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Quality aspects of oven-dried whole (full-fat) and d-oiled mealworm stored at room temperature

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ABSTRACT: Mealworms (*Tenebrio molitor*) are considered the potential novel alternative source of protein for sustainable food production and also have a low ecological footprint. Nonetheless, fresh mealworms have a shorter shelf-life, therefore, to preserve their nutrient quality and safety it is of utmost importance to optimize the post-harvest processing techniques. Therefore, in the present study two technological forms of oven-dried (60 °C for 2 h) mealworms namely whole (full-fat) mealworms (WMW) and de-oiled mealworms (D-OMW) were analysed for nutrient quality, microbial count, and aflatoxin B1 safety levels. Both WMW and D-OMW were stored for 56 days at room temperature (25 to 30 °C) and were sampled on alternate weeks (days 0, 14, 28, 42, and 56, respectively) for analysis. In comparison to WMW, the D-OMW contains high ($P < 0.001$) contents of crude protein (CP) and dry matter (DM). Notably, at 56 days of storage interval, the D-OMW is more stable with CP and DM contents having DM (87.3 vs. 77.3%) and CP (76 vs. 46.0%) contents. In contrast, WMW had high ($P < 0.001$) content of EE than D-OMW (24.5 vs. 3.30%) at 56 days of storage. Moreover, WMW and D-OMW are rich sources of minerals, particularly potassium and phosphorus, with no significant ($P > 0.05$) effect during storage interval. Notably, leucine, lysine, and valine are the abundant amino acids in WMW and D-OMW. During the entire storage period, no growth of *Escherichia coli*, *Salmonella*, *Campylobacter*, and *Clostridium perfringens* were found. However, low levels of other coliforms, *Enterococci*, and *Staphylococcus aureus* were detected in D-OMW than in WMW. Likewise, the aflatoxin B1 contamination level was much lower in D-OMW during the entire storage time. This study concluded that storage times affected the nutrient profile and safety level of WMW. Nonetheless, the D-OMW was found almost stable and safe even at the storage of 56 days at room temperature.

Keywords: *Tenebrio molitor*; nutrient composition; microbial load; aflatoxin B1

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INTRODUCTION

The yellow mealworms (*Tenebrio molitor* L., Coleoptera: Tenebrionidae) have been considered a promising candidate for the cultivation and large-scale industrial production owing to their ubiquitous existence and high rate of consumption (Van Huis, 2013; EFSA, 2015). It is an edible insect that is progressively becoming the focus of attention due to its high protein contents, vitamins, and mineral profile (Rumpold and Schluter, 2013; Van Huis, 2013). Additionally, the *Tenebrio molitor* L. can easily be produced in a wide range of environmental states and it does not require a huge area for production (Makkaret *et al.*, 2014). Likewise, a mealworm-based diet has been successfully practiced in poultry production (Hussain *et al.*, 2017). Nonetheless, the fresh mealworms are highly perishable (short shelf-life) and during the post-harvest stage, the mealworms provide a favorable environment for microbial growth, mycotoxin production, and lipid peroxidation (Klunderet *et al.*, 2012; Kronckeet *et al.*, 2019). These changes in mealworms are mainly connected with rancidity and alteration of nutrient profile (Ramashia *et al.*, 2020). Therefore, fresh stock of the mealworms is required to be stabilized in terms of microbial count, nutrient quality, and safety.

Alternatively, chemically and microbiologically stable mealworms are produced by freezing and freeze-drying method after harvesting (EFSA, 2015; Kronckeet *et al.*, 2019). However, these methods are expensive because of the long processing for small holder insect-rearing farmers. Consequently, alternate cost-effective methods are needed to be explored that efficiently retain its nutrient profile and safety levels for a long period. The available literature on the storage of mealworms and/ or their products developed from whole (full-fat) mealworms (WMW) and de-oiled mealworms (D-OMW) is scarce. Therefore, the present study was conducted to evaluate the effect of storage interval on nutrient quality, microbiological safety, and aflatoxin B1 contamination of two technological forms of oven-dried mealworms namely WMW and D-OMW under room temperature.

