C3 (7 cm)	3.47 C	0.80 A	23.79 B
M0 (0 mg)	3.56 A	0.78 B	24.02 A
M60 (60 mg)	3.54 B	0.74 C	23.91 A
M120 (120 mg)	3.46 C	0.80 A	23.73 B
Two-way interaction: V x C			
V1C1	2.92 b	0.60 d	16.05 c
V1C2	2.83 bc	0.70 c	15.94 c
V1C3	2.80 c	0.69 c	16.08 c
V2C1	4.22 a	0.89 ab	31.65 b
V2C2	4.23 a	0.85 b	32.10 a

Treatment	B6	B9	Vit. C
V2C3	4.14 a	0.91 a	31.50 b
Two-way interaction: V x M			
V1M0	2.86 c	0.66 c	16.26 b
V1M60	2.86 c	0.64 c	15.95 c
V1M120	2.82 c	0.68 c	15.86 c
V2M0	4.27 a	0.89 ab	31.78 a
V2M60	4.21 a	0.85 b	31.87 a
V2M120	4.10 b	0.91 a	31.60 a
Two-way interaction: C x M			
C1M0	3.52 a	0.75 a	23.98 a
C1M60	3.63 a	0.69 a	23.81 a
C1M120	3.55 a	0.79 a	23.76 a
C2M0	3.61 a	0.73 a	24.12 a
C2M60	3.62 a	0.78 a	24.13 a
C2M120	3.36 a	0.80 a	23.82 a
C3M0	3.56 a	0.85 a	23.97 a
C3M60	3.37 a	0.76 a	23.79 a
C3M120	3.48 a	0.80 a	23.61 a
Three-way interaction: V x C x M			
V1C1M0	2.81 i	0.60 h	16.23 ef
V1C1M60	2.97 f	0.55 i	15.88 fg
V1C1M120	2.97 f	0.63 g	16.04 efg
V1C2M0	2.89 g	0.65 g	16.17 ef
V1C2M60	2.91 g	0.74 e	15.93 fg
V1C2M120	2.68 k	0.70 f	15.72 g
V1C3M0	2.87 h	0.73 e	16.38 e
V1C3M60	2.72 j	0.61 h	16.04 efg
V1C3M120	2.81 i	0.71 f	15.82 fg
V2C1M0	4.23 c	0.90 bc	31.72 bcd
V2C1M60	4.29 b	0.82 d	31.74 bcd
V2C1M120	4.13 d	0.95 a	31.48 d
V2C2M0	4.33 a	0.82 d	32.06 ab
V2C2M60	4.34 a	0.82 d	32.33 a
V2C2M120	4.03 e	0.90 bc	31.92 bc
V2C3M0	4.25 c	0.96 a	31.55 cd
V2C3M60	4.01 e	0.91 b	31.54 cd
V2C3M120	4.15 d	0.88 c	31.41 d

* V1 = white isolate; V2 = brown isolate; C1 = 3 cm casing layer; C2 = 5 cm casing layer; C3 = 7 cm casing layer; M0 = 0 mg Mo experimental unit^{-1}; M60 = 60 mg Mo experimental unit^{-1}; M120 = 120 mg Mo experimental unit^{-1}; DW = dry weight.

* Within each factor or interaction section and column, means followed by different letters differ significantly according to Duncan's multiple range test at $P \leq 0.05$.

Table (3). Correlation coefficients among water-soluble vitamin contents in the fruiting bodies of the mushroom A. bisporus.

Trait	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin B5	Vitamin B6	Vitamin B9
Vitamin B2	0.965***					
Vitamin B3	0.962***	0.987***				
Vitamin B5	0.965***	0.996***	0.986***			
Vitamin B6	0.979***	0.984***	0.973***	0.988***		
Vitamin B9	0.826***	0.901***	0.895***	0.891***	0.854***	
Vitamin C	0.966***	0.997***	0.986***	0.998***	0.989***	0.886***

n = 54 observations. *, **, and *** indicate significant correlation coefficients at probability levels of 0.05, 0.01, and 0.001, respectively, while values without asterisks indicate non-significant correlations at the 0.05 probability level.

Isolate-related variation in vitamin accumulation

Isolate identity was the most consistent determinant of the water-soluble vitamin profile. V2 exceeded V1 for all seven vitamins, indicating that the isolate effect extended across compounds with different biochemical functions rather than being restricted to a particular vitamin. Cultivated mushrooms, including Agaricus spp., are recognized sources of riboflavin, niacin, and folate, although their concentrations vary with fungal material and cultivation conditions (Mattila et al., 2001; Furlani and Godoy, 2008; Bernaś and Jaworska, 2016). Çağlarırmak (2011) also reported relatively high riboflavin and niacin concentrations in brown Portobello mushrooms compared with other cultivated mushrooms examined. Together with evidence that *A. bisporus* strains differ in crop performance and chemical composition (Andrade et al., 2008), the present results suggest a broad isolate-dependent difference in nutrient accumulation.

The physiological basis of this difference cannot be determined from the present experiment. Variation in substrate utilization, nutrient acquisition, mycelial physiology, or allocation of metabolic precursors during fruiting-body development may have contributed to the response. Changes in the nutritional composition of Agaricus fruiting bodies under different substrates further indicate that fungal material and the cultivation environment can act jointly in determining chemical composition (Rózsa et al., 2019; Wang et al., 2023b).

The magnitude of the isolate effect should also be considered in relation to the basis on which vitamin concentrations were expressed. Values were determined on a dry-weight basis and therefore describe vitamin concentration in fruiting-body dry matter rather than total vitamin production per experimental unit. Higher concentrations in V2 do not necessarily indicate greater commercial productivity because fresh yield, biological efficiency, crop duration, dry-matter production, and total vitamin yield would also need to be considered. Comparisons among studies may also be affected by analytical and postharvest conditions because extraction procedures can influence B-vitamin recovery, while vitamin C is particularly sensitive to oxidation and handling (Mattila et al., 2001; Furlani and Godoy, 2008; Bernaś and Jaworska, 2016).

Effects of casing-layer thickness

Casing-layer thickness did not affect all vitamins in the same direction. The 5-cm layer was most favorable for B1 and vitamin C and was among the higher treatments for B2, whereas the 7-cm layer favored B3 and B9. B6 was highest at 3 cm, while B5 showed only a limited response, with the 3-cm layer producing the highest mean. This pattern suggests that the effect of casing thickness was associated with the microenvironment created around the developing mycelium rather than with increasing or decreasing thickness alone.

The casing layer influences water availability, porosity, gas exchange, mycelial distribution, and microbial activity. Physical properties of casing materials are known to affect water relations and crop development (Noble et al., 1999; Pardo et al., 2010), while studies of *A. bisporus* cultivation emphasize the combined importance of casing water content, aeration, and management practices (Baars et al., 2020; Dias et al., 2021; Navarro et al., 2021). Ghasemi et al. (2020) likewise reported that casing material and thickness affected yield, quality characteristics, and nutrient concentrations in *A. bisporus*.

Changes in casing-associated microbial communities and volatile compounds during fruiting have also been reported (Wang et al., 2023a). Vieira et al. (2023) showed that manipulation of casing microbial communities altered community composition and influenced the timing of fruiting-body development. The present differences among casing thicknesses may therefore reflect changes in the physical and biological environment surrounding the mycelium, although these mechanisms were not measured directly. Studies published in the Iraqi Journal of Agricultural Sciences have similarly shown that substrate and casing choices can modify mushroom productivity and fruiting-body quality in other edible species (Abdulrazzaq et al., 2017a; Abdulrazzaq et al., 2017b; Mardiana et al., 2021).

Effects of molybdenum

Molybdenum produced a non-linear and vitamin-specific response. M0 or the lower supplementation level was associated with the higher values of B1, B2, B3, and vitamin C, whereas M60 was most favorable for B5. B6 decreased as molybdenum level increased, while B9 showed the opposite general pattern and reached its highest main-effect mean at M120. These contrasting responses indicate that no single molybdenum level was most favorable for the complete vitamin profile.

Molybdenum becomes biologically active after incorporation into the molybdenum cofactor (Moco), which is required for the catalytic activity of several molybdenum-dependent enzymes (Mendel and Oliphant, 2024). In fungi, nitrate reductase is Moco-dependent, and biochemical studies with *Neurospora crassa* have demonstrated the importance of Moco for nitrate-reductase structure and activity (Ringel et al., 2013). Moco biosynthesis is also regulated in relation to nitrogen status and nitrate induction (Wajmann et al., 2020). Molybdenum availability could therefore influence vitamin accumulation indirectly through changes in nitrogen-related metabolism and the availability of metabolic precursors.

The observed response, however, cannot be attributed to nitrate reductase alone. Baars et al. (1996) reported that ammonium assimilation in *A. bisporus* mycelium occurred mainly through the glutamine synthetase/glutamate synthase pathway under the conditions they examined. The present results therefore suggest an association with broader metabolic responses rather than a single biochemical pathway. Moreover, molybdenum concentrations in the compost, casing layer, and fruiting bodies were not measured. The comparatively favorable vitamin concentrations observed under M0 for several vitamins should therefore not be interpreted as evidence of molybdenum deficiency or toxicity at any of the tested supplementation levels.