MATERIALS AND METHODS

Experimental setup and ethical consideration

Tenebriomolitor larvae, a species of darkling beetle, were kept in a tray (80 x 40 cm²) at a density of 5 larvae per cm square in a worm-growth chamber. The mealworms were maintained under standard room

conditions (set to 25 ± 2 °C and 70% relative humidity) and a photoperiod of 14 (L):10 (D) h, and were fed on 70% wheat bran, and 10% each corn flour, canola meal, and fresh potatoes. The *T. molitor* larvae were reared for 12 weeks until harvest.

At harvest time, the individual larva of mealworms was carefully separated and frozen at -20°C for 24 h, and subsequently, blanched in boiling water for 20 Sec and then oven-dried. The research was prior approved by the Faculty ethical committee of The University of Agriculture, Peshawar, Pakistan for a procedure involving animal welfare and standard laboratory protocols.

Mealworms preparation

Mealworms (*Tenebrio molitor* L.) were prepared by the method described by Khan *et al.* (2016; 2018) in the mealworms research lab at the University of Agriculture Peshawar. Briefly, the mealworms were oven-dried at 60°C for 2 hours. The mealworms were further divided into two main fractions namely whole (full-fat) mealworms (WMW) and de-oiled mealworms (D-OMW) followed by the drying. The former one was crushed as full-fat while the latter one was de-oiled using a screw press machine (300 MPa/minute). Both the fractions were stored in sterile cloth bags at room temperature (25-30 °C; humidity, 45-50%) in September and October. The study lasted for 8 weeks. Duplicate samples from WMW and D-OMW were taken on alternate weeks (days 0, 14, 28, 42, and 56, respectively) for onward laboratory analysis.

Chemical composition of WMW and D-OMW

The dried samples of WMW and D-OMW were ground through a 1 mm mesh sieve, and were analyzed for the contents of dry matter (DM), CP, ash, fiber, and ether extract (EE) according to the standard methods of AOAC (1990). The content of DM was calculated by drying the sample in the oven (at a temperature of 103 °C; method 930.15) till constant weight, and the EE content was determined by the standard Soxhlet extraction technique (method 920.39). Likewise, the N content was determined by the Kjeldahl procedure (method 984.13; by a KjeltectTM 2400 auto-analyzer; Foss Analytical A/S, Hillerod, Denmark) and CP was calculated as N × 6.25. The content of ash in the dried insect sample was determined by the incineration at 550 °C (method 942.05) as stated previously by Khan *et al.* (2017). The fiber content was determined followed by digestion with 72% sulphuric acid for 3 hours. Each analysis was conducted in duplicate.

The mineral contents were analyzed by atomic absorption spectrophotometer (Buck Scientific 240VGP, Milan, Italy) after digestion of the WMW and D-OMW samples with a mixture of nitric, per-chloric, and sulphuric acids according to the procedures described by AOAC (1995).

Amino acid and vitamins analysis

For determination of amino acid contents analysis of WMW and D-OMW, ion exchange column chromatography method using amino acid analyzer was used. Briefly, the dried grounded samples (500 microns) of WMW and D-OMW have oxidized with hydrogen peroxide-formic acid-phenol solution. After oxidation, the samples were hydrolyzed by 6 M HCL/phenol for 24 h. The hydrolysate pH was adjusted at 2.20, using 7.5 M NaOH. After that the mealworms samples were diluted first and then filter it to the sample vials. The split-up of the different amino acids was performed by an amino acid analyzer, and their amount was calculated based on elution and standard solution volumes as reported earlier by Shang-guiet *al.* (2004).

Water- and fat-soluble vitamins were determined through HPLC adopting the procedure of Kartsova and Koroleva, (2007).

Microbiological analysis

Microbiological analysis of WMW and D-OMW was executed by standard plate methods as described by Stastniket *al.* (2021). Briefly, the samples (10 g) of WMW and D-OMW were prepared in duplicates. The growth of the following groups of microorganisms was determined by standard procedures. The Rapid *E. coli* Agar was used for the *E. coli* and other coliforms at 37 °C for 24 h and *Campylobacter* on Mueller-Hinton (MH) agar. The *Enterococci* growth was observed at 42 °C for 24 h on Compass *Enterococcus* agar, while *Clostridium perfringens* at 45 °C for 24 h on TSN Agar plate, *Staphylococcus aureus* at 37 °C for 24 h on Baird-Parker agar with rabbit plasma, *Salmonella* at 37 °C for 24 h by double enrichment method on Rapid Salmonella Agar plate. The plates were counted using a colony counter for each microorganism. The counts were shown as colony-forming units per gram CFU/g.

Aflatoxin B1 analysis

Contamination levels of Aflatoxin B1 were determined according to the procedure of Gilbert and Anklam, (2002). Briefly, for AflatoxinB1 analysis,

ground samples of WMW and D-OMW (10 g each; 1 mm particle size) were mixed in methanol and water (80:20), and were shaken for 30 min, and then filter (Whatman \neq 1). The filtrate was than mixed with acetonitrile (1:1) and pass through MycoSepa clean-up columns. Finally, it was diluted in the water and methanol (mobile phase) and used for HPLC analyses. Mycotoxins in mealworms larvae were analyzed in duplicates.

Statistical analysis

Data on the effect of storage interval on nutrient quality and safety of WMW and D-OMW was conducted using an ANOVA of statistical software SAS. In case of significant difference ($P < 0.05$) between treatments, then Duncan's Multiple Range test was used for analysis.

RESULTS

The effects of post oven-drying interval on the basic chemical composition of the WMW and D-OMW are summarized in Table 1. Compared to WMW, The D-OMW had high ($P < 0.001$) contents of DM (87.3 vs. 77.3%) and CP (76 vs.46.0%) on day 56 of storage. Furthermore, The D-OMW is more stable in terms of DM and CP during storage intervals. On the other hand, WMW had high($P < 0.001$)content of EE than D-OMW (24.5 vs.3.30%) at 56 days of storage. Moreover, the contents of ash and fiber did not vary ($P > 0.05$)during the storage period for both WMW and D-OMW.

The effects of storage intervals on the mineral profile (mg/kg) of WMW and D-OMW are presented in Table 2. Importantly, the mineral profile of mealworms during storage times did not vary ($P > 0.05$). Notably, both the fractions of mealworms were found to be rich in most nutritive elements, especially phosphorus and potassium in terms of macronutrients, and Zn in terms of micronutrients. Whereas, lower contents of calcium, manganese, iodine, and selenium were found in WMW and D-OMW.

The effects of storage intervals on the amino acid contents of WMW and D-OMW are presented in Table 3. The storage intervals had no significant ($P > 0.05$) effects on the amino acid profile of both frictions of mealworms. Notably, the levels of the important amino acids were found to be considerable, especially leucine, lysine, and valine in both WMW and D-OMW.

Table 1. Effects of storage intervals(days 0 to 56) on the chemical composition of oven-dried whole (full-fat) mealworms (WMW) and de-oiled mealworms (D-OMW)

	Days	Nutrients (% DM basis)				
		DM	CP	EE	Crude fiber	Ash
WMW	0	90.0	50.0	35	5.51	3.51
	14	87.5	49.1	30	5.5	3.03
	28	84.6	48.5	28	5.07	2.81
	42	80.6	47.5	25.7	4.90	2.51
	56	77.3	46.0	24.5	4.58	2.37
D-OMW	0	90.1	76.5	5.01	5.50	3.5
	14	89.5	76.2	5	5.5	3.23
	28	88.6	76.0	4.8	5	2.8
	42	87.6	76.0	4.03	4.81	2.61
	56	87.3	76.0	3.30	4.58	2.57
SEM		.016	0.028	.013	0.011	0.016
Significance		***	***	***	ns	ns

Data on day 0 indicated the value after oven-drying
 DM, dry matter, CP, crude protein, EE, ether extract
 SEM, standard error of the mean
 ns, non-significant ($P > 0.05$), ***, $P < 0.001$

Table 2. Effects of storage interval (days 0 to 56) on the mineral profile (mg/kg) of oven-dried whole fat mealworm and de-oiled mealworm