Interpretation of the interaction effects

The two-way interactions further emphasized the dominant influence of isolate identity. Across the isolate ×molybdenum combinations, V2 maintained higher concentrations of B1, B2, B3, B5, ,C, while changes in molybdenum level within V2 had relatively little influence on these vitamins. More pronounced responses were observed for B6 and B9 . A similar pattern occurred for isolate× casing-layer thickness. V2 remained associated with the higher vitamin concentrations, but the most favorable casing thickness varied among vitamins, with 5 cm particularly favorable for B1 and vitamin C and 7 cm for B9. These results indicate that casing-layer thickness and molybdenum modified individual components of the vitamin profile without overriding the general isolate effect. Such responses are consistent with previous evidence that fungal material can interact with growth medium, casing properties, and cultivation amendments in determining fruiting-body composition (Andrade et al., 2008; Ghasemi et al., 2020; Wang et al., 2023b ; Hammad et al., 2023; Mustaf et al., 2025).

In contrast, the casing-layer thickness × molybdenum interaction was non-significant for all seven vitamins. When averaged across isolates, there was no consistent pattern indicating that the effect of molybdenum depended on casing thickness within the tested ranges. This lack of statistical interaction does not imply that the two factors were physiologically independent. Compost and casing are dynamic environments in which moisture conditions, nutrient transformations, enzyme activities, and microbial communities change during cultivation (Carrasco and Preston, 2020; Thai et al., 2022). Rather, the statistical response indicates that any combined effects of casing thickness and molybdenum were not sufficiently consistent across isolates to generate a significant two-factor interaction.

The three-way interaction also demonstrated that no single treatment combination maximized all seven vitamins. Most of the higher values occurred in V2 treatments, but the associated casing thickness and molybdenum level differed among vitamins. V2C2M60 was particularly favorable because it produced the highest B1, B6, and vitamin C concentrations and the highest numerical B3 concentration, while remaining in the highest statistical group for B2. In contrast, the highest B5 and B9 concentrations occurred under V2C1M120 and V2C3M0, respectively. A 5-cm casing layer combined with 60 mg Mo experimental unit^{-1} therefore provided a comparatively balanced response across several vitamins, but it was not the most favorable treatment for every vitamin. This pattern again indicates that isolate identity was the most consistent factor, whereas casing-layer thickness and molybdenum acted in a vitamin-dependent manner.

Relationships among vitamin concentrations

The correlation analysis provided additional evidence that vitamin concentrations varied in a coordinated manner across the experimental treatments. All correlations were positive, with the strongest relationship occurring between B5 and vitamin C ($r = 0.998$), followed by B2 with vitamin C ($r = 0.997$) and B5 ($r = 0.996$). Even the lowest coefficient, between B1 and B9, remained high at $r = 0.826$.

Because V2 increased the concentrations of all seven vitamins, the common response to isolate identity likely contributed substantially to these strong positive correlations. The coefficients should therefore be interpreted as evidence that the vitamins tended to vary together under the tested treatments, rather than as evidence of direct biochemical dependence among their biosynthetic pathways.

Practical implications

From a practical perspective, isolate selection appears to be the first consideration when the objective is to modify the water-soluble vitamin profile of *A. bisporus* fruiting bodies. Casing-layer thickness and molybdenum supplementation can then be adjusted according to the vitamin or nutritional profile being targeted. V2C2M60 represents a suitable treatment for further evaluation because of its comparatively balanced performance for B1, B2, B3, B6, and vitamin C. Commercial recommendations, however, require confirmation across additional flushes and cultivation cycles and should incorporate fresh yield, biological efficiency, total vitamin yield, mineral accumulation, and production cost. Such measurements would distinguish treatments that increase vitamin concentration from those that increase the total recoverable amount of each vitamin.

CONCLUSION

The water-soluble vitamin profile of *A. bisporus* fruiting bodies was determined mainly by isolate identity, with additional effects from casing-layer thickness and molybdenum level. The brown isolate V2 consistently produced higher concentrations of B1, B2, B3, B5, B6, B9, and vitamin C than the white isolate V1. Responses to casing-layer thickness and molybdenum were vitamin-specific and non-linear, while the casing-layer thickness × molybdenum interaction was non-significant for all measured vitamins. Among the three-way treatment combinations, V2C2M60 provided the most balanced response for B1, B2, B3, B6, and vitamin C, whereas other V2 combinations were more favorable for B5 and B9. The consistently positive correlations among vitamin concentrations ($r = 0.826$-0.998) indicated coordinated variation across the experimental treatments. Under the conditions of this study, isolate selection appears to be the primary consideration when targeting the vitamin composition of fruiting bodies, while casing-layer thickness and molybdenum supplementation may be adjusted according to specific nutritional objectives . Further evaluation should relate vitamin concentration to fresh yield, biological efficiency, total vitamin recovery, production cost, and response stability across successive flushes and cultivation cycles.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

S.A.A.: conceptualization, methodology, formal analysis, data curation, supervision, and writing - review and editing. K.I.M.: methodology, investigation, formal analysis, data curation, writing - original draft, and writing - review and editing. Both authors read and approved the final version of the manuscript.

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