Minerals	WMW					D-OMW					SEM	Sig
	day-0	d-14	d-28	d-42	d-56	day-0	d-14	d-28	d-42	d-56		
Calcium	262	262	260	260	260	262	262	260	260	260	.224	ns
Phosphorus	2,190	2,100	2,092	2,085	1,990	2,190	2,160	2,092	2,090	2010	1.83	ns
Magnesium	435	435	430	430	430	435	435	430	430	430	.236	ns
Sodium	388	388	386	386	386	388	388	385	385	385	0.16	ns
Potassium	2950	2950	2950	2950	2940	2950	2950	2940	2935	2930	0.149	ns
Chloride	1620	1610	1610	1600	1600	1620	1610	1610	1600	1600	0.031	ns
Iron	19.00	17.00	16.00	13.00	13.00	19.00	17.00	16.00	15.00	15.00	0.21	ns
Zinc	30.2	30.2	30.2	30.2	30.0	30.2	30.2	30.2	30.0	30.0	0.01	ns
Copper	3.6	3.5	3.5	3.5	3.5	3.6	3.6	3.5	3.5	3.5	0.04	ns
Manganese	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	0.02	ns
Iodine	0.12	0.10	0.10	ND	ND	0.12	0.10	ND	ND	ND	.001	ns
Selenium	0.100	0.100	0.100	ND	ND	0.100	0.100	0.100	ND	ND	.000	ns

WMW; whole (full-fat) mealworms

D-OMW; De-oil mealworms

ns, non-significant ($P > 0.05$); SEM, standard error of mean; Sig, significance

ND, not detected

Effects of storage interval on the vitamin levels of WMW and D-OMW are presented in Table 4. Both WMW and D-OMW contained substantial detectable levels of various vitamins particularly B-complex vitamins. Both meal worms contained significant quantities of inositol and choline. However, lower contents of folic acid, biotin, and pyridoxine were found.

In the present study, no growth of *E. coli*, *Salmo-*

nella, *Campylobacters*, and *Clostridium perfringens* contamination was noted in WMW and D-OMW throughout the trial. However, significantly low levels of other coliforms, *Enterococci*, and *Staphylococcus aureus* were detected in D-OMW than in WMW. Nonetheless, the aflatoxin B1 contamination levels were lower in D-OMW than that of WMW (Table 5).

Table 3. Effects of storage interval (days 0 to 56) on the amino acid contents (g/kg crude protein) of oven-dried whole (full-fat) mealworms (WMW) and de-oiled (D-OMW) mealworms

	WMW					D-OMW					SEM	Sig
	day-0	d-14	d-28	d-42	d-56	day-0	d-14	d-28	d-42	d-56		
Isoleucine	8.82	8.82	7.64	6.89	6.83	8.81	8.80	8.63	7.89	6.87	.008	ns
Leucine	13.6	13.4	11.6	10.7	9.37	13.6	13.4	12.9	11.7	9.9	.025	ns
Lysine	10.8	9.98	9.96	8.79	7.94	10.7	9.98	9.93	9.76	8.98	.018	ns
Methionine	3.15	2.89	2.59	2.57	2.38	3.12	3	2.98	2.95	2.79	.005	ns
Phenylalanine	8.67	8.56	8.45	8.29	8.27	8.72	8.62	8.53	8.35	8.34	.026	ns
Proline	11	11	10.7	9.86	8.67	11	11	10.6	9.62	9.78	.038	ns
Serine	8.5	8.15	7.97	7.58	6.89	8.5	8.05	7.94	7.59	7	.027	ns
Threonine	7.55	7.35	6.9	5.96	5.88	7.5	7.45	6.89	6.02	5.94	.032	ns
Tryptophan	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	.000	ns
Tyrosine	13.1	13	11.9	10.9	10.6	13	13	11.8	11.6	10.9	.035	ns
Valine	12.5	12.1	10.6	10.5	9.86	12.5	12.1	11.6	10.7	10.5	.023	ns

ns, non-significant ($P > 0.05$); SEM, standard error of mean; Sig, significance

Table 4. Effects of storage interval (days 0 to 56) on the vitamins profile of oven-dried whole fat mealworms (WMW) and de-oiled mealworms (D-OMW)

Vitamins	WMW					D-OMW					SEM	Sig
	day-0	d-14	d-28	d-42	d-56	day-0	d-14	d-28	d-42	d-56		
Vitamin A (IU/kg)	3400	3400	3370	3370	3370	3400	3400	3400	3380	3370	2.43	ns
Vitamin D ₂ (IU/kg)	520	520	510	510	510	510	500	500	500	500	.012	ns
Vitamin E (mg/kg)	163	160	155	155	155	163	160	155	155	150	1.13	ns
Vitamin C (mg/kg)	100	100	90	90	90	100	100	100	100	90	.034	ns
Thiamine (mg/kg)	1.7	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	.002	ns
Riboflavin (mg/kg)	11.4	10.3	10	10	9.70	11.4	10.3	9.38	9.38	9.15	1.13	ns
Niacin (mg/kg)	35.3	35.3	34.1	34.0	33.8	35.3	35.3	34.8	34.5	34.1	1.21	ns
Pyridoxine (mg/kg)	3.70	3.70	3.70	3.70	3.70	3.70	3.70	3.70	3.70	3.70	.001	ns
Folic Acid (mg/kg)	0.65	0.65	0.64	0.59	0.59	0.65	0.65	0.62	0.62	0.62	.001	ns
Biotin (mg/kg)	0.38	0.34	0.34	0.25	0.25	0.38	0.30	0.30	0.25	0.25	.014	ns
Choline (mg/kg)	1,246	1,242	1240	1240	1240	1250	1250	1247	1245	1245	.051	ns
Inositol (mg/kg)	225	220	220	215	210	225	225	220	220	220	1.23	ns

ns, non-significant ($P > 0.05$); SEM, standard error of mean; Sig, significance

Table 5: Effects of storage interval (days 0 to 56) on microbiological count (CFU/g) and aflatoxin B1 ($\mu\text{g}/\text{kg}$) contamination of WMW and D-OMW

	WMW					D-OMW					SEM	Sig
	day-0	d-14	d-28	d-42	d-56	day-0	d-14	d-28	d-42	d-56		
<i>E. coli</i>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
<i>Salmonella</i>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
<i>Other coliforms</i>	2.5	3	5.5	6.5	7	2.5	3	4.5	6.5	6	.001	***
<i>Enterococci</i>	2.3	4.3	4.8	5.2	5.3	2.30	4.30	4.20	4.80	5.1	.002	***
<i>Campylobacters</i>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
<i>S. aureus</i>	2.1	5.1	6.1	6.1	7.1	2.1	5.1	5.1	5.1	5.5	.013	**
<i>Cl. perfringens</i>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
Aflatoxin-B1	.01	.81	1.7	2	3.01	.01	.21	.70	.903	2.01	.021	***

WMW; whole (full-fat) mealworms

D-OMW; De-oil mealworms

SEM, standard error of mean; Sig, significance; ***, $P < 0.001$

DISCUSSION

Mealworms have been identified as a potential source of protein, fats, amino acids, and fatty acids (Food and Agriculture Organization (FAO) in 2012 and 2013). Due to their nutrient-rich composition, mealworms are considered a valuable supplement for animal feed and can be used to address under nutrition in certain populations, such as children (Chakravarthy et al., 2016). Compared to conventional livestock species, mealworms are generally considered to emit fewer greenhouse gases (Oonincx et al., 2010). Owing to the short generation interval of insects and their superior nutritional quality make them a promising tool to address issues of hunger and poverty, particularly in developing countries such as Pakistan. Insect rearing has the potential to produce natural, high-protein animal feed products that can help address protein deficiencies in both animal and human diets. However, safety is a major concern for the insect-rearing community, especially when it comes to harvesting and processing insects for human consumption. In this context, drying is considered the most commonly used methods for processing insect biomass, and it has been shown to be effective in preserving the nutrient properties and safety of insect-based products (Hernandez-Alvarez et al., 2021; Parniakov et al., 2022). In the present study, we examined the stability and safety of post-oven drying of WMW and D-OMW stored under room temperature for 56 days.

It is interesting to compare the high nutritional value of mealworms with the nutrient content of oven-dried WMW and D-OMW. Both of these by-products are also rich sources of nutrients, particularly proteins and fats, and fall within the reported range of values in the literature for mealworms (Nowak et al., 2016). Based on the evidence published to date, mealworms have been found to contain 30% to 65% CP (Rumpold and Schluter, 2013; Van Huis, 2013), which is comparable to soybean meal. This highlights the potential of using these by-products as alternative protein sources for animal feed (Jin et al., 2016). However, as mentioned earlier, the DM content of WMW and D-OMW can change during storage, which can affect the nutrient content. In terms of CP content, the stability of D-OMW during storage suggests that it may be a more reliable source of protein compared to WMW, which experiences some reduction in CP content over time. This reduction could be due to various factors such as microbial growth, enzymatic activity, and the oven-drying process used to produce WMW.

Literature shows that the differences in the mineral composition of insect-based products may be due to various factors, including the substrate used, rearing conditions, and analytical protocols (Bonazzi and Dumoulin, 2011). However, this study found that both fractions of oven-dried mealworms were rich sources of minerals, and also the mineral profile of these products did not significantly change during the storage interval. Despite this, literature suggests that most edible insects are good sources of vitamins, particularly vitamin B complex (Finke, 2002; Bukkens 2005). In this study, the thiamine (vitamin B1) content in both WMW and D-OMW was found to range from 1.4 to 1.7 mg per kg of dry tissue. However, edible insects are not considered to be good sources of vitamin A and α -tocopherol contents, according to available literature (Bukkens, 2005). Additionally, moderate levels of choline and inositol were also found in mealworms, further highlighting their potential as a valuable source of nutrients for animal feed.

It is widely accepted in the literature that the quality of protein is determined by its amino acid profile (Rumpold and Schluter, 2013). The *Tenebrio molitor* meal has been reported to contain a higher concentration of lysine, methionine, and threonine (De Marco et al., 2015). In this study, both of the oven-dried mealworms were found to be a good source of most essential amino acids. Interestingly, the amino acid profile of drying mealworms in this study was found to be comparable to that of soybean and fishmeal (Hussain et al., 2017). However, in both WMW and D-OMW, methionine was the limiting amino acid in the drying sample and during the entire storage period. This means that commercial synthetic methionine must be incorporated into the diet of poultry if mealworms are used as a protein supplement.

Recently various research studies have demonstrated the impact of different drying procedures on microbial testing of mealworms (Grabowski and Klein, 2017; Kroncke et al., 2019). Importantly, spore-forming bacteria such as *Clostridium* species that can cause food poisoning in humans have been reported in yellow mealworms (Swickett et al., 2016; Vandeweyer et al., 2017). Microbiological analysis of mealworms, in this study, shows that there was no detection of microbial growth for *E. coli*, *Salmonella*, *Campylobacter*, and *Clostridium perfringens* in WMW and D-OMW throughout the trial.

According to our results, there was no detection of microbial growth for *E. coli*, *Salmonella*, *Campy-*

lobacters, and *Clostridium perfringens* in either over-dried WMW or D-OMW throughout the trial. The absence of microbial growth in both fractions of the mealworms in this study indicates that the drying and storage conditions used were effective in preventing the growth of these harmful bacteria. The absence of pathogenic bacteria such as *E. coli* and *Salmonella* in mealworms, as reported in both Ravzanaadii et al. (2012) and the present study, suggests that they can be safely consumed by humans or used as animal feed. However, it is important to note that other microorganisms, such as coliforms, Enterococci, and *Staphylococcus aureus*, were detected in some samples in the present study. Therefore, proper processing, handling and storage of mealworms are still necessary to ensure food safety.

The lower contamination levels of aflatoxin B1 in D-OMW compared to WMW could be attributed to the lower moisture content in D-OMW. High moisture content in WMW may facilitate higher microbial growth, which in turn increases the risk of aflatoxin contamination. Although there is no regulation for aflatoxin contamination in insects, the EU has set a maximum safe level (10-15 µg kg⁻¹) for foodstuffs of plant origin. Interestingly, a study by Bosch et al. (2017) reported that *Tenebrio molitor* larvae have high tolerance against aflatoxin B1, which could also contribute to the lower contamination ratio of aflatoxin in this study. However, further research is needed to better understand the factors influencing aflatoxin contamination in insects and to establish safe limits for consumption.

CONCLUSION

Based on the results of this study, it can be concluded that D-OMW is a safe and stable source of nutrients even after storage for extended periods at room temperature without any preservatives. The nutrient profile of D-OMW remained almost stable during the entire storage period, and there was no significant change in the mineral and amino acid profiles. Moreover, the absence of microbial growth for *E. coli*, *Salmonella*, *Campylobacter*, and *Clostridium perfringens* in both fractions of mealworms indicated that the drying and storage conditions used were effective in preventing the growth of harmful bacteria, and they are safe for consumption or use as animal feed. However, it is important to note that aflatoxin B1 contamination levels were higher in WMW than D-OMW. The high toxins load may be associated with the high fat contents being attacked by the fungal spores. The same spores in the deoiled samples may have not thrived well due to a lack of fat substrate for their growth. Overall, D-OMW can be considered a safe and stable source of nutrients for human food consumption or animal feed.

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CONFLICT OF INTEREST

None declared.

REFERENCES

- AOAC (1990). Methods of Analysis of the Association of Official Analytical Chemists, Association of Official Analytical Chemists, Washington, DC, USA, 1990.
- AOAC (1995). Association of Official Analytical Chemists, 16th ed. Arlington, VA, USA.
- Bonazzi C, Dumoulin E (2011). Quality changes in food materials as influenced by drying processes. *Modern Dry Technol* 3: 1-20.
- Bosch G, van der Fels-Klerx HJ, de Rijk TC, Oonincx DGAB (2017). Aflatoxin B1 tolerance and accumulation in black soldier fly larvae (*Hermetia illucens*) and yellow mealworms (*Tenebrio molitor*). *Toxins* 9: 185. <https://doi.org/10.3390/toxins9060185>
- Bukkens SGF (2005). Insects in the human diet: nutritional aspects. Ecological implications of mini livestock, role of rodents, frogs, snails, and insects for sustainable development. Science Publishers, New Hampshire. 545-577.
- Chakravarthy AK, Jayasimha GT, Rachana RR, Rohini G (2016). Insects as human food. In: Chakravarthy A, Sridhara S, (eds) Economic and ecological significance of arthropods in diversified ecosystems. Springer, Singapore, 133-146. https://doi.org/10.1007/978-981-10-1524-3_7
- De Marcoa M, Martinez S, Hernandez F, Madrid J, Gai F, Rotolo L, Belforti M, Bergeroa D, Katz H, Dabboud S, Kovitvadhi A, Zoccarato I, Gascoc L, Schiavone A (2015). Nutritional value of two insect larval meals (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens: apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. *Anim Feed Sci Technol* 209: 211-218.
- Ravzanaadii N, Kim SH, Choi WH, Hong SJ, Kim NJ (2012). Nutritional value of mealworm, *Tenebrio molitor* as food source. *Int J Indust Entomol* 25(1): 93-98.
- EFSA Scientific Committee (2015). Risk profile related to production and consumption of insects as food and feed. *EFSA Journal* 13(10): 4257. <https://doi.org/10.2903/j.efsa.2015.4257>
- FAO (2012). Assessing the potential of insects as food and feed in assuring food security. Food and Agriculture Organization of the United Nations (FAO), Summary Report, Rome, Italy, 1: 1-38.
- FAO (2013). Edible insects: future prospects for food and feed security. Food and Agriculture Organization of the United Nations, (FAO),

- Rome, Italy. <http://www.fao.org/i3253e.pdf>
- Finke MD (2002). Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biology*: Published in Affiliation with the American Zoo and Aquarium Association 21(3): 269-285.
- Parniakov O, Mikhrovska M, Wiktor A, Alles M, Ristic D, Bogusz R, Nowacka M, Devahastin S, Mujumdar A, Heinz V, Smetana S (2022). Insect processing for food and feed: A review of drying methods. *Drying Technology*, 40(8), 1500-1513.
- Hernandez-Alvarez AJ, Mondor M, Pina-Dominguez IA, Sanchez-Velazquez OA, Melgar-Lalanne G (2021). Drying technologies for edible insects and their derived ingredients. *Drying Technology*, 39(13), 1991-2009.
- Gilbert J, Anklam E (2002). Validation of analytical methods for determining mycotoxins in foodstuffs. *Trends Anal Chem* 21(6-7): 468-86.
- Grabowski NT, Klein G (2017). Bacteria encountered in raw insect, spider, scorpion, and centipede taxa including edible species, and their significance from the food hygiene point of view. *Trends Food Sci Technol* 63: 80-90.
- Hussain I, Khan S, Sultan A, Chand N, Khan R, Alam W, Ahmad N (2017). Mealworm (*Tenebrio molitor*) as potential alternative source of protein supplementation in broiler. *Int J Biosci* 10(4): 225-262.
- Jin XH, Heo PS, Hong JS, Kim NJ, Kim YY (2016). Supplementation of dried mealworm (*Tenebrio molitor* larva) on growth performance, nutrient digestibility and blood profiles in weaning pigs. *Asian-Australas J Anim Sci* 29: 979-986.
- Kartsova LA, Koroleva OA (2007). Simultaneous determination of water and fat soluble vitamins by high-performance thin-layer chromatography using an aqueous micellar mobile phase. *J Anal Chem* 62: 255-259.
- Khan S, Khan RU, Alam W, Sultan A (2018). Evaluating the nutritive profile of three insect meals and their effects to replace soya bean in broiler diet. *J Anim Physiol Anim Nutr* 102(2): e662-e668.
- Khan S, Khan RU, Sultan A, Khan M, Hayat SU, Shahid MS (2016). Evaluating the suitability of maggot meal as a partial substitute of soya bean on the productive traits, digestibility indices and organoleptic properties of broiler meat. *J Anim Physiol Anim Nutr* 100: 649-656.
- Khan K, Khan S, Ullah S, Khan NA, Khan I, Ahmad N (2017). Nutritive value, fiber digestibility and methane production potential of tropical forages in rabbits: effect of species and harvest maturity. *J Anim Plant Sci* 27: 1094-1100.
- Klunder HC, Wolkers-Rooijackers J, Korpela JM, Nout MJR (2012). Microbiological aspects of processing and storage of edible insects. *Food Control* 26: 628-631.
- Kroncke N, Grebenteuch S, Keil C, Demtroder S, Kroh L, Thunemann AF, Benning R, Haase H (2019). Effect of different drying methods on nutrient quality of the yellow mealworm (*Tenebrio molitor* L.). *Insects* 10: 84.
- Makkar PSH, Tran G, Heuze V, Ankers P (2014). State of the art on use of insects as animal feed. *Anim Feed Sci Technol*, 197: 1-33.
- Nowak V, Persijn D, Rittenschober D, Charrondiere UR (2016). Review of food composition data for edible insects. *Food Chem* 193: 39-46.
- Oonincx DGAB, Van Itterbeeck J, Heetkamp MJW, Van den Brand H, Van Loon JJA, Van Huis A (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS One* 5(12): e14445.
- Ramashia SE, Tangulani T, Mashau ME, Nethathe B (2020). Microbiological quality of different dried insects sold at Thohoyandou open market, South Africa. *Food Res* 4: 2247-2255.
- Rumpold BA, Schluter OK (2013). Nutritional composition and safety aspects of edible insects. *Mol Nutr Food Res* 57: 802-823.
- Shang-Gui D, Zhi-Ying P, Fang C, Ping Y, Tie W (2004). Amino acid composition and anti-anaemia action of hydrolyzed offal protein from Harengula Zunasi Bleeker. *Food Chem* 87(1): 97-102.
- Stastnik O, Novotny J, Roztocilova A, Kouril P, Kumbar V, Cernik J, Kalhotka L, Pavlata L, Lacina L, Mrkvicova E (2021). Safety of mealworm meal in layer diets and their influence on gut morphology. *Anim* 11: 1439.
- Swick MC, Koehler TM, Driks A (2016). Surviving between hosts: sporulation and transmission. *Microbiol Spectr* 4(4): 567-591.
- Van Huis A (2013). Potential of insects as food and feed in assuring food security. *Annu Rev Entomol* 58: 563-583.
- Vandeweyer D, Lenaerts S, Callens A, Van Campenhout L (2017). Effect of blanching followed by refrigerated storage or industrial microwave drying on the microbial load of yellow mealworm larvae (*Tenebrio molitor*). *Food Contr* 71: 311-314